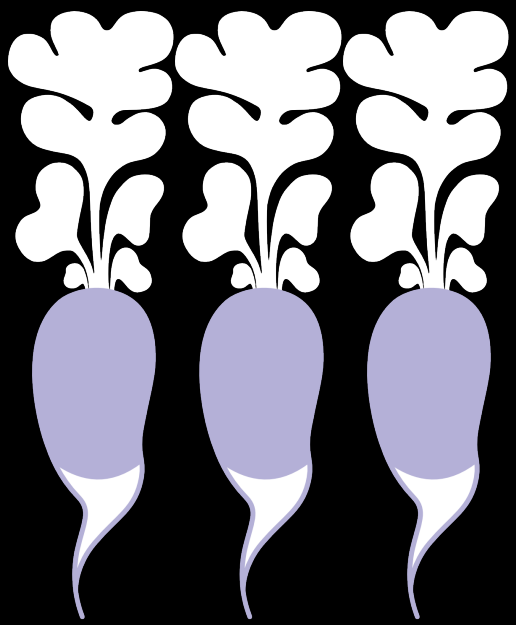
2024

The Enteric Immune Landscape in Response to High-Fat High-Sugar Diet

Kristin Fischer



Dissertation presented to obtain the **Ph.D degree in Neuroscience**

Oeiras, December, 2024

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER | UNIVERSIDADE NOVA DE LISBOA

The Enteric Immune Landscape in Response to High-Fat High-Sugar Diet

Cover: "Enteric Sugar Beets" by 4tin.

The Enteric Immune Landscape in Response to High-Fat High-Sugar Diet

Kristin Fischer

A dissertation presented to the Universidade Nova de Lisboa

in candidacy for the degree of

Doctor of Philosophy in Neuroscience

Supervised by Dr. Henrique Veiga-Fernandes

International Neuroscience Programe

Champalimaud Research

Lisbon, Portugal

2024

Acknowledgement

I would like to thank my supervisor, Henrique, for giving me the opportunity to pursue a PhD in his lab. I'm also thankful to all lab members for sharing protocols: Masha, Cristina, Roksana, Miguel, David, Ana, Inês, Raquel, Patricia, Beatriz and Maria B. I'm especially thankful to María M. for her help and contribution and for showing me the beautiful world of dendritic cells. This thesis would not have been possible without your help! And to Bruno for always being there with a helping hand.

I would also like to thank the jury members, Salvador, Manuela, Carlos and Inês, for accepting to be part of this last step and reading through this thesis. I'm also thankful to my Thesis Committee Members, Marc and Carlos.

To Oihane, who majorly contributed to data analysis and enthusiastically helped me through the last year of my PhD. Thank you for lending me your brain, computer, space and patience.

To my INDP class of 2018, especially Mirjam, Nuno, Matheus and Teresa, who directly contributed to this PhD.

To the platforms, especially Flow Cytometry, MGP, and the Vivarium, without none of this work would have been possible. But also Teaching lab and Microscopy, who helped out with computing powers.

To all the mice that participated in the study and were sacrificed to gain knowledge. And to Thomas, my first PI in Vienna, who taught me how to handle mice and keep their welfare in mind.

I'm thankful to FCT for the financial support and ITQB for their logistical support. And to everyone in the GSO, especially Teresa.

It takes a village to write a thesis, and therefore, I'm thankful to Megha, Inês, Cecilia, Francisco and Diego for their friendship and support during these years in Lisbon. And to Pascal, who encouraged me to start a PhD in Portugal.

To my parents, Andrea and Werner, who patiently read countless 'Tu-Was' books to me when reading was still too difficult, but my curiosity about all cool things from nature couldn't be contained. They nurtured my love for science by teaching me about animals, providing me with my beloved Kosmos experiment kits, and graciously accepting that their tools and devices might become victims of my endless quest to understand how things work.

Lastly, to my 19-year-old self, who sat in her room making a mind map of all her skills and interests, ultimately discovering that Biomedicine and Biotechnology was the perfect path. Thank you for having the clarity to make that decision and the perseverance to push through 13 years of higher education.

ABSTRACT

Obesity, often induced by diet high in fat and sugar (HFHSD), is a growing global health concern associated with various metabolic and immunological alterations and profound consequences for the nervous system. HFHSD and resulting obesity have been linked to cognitive decline, increased risk of neurodegenerative diseases, and alterations in the gut-brain axis. These neurological impacts underscore the systemic nature of obesity's effects and highlight the interconnectedness of metabolic, immune, and nervous systems in the context of diet-induced obesity.

The present study investigated the impact of an HFHSD on the enteric immune system and the host physiology, with a particular focus on conventional dendritic cell type 1 (cDC1) and their role in food tolerance. Male C57BL/6J mice were fed either a standard chow or HFHSD for 4, 8 and 16 weeks. Flow cytometry analysis revealed a distinct immune composition in the small intestine dependent on diet and intestinal segment but not dependent on duration of diet. Notably, the frequency of cDC1s was increased in the obese lamina propria across all intestinal segments. This increase was consistent over time. Despite the increase in cDC1 frequency, their activation status and cytokine expression

profiles remained largely unchanged, except for decreased *Il10* mRNA expression. Analysis of cDC1 precursors and gut-homing markers showed no significant alterations, suggesting that the increased cDC1 presence may not result from enhanced production or specific homing. The oral tolerance model demonstrated that HFHSD-fed mice exhibited enhanced food tolerance, characterised by a significant increase in regulatory T cell (Treg) frequency upon antigen challenge. This finding suggests that the diet-induced shift in the enteric immune landscape may promote a more tolerogenic environment in the gut.

These results provide new insights into the complex interplay between diet, the immune system, and tolerance mechanisms. The observed enhanced tolerance to food antigens demonstrates that despite the typically pro-inflammatory state associated with obesity, the enteric immune system can maintain and even enhance regulatory functions. This observation aligns with our current understanding of the immune system that adapts to maintain homeostasis under varying dietary conditions. The increased frequency of cDC1, coupled with enhanced Treg induction, may represent an adaptive mechanism to cope with chronic metabolic stress while preventing excessive inflammation in the intestine. This work advances our understanding of how dietary choices shape immune function and suggests that some adaptations to an obesogenic diet may serve protective functions. These findings open new avenues for understanding the rising prevalence of both obesity and food intolerances worldwide while highlighting the remarkable plasticity of the intestinal immune system in response to environmental changes.

Keywords: Obesity, Enteric immune system, cDC1, Oral tolerance, Mouse, Diet

RESUMO

A obesidade, frequentemente induzida por dietas ricas em gorduras e açúcares (HFHSD), é um problema crescente para a saúde global, estando associada a várias alterações metabólicas e imunológicas com consequências profundas para o sistema nervoso. HFHSD e consequente obesidade têm sido associadas ao declínio cognitivo, aumento do risco de doenças neurodegenerativas e alterações no eixo intestino-cérebro. Estes impactos neurológicos frisam a natureza sistêmica dos efeitos da obesidade e destacam a interconexão dos sistemas metabólico, imune e nervoso no contexto da obesidade induzida pela dieta.

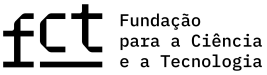
Este estudo investiga o impacto de uma HFHSD no sistema imune no intestino e na fisiologia do hospedeiro, com foco particular nas células dendríticas convencionais tipo 1 (cDC1) e o seu papel na tolerância alimentar. Ratinhos machos C57BL/6J foram alimentados com uma dieta padrão ou HFHSD durante 4, 8 e 16 semanas. A análise por citometria de fluxo revelou uma composição imune distinta no intestino delgado, dependente da dieta e do segmento intestinal, mas não dependente da duração da dieta. Em particular, a frequência de cDC1s aumentou na lâmina própria em todos os segmentos intestinais. Este aumento foi consistente ao longo do tempo. Apesar do aumento

na frequência de cDC1, o seu estado de ativação e perfis de expressão de citocinas permaneceram maioritariamente inalterados, com exceção da diminuição da expressão de *Il10* mRNA. A análise dos precursores de cDC1 e receptores de migração específica para o intestino não mostraram alterações significativas, sugerindo que o aumento da presença de cDC1 pode não resultar de produção aumentada ou migração específica. Ensaios funcionais usando o modelo OVA/OT-II demonstraram que ratinhos alimentados com HFHS exibiram tolerância alimentar aumentada, caracterizada por um aumento significativo na frequência de células T reguladoras (Treg) após desafio antigénico. Estes resultados sugerem que o aumento de cDC1 induzido pela dieta poderá promover um ambiente mais tolerante no intestino. Estes resultados fornecem novas perspetivas sobre a complexa interação entre dieta, sistema imunológico e mecanismos de tolerância. A maior tolerância que observámos a antigénios alimentares demonstra que, apesar do estado tipicamente pró-inflamatório associado à obesidade, o sistema imune do intestino pode manter e até aumentar funções regulatórias. Esta observação alinha-se com a nossa compreensão atual do sistema imune que se adapta para manter a homeostase sob diferentes condições dietéticas. O aumento da frequência de cDC1s, juntamente com a indução aumentada de Tregs, pode representar um mecanismo adaptativo para lidar com o stress metabólico crónico enquanto previne inflamação excessiva no intestino. Este trabalho contribui para uma melhor compreensão sobre como as escolhas dietéticas moldam a função imune e sugere que algumas adaptações a uma dieta obesogénica podem servir funções protetoras. Estas descobertas abrem novas possibilidades para compreender a crescente prevalência tanto da obesidade como das intolerâncias alimentares a nível mundial, destacando a notável plasticidade do sistema imune intestinal em resposta a mudanças ambientais.

Palavras-chave: Obesidade, Sistema imune do intestino, cDC1, Tolerância oral, Ratinho, Dieta

Financial Support

The work described in this thesis was performed under the International Neuroscience Doctoral Programme (INDP 2018), funded by the Fundação para a Ciência e Tecnologia (FCT) with a doctoral fellowship (SFRH/BD/148058/2019) and the Champalimaud Foundation.



CONTENTS

1 General Introduction **1**

1.1 The immune system 1

1.1.1 A brief history of the discovery of immune functions 1

1.1.2 Overview of the innate and adaptive immune system 5

1.1.3 Dendritic cells 7

1.1.3.1 Heterogeneity of conventional dendritic cells 9

1.1.3.2 Functions of cDCs 13

1.1.4 T cells 23

1.1.4.1 Regulatory T cells 25

1.1.5 The immune system of the small intestine 30

1.1.6 Food tolerance 33

1.1.6.1 Mechanistic pathways to acquire food tolerance 34

1.2 Obesity 38

1.2.1 Diet-induced obesity 39

1.2.2 General health consequences of obesity 40

1.2.3 The immune system of the obese host 43

1.2.3.1 cDC1 in obesity 45

1.2.3.2 The enteric immune system in obesity 49

1.2.3.3 Food tolerance in obesity 56

2 Research Objective and Hypothesis 59

2.1 Significance of the study 59

2.2 Hypothesis 60

2.3 Objectives 60

3 Materials and Methods 61

3.1 Experimental Animals 61

3.2 Dietary Intervention 62

3.3 Glucose Tolerance Test 63

3.4 Cell Isolation and purification 63

3.4.1 Small intestine 64

3.4.2 Liver 66

3.4.3 Pancreas 66

3.4.4 Spleen 67

3.4.5 Colon 67

3.4.6 Fat 68

3.4.7 Lymph nodes 68

3.4.8 Bone marrow 69

3.4.9 Blood 69

3.5 Flow cytometry 70

3.5.1 Cell stimulation and staining 70

3.5.2 Cell markers and gating strategy 71

3.5.3 Flow cytometry and cell sorting 75

3.6 Elisa 77

3.6.1 Tissue preparation 78

3.6.2 Elisa 78

3.7 Food tolerance 79

3.7.1 Adoptive T cell transfer 80
3.7.2 Oral antigen administration 80
3.8 Dimensionality reduction 80
3.9 Multi-linear regression 81
3.10 Statistical analysis 82
3.11 Analysis of scRNA-seq dataset 83

4 Results 85

4.1 Obesogenic diet reshapes the enteric immune system of the host 85
4.1.1 Mice on HFHS diet develop obesity 85
4.1.2 The phenotype of the enteric immune system is dependent on intestinal segment, age and dietary choices of the host 87
4.1.3 Effect of age, diet and duration of dietary intervention 89
4.1.4 Effect of diet and intestinal segments 93
4.1.5 Enteric cDC1s are consistently increased throughout long-term exposure to HFHSD 96
4.1.6 Phenotype of the enteric immune landscape is not random 99
4.1.7 Diet-induced alterations in enteric immune compartments are reproducible 101
4.2. cDC1s in the obese host 104
4.2.1 Ontogeny and homing of cDC1 are similar in the steady stat and metabolically stressed host 104
4.2.2 Enteric cDC1s exhibit functional similarities in the steady stat and metabolically stressed host 108
4.3 Food tolerance in obesity 111
4.3.1 Interaction between intestinal cDC1 and CD4+ T cells is altered in the obese host 111
4.3.2 HFHSD increases food tolerance 114

5 Discussion **116**

5.1 Regional and temporal dynamics of diet-induced immune adaptation 116

5.1.1 Considerations in experimental design 116

5.1.1.1 The choice of age and sex 116

5.1.1.2 The choice of diet 118

5.1.1.3 Definition of immunophenotypes 120

5.1.2 Plasticity of the enteric immune landscape 122

5.1.2.1 General regional and temporal effects on enteric immune composition 122

5.1.2.2 Cell type-specific responses to dietary intervention 127

5.2 cDC1 regulation and function in obesity 134

5.2.1 Selective accumulation of cDC1s in the small intestine despite unaltered ontogeny 134

5.2.2 Preserved cDC1 function in obesity 137

5.2.3 cDC1/T cell interaction in obesity 141

5.3 Physiological implications and evolutionary perspective 146

6 Conclusion **149**

7 Appendices **152**

7.1 Two-way repeated measures ANOVA between subjects comparing treatment and duration of treatment 152

7.2 Mixed-repeated measures ANOVA between and within subjects comparing treatment and intestinal segment 163

7.3 Ontogeny and distribution of cDC1 in obesity 173

7.4 Weight gain of mice on HFHSD 174

7.5 Spacial description of the enteric immune landscape 174

8 Bibliography **175**

FIGURES

Figure 1	Mouse cDC poiesis	11
Figure 2	cDC function	21
Figure 3	cDC1 involvement in food tolerance	37
Figure 4	HFHSD leads to obesity	86
Figure 5	The adult enteric immunophenotype is dependent on diet of the host and intestinal segment	88
Figure 6	Diet, duration of diet and age shape the enteric immune landscape	92
Figure 7	Diet, duration of diet and intestinal segment shape the enteric immune landscape	95
Figure 8	Kinetics of effect of diet and location on immunophenotype	98
Figure 9	Relationship between enteric immunophenotype and diet of the host	101

Figure 10	Comparison of scRNA-seq dataset from Wang et al. and our flow cytometry dataset	103
Figure 11	Similar ontogeny of cDC1s in the obese and stead-state host	107
Figure 12	HFHSD does not alter status of cDC1 in the small intestine	110
Figure 13	Functional impact of HFHSD on cDC1/T cell interaction	113
Figure 14	Functional impact of HFHSD-shaped enteric immune landscape in food tolerance	115
Figure S1	Ontogeny and distribution of cDC1s in the obese and stead-state host	173
Figure S2	Regional comparison of immune cell types in steady-state	174

TABLES

Table 1	Experimental animal models	62
Table 2	Reagents for dietary intervention	62
Table 3	Reagents and materials for intraperitoneal glucose tolerance	63
Table 4	Reagents and materials for cell isolation and purification	64
Table 5	Reagents for cell stimulation and staining	70
Table 6	Antibodies for cell staining	75
Table 7	Reagents for RNA extraction and RT-qPCR	76
Table 8	Reagents for ELISA	77
Table 9	Reagents for food tolerance experiments	79
Table S1	Effect of diet and duration of diet on ratio of cell types in $CD45^+$ cells	157
Table S2	Pairwise comparison of effect of diet and duration of diet on ratio of cell types in $CD45^+$ cells	162
Table S3	Effect of diet and intestinal segment on ratio of cell types in $CD45^+$ cells	167
Table S4	Pairwise comparison of effect of diet and intestinal segment on ratio of cell types in $CD45^+$ cells	173

Table S5 Comparison of body weight of HFHSD-fed mice throughout time 174

ABBREVIATIONS

APC	Antigen presenting cell
ASC	Antibody-secreting cell
ATM	Adipose tissue macrophage
BCR	B cell receptor
BMI	Body Mass Index
cAMP	Cyclic adenosine monophosphate
CD	Chow or control diet
CD#	Cluster of differentiation + number
CD103	E-cadherin–binding integrin αE
CD62L	L-selectin
cDC	Conventional dendritic cell
cDC1	Conventional dendritic cell type 1
cDC2	Conventional dendritic cell type 2
CDP	Common DC progenitor
CLEC9A	C-type lectin receptor DNGR-1
CLT	C-type lectin receptor
cRPMI	Complete RPMI

CTL	Cytotoxic T lymphocyte
DAMPs	Damage-associated molecular pattern
DC	Dendritic cell
DEG	Differentially expressed gene
DIO	Diet-induced obesity
dLN	Draining lymph node
dpDC	Double positive conventional dendritic cell
dpDC	double positive cDC
dpIEL	Double positive intraepithelial lymphocyte
EDTA	Ethylenediaminetetraacetic acid
FAE	Follicle-associated epithelium
Flt3	FMS-like tyrosine kinase 3, CD135 or Flk2
Flt3L	FMS-like tyrosine kinase 3 ligand
GALT	Gut-associated lymphoid tissue
Gzmb	Granzyme B
HDL	High-density lipoprotein
HFD	High-fat diet
HFHSD	High-fat high-sugar diet
HSC	Hematopoietic stem cells
IBD	Inflammatory bowel disease
IDO	Indoleamine 2,3-dioxygenase
IEL	Intraepithelial lymphocyte
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IGTT	Intraperitoneal glucose tolerance test
IL	Interleukin
ILC	Innate lymphoid cell
iNKT	NK1.1^{+} invariant natural killer cells

LDL	Low-density lipoprotein
LN	Lymph node
LXR	Liver X receptor
M cell	Microfold cell
MHC	Major histocompatibility complex
MHC I	Major histocompatibility complex class I
MHC II	Major histocompatibility complex class II
mLN	Mesenteric lymph ode
mregDC	Mature regulatory DC
Mφ	Macrophage
NAFLD	Non-alcoholic fatty liver disease
NASH	Alcoholic steatohepatitis
NK	Natural killer cell
OVA	Ovalbumin
PAMPs	Pathogen-associated molecular pattern
PBS	Phosphate-buffered saline
PCA	Principal component analysis
pDC	Plasmacytoid dendritic cells
pMHC	peptides presented on major histocompatibility complex
pre-cDC	Pre conventional dendritic cell
pre-cDC1	Pre conventional dendritic cell type 1
pre-cDC2	Pre conventional dendritic cell type 2
PRR	Pattern recognition receptor
pTreg	Peripheral or induced regulatory T cell
RAG	Recombination activating gene
SC	Stem cell
SCFAs	Short-chain fatty acids
scRNA-seq	Single-cell RNA sequencing

TCR	T cell receptor
Tfh	Follicular helper T cell
TGF-β	Transforming growth factor beta
Th	T helper lymphocyte
Th3	T helper 3 cell
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor α
Tr1	Type 1 regulatory T cell
Treg	Regulatory T cell
tTreg	Thymic or natural regulatory T cell
WAT	White adipose tissue

1

General Introduction

1.1 The immune system

1.1.1 A brief history of the discovery of immune functions

The immune system is a complex and dynamic entity in multicellular organisms that has long been recognised as crucial for human health. However, our understanding of its function has undergone significant transformations since the emergence of immunology as a scientific discipline.

The initial understanding of immune function was deeply rooted in the germ theory of disease, developed by Louis Pasteur and Robert Koch in the late 19th century, according to which germs (pathogens) are the cause of disease (Koch, 1982). The immune system was primarily viewed as a defence mechanism, protecting the body against pathogenic microorganisms. This perspective was reinforced by Emil von Behring and Kitasato Shibasaburo's discovery in 1890 that serum from Diphtheria-infected animals can be used to prevent and treat infection in other animals (Behring and Kitasato, 1890). Their work led to the concept of humoral immunity and antibodies, and a few years later, Paul Ehrlich

offered an early mechanistic explanation for how the immune system might recognise and neutralise pathogens (Ehrlich, 1091, 1997). His side-chain theory introduced the concept of specific recognition, a fundamental principle that continues to shape our understanding of immune function.

The mid-20th century brought a significant expansion in the ideas about immune functions. One of the foundational concepts from this time is the clonal selection theory, introduced by Macfarlane Burnet in 1957 (Burnet, 1957, 1959). He proposed that lymphocytes with receptors specific to antigens develop spontaneously and proliferate and develop in response to their recognition in a clonal manner. Now, the generation and selection of a vast array of potential specificities in the immune repertoire, crucial for adaptive immune responses, could be explained. This idea of specific recognition and response forms the basis of our understanding of how the body can target and remember specific pathogens, a feature primarily attributed to the adaptive immune system. It also introduced the critical concept of self/non-self discrimination, according to which the immune system's primary function is to distinguish between "self" and "non-self", eliminating foreign entities while preserving the body's tissues (Burnet, 1957). Yet another major milestone in immunology was the discovery of T cells and their role in cellular immunity by Jacques Miller in 1961 (Miller, 1961). The immune system was now seen as capable of targeting not just extracellular pathogens but also intracellular threats and altered self-cells, such as tumours.

As research progressed, it became evident that the function of the immune system extended beyond simple self/non-self discrimination. The discovery of suppressor T cells (later renamed regulatory T cells) by Richard Gershon and Kazunari Kondo in the 1970s introduced the concept of active immune regulation (Gershon and Kondo, 1970). This shifted the paradigm from viewing the immune system as purely a defence mechanism to understanding it as a carefully

regulated system capable of both activation and suppression. It shall be noted that the concept of immunological tolerance, however, was already proposed several decades earlier, when Ray Owen could observe natural chimerism in twin cattle that often have two distinct blood types — their own and also the type of their twin (Owen, 1945).

The late 20th century brought about another major shift in our understanding of immune function. Research began to reveal that the immune system plays crucial roles in processes seemingly unrelated to host defence. For instance, Polly Matzinger proposed the danger model in 1994, which postulates the immune response to danger signals (Matzinger, 1994). According to this model, the immune system is activated by signals from cells in distress, not just by the presence of non-self molecules. This approach helped integrate the immune system's role in tissue integrity and repair processes to maintain organismal balance. Complementing the danger model, the tolerance model described by Ruslan Medzhitov and colleagues in 2012 emphasises the immune system's capacity for disease tolerance (Medzhitov et al., 2012). It recognises the system's ability to reduce the impact of infection on the host's fitness, protecting the organism from infectious diseases via mechanisms that do not directly attack the pathogen but rather mitigate its effects. Both the danger and tolerance models suggest that the immune system is activated by detecting damage inflicted on cells and tissues by microbes or other sources to maintain overall physiological homeostasis. However, microbial products like LPS can activate the immune system without causing direct damage, acting as indicators of potential danger. Building on this, Thomas Pradeu and colleagues proposed in 2013 that the immune system is fundamentally activated by "discontinuity" or changes in normal physiological states rather than by non-self or danger alone (Pradeu et al., 2013). Conversely, immune cells become tolerant to stable or continuously present motifs, while an effector response is triggered by rapid, discontinuous

changes in molecular motifs. Here, the magnitude of immune response is determined by the rate of these changes. This perspective underscores the importance of immune history and the adaptive nature of the immune system in responding to evolving environmental challenges. In their seminal work from 2017, Henrique Veiga-Fernandes and António Freitas proposed the S(c)ensory immune system theory (Veiga-Fernandes and Freitas, 2017). They describe the immune system as a sensory organ that perceives, integrates and responds to environmental and intra-organismal cues to re-establish homeostasis within its soma and frontiers, ensuring cell survival and perpetuation – similar to the nervous system. This way, it is seen as part of the larger sensory interface of vertebrates.

In the meantime, the rapidly expanding field of microbiome research has led to yet another reconsideration of immune system function. The holobiont concept, which views a host and its associated microorganisms as a single ecological unit, has profound implications for our understanding of immunity (Bordenstein and Theis, 2015). In this context, a key function of the immune system is to manage the host's relationship with its microbiome, fostering beneficial interactions while preventing harmful overgrowth or invasion. This involves maintaining a delicate balance between tolerance and responsiveness, further highlighting the immune system's role in homeostasis (Belkaid and Hand, 2014).

Our understanding of the role of the immune system has evolved dramatically from a static, defence-only perspective to a dynamic, integrated view that encompasses not just defence but also the maintenance of homeostasis and adaptation to environmental changes. Today, we recognise that the immune system performs a multitude of functions: it defends against pathogens, maintains self-tolerance, regulates tissue repair and regeneration, influences

metabolism, and manages microbial symbiosis. This evolving perspective has profound implications for medicine and human health. It suggests that allegedly harmless environmental changes might reshape the immune system and result in immune dysfunction. It may also underlie a much broader range of diseases than previously thought, from metabolic disorders to neurodegenerative conditions. As our understanding continues to grow, it promises to open new avenues for therapeutic interventions and strategies for promoting health and longevity.

1.1.2 Overview of the innate and adaptive immune system

The immune system of vertebrates consists of two primary arms: the innate (general) and adaptive (specialised) immune systems (Medzhitov and Janeway, 2000). The innate immune system displays highly conserved and ready-to-use environmental sensors. It comprises physical barriers such as epithelial surfaces and mucosal linings, as well as a series of cellular and molecular antimicrobial components. Among these are the complement system, antimicrobial peptides, and a variety of innate immune cells, including phagocytes (such as dendritic cells (DC), macrophages (Mφ), monocytes, and the granulocytes), natural killer cells (NK), innate lymphoid cells (ILC), basophils, eosinophils, neutrophils and mast cells. The initiation of the immune response is governed by non-specific markers that are shared amongst pathogens, known as pathogen associated molecular patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs) that can occur during tissue or cell damage (Matzinger, 2002). PAMPs and DAMPs are recognised by pattern recognition receptors (PRRs) located on or within phagocytic cells of the host (Takeuchi and Akira, 2010). The innate immune response occurs rapidly after activation. It blocks pathogen invasion or initiates an inflammatory response within minutes to hours after infection. The innate response has a memory effect called trained immunity, by

which a primary stimulus can alter innate immune cells metabolically or epigenetically (Netea et al., 2016). A secondary stimulus then leads to either increased or decreased response. Another important role of innate immune cells is the connection between the innate and adaptive immune system. This function was first posited by Charles Janeway in 1989 but only well accepted about a decade later (Janeway, 1989). Professional antigen-presenting cells (APCs), especially DCs, were demonstrated to be responsible for the initiation of antigen-specific T cell responses (Banchereau and Steinman, 1998). DCs endocytose and digest pathogens and present their antigens on the cell surface to T cells via major histocompatibility complexes (MHC), activating the adaptive immune system.

In addition to innate defences, vertebrates have developed the adaptive immune system (Medzhitov and Janeway, 2000). It is evolutionarily younger than the innate system and mediated by lymphocytes. T and B lymphocytes are equipped with receptors that are capable of recognising self and non-self structures in an antigen-specific manner. The vast diversity of receptors on these cells is generated in the germline through rearrangement and recombination of immunoglobulin and T cell receptor genes in lymphocytes. This process is conducted by recombination enzymes that are encoded in recombination activating genes (RAG). RAGs are currently viewed as core to the adaptive immune system (Schatz and Ji, 2011). T lymphocytes confer cell-mediated immunity, while B cells give humoral immunity. B cells develop and mature in the bone marrow and express B cell receptors (BCR), which are specific for antigens due to RAGs. Activated B cells proliferate and differentiate into either plasma cells, which secrete vast quantities of antibodies against the antigen, or into memory B cells, which maintain long-lasting immunity against the specific antigen. Precursors of T cells are also generated in the bone marrow. However, T cells mature in the thymus, where they undergo selection and differentiation to

ensure they display functional and non-self-reactive T cell receptors (TCRs) that rise based on RAGs (Nemazee, 2006). Naïve lymphocytes encounter antigens displayed on APCs in peripheral lymphoid organs and undergo maturation. This results in the differentiation of T cells into either cluster of differentiation (CD) 4^+ T helper (Th) lymphocytes or $CD8^+$ cytotoxic T lymphocytes (CTLs) (Zhu et al., 2010). Unlike the innate immune response, the adaptive immune response becomes effective only a few days after infection, as this time is required for T and B cells to interact with the antigen, proliferate, and differentiate into effector cells (Bonilla and Oettgen, 2010). Once activated, these cells can give rise to memory cells, enhancing the efficiency of immune responses upon subsequent encounters with the same antigen, which is the fundamental principle of vaccination (Pulendran and Ahmed, 2011). The adaptive immune system also harbours strategies aiming at reducing the negative impact of immune reaction to infection and protecting the host's fitness through disease tolerance (Medzhitov et al., 2012). A growing body of evidence also indicates that nutritional resources can be sensed by lymphocytes and influence their state (Goverse et al.; 2016 Pavert et al., 2014).

1.1.3 Dendritic cells

DCs are sentinel cells of the immune system, uniquely positioned at the crossroads of innate and adaptive immunity. Their discovery by Ralph Steinman and Zanvil Cohn in the 1970s marked a significant breakthrough in immunology, and Ralph Steinman's contributions to this field were later honoured with several prestigious awards, including the Nobel Prize in Physiology or Medicine in 2011 (Nobel Media, 2011; Steinman and Cohn, 1973).

DCs are characterised by their distinct stellar or dendritic morphology, which inspired their name, and by their ability to capture, process, and present antigens

to T cells. They have the unique ability to initiate adaptive immune responses against foreign antigens but also to maintain immune homeostasis or tolerance to prevent unwanted immune reactions (Banchereau and Steinman, 1998). DCs are positioned strategically throughout the body in both lymphoid and non-lymphoid tissues (1-5% of tissue cells) (Austyn, 1987). As antigen presenting cells, they detect pathogens, dead cells, and other environmental signals and initiate appropriate immune responses. Upon activation, DCs undergo significant changes, including the up-regulation of MHC class II (MHC II) and co-stimulatory molecules, preparing them for T cell interaction (Cella et al., 1997). In this process, they migrate to T cell rich regions and especially this migration capacity makes them irreplaceable in their ability to mount immune responses. However, the role of DCs extends beyond mere antigen presentation. They are also pivotal in determining the nature of the immune response — whether it will lead to tolerance or active immunity. Their ability to produce various cytokines enables them to influence the differentiation of T cells into diverse subsets like Th1, Th2, Th17, and regulatory T cells (Tregs), thus tailoring immune responses to specific signals (Kapsenberg, 2003).

DCs originate from a distinct haematopoietic lineage, differentiating them from other leukocytes. They can be broadly categorised into two main subsets: classical dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs) (Liu and Nussenzweig, 2010). cDCs are known for their potent antigen-presenting capabilities, crucial for the activation of T cells and the initiation of adaptive immunity. In contrast, pDCs are recognised for their ability to produce large quantities of type I interferons in response to viral infections, playing a critical role in the body's antiviral defence (Reizis, 2019; Swiecki and Colonna 2015; Colonna et al., 2004).

The realm of DCs is complex, partially due to the diverse types of cells often grouped under the DC umbrella, including not only plasmacytoid DCs but also Langerhans cells and monocyte-derived DCs. At times, this diversity has led to confusion about what constitutes a bona fide DC and how to describe its various functions (Merad et al., 2013). This thesis is focused on conventional or classical DCs, which are closest to the cells originally described by Steinman and Cohn and are distinct from before mentioned pDCs, Langerhans and monocyte-derived DCs in their ontogeny, gene expression, and function (Guilliams et al., 2014).

1.1.3.1 Heterogeneity of conventional dendritic cells

In mice, cDCs are primarily categorised into two subsets: cDC1 and cDC2, each with distinct phenotypes, and developmental pathways (Cabeza-Cabrerizo et al., 2021). The differentiation of these cDC subsets from haematopoietic stem cells (HSCs) starts in the bone marrow. HSCs possess the distinctive capability to self-renew and provide sustained, multi-lineage reconstitution of the haematopoietic system (Weissman, 2000). Traditionally positioned at the pinnacle of a cellular differentiation hierarchy, HSCs generate progressively lineage-specific progenitor cells, which eventually lead to the formation of fully differentiated blood and immune cells. Recent perspectives on haematopoiesis, however, propose that HSCs can also differentiate more directly, potentially skipping intermediary progenitor stages or moving along a continuum of lineage commitment (Laurenti and Göttgens, 2018; Zhang et al., 2018). As current research finds some previously described intermediate stages of cDC development controversial, the earliest precursor whose potential is restricted to the cDC lineage is nowadays claimed to be the "common" DC progenitors (CDPs) (Naik et al., 2007). The differentiation from CDPs to pre-classical dendritic cells (pre-cDCs) is marked by an increase in CD11c expression. Pre-cDCs are the cells

that depart from the bone marrow through the bloodstream, populating both lymphoid and non-lymphoid tissues to produce cDCs (Liu et al., 2009). It was initially believed that pre-cDC had oligopotency to develop into cDC1 or cDC2. Recent findings, however, indicate that subset specification can occur during the development from CDP to pre-cDC, leading to the formation of either committed pre-cDC1s or pre-cDC2s (Grajales-Reyes et al., 2015; Schlitzer et al., 2015). Research has shown that in mouse bone marrow, uncommitted pre-cDCs do not express CD115 and CD117, while pre-cDC1s carry CD117 and pre-cDC2s express CD115 (Grajales-Reyes et al., 2015). Another study identified Ly6C and SiglecH as markers for subtype commitment, revealing that uncommitted pre-DCs that are developmentally closer to CDPs, and still have the capacity to differentiate into cDCs and pDCs, are $Ly6C^{-}$ and $SiglecH^{+}$, pre-cDC1s lack both markers, and pre-cDC2s are $Ly6C^{+}$ and $SiglecH^{+}$ (Schlitzer et al., 2015). cDC2s can further be subdivided into cDC2As and cDC2Bs, and a recent study showed that distinct pre-cDC2 subsets give rise to those subclasses (Minutti et al., 2024).

The cytokine Flt3L (FMS-like tyrosine kinase 3 ligand) plays a crucial role in regulating cDC commitment during haematopoiesis (McKenna et al., 2000). Produced broadly by tissue stroma, endothelial cells, and activated T cells, Flt3L interacts with its receptor Flt3 (also known as CD135 and Flk2), which is found on HSCs and continues to be expressed on the cDC progenitors CDPs and pre-cDCs. As these precursors progress towards the cDC lineage, their reliance on Flt3L intensifies, as demonstrated by the significant decrease in CDPs and pre-DCs (but not earlier progenitors) in Flt3L-deficient mice (Kingston et al., 2009). Beyond its influence on cDC differentiation, Flt3L also controls the proliferation of peripheral cDCs, ensuring the maintenance of stable cDC numbers. Experiments with parabiotic mice have demonstrated that most cDCs in tissues have a lifespan of 10-14 days, replenished by bone marrow progenitors, except for some subsets of cDCs in the lung, which can last over 30 days (Ginhoux et al.,

2009). This suggests that cDCs are largely post-mitotic and short-lived, continually renewed by circulating pre-cDCs. However, fully differentiated cDCs can proliferate in the presence of Flt3L (Waskow et al., 2008).

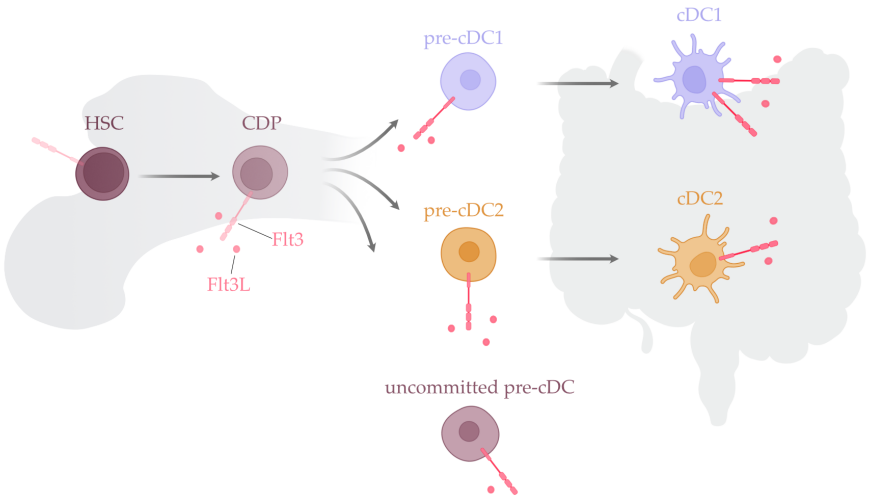


Figure 1. Mouse cDC poiesis. Pathway of hematopoietic commitment of cDCs and dependency on Flt3L. CDP is the earliest precursor whose potential is restricted to the cDC lineage. pre-cDCs exit the bone marrow and home to tissues through the bloodstream, where they develop into cDCs. Dependency on Flt3L increases as precursors progress towards the cDC lineage. cDC1s express more Flt3 than cDC2. High local Flt3L levels can induce peripheral proliferation of cDCs.
HSC: hematopoietic stem cell; CDP: common or conventional dendritic cell progenitor.

cDCs in the mouse can be identified by the presence of surface markers CD45, CD135, CD11c, and MHC II in the absence of specific markers for T cells, B cells, NK cells, plasmacytoid cells and erythrocytes (Guilliams et al., 2016). In lymph nodes (LNs), cDC1 and cDC2 subsets can either be resident cDCs derived from pre-cDCs that have travelled through the bloodstream or migratory cDCs that have travelled from tissues via afferent lymph. Resident cDCs are characterised by lower levels of MHC II and higher levels of CD11c compared to migratory

cDCs (Henri et al., 2010). Similarly, cDCs in peripheral tissues can either be of resident or migratory phenotype, and these can be distinguished by elevated levels of co-stimulatory molecules like CD80, CD86, and chemokine receptor CCR7 for the latter (Ohl et al., 2004).

In peripheral tissues, cDC1s constitute around 30% of all cDCs, while their proportion increases to 40% in lymphoid organs. Exceptions in the body to these proportions are the Peyer's patches and the thymus, where cDC1s are more prevalent (Guilliams et al., 2016). cDC1s in the spleen, LNs, and thymus, but not in non-lymphoid organs, express the CD8$\alpha\alpha$ homodimer. Those in non-lymphoid organs express either the E-cadherin–binding integrin αE (CD103) or CD24, although subsets of cDC2 in specific organs can share these markers (Edelson et al., 2010). cDC1s carry the chemokine receptor XCR1 and CLEC9A (also known as the C-type lectin receptor DNGR-1) CD205, and CD207. However, the latter is absent in intestinal and pancreatic cDC1s (Bachem et al., 2012). In comparison to cDC2s, cDC1s show higher levels of Flt3 and lower expression of Sirpα and CD11b (Crozat et al., 2010).

More populous and varied than cDC1s are cDC2s. Almost all cDC2s show high expression of Sirpα and CD11b and an absence of markers common to cDC1s such as CD8α, XCR1, CLEC9A and, except in the intestine, CD103 (Guilliams et al., 2016). The expression of CD4 is restricted to a fraction of cDC2s in lymphoid tissues. In the gut, both $CD11b^{+}CD103^{+}$ and $CD11b^{+}CD103^{-}$ cDC2s populations exist alongside $CD11b^{-}CD103^{+}$ cDC1s (Scott et al., 2015). cDC2s are subdivided into two groups defined either by their dependence on different factors during development (Notch2 receptor or Klf4 transcription factor) or by T-bet and RORγt expression (Schlitzer et al., 2013; Tussiwand et al., 2015). Subgroups of cDC2 are called cDC2As and cDC2Bs (Brown et al., 2019).

As in mice, human pre-cDCs were found in the bone marrow, the bloodstream and peripheral tissues. It is believed that pre-cDCs leave the bone marrow and seed tissues, where they mature to cDC (See et al., 2017). Similar to murine pre-cDCs, human pre-cDCs can be divided into uncommitted pre-cDCs, pre-cDC1 and pre-cDC2s. Human cDC2s and cDC1s are present in blood, lymphoid, and non-lymphoid tissues, with cDC1s being less common than cDC2s, similar to their distribution in mice (Collin and Bigley, 2018). Human cDC2s exhibit greater diversity and distinct gene expression profiles across various tissues compared to cDC1s (Dutertre et al., 2019). Transcript analysis of single myeloid cells has led to the proposal of additional subsets of cDCs, such as cDC3s, cDC4s, and cDC5s. However, it is still to be investigated if these subgroups may resemble additional subpopulations of cDCs or correspond to precursors of cDCs or variants of other macrophages or activated cDCs (Villani et al., 2017). Along with this, cDC3 is identified as a subgroup of myeloid cells similar to inflammatory cDC2s. cDC4s appear to belong to the family of monocytes, while cDC5s share characteristics with pre-cDCs and have been recognised as progenitors for cDC2s (Dress et al., 2019)

1.1.3.2 Functions of cDCs

cDCs as environmental sensors

cDCs are exceptional environmental sentinels, capable of detecting a wide range of stimuli in both steady-state conditions and during infection or tissue damage. They rely on tonic signals such as Flt3L and Type I Interferons for their survival, proliferation, and maintenance of their responsive state (Merad et al., 2013; Shortman and Heath, 2010). The sensing abilities of cDCs are facilitated by an array of PRRs, including Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). These receptors recognise PAMPs and DAMPs, triggering activation

pathways within the cDCs (Reis e Sousa, 2004).

Both subgroups of cDCs express CD36, which can bind to dead cells (Albert et al., 1998). cDC1s can detect viral infections through receptors specialised in recognising viral RNA and DNA, such as TLR3. This capability enables them to cross-prime $CD8^+$ T cells against infected cells (Schulz et al., 2005). Additionally, cDC1s express TLR11 and TLR12, which recognise antigens from *Toxoplasma gondii* and stimulate interleukin (IL) -12 production (Yarovinsky et al., 2005). The high expression of CLEC9A, a receptor for necrotic bodies, allows cDC1s to induce CTL responses, and signalling through this receptor is important for effector or memory responses (Sancho et al., 2009).

cDC2s are thought to be more specialised in detecting intracellular pathogens. They can sense viral infections via TLR7, RIG-I, and MDA-5 (Kato et al., 2005). However, they are also attuned to bacterial components and extracellular signals through receptors like TLR5 and TLR4 (Uematsu et al., 2006). For instance, the activation of TLR5 by bacterial antigens prompts cDC2s to produce IL-23 and induce Th17 cell responses (Kinnebrew et al., 2012).

According to their function as sensors, cDCs are found at distinct locations at various interfaces between the host and the external world. While cDC1s are found closer to the epithelial layers of mucosal barriers, interdigitating the epithelial layer with their dendrite-like protrusions, cDC2s are located deeper in the tissues, accessing antigens that cross the epithelial barrier (Ginhoux et al., 2009; Rescigno et al., 2001).

Activation of cDCs

Two important properties of cDC function are the induction of adaptive immunity and, conversely, the silencing of an immune response and priming of self-tolerance (Steinman et al., 2003). To acquire these abilities, cDCs can

transition from resting to activated states, a process also known as cDC maturation or activation. Upon antigen recognition, cDCs undergo a transformation from an antigen-capturing state to an antigen-presenting state, defined by their enhanced ability to interact with other immune or non-immune cells (Reis e Sousa, 2006). This transition occurs in both steady-state and inflammatory conditions. It is characterised by phenotypic changes such as the up-regulation of expression of co-stimulatory molecules and MHC molecules on their surface, including CD80, CD86, and CD40, as well as the extension of their dendrites to facilitate interaction with T cells (Banchereau and Steinman, 1998). Migratory cDCs also up-regulate the expression of chemokine receptors like CCR7 that enable migration to lymph nodes (Förster et al., 1999).

As already mentioned and depending on the context, cDCs can produce various cytokines that guide T cell differentiation into effector T cells, including Th1, Th2, and Th17 cells, as well as Tregs (Kapsenberg, 2003). Interestingly, cDCs can also enter a phenotypically mature state without PRR triggers solely through cytokine stimulation. However, these cytokine-matured cells cannot produce cytokines that direct T cell differentiation (Spörri and Reis e Sousa, 2005). Along the same lines, even in the absence of pathogens or under germ-free conditions, cDCs undergo a maturation process and actively present self-antigens to auto-reactive T lymphocytes that have evaded thymic elimination (Ardouin et al., 2016; Wilson et al., 2008). This phenomenon, termed "homeostatic" or "tolerogenic" maturation, plays a crucial role in maintaining central and peripheral tolerance (Ardouin et al., 2016). The mechanisms by which this process preserves immune homeostasis include T cell elimination, anergy or facilitating their conversion to Treg cells. At the heart of homeostatic cDC maturation lies the engulfment of apoptotic cells, a process that has emerged as a key driver of tolerogenic programming, especially in cDC1s (Bosteels et al., 2023; Cummings et al., 2016). The mere influx of lipids and cholesterol appears

sufficient to trigger this maturation pathway, while nucleic acid sensing seems dispensable (Bosteels et al., 2023). The homeostatic maturation program is characterised by a distinct transcriptional signature, including the activation of liver X receptor (LXR) and sterol regulatory element-binding protein 2 (SREBP2) pathways (Gainullina et al., 2023; Plebanek et al., 2024). Notably, LXR signalling not only orchestrates cholesterol efflux but also acts as a repressor of interferon-stimulated genes, effectively dampening inflammatory responses (Tall and Yvan-Charvet, 2015). This metabolic reprogramming appears to be a crucial checkpoint in the decision between tolerance and immunity, with cholesterol homeostasis playing a central role. These recent findings emphasise that tolerance induction is an active process rather than merely a default state in the absence of danger signals, as was proposed earlier. This lipid-centric initiation of tolerance aligns with ancient evolutionary pathways predating many receptor-ligand interactions, suggesting a fundamental link between lipid metabolism and immune regulation (Bosteels and Janssens, 2024).

While cDCs are primarily known for their role in interacting with T cells, they can also present antigens to B cells, leading to antibody production (Qi et al., 2006). Additionally, they significantly contribute to innate immune responses. For example, activated cDCs interact with neutrophils to shape early bacterial responses (Del Fresno et al., 2018) and can also enhance NK cell cytotoxicity and proliferation through direct interactions (Andrews et al., 2003). Beyond their interactions with immune cells, cDCs also communicate with non-immune cells. Recent findings have suggested that cDCs can influence sensory neurons; for instance, IL-31 released by activated dermal cDC2s during wound healing can intensify the itching response that accompanies the healing process (Xu et al., 2020).

Activation of tissue cDCs involves the migration from peripheral tissues to the draining lymph nodes (dLNs), a process driven by the expression of chemokine receptors such as CCR7, which follows a gradient of chemokines like CCL19 and CCL21 that are present in the lymphatic vessels (Förster et al., 1999; Ohl et al., 2004). Antigens that freely enter afferent lymph can also be passively drained to dLNs and be indulged by resident cDC and induce T cell response (Hickman et al., 2008), a process that could have a kinetic advantage to antigen presentation by migratory cDCs (Gerner et al., 2015). It is suggested that induction of initial but weaker T cell priming is mediated by LN-resident cDCs, and later, but more potent effector responses are mediated by migratory cDCs that sample locally constrained antigens in tissue (Itano et al., 2003). Upon arrival in the LNs, cDCs settle in the T cell zones, where they present antigens to naïve T cells. The location of subgroups of cDCs has been described as cDC1s being found predominantly in deeper T cell zones and cDC2s mostly residing in the outer T cell zone and the inter-follicular zone in steady state. However, we know that, especially in the inflamed state, segregation is dynamic and involves relocation within the dLN (Gerner et al., 2012).

cDC antigen presentation to T cells

The core function of DCs lies in their ability to process and present antigens on both MHC class I (MHC I) and class II (MHC II) molecules, a dual capacity that enables them to activate both $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells, respectively (Dudziak et al., 2007). cDC1s are particularly proficient in cross-presenting antigens on MHC I molecules, a crucial mechanism for inducing cytotoxic responses against tumours and virally infected cells (Den Haan et al., 2000; Pooley et al., 2001). In contrast, cDC2s excel in presenting antigens on MHC II molecules, pivotal for helper T cell activation and the subsequent

orchestration of humoral and cellular immunity (Maldonado-López et al., 1999; Pulendran et al., 1999).

The interaction between cDCs and T cells does not merely hinge on antigen presentation but also involves the engagement of co-stimulatory molecules as well as cytokines. These components of a successful cDC-T cell interaction are categorised into three signals (Kaliński et al., 1999; Reis e Sousa, 2006):

- Signal 1: peptides presented on MHC (pMHC) molecules on cDCs engage with TCRs of T cells, which results in a stable TCR-pMHC complex, also referred to as immunological synapse. Additional adhesion molecules can stabilise this complex, like ICAM-1 of cDCs that can bind to LFA-1 of T cells (Grakoui et al., 1999).

- Signal 2: co-stimulatory molecules expressed by cDCs that interact with CDs on T cells to mediate T cell survival, expansion and activation. These molecules belong either to the immunoglobulin superfamily like CD80 and CD86 that interact with CD28 on naïve T cells (Harding et al., 1992) or to the tumour necrosis factor (TNF) superfamily, for example, CD40L that can bind to CD40 (Ahonen et al., 2004). Activation of both named examples, CD28 and CD40L, are supporting T cell expansion (Ahonen et al., 2004; June et al., 1987).

- Signal 3: Immunomodulatory mediators secreted by cDCs that navigate T cell expansion and differentiation from naïve to effector T cells (Curtsinger et al., 2005, 2003; Kaliński et al., 1999). For example, IL-12 and type I interferon (IFN) are necessary for $CD8^+$ T cell memory formation (Xiao et al., 2009).

Activation of naïve CD8$^+$ T cells in immunity

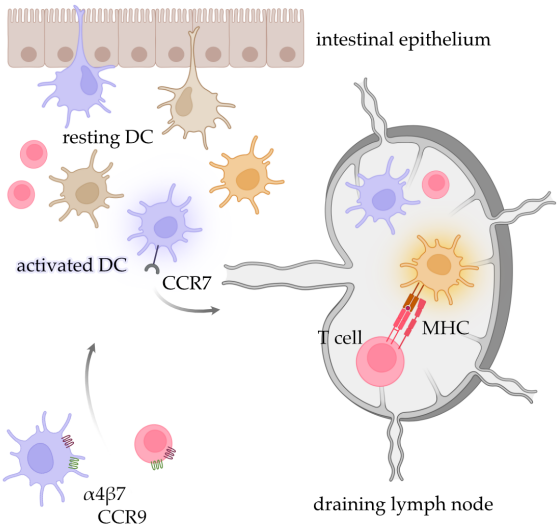
The capability of cDC1s to process and present extracellular antigens via MHCI molecules was described over two decades ago (Den Haan et al., 2000). This phenomenon is called cross-presentation. It is of particular importance as exogenous antigens are typically presented by MHCII, and the MHCI pathway ordinarily displays endogenous antigens from within infected cells. Though several cell types of APCs are able to cross-present, cDC1s are the dominant cross-presenting APCs (Jung et al., 2002). Through cross-presentation, cDC1s can initiate CTL responses. This function of cDCs seems to be crucial during infection as *Batf3* knockout mice that lack cDC1s show impaired CD8$^+$ T cell response during Sendai virus, West Nile virus and Vaccinia virus infection (Edelson et al., 2010; Hildner et al., 2008; Iborra et al., 2012).

Additionally, cDC1s show a notable ability to prime CD8$^+$ T cells independently of their capacity to cross-present. They are unique among haematopoietic cells in expressing the chemokine receptor XCR1. The corresponding ligand, XCL1, is produced by CD8$^+$ T cells after antigen recognition (Dudziak et al., 2007). This expression enables cDC1 to facilitate interactions between previously activated CD8$^+$ T cells and cDC1s, thereby enhancing the differentiation of CTLs (Crozat et al., 2010; Dudziak et al., 2007). Besides direct induction of CTLs via cell-cell contact, cDC1s have also been shown to be the main producers of IL-12 (Farrand et al., 2009; Hochrein et al., 2001; Maldonado-López et al., 1999) and IL-15 (Mattei et al., 2001) during infection. These cytokines are additionally triggering the differentiation of CTLs (Pulendran, 2004). Notably, inflammatory cDC2s have been shown to also efficiently cross-present to CD8$^+$ T cells and produce IL-12 (Bosteels et al., 2020; Desch et al., 2014).

Activation of naïve CD4$^+$ T cells in immunity

As mentioned earlier, both cDC1s and cDC2s play significant roles in the activation and differentiation of CD4$^+$ T cells, which is crucial for orchestrating adaptive immune responses and shaping the overall immune landscape. Both cDC1s and cDC2s, like other APCs, express MHCII molecules, which are essential for presenting exogenous antigens to CD4$^+$ T cells. This classical pathway of antigen presentation allows these cDC subsets to initiate CD4$^+$ T cell responses against a wide array of pathogens and foreign antigens (Cabeza-Cabrerizo et al., 2021). Interestingly, cDC1s and cDC2s show distinct patterns in promoting CD4$^+$ T cell differentiation. The role of cDC1s in the activation of CD4$^+$ T cells is not as clear as their role in the activation of CD8$^+$ T cells. However, cDC1s have been shown to excel at promoting IFN-γ-producing Th1 differentiation of CD4$^+$ T cells, largely attributed to their superior ability to produce IL-12, a key cytokine for Th1 polarisation (Reis e Sousa et al., 1997). This has been shown to be critical for cell-mediated immunity against intracellular pathogens (Martínez-López et al., 2015).

In contrast, cDC2s are more versatile in their ability to induce different CD4$^+$ T cell subsets. They are particularly adept at promoting Th2 and Th17 responses. cDC2s express higher levels of Interferon regulatory factor 4 (IRF4), which is crucial for their development and function, including their ability to induce Th2 responses (Schlitzer et al., 2013). They are also major producers of IL-23, a cytokine essential for Th17 differentiation and maintenance (Persson et al., 2013). Additionally, both cDC subsets contribute to the development of Tfh cells, which are essential for supporting B cell responses and antibody production (Krishnaswamy et al., 2018).



	cDC1	dpDC	cDC2
Treg	++	+	
Th1	+	+	+
Th2		+	+
Th17		++	+
X-presentation	+		

Figure 2. cDC function. Gut-homing of pre-cDCs and T cells is mediated by $\alpha 4\beta 7$ and CCR9. Activated cDCs up-regulate CCR7 and migrate to the dLNs, where they interact with T cells. Different cDC subsets can activate specific T cells.

cDC and T cell interaction in the context of immune tolerance

While the mechanisms by which cDCs initiate immune responses are well-characterised, their function in promoting tolerance is less known. In homeostatic conditions, cDCs play a crucial role in both central and peripheral tolerance. Within the thymus, they contribute to the elimination of self-reactive T cells during development (Perry et al., 2018, 2014). In peripheral tissues, tolerogenic cDCs employ various strategies to maintain immune quiescence, including the induction of T cell anergy, deletion of auto-reactive cells, and the generation and activation of Tregs (Kurts et al., 2001).

The process of T cell priming by tolerogenic cDCs shares some similarities with T cell activation in immunity, particularly the requirement for TCR engagement. However, tolerogenic cDCs show lower levels of MHC and co-stimulatory molecules like CD80 and CD86 (Qureshi et al., 2011; Wing et al.,

2008). This reduced co-stimulation through the CD28 pathway on T cells can lead to the activation of anergy-associated genes regulated by Nuclear factor of activated T-cells (NFAT), ultimately resulting in T cell unresponsiveness (Hui et al., 2017; Sharpe and Pauken, 2018). Additionally, co-stimulation through CD86 may lead to Treg induction (Halliday et al., 2020).

Tolerance can also be achieved by converting $CD4^+$ T cells into Tregs. In this context, cDC1s play a pivotal role in inducing Tregs through the coordinated action of transforming growth factor beta (TGF-β) and retinoic acid (RA), the latter derived from dietary vitamin A (Scott et al., 2011). This process establishes a positive feedback loop, with Treg-derived signals like IL-10 that further enhance the tolerogenic functions of cDCs (Mahnke et al., 2007).

The tolerogenic capabilities of DCs can be detrimental in the context of cancer, where they may contribute to immune suppression within the tumour microenvironment, hindering effective anti-tumour responses. In this context, it is important to mention a recently described population known as mature regulatory DC (mregDC) that have been distinguished from tolerogenic cDCs in the tumour. These cells have been characterised by a specific gene expression profile enriched for immunoregulatory molecules (Maier et al., 2020). They are particularly prevalent in the tumour microenvironment and are defined by their activated state, migratory capabilities, and immunomodulatory functions while lacking key markers associated with conventional DC subsets (Li et al., 2023). However, it remains to be investigated if mregDCs actually represent a distinct subset of cells or merely an activation status of DCs.

1.1.4 T cells

In the early 1970s, Jacques Miller and Max Cooper first described two distinct classes of lymphocytes: B cells and T cells (Cooper, 2010; Miller, 1961). T cells are a central component of the adaptive immune system, providing an essential role in immune regulation, pathogen defence, and immune homeostasis. They are crucial for activating and coordinating other components of the immune system, notably B cells and macrophages, to ensure an effective defence mechanism. T cells are also responsible for establishing immune memory, which facilitates quicker and more efficient responses to subsequent encounters with the same antigens (Nicholson, 2016). In addition, T cells are essential players in maintaining tissue homeostasis; for instance, they select specific organisms in the intestinal microflora or provide growth factors during pregnancy (Komano et al., 1995; Zhang et al., 2018).

T cells derive from HSCs in the bone marrow but then migrate to the thymus, where they undergo critical maturation and differentiation processes, hence their name "T" cells. This process involves complex interactions with APCs and the selective rearrangement of TCRs, which are pivotal for recognising specific antigens presented on MHC molecules by APCs (Dutta et al., 2021; Kisielow et al., 1988). During thymic maturation, a selection process takes place to ensure self-tolerance (Kisielow et al., 1988; Klein et al., 2014; Starr et al., 2003) and the capability to respond to foreign antigens (Kisielow et al., 1988). Due to this selection process, only a fraction of generated T cells leave the thymus and enter through the bloodstream into the periphery (Egerton et al., 1990).

The TCRs are built in a heterodimeric structure and consist of a disulfide-linked and either α and β combined polypeptide chain (TCR$\alpha\beta$) or a γ and δ combined chain (TCR$\gamma\delta$). TCRs are associated with the proteins of the CD3

complex, and, notably, the majority of T cells express TCR$\alpha\beta$. Both $\alpha\beta$- and $\gamma\delta$-chains are highly variable and enable the recognition of a vast array of antigens. This diversity is generated through somatic recombination of gene segments in developing T cells (Acuto et al., 1984; Brenner et al., 1986; Chien et al., 1987; Hedrick et al., 1984; Saito et al., 1984). The recognition of antigenic peptides by TCRs is highly specific, leading to a relatively low occurrence of T lymphocytes that recognise a specific antigen (Obar et al., 2008).

T cells are subdivided based on the expression of the surface molecules CD4 and CD8, which are associated with helper and cytotoxic functions, respectively (Taniuchi, 2018; Teh et al., 1988). CD4 recognises peptides that are presented on MHC II, and $CD4^+$ T cells assist other cells of the immune system in mounting and regulating immune responses. The receptor CD8 can engage with peptides presented on MHC I, and $CD8^+$ T cells are primarily involved in directly killing infected or malignant cells. While MHC I is expressed on all nucleated cells, MHC II is mainly expressed on APCs (Gabert et al., 1987; Gay et al., 1987). Maturation of T cells into $CD4^+$ or $CD8^+$ cells is principally defined by a balance between two transcription factors: Thpok dedicates $CD4^+$ T cell development while Runx3 facilitates $CD8^+$ lineage commitment (Egawa and Littman, 2008; Taniuchi et al., 2002). Upon encountering their specific antigen, stimulation through co-stimulatory molecules and cytokine signalling, naïve $CD4^+$ or $CD8^+$ T cells become activated and differentiate into various effector subtypes, each characterised by distinct functions and cytokine profile (Davis et al., 1998; Kaech and Wherry, 2007; Ruterbusch et al., 2020; Zhu et al., 2010). The differentiation into distinct subtypes is regulated by specific transcription factors that activate genes necessary for the effector functions and also suppress the development of alternative T cell fates (Luckheeram et al., 2012). The complexity of T cell functions in immune responses underscores their importance not only in the

defence against pathogens but also in the development of immunological tolerance and autoimmunity (Sun et al., 2023).

1.1.4.1 Regulatory T cells

Immunosuppressive functions of Tregs

Regulatory T cells are $CD25^+$ and $CD4^+$ T cells that are fundamental for maintaining tolerance and for the resolution of immune responses by suppressing effector T cell function. They execute their suppressive functions through metabolic disruption, cytolysis, and modulation of APCs. However, their major suppressive mechanism acts through the secretion of inhibitory cytokines, primarily IL-10, TGF-β, and IL-35 (Plitas and Rudensky, 2016). IL-10 is a strong inhibitory cytokine that suppresses pro-inflammatory responses and mediates homeostasis by inhibiting Th1 and other immune cells (Couper et al., 2008), and both IL-10 and TGF-β, effectively inhibit Immunoglobulin E (IgE) production, thereby attenuating allergic responses (Jutel and Akdis, 2008). Cytolysis is facilitated by Granzyme B (Gzmb) (Gondek et al., 2005) while metabolic disruption results from IL-2 deprivation, leading to apoptosis (Vignali et al., 2008), activation of the adenosine receptor 2A (Tang and Bluestone, 2008) or insertion of the inhibitory second messenger cAMP (cyclic adenosine monophosphate) into effector T cells (Sojka et al., 2008). Alteration of APC function is mediated by interaction of Tregs with CD80, CD86 and MHC II molecules on APCs, resulting in a diminished ability of APCs to activate T effector cells (Sojka et al., 2008). Tregs can also influence the production of the immunoregulatory and tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) of cDCs (Mellor and Munn, 2004).

In contrast to effector T cells that require antigen recognition for secretion of anti-inflammatory molecules, Tregs continuously express a high amount of some of their effector molecules like IL-10, Gzmb, and others. Due to this baseline activity, Tregs have a head start in their suppressive activity in comparison to other effector T cells that express the same molecules but later in their activation. Although their secretion of immunosuppressive molecules facilitates the resolution of immune reactions, other T cells alone, in the absence of Tregs, cannot prevent autoimmunity and inflammation (Dikiy and Rudensky, 2023).

Compared to other $CD4^+$ T cells, Treg cells harbour a distinct TCR repertoire, highly enriched for "self" recognising receptors, which is important for their ability to suppress inappropriate- as well as auto-immune reactions (Hsieh et al., 2006; Nishio et al., 2015). Additionally, Tregs specific to a particular antigen effectively inhibited the activity of $CD4^+$ T cells that recognise the same peptide epitope while not affecting those T cells that responded to different, non-cross-presented peptides presented by the same cDC cell on MHC II molecules. This suppression occurred through a process that involves the direct elimination of peptide-MHC complexes from the cDCs facilitated by the TCRs on Tregs (Akkaya et al., 2019). However, this precise TCR-mediated targeting of specific immune responses does not imply that the following suppressive mechanisms have to be TCR-dependent. Conversely, activated Tregs display important and non-redundant TCR-dependent and -independent effector functions (Dikiy and Rudensky, 2023).

Activated and migrating Tregs express CCR7 and L-selectin (CD62L), guiding them to secondary lymphoid organs (Huehn et al., 2004). They also express high amounts of CCR4, a chemokine receptor that binds to CCL22, expressed by activated cDCs (Rapp et al., 2019). Through coordination of these interaction-promoting receptors, Tregs can regulate the immune response in a time-sensitive

manner and preclude over-reactivity to antigens in secondary lymphoid tissue. Treg-mediated suppression of inflammation is of special importance at barrier tissues that are constantly exposed to exogenous antigens in physiological conditions. The intestinal environment, for example, presents a unique challenge for the immune system, requiring a delicate balance between tolerance to food antigens and commensal microbiota while maintaining the ability to respond to pathogens. To achieve this balance, activated Tregs also express a variety of other inducible chemokines, integrins and tissue-homing receptors that guide Treg migration in response to infection or injury to specific locations, aside from secondary lymphoid tissues (Mempel and Marangoni, 2019). Another strategy of Tregs to co-localise with effector T cells is the expression of CCL3 and CCL4. These molecules attract pro-inflammatory T cells to Tregs, which allows them to suppress their inflammatory response subsequently (Patterson et al., 2016).

Besides their immune suppressive functions, Tregs have also been shown to be directly involved in tissue repair. For instance, Tregs in the skin stimulate Notch or TGF-β receptor signalling in hair follicle stem cells (SCs), supporting the SC niche and promoting regrowth of hair follicles and epithelial barrier (Ali et al., 2017; Mathur et al., 2019).

Heterogeneity in the Treg family

There are two main subsets of Tregs, defined by their location of development (Hori et al., 2003): natural or thymic Treg (nTreg or tTreg) cells derived from the thymus and induced or peripheral Treg (iTreg or pTreg) cells derived from peripheral $CD4^+$ T after antigen and TGF-β and IL-2 stimulation (Josefowicz et al., 2012; Kanamori et al., 2016). tTreg cells are characterised by high expression of CD25 and their master regulator FoxP3, which are essential for their suppressive function. They develop in response to self-antigen, which makes them particularly responsive to autoimmune reactions (Zheng et al., 2004). This

characteristic is especially evident during events that involve damage to the body's tissues (Wyss et al., 2016). They require co-stimulation through CD28 (Sempowski et al., 2004) and are dependent on IL-2 and TGF-β for survival and growth (Furtado et al., 2002; Marie et al., 2005). In secondary lymphoid organs, tTregs constitute the majority of the regulatory T cell population. Following their activation by DCs through antigen-specific interactions in lymph nodes, tTregs can experience changes in their expression of trafficking receptors, such as an up-regulation of chemokine receptors like CCR4, CCR8, or CCR9 (Lee et al., 2007). Such modifications facilitate the movement of tTregs from lymphoid tissues to various peripheral sites in the body. The transcription factor Helios has been proposed as a marker to distinguish tTregs from pTregs (Thornton et al., 2010). However, the specificity of this marker remains a topic of debate, as some researchers have reported Helios expression in other cell types (Thornton and Shevach, 2019). The gut harbours a distinct intestinal tTreg subpopulation which appears to possess a specialised functional role in the suppression of ongoing inflammation (Wohlfert et al., 2011). $Gata3^{+}Helios^{+}$ Tregs display major immunosuppressor function during intestinal inflammation. A deficiency in Gata3 was shown to hinder Treg infiltration to the inflamed intestine and mesenteric LN (mLN), while T effector cells were unaffected.

pTreg cells are crucial for maintaining tolerance to non-self antigens, including commensal bacteria and dietary antigens, which the immune system encounters post-thymically (Workman et al., 2009). Therefore, they are predominantly found in peripheral tissue barriers. In comparison to tTregs, the development of pTregs is dependent on weaker TCR stimulation and peripheral exogenous antigens (Apostolou and von Boehmer, 2004). They are primarily generated in the presence of TGF-β in the presence of IL-2 and, as opposed to tTreg development, are not dependent on CD28 stimulation (Kretschmer et al., 2005). For their generation, APCs, including cDCs, pDCs and monocyte-derived DCs, have been

shown to play an important role (Moseman et al., 2004; Mucida et al., 2007; Steinbrink et al., 2002; Sun et al., 2007). Importantly, cDC1 but not cDC2 have the capacity to generate pTregs (Verginis et al., 2005). Both tTregs and pTregs have similar phenotypes and secrete IL-10 and TGF-β amongst other cytokines (Povoleri et al., 2013). However, even though they share similar functions, it has been shown that functions are not completely redundant. In this sense, e.g. pTreg depletion in mice resulted in inflammation and weight loss (Haribhai et al., 2012). Some pTregs express Rorγt. They occur preferentially in the colon and small intestine but not in other non-lymphoid tissue or splenic Tregs (Burzyn et al., 2013). Rorγt$^+$ Tregs down-regulate T effector cell-derived IL-17α and IFN-γ, and ablation of those results in severe colitis (Sefik et al., 2015).

Additionally to FoxP3 expressing Tregs, other inducible T cells with regulatory properties have been described. These include CD4$^+$ (Th3, Tr1, and iTr35) and CD8$^+$ (CD8$^+$CD28$^-$) cells. For example, Tr1 differentiation can be induced by IL-10, and they repress T effector cell function by secretion of IL-10 and TGF-β (Groux et al., 1997; Levings et al., 2005).

In the small intestinal Treg population, 30% of all Tregs are Gata3$^{+,}$ and 15% are Rorγt$^+$ compared to around >10% of Tregs in Peyer's patches or mLNs (Sefik et al., 2015; Wohlfert et al., 2011). The expression of Gata3 and Rorγt in colonic and small intestinal Tregs are mutually exclusive (Sefik et al., 2015; Wohlfert et al., 2011). Taken together, enteric Tregs can be divided into three distinct phenotypes: Gata3$^+$ tTregs, Rorγt$^+$ and Rorγt$^-$ pTregs.

As already mentioned, both tTregs and pTregs are characterised by their expression of the transcription factor FoxP3. However, tTregs possess a unique epigenetic signature in the *FOXP3* locus known as the Treg-specific demethylated region (TSDR). This region remains largely unmethylated in tTregs, whereas it is

heavily methylated in pTregs (Kim et al., 2012). The demethylated state of the TSDR is associated with more stable FoxP3 expression, suggesting that tTregs may maintain their suppressive phenotype more consistently than pTregs (Polansky et al., 2010). While TGF-β has been extensively studied for its role in pTreg differentiation from conventional T cells, research has shown that it is also crucial for the development of tTregs in the thymus. This highlights the importance of TGF-β signalling across different Treg subsets, which induced FoxP3 expression (Chen and Konkel, 2015).

1.1.5 The immune system of the small intestine

The small intestine presents a tight regulation of the immunological frontier at the interface between host and environment (Mowat and Agace, 2014). Here, the immune system faces a constant barrage of dietary antigens, commensal microbiota, and potential pathogens, necessitating a delicate balance between tolerance and defence.

The gut-associated lymphoid tissue (GALT) forms the cornerstone of small intestinal immunity (Mowat and Agace, 2014). This network of organised lymphoid structures and diffuse lymphoid cells permeate the intestinal mucosa and submucosa, creating a robust defence system. At the forefront are the Peyer's patches, macroscopic lymphoid aggregates primarily found in the ileum. These structures, covered by a specialised follicle-associated epithelium (FAE), serve as critical sites for antigen sampling and immune induction. Complementing the Peyer patches are the isolated lymphoid follicles and cryptopatches, smaller lymphoid aggregates scattered throughout the intestine that contribute to local immune responses and tissue homeostasis (Knoop and Newberry, 2012; Pabst et al., 2005).

The epithelial barrier of the small intestine represents the first line of defence against luminal threats (Peterson and Artis, 2014). Enterocytes, the predominant cell type, not only facilitate nutrient absorption but also express PRRs that detect microbial products, alerting the immune system to potential threats. Interspersed among the enterocytes are specialised cell types, each contributing uniquely to intestinal immunity. Goblet cells secrete mucus, forming a physical and chemical barrier against microbes (Johansson et al., 2013). Paneth cells, nestled at the base of intestinal crypts, produce an arsenal of antimicrobial peptides, bolstering innate immunity (Clevers and Bevins, 2013). The rare but significant tuft cells play a crucial role in type 2 immune responses and parasite detection (Gerbe et al., 2016). Within the FAE of Peyer's patches, microfold (M) cells facilitate the sampling and transport of antigens from the lumen to underlying immune cells, bridging the gap between the external environment and the immune system (Mabbott et al., 2013).

Beneath the epithelium lies the lamina propria, a region harbouring diverse immune cells (Mowat and Agace, 2014). Here, T cells of various subsets – including Th1, Th2, Th17, and Tregs – orchestrate adaptive immune responses and maintain tolerance. B cells, particularly plasma cells, produce copious amounts of Immunoglobulin A (IgA), which is transported across the epithelium to provide humoral defence in the lumen (Macpherson et al., 2008). ILCs, especially ILC3s, play pivotal roles in maintaining epithelial barrier integrity and coordinating immune responses (Artis and Spits, 2015). Phagocytes, including DCs and macrophages, serve as sentinels, presenting antigens and initiating adaptive immune responses (Mowat and Agace, 2014). Granulocytes such as mast cells and eosinophils contribute to host defence and mediate allergic responses (Kurashima and Kiyono, 2014). This intricate network of specialised structures and diverse cell types forms a highly sophisticated immune system uniquely adapted to the challenges of the small intestinal environment.

Interestingly, the small intestinal immune system exhibits significant regional differences along its length (Mowat and Agace, 2014). The proximal small intestine is characterised by a thinner mucus layer, higher oxygen levels, a more acidic pH, and a relatively fast transit time, which limits bacterial growth. This region has the highest concentrations of luminal IgA, which decreases along the intestine. The duodenum and jejunum also show an increased frequency of ILC2s, Th17 cells, NKs, ILCs, eosinophils, pDCs and mast cells. In contrast, the distal small intestine, primarily the ileum, has shorter villi, a thicker mucus layer, and higher numbers of Paneth cells and goblet cells to cope with increasing bacterial loads. The ileum exhibits an increasing gradient of Rorγt^{+} pTreg cells and ILC3s. Additionally, the ileum has more Peyer patches, as they gradually increase towards the distal gut. The spacial immune landscape has been reviewed extensively. However, a complete and systematic comparison of all major immune cells in all intestinal segments is missing (Mowat and Agace, 2014; Brown and Esterházy, 2021). Regional differences in the gut are influenced by a combination of intrinsic tissue properties, extrinsic environmental factors, and specialised immune cell populations, all of which contribute to the unique immunological niches found along the small intestine. The duodenum receives food components mixed with secretions from the pancreas, liver, and stomach. Moving along to the jejunum and ileum, one finds differences in tissue permeability and nutrient absorption through the intestinal wall, pH, transport proteins, signalling proteins and immune responses (Spohn and Young, 2018). Studies have shown that the microbial populations vary among these different parts of the intestine (Donaldson et al., 2016; Kastl et al., 2020). Similarly, the metabolic environment changes through the gut, with each section having its unique characteristics (Meier et al., 2023).

1.1.6 Food tolerance

The interface between an organism and its environment is critically important for survival, with barrier tissues being constantly exposed to exogenous antigens. In animals, the gastrointestinal tract represents the largest and most dynamic interface with environmental antigens.

The mucosal area of the human small intestine is estimated to span approximately 300 m^2 and is, therefore, 100-fold larger than the skin. Each day, around 130 to 190 grams of dietary proteins are absorbed within the intestinal tract (Brandtzaeg, 1998). While the majority of dietary macromolecules are broken down by the time they enter the small intestine, some proteins that are un- or only partially degraded still manage to cross the intestinal barrier and are absorbed into the bloodstream (Bruce et al., 1987; Husby et al., 1985). Even though each meter of the intestinal mucosa houses about a trillion lymphoid cells (Moog, 1981), the most common response to dietary antigens is tolerance.

Tolerance to ingested food antigens is a fundamental immunological process that prevents adverse immune reactions to dietary proteins and maintains homeostasis (Faria et al., 2003). It is characterised by a state of systemic unresponsiveness or hypo-responsiveness to food antigens that the immune system encounters through the gastrointestinal tract. The concept of so-called oral tolerance stems from animal experiments. First described in 1911, Wells and Osborne observed that guinea pigs could not develop an anaphylactic reaction to antigens that these animals were already exposed to through their diet (Wells and Osborne, 1911).

1.1.6.1 Mechanistic pathways to acquire food tolerance

To date, several mechanisms have been proposed for the induction of oral tolerance. They are mainly dependent on type, dose and administration form of antigens (Faria et al., 2003; Mowat et al., 1982). While repeated exposure to low doses of antigen results in induction of Tregs, exposure to high doses of antigens leads to anergy or clonal deletion (Weiner et al., 1994). Notably, some theories implicate the skin, rather than the digestive tract, as the crucial site for the breakdown of food tolerance (Lack 2012).

The first step in the induction of oral tolerance is the sampling of antigens from the gut lumen. This process is facilitated by specialised cells, including M cells in Peyer patches and goblet cells and CX3CR1^{+} macrophages in the intestinal epithelium (Mazzini et al., 2014; McDole et al., 2012). Once sampled, antigens are processed and presented by APCs, primarily cDCs, which subsequently migrate in a CCR7-dependent manner to the mLNs. In the GALT, they present the antigens to naïve T cells and initiate the tolerogenic response. The induction of pTregs is a central mechanism in oral tolerance and is generated in the periphery from naïve CD4^{+} T cells under the influence of TGF-β and RA (Chen et al., 2003; Coombes et al., 2007). FoxP3^{+} pTregs play a critical role in suppressing effector T cell responses and maintaining tolerance to food antigens through various mechanisms, including the production of anti-inflammatory cytokines such as IL-10 and TGF-β, as well as cell contact-dependent mechanisms (Sakaguchi et al., 2008).

cDC1s play a crucial role in the induction of pTregs (Esterházy et al., 2016). They have the ability to activate latent TGF-β through the integrin αVβ8 and to metabolise vitamin A into RA, which is essential for the differentiation of pTregs and facilitates the imprinting of gut-homing receptors on T cells (Coombes et al.,

2007). As TGF-β, along with RA, can increase the expression of integrin αVβ8 expression in cDC1s, this creates a positive feedback loop which strengthens the regulatory function of cDC1s (Boucard-Jourdin et al., 2016). cDC1s also express the enzyme IDO, which is involved in tryptophan catabolism. It reduces local concentrations of tryptophan and produces tryptophan metabolites which act as immunomodulators that can induce pTreg cell conversion and thereby support oral tolerance (Matteoli et al., 2010).

As mentioned earlier, high doses of antigen can lead to anergy or clonal deletion of antigen-specific T cells. Anergy refers to a state of functional unresponsiveness in T cells, characterised by their inability to produce IL-2 and to proliferate subsequently to antigen encounters (Schwartz, 2003). This state is induced when T cells receive antigen stimulation in the absence of adequate co-stimulatory signals. Clonal deletion, on the other hand, involves the elimination of antigen-specific T cells through apoptosis (Chen et al., 1995). This process is mediated by Fas-FasL interactions and is particularly important in maintaining central tolerance in the thymus. However, it also plays a role in peripheral tolerance, including oral tolerance.

Cytokines have a crucial role in orchestrating the tolerogenic response. TGF-β is particularly important, as it promotes the differentiation of naïve T cells into pTregs and suppresses the differentiation of pro-inflammatory Th1 and Th17 cells (Li et al., 2006). IL-10 is another key cytokine in oral tolerance, suppressing the activation and effector functions of T cells and promoting the development of regulatory cells (Moore et al., 2001). Interestingly, recent research has highlighted the role of IL-27 in oral tolerance. IL-27 has been shown to induce IL-10-producing Tr1 cells, which contribute to the maintenance of intestinal homeostasis (Awasthi et al., 2007).

Additionally to food antigens, the human body hosts an immense microbial population, making up for around 0.3% of the overall body weight. Most of these bacteria live in the gut, and while they constitute another source of exogenous and physiological antigens, they also play a significant role in shaping the immune response and contribute to oral tolerance (Sender et al., 2016). Commensal bacteria produce metabolites, such as short-chain fatty acids (SCFAs), which have been shown to indirectly promote the differentiation and function of pTregs (Arpaia et al., 2013). Moreover, certain bacterial species, such as Clostridium clusters IV and XIVa, have been directly linked to the induction of colonic Tregs (Atarashi et al., 2011).

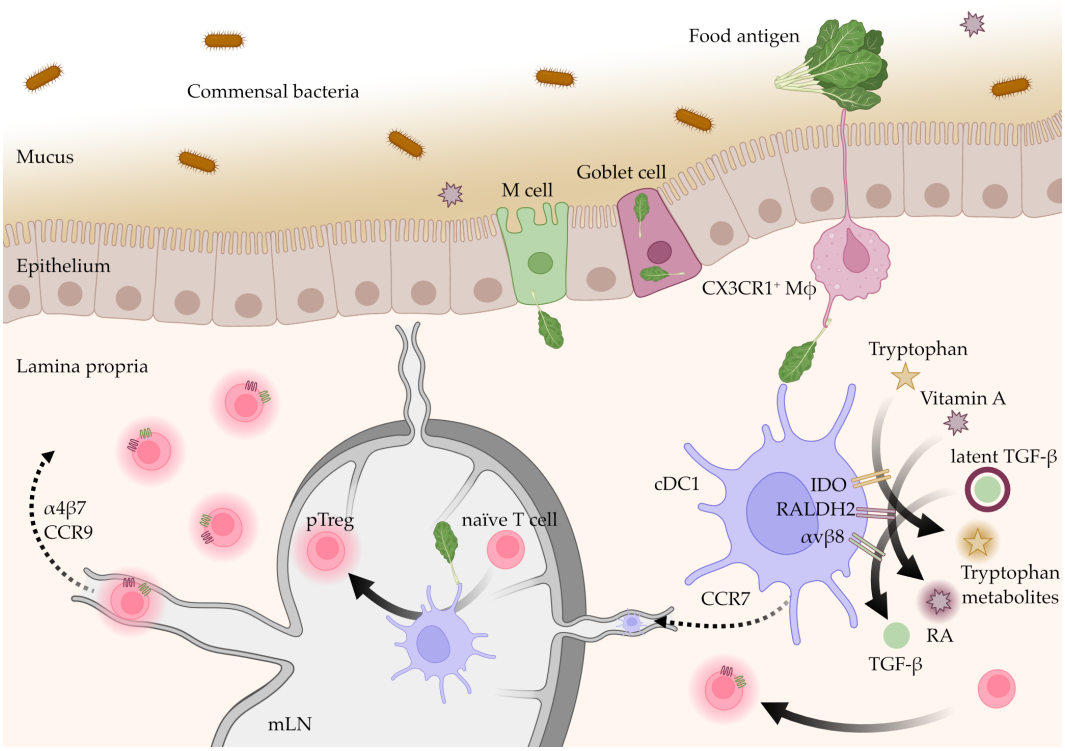


Figure 3. cDC1 involvement in food tolerance. Specialised cells like M cells, goblet cells and CX3CR1$^+$ macrophages sample luminal food antigens and deliver them to cDC1s. Commensal bacterial metabolites, vitamin A and TGF-β imprint tolerogenic properties on cDC1s. cDC1s migrate to mLNs through afferent lymphatic vessels where they induce differentiation of naïve CD4$^+$ T cells into pTreg cells. Differentiation and gut-homing (through CCR9 and α4β7) is facilitated by TGF-β, RA and tryptophan metabolites. mLN, mesenteric lymph node; RA, retinoic acid; Mϕ, macrophage; IDO, indoleamine 2,3-dioxygenase.

Image adapted from Liu et al., 2021

1.2 Obesity

Throughout history, perceptions of body weight have evolved dramatically. In many ancient societies, excess weight was often viewed as a symbol of wealth and prosperity (Marghoub et al., 2023). Ancient Greek and Roman cultures generally considered a rounded figure attractive for women, as it signified fertility and abundance. In the Middle Ages, plumpness was often associated with health and wealth, especially in Europe. This ideal persisted into the Renaissance, where art frequently portrayed curvaceous bodies as the pinnacle of beauty. In stark contrast, contemporary views on body weight have shifted significantly. Nowadays, most people in the Western world do not associate obesity anymore with health but rather view it as a social stigma and reduced quality of life. Modern science has further illuminated the link between obesity and a range of health issues, from respiratory problems, autoimmunity or diabetes to cancer and neurodegenerative and cardiovascular diseases (Hruby and Hu, 2015).

The World Health Organisation (WHO) describes overweight as a condition of excess fat deposits in the body, while obesity is defined as a chronic complex disease with negative implications on health due to being overweight (WHO, 2024). The diagnosis of obesity often relies on the Body Mass Index (BMI), a formula that calculates body fat based on an individual's weight and height [(weight in kg)/((height in m)^2)]. The BMI was developed in the 1830s by Lambert Adolphe Jacques Quetelet, a Belgian mathematician and sociologist. It classifies human adults into either "underweight", "overweight", or "obese" (Eknoyan, 2008). For adults, a BMI of 25 to 29.9 is nowadays considered overweight, while a BMI of 30 or more defines obesity (WHO, 2024). Importantly, the BMI is a surrogate marker and does not always accurately reflect body fat

distribution. Additional measures, such as waist circumference, can help refine the diagnosis by indicating central obesity (Ross et al., 2020).

The current global trend in obesity is alarming, with substantial increases observed across all age groups and regions. According to the WHO, in 2022, 2.5 billion adults (43% of the global adult population) were overweight, of whom 890 million (16% of the global adult population) were living with obesity (WHO, 2024). This represents a significant increase from 1990, when only 25% of adults were overweight. Among children and adolescents, the prevalence of overweight has risen from 8% in 1990 to 20% in 2022, with 390 million people affected. Of these, 31 million are obese. The rates of overweight individuals seem to be comparable between genders, with 43% of men and 44% of women and 19% of girls and 21% of boys affected in 2022.

Simultaneously to health concerns, the economic impacts of the obesity epidemic are growing, with predictions suggesting global costs could reach 4 trillion US$ per year by 2035 (World Obesity Federation, 2023). The rise in obesity in lower- and middle-income countries is particularly concerning, as these regions now face the double burden of malnutrition and obesity.

1.2.1 Diet-induced obesity

At the core, obesity results from an energy imbalance where caloric intake surpasses energy expenditure, leading to excessive fat accumulation (Hill et al., 2012). This basic principle, however, is influenced by a range of psycho-social factors, genetic factors and an obesogenic environment (Swinburn et al., 2011). A subgroup of obese patients suffer from obesity that is not strictly induced by diet but a result or side-effect of certain diseases or syndromes, medications, immobilisation and iatrogenic procedures (Heymsfield and Wadden, 2017).

Though diet-induced obesity (DIO) is the most prevalent form of obesity (WHO, 2024), its aetiology is a complex and multifaceted issue, reflecting an intricate interplay of genetic, environmental, and lifestyle factors.

Genetically, obesity is highly heritable, with estimates indicating that between 40% and 70% of individual differences in adiposity are attributed to genetic factors (Herrera and Lindgren, 2010). These genetic predispositions interact with environmental triggers to manifest as obesity. For instance, certain genetic variants can influence appetite, metabolism, and fat storage, predisposing individuals to gain weight more easily.

Environmental factors are critical in the recent DIO rates (WHO, 2024). These include the increased availability of high-calorie, nutrient-poor foods coupled with aggressive marketing strategies. Urbanisation has also led to changes in lifestyle, with more sedentary behaviours and less physical activity, further exacerbating the risk of obesity. Additionally, socioeconomic factors such as lower education levels and income can limit access to healthy food options and safe spaces for physical activity, thereby increasing obesity prevalence. Other factors like smoking and stress can indirectly influence weight by altering metabolism or increasing the likelihood of seeking comfort in food.

1.2.2 General health consequences of obesity

Obesity is a complex health condition that leads to a wide range of complications, affecting various systems in the body. It is not only a risk factor for numerous chronic diseases but also exacerbates existing health issues, leading to diminished quality of life and increased mortality (Guh et al., 2009).

One of the most significant effects of obesity is decreased cardiovascular health. The excess of fat in the body contributes to the development of

atherosclerosis, a process characterised by the buildup of fatty deposits in the arteries, leading to reduced blood flow and increased risk of coronary artery disease (Poirier et al., 2006). Obesity also predisposes individuals to hypertension by increasing the resistance in blood vessels and altering kidney function, which in turn can lead to heart failure (Hall et al., 2015). Additionally, the presence of metabolic syndrome, a cluster of conditions including high blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol levels, is more common in obese individuals and further elevates the risk of cardiovascular diseases (Grundy, 2016).

In the realm of metabolic disorders, obesity is intrinsically linked with type 2 diabetes due to the role of excessive fat in inducing insulin resistance (Kahn et al., 2006). The body's inability to effectively use insulin leads to elevated blood glucose levels, necessitating medical intervention and lifestyle changes to manage the disease.

Non-alcoholic fatty liver disease (NAFLD) is another significant complication of obesity and is rapidly becoming the most common liver disorder worldwide (Younossi et al., 2016). Characterised by the accumulation of fat in liver cells in individuals who consume little or no alcohol, NAFLD can progress to non-alcoholic steatohepatitis (NASH), leading to liver inflammation and damage. This condition can further evolve into cirrhosis, liver failure, or hepatocellular carcinoma (Chalasani et al., 2018). Obesity-induced insulin resistance plays a crucial role in NAFLD development, as it increases free fatty acid flux to the liver and promotes lipotoxicity and oxidative stress, which in turn triggers liver injury and fibrosis (Buzzetti et al., 2016).

In addition to NAFLD, obesity is intimately linked to a range of metabolic diseases beyond type 2 diabetes. These include dyslipidemia, characterised by elevated levels of triglycerides, decreased high-density lipoprotein (HDL)

cholesterol, and increased low-density lipoprotein (LDL) cholesterol, which heightens the risk of atherosclerosis and cardiovascular diseases (Klop et al., 2013). Furthermore, obesity can lead to an increased incidence of gout and kidney diseases due to systemic metabolic stresses and the strain of carrying excess weight, which disrupts normal renal function and uric acid metabolism (Kovesdy et al., 2017).

Cancer risk is also heightened by obesity, with increased incidences of breast, colon, endometrium, kidney, and oesophagal cancers, among others (Lauby-Secretan et al., 2016). The mechanism behind this association involves the hormonal imbalances and chronic inflammation induced by excessive adipose tissue. Adipokines and cytokines released by adipocytes can promote tumourigenesis and alter cellular signalling pathways, contributing to the initiation and progression of cancer (Iyengar et al., 2016).

Respiratory problems are another critical complication of obesity. Obstructive sleep apnea, characterised by intermittent airflow blockage during sleep, is significantly more prevalent in obese individuals (Peppard et al., 2013). This is primarily due to the mechanical obstruction from excessive neck fat, as well as the altered respiratory mechanics from the increased abdominal girth, which can compromise lung capacity and function. This condition not only disrupts sleep quality but also increases the risk for further cardiovascular complications (Drager et al., 2013).

The musculoskeletal system is also severely impacted by obesity. The additional weight places excessive stress on weight-bearing joints, accelerating the wear and tear that leads to osteoarthritis (King et al., 2013). This is compounded by the systemic inflammation associated with obesity, which can further degrade cartilage and joint function (Berenbaum et al., 2017).

Psychologically, obesity is associated with a spectrum of complications, including increased risks of depression, anxiety, and lower self-esteem (Luppino et al., 2010). The stigma and discrimination faced by obese individuals can lead to social isolation and diminished quality of life, exacerbating mental health issues (Puhl and Heuer, 2009). Eating disorders, particularly binge eating disorders, are more prevalent in obese populations, often as a result of psychological stress and poor body image (Kessler et al., 2013). Moreover, the chronic stress from coping with obesity and its associated health issues can lead to mood disturbances and further complicate the management of obesity (Tomiyama, 2019).

Taken together, obesity affects the body in various ways and can lead to a wide range of serious health complications that are also termed non-communicable diseases.

1.2.3 The immune system of the obese host

The obese status is characterised by a chronic, low-grade inflammatory condition of the host, often termed metabolic inflammation (Fantuzzi, 2005; Xu, 2013). This condition is marked by the infiltration of myeloid cells to metabolism-related organs, such as the white adipose tissue (WAT) (Fujisaka et al., 2013). Adipose tissue consists of a diverse array of cells, including adipocytes, stroma cells, and resident immune cells (Lenz et al., 2020). Besides its role as an energy reservoir, adipose tissue functions as an endocrine organ, secreting adipokines that can modulate metabolism and influence inflammation (Chan et al., 2017). In obesity, the expansion and altered function of adipose tissue leads to significant changes in both local and systemic metabolism and resident cell populations, contributing to adipose tissue inflammation (Chan et al., 2017; Crewe et al., 2017). This localised inflammation can become systemic, affecting the homeostasis of other cells and tissues and fostering the development of various chronic

comorbidities, as mentioned before. For instance, obesity-induced changes in adipose tissue macrophages have been shown to play a crucial role in metabolic inflammation. These cells undergo a phenotypic switch from an anti-inflammatory M2 state to a pro-inflammatory M1 state, contributing to insulin resistance and metabolic dysfunction (Castoldi et al., 2015). Additionally, the recruitment of $CD8^+$ T cells to adipose tissue has been found to precede macrophage infiltration and may be instrumental in initiating and perpetuating adipose tissue inflammation (Nishimura et al., 2009). The systemic effects of obesity-induced inflammation extend beyond adipose tissue. Levels of tumour necrosis factor α (TNF-α), IL-1, and IL-6 in the circulation in obese humans and mice are increased and contribute to the development of insulin resistance and, therefore, type 2 diabetes (Hotamisligil, 2010; Hotamisligil et al., 1993; Olefsky and Glass, 2010). Moreover, recent studies have identified new players in obesity-related inflammation, such as inflammasome activation and the role of DAMPs in triggering inflammatory responses (Cardoso et al., 2021; Christ et al., 2018).

Several other organs besides the WAT exhibit low-grade chronic inflammation in the obese status of the host. These include the liver, muscle, pancreas, brain, and both the small and large intestines. The gut, in particular, houses a vast immune system due to its constant exposure to microbial and dietary antigens. Research has revealed significant alterations in gut immunity and barrier function in obesity. For instance, obesity has been associated with changes in the composition and function of the gut microbiome, which can influence intestinal permeability and result in systemic inflammation (Cani et al., 2007). Furthermore, obesity-induced alterations in intestinal immune cells, such as increased pro-inflammatory Th17 cells and decreased Treg cells, have been linked to metabolic dysfunction and insulin resistance (Winer et al., 2011). The impact of obesity on the immune system extends beyond metabolic inflammation. Studies have shown that obesity can impair immune responses to infections. For example,

obesity has been associated with increased susceptibility to viral infections, including influenza and SARS-CoV-2, possibly due to impaired T cell and B cell responses (Green and Beck, 2017; Popkin et al., 2020). Additionally, obesity has been linked to reduced vaccine efficacy, particularly for influenza vaccines, highlighting the broader implications of obesity on immune function (Sheridan et al., 2012).

Understanding the complex interactions between obesity and the immune system is crucial for developing targeted therapies to address obesity-related complications. Recent research has explored potential interventions, such as targeting specific inflammatory pathways or modulating the gut microbiome, to mitigate the negative effects of obesity on immune function and metabolic health (Goldfine and Shoelson, 2017; Zmora et al., 2019)

1.2.3.1 cDC1 in obesity

cDCs play a crucial role in regulating metabolic homeostasis and immune responses in obesity and high-fat diet (HFD) conditions. As the present thesis focuses on cDC1s, the following chapter will mainly discuss this cDC subtype.

cDC1 development and homeostasis

In the context of obesity, the homeostasis of cDC1s is disrupted. Hernández-García and colleagues demonstrated that HFD feeding leads to a significant decrease in the percentage of cDC1s in visceral adipose tissue (Hernández-García et al., 2022). This reduction is likely mediated by alterations in the Flt3L-STAT3 signalling axis, which is critical for cDC1 development and maintenance (Waskow et al., 2008). Research conducted by Deczkowska and colleagues in 2021 demonstrated that when mice were fed an HFD lacking methionine and choline, there was an observed increase in the proliferation of circulating cDC progenitors

in their bloodstream (Deczkowska et al., 2021). Human studies also suggest a positive relationship between BMI and DCs in subcutaneous adipose tissue (Hildreth et al., 2021). It has also been shown that obesity enhances the expression of certain surface molecules on adipose tissue DCs, including MHC, CD40, CD80, and CD86 (Lee and Dixit, 2020). This suggests that these cells become activated in obese conditions and may contribute to the progression of obesity-related disorders. Another study demonstrated that specific dietary elements can influence the inflammatory state of cDCs in laboratory settings (Jaiswal et al., 2019). Interestingly, the study found that the pro-inflammatory responses of cDCs in high-fructose environments were not mediated by major known metabolic regulators or glycolytic control. Instead, acute fructose exposure activated cDCs through the receptor for advanced glycation end-products.

cDC1s and whole-body metabolic regulation

Studies have shown that mice lacking the *Flt3l* gene, which results in the absence of DCs, do not become obese when fed an HFD (Choi et al, 2011; Stefanovic-Racic et al., 2012). In a related study, Cho and colleagues examined mice deficient in the *Ccr7* gene. These mice exhibited reduced numbers of cDCs in adipose tissue during DIO (Cho et al., 2016). Interestingly, these CCR7-deficient mice showed decreased inflammation and lower fasting glucose and insulin levels compared to their wild-type counterparts. Notably, *Flt3l* and *Ccr7* knockouts are also affecting several other cell types besides cDCs (Rasko et al., 1995; Förster et al., 2008).

Contrary to these results, a systemic increase in the expression of Flt3L, and therefore the number of cDCs, reduces the weight gain of WT mice induced by an HFD (Hernández-García et al., 2022). This effect is dependent on *Batf3* and at least partially mediated by NK cells. The same study also showed that *Batf3*-

deficient mice, which lack cDC1s, spontaneously increase body weight and adiposity during ageing. Researchers observed reduced energy expenditure, altered oxygen consumption and increased food intake by these mice. This was associated with a higher respiratory exchange ratio, indicating a shift from fatty acid oxidation to carbohydrate metabolism. At the molecular level, the absence of cDC1s led to decreased expression of genes involved in gluconeogenesis (*G6pc* and *Pck1*) and lipid metabolism (*Cpt1a*) in the liver. Conversely, lipogenic genes such as *Srebf1* and *Scd1* were up-regulated. These changes suggest that cDC1s play a crucial role in maintaining metabolic flexibility and preventing the development of hepatic steatosis. It is notable that HFD-fed *Batf3*−/− mice do not become more obese than their WT counterparts.

cDC1s and hepatic steatosis

The role of cDC1s in hepatic steatosis is complex and context-dependent. Heier and colleagues demonstrated that cDC1s play a protective role against steatosis progression (Heier et al., 2017). They found that cDC1s in the liver produce IL-15, which promotes the survival and function of NK cells and memory T cells. These cells, in turn, help maintain liver homeostasis by eliminating stressed hepatocytes and regulating lipid metabolism. Another study demonstrated that *Batf3*−/− mice fed a chow diet developed liver steatosis with age, and an increase in cDC numbers due to Flt3L treatment resulted in decreased hepatomegaly (Hernández-García et al., 2022). However, Deczkowska and colleagues presented contrasting findings, showing that mice depleted of cDC1s develop less hepatic steatosis on a choline-deficient and HFD and stated that cDC1s drive liver pathology in non-alcoholic steatohepatitis (NASH) (Deczkowska et al., 2021). They found that in NASH, cDC1s up-regulate the expression of oxidised phospholipid receptors like CD36 and MSR1. This leads to

increased uptake of oxidised lipids, triggering a pro-inflammatory response characterised by the production of TNF-α and IL-1β.

cDC1s and adipose tissue

In lean adipose tissue, cDC1s contribute to maintaining a balanced immune environment. Hernández-García and colleagues showed that cDC1s support the presence of NK1.1^{+} invariant NKT (iNKT) cells in adipose tissue (Hernández-García et al., 2022). iNKT cells produce IFN-γ, which licenses NK cells to efficiently remove apoptotic macrophages, thereby preventing excessive inflammation. The absence of cDC1s in *Batf3*$-/-$ mice leads to increased inflammation in adipose tissue with age, with accumulation of pro-inflammatory macrophages and increased TNF-α expression. The researchers showed that HFD feeding leads to a significant decrease in the percentage of cDC1s in visceral adipose tissue, which might explain adipose tissue inflammation in obesity.

cDC1s and the small intestine

The intestinal barrier plays a critical role in metabolic health, and cDC1s are essential for maintaining its integrity. cDC1s were demonstrated to be crucial for the proper localisation of tight junction proteins in intestinal epithelial cells (Ohta et al., 2016). In the absence of cDC1s, mice exhibit increased intestinal permeability and susceptibility to colitis. Mechanistically, cDC1s in the intestine produce IL-12 and IL-15, which stimulate IFN-γ production by intraepithelial lymphocytes. This IFN-γ then acts on intestinal epithelial cells to induce the expression of tight junction proteins and antimicrobial peptides, reinforcing the intestinal barrier. According to findings from Lécuyer and colleagues, cDC1s appear to play a protective role against obesity and metabolic dysfunction connected to improved gut health (Lécuyer et al., 2021). Specifically, the researchers found that mice with enhanced cDC1 survival and function due to

Bcl-2 over-expression were resistant to DIO and had improved metabolic parameters, which was associated with shaping a protective gut microbiota characterised by lower immunogenicity and higher butyrate production. A study by Hamade and colleagues revealed that *Batf3*−/− mice developed metabolic syndrome and showed changes in intestinal epithelial tight junction protein distribution, leading to increased gut permeability (Hamade et al., 2022). Antibiotic treatment in these mice prevented both metabolic syndrome and impaired intestinal barrier function. The *Batf3*-deficient mice exhibited alterations in IgA-coated faecal bacteria and microbial imbalance, characterised by reduced levels of *Akkermansia muciniphila* and *Bifidobacterium*. These findings suggest that cDC1s might contribute to maintaining an optimal intestinal barrier, potentially fostering the growth of microbiota that protect against obesity.

Wang and colleagues demonstrated the increase of cDC1 proportion within $CD45^+$ cells in the small intestine of high-fat, high-sugar diet (HFHSD) fed mice (Wang et al., 2023). They identified a potential immunoregulatory transformation of cDCs in the obese host, with gene expression profiles indicating negative regulation of pro-inflammatory activation of cDCs and facilitated Treg induction.

1.2.3.2 The enteric immune system in obesity

The small intestine displays the largest immune compartment in mammals and is also the main organ responsible for nutrient absorption (Mowat and Agace, 2014). As the immune system of the gut is shaped by the dietary choices of the host, it is not surprising that the immune landscape in obesity differs from steady-state (Khan et al., 2021; Sullivan et al., 2021; Wang et al., 2023). Several groups have investigated the enteric immune response to obesogenic diet. In 2023, Wang and colleagues contributed with a high-resolution, high-dimensional

single-cell intestine atlas of the lean and obese intestine to the field (Wang et al., 2023). They provided a comprehensive characterisation of the cellular composition and cell type-specific signalling networks in the small intestine, encompassing intraepithelial immune cells, lamina propria immune cells, and epithelial structural cells (defined as CD45- cells). This work was based on Single-cell RNA sequencing (scRNA-seq) data, and results were validated using flow cytometry on independent cell cohorts. Through ligand-receptor analysis, they mapped both homeostatic and inflammatory circuits in the intestine, establishing a detailed signalome across various cell types, which uncovered obesity-associated inflammatory and immunoregulatory pathways that were enriched in the HFHSD-fed intestine.

In the innate immune compartment, obesity induces significant changes in ILC populations. Wang and colleagues could identify an increased proportion of ILC1s, ILC2s and ILC3s in HFHSD (Wang et al., 2023). Other studies showed that the frequency of IL-22- and IL-17-secreting ILC3s is reduced in HFD-fed mice compared to lean controls. This reduction correlated with decreased epithelial barrier integrity and increased serum lipopolysaccharide levels, suggesting a potential link to metabolic endotoxemia (Luck et al., 2015). The underlying mechanism for this reduction remains unclear, but a defective IL-23-to-IL-22 axis has been proposed based on observations of reduced IL-23 expression in obese mice following *Citrobacter rodentium* infection (Wang et al., 2014; Zheng et al., 2008). Interestingly, small intestinal ILC2s appear to play a pathogenic role in obesity-related metabolic syndrome. Studies have shown that ILC2-deficient mice are protected from metabolic syndrome, while adoptive transfer of these cells into ILC2-deficient mice recapitulates disease parameters (Sasaki et al., 2019). However, the role of intestinal ILC2s is unclear. They are a prominent source of the anti-inflammatory cytokine IL-10 in steady-state and might become pathogenic during obesity (Bando et al., 2020). Human studies have revealed an

inverse relationship between BMI and the number of intestinal ILCs (Yudanin et al., 2019). However, the functional implications of this observed correlation remain to be fully elucidated, highlighting the need for further research to understand the physiological consequences of reduced ILC numbers in obesity.

The T cell compartment in the intestine also undergoes significant remodelling during obesity. In the epithelium, changes in intraepithelial lymphocyte (IEL) populations have been observed. Some studies report an increase in IL-17-producing TCR$\gamma\delta^+$ T cells in the small bowel after prolonged HFD feeding (Luck et al., 2015), while others note a reduction in both TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ IELs (Park et al., 2019). The latter study showed that mechanisms underlying these changes appear to differ between TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ IEL populations, with TCR$\alpha\beta^+$ IELs showing reduced homeostatic proliferation and both populations exhibited a down-regulation of CD103 and CCR9 expression. Tanaka and colleagues also found that TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ IELs, CD4$^-$CD8$\alpha\beta^-$TCR$\alpha\beta^+$ and CD4$^+$ T cells were reduced upon HFD feeding (Tanaka et al., 2020). Wang and colleagues saw a decrease in CD8$\alpha\alpha^+$TCR$\alpha\beta^+/\gamma\delta^+$ and CD8$\alpha\beta^+$TCR$\alpha\beta^+/\gamma\delta^+$ T cell proportions in HFHSD conditions, primarily coming from CD8$\alpha\alpha^+$TCR$\gamma\delta^+$, CD8$\alpha\beta^+$TCR$\gamma\delta^+$ and CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells that showed increased cell death (Wang et al., 2023). They also observed a shift in frequency from effector-like CD8$^+$ IELs in chow diet intestines to memory-like CD8$^+$ IELs in HFHSD intestines, which may be due to an increase in differentiation from proliferating CD8$\alpha\alpha^+$ IELs. Oppositional to that, they found CD4$^+$TCR$\alpha\beta^+$ T cells increased and a shift towards higher frequencies of memory-like CD4$^+$ T cells and Tregs while Th1 frequencies were decreased.

In the lamina propria, obesity is associated with an elevation in pathogenic IFN-γ-producing Th1 and CD8$\alpha\beta^+$ T cells, alongside a reduction in protective IL-17-producing Th17 cells and IL-10-secreting Tregs (Garidou et al., 2015; Hong

et al., 2017; Luck et al., 2015; Monteiro-Sepulveda et al., 2015). However, the timing and extent of these changes vary between studies, likely due to differences in experimental conditions and duration of HFD feeding. One study reported a change in T cells only at 12 weeks but not after 3 weeks of an obesogenic diet. Here, the proportions of IFN-γ-producing Th1 cells and $CD8^+$ T cells increased, and percentage of Tregs reduced upon HFD feeding (Luck et al., 2015). Another study identified reduced frequency and numbers of Th17 cells in the ileum after 4 weeks of HFD feeding, which may contribute to increased intestinal permeability through altered antimicrobial peptide production (Garidou et al., 2015). Tanaka and colleagues reported a reduction in $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells, $CD4^-CD8\alpha\beta^-TCR\alpha\beta^+$, $CD4^+$ and $CD8^+$ T cells (Tanaka et al., 2020). Wang and colleagues observed a reduction in $CD8\alpha\alpha^+TCR\alpha\beta^+/\gamma\delta^+$, $CD8\alpha\beta^+TCR\alpha\beta^+/\gamma\delta^+$ T cells, which primarily resulted from the reduction of $CD8\alpha\alpha^+TCR\gamma\delta^+$, $CD8\alpha\beta^+TCR\gamma\delta^+$, and $CD8\alpha\beta^+TCR\alpha\beta^+$ T cells due to increased cell death (Wang et al., 2023). Like in the epithelium, they also saw an increase in $CD4^+TCR\alpha\beta^+$ T cells in the lamina propria with the same trends of increased memory-like $CD4^+$ T cells and Tregs and decreased Th1 frequencies. However, absolute numbers of Th17 cells were slightly increased, and Th17 cells significantly increased in obese mice. Human studies have largely corroborated findings from animal models. Obese individuals show increased T-bet^+ Th1 and ILC1s and $CD8^+$ T cells and reduced Treg cells in the small intestine compared to lean subjects (Luck et al., 2015). Notably, an increase in lamina propria and intraepithelial $CD8^+$ T cells (especially $CD8\alpha\beta^+$ T cells) has been observed, which may directly influence epithelial barrier function due to their proximity (Monteiro-Sepulveda et al., 2015).

Research has also revealed alterations in B cell populations and antibody-secreting cells (ASCs) within the intestine during obesity (Luck et al., 2019). Specifically, investigators have observed a decline in the number of these

populations in the intestinal tissues of obese mice. This reduction was associated with lower levels of secretory IgA in the colon and is potentially contributing to insulin resistance. Oppositional, Wang and colleagues observed an increase of B cell proportion in lamina propria and epithelium of HFHSD mice compared to lean mice (Wang et al., 2023).

The myeloid compartment is also affected by obesity, although findings have been somewhat inconsistent. Jonhson and colleagues reported no changes in total macrophage and DC numbers but an increase in frequency early in HFD feeding (Johnson et al., 2015), while others note an increase in pro-inflammatory $CCR2^{+}$ macrophages (Kawano et al., 2016) and a decrease in $CX3CR1^{+}$ lamina propria macrophages (Luck et al., 2019; Hong et al., 2017). However, a direct relationship between the latter results has not been assessed. Wang and colleagues identified several macrophage subpopulations, namely Ly6C high ($Ly6C^{hi}$) CX3CR1 low ($CX3CR1^{lo}$) monocyte-derived macrophages and, within the high CX3CR1 ($CX3CR1^{hi}$) population, $CX3CR1^{hi}$ inflammatory and $CX3CR1^{hi}$ regulatory macrophages (Wang et al., 2023). In HFHSD conditions, they observed a slight increase in $CX3CR1^{hi}$ inflammatory macrophages, which express high levels of the co-stimulatory ligands CD86 and CD80, as well as the chemokine CXCL9, which are critical for activation and recruitment of T cells. Conversely, they detected a decrease in $CX3CR1^{hi}$ regulatory macrophages, which express high levels of immunoregulatory genes like *Mrc1* or *Clec12a* and also produce growth factors like IGF-binding proteins (Igfbp4) and growth and differentiation factor 15 (Gdf15). These could influence whole-body metabolism when entering the bloodstream. The $Ly6C^{hi}CX3CR1^{lo}$ populations showed no differences between HFHSD and CD animals. Another study examined total APCs ($MHCII^{+}CD19^{-}$ gating) in mice fed an HFD for 10 or 30 days (Garidou et al., 2015). On the one hand, the cells exhibited reduced expression of activation markers, including CD86, and demonstrated a diminished capacity to promote Th17 cell

differentiation *in vitro*. On the other hand, these APCs showed increased expression of genes associated with inflammatory pathways. This seemingly paradoxical profile suggests that HFD induces nuanced changes in APC function, potentially altering their role in intestinal immune regulation and inflammation during the development of obesity.

Changes in DC subsets have been observed, in particular, a lower ratio of pro-IgA intestinal-specific double-positive cDCs (dpDC) within total cDCs was observed (Luck et al., 2019). Wang and colleagues detected an increase in proportions of cDC1 and cDC2A in total $CD45^+$ cells, while cDC2B were decreased in HFHSD animals (Wang et al., 2023). They saw a potential immunoregulatory transformation of cDCs in the intestine of the obese host as HFHSD-induced shifts in upstream regulators of differentially expressed genes (DEGs) between all CD and HFHSD cDCs towards genes that negatively regulate the pro-inflammatory activation of cDCs and facilitate Treg induction. Interestingly, they also demonstrated that cDC1s increased their cell-cell interactions in HFHSD conditions and identified these cells as central communication hubs in the intestine of the obese host. Their interaction analyses also indicated that both $CD8\alpha\alpha^+$ T cells and $CD\alpha\beta^+$ T cells significantly increased the production of the chemokine XCL1, while frequencies of these populations decreased. XCL1 is the only known ligand for XCR1, which is present on cDC1s and mediates XCL1–XCR1-mediated recruitment of cDC1s. In contrast to animal studies, research on humans reported an elevated ratio of macrophages and up-regulated numbers of activated cDCs and NK cells compared to overweight but metabolically healthy and lean patients (Monteiro-Sepulveda et al., 2015).

The role of granulocytes in HFD-induced intestinal inflammation remains incompletely understood. Current evidence suggests a limited involvement of neutrophils in this process. Histological analyses of intestinal specimens from

HFD-fed mice and obese humans have not revealed signs of active ileitis, which is typically characterised by neutrophil infiltration (Luck et al., 2015). Eosinophils, on the other hand, appear to be more significantly affected by HFD. Studies have shown a reduction in both the number and frequency of eosinophils in the intestine after just one week of HFD feeding (Johnson et al., 2015) and after 8 weeks of HFHSD feeding (Wang et al., 2023). However, the long-term implications of this eosinophil reduction require further investigation to fully understand their significance in obesity-related intestinal dysfunction.

It is important to note that the intestinal inflammation observed in obesity appears to be more subtle compared to that seen in other metabolic tissues like adipose tissue or liver (Luck et al., 2015). This suggests that obesity-related intestinal alterations manifest through changes in immune cell numbers, localisation, and functional states rather than large inflammatory infiltrates.

In conclusion, the enteric immune system undergoes complex and multifaceted changes in response to obesity. These alterations involve shifts in the proportions and functions of various immune cell populations, as well as remodelling of cellular communication networks. This might contribute to a state of low-grade inflammation and deregulated intestinal homeostasis. However, studies disagree about exact timing and even effects of an obesogenic diet. Results may vary depending on factors such as diet composition, microbiota, animal housing conditions, and host genetic background (Khan et al., 2021; Ussar et al., 2015).

At the same time, studies in the lean host have shown that the immune system in the small intestine is not homogeneous along the intestine, but duodenum, jejunum and ileum differ in the composition of their immune landscape (Mowat and Agace, 2014). The small intestine's various segments play distinct roles in digestion and absorption and might be affected differently by an obesogenic

diet. Importantly, each segment of the gut responds differently to acute and chronic nutrient exposure. For example, de Wit and colleagues found that different fat contents in diet lead to differential gene expression and thickness of mucosal lining along the length of the gut (De Wit et al., 2008). However, studies that aimed to investigate the impact of an obesogenic diet on the enteric immune system most often did not consider the regional specialisation of the gut. Oppositional adaptations in different segments might even be balancing each other out, and analysing the intestine as a whole might not uncover certain diet-induced adaptations.

1.2.3.3 Food tolerance in obesity

Obesity has become a global health concern, with its prevalence reaching epidemic proportions in many countries. At the same time, food intolerances are on the rise (Renz et al., 2018). It is unclear if these phenomena are in mechanistic relationship to each other or merely correlate, as it could be that unfavourable dietary choices driven by food intolerance are leading to obesity (Hayashi et al., 2021; Lee et al., 2021). Even though there has been a lot of research done in the fields of obesity and food tolerance separately, we do not have a clear understanding of how obesity affects oral tolerance.

One study demonstrated that intake of dietary lipids impaired the induction of oral tolerance (Streich et al., 2022). Researchers observed a decreased hypersensitivity response to ovalbumin (OVA) in naïve obese mice compared to lean mice but an increased response in tolerised obese mice compared to tolerised lean mice.

The pro-inflammatory and tolerogenic immune responses in the gut depend on interactions between APCs and T cells (Rezende and Weiner, 2022). Since

obesity shifts the immune landscape of the small intestine (Wang et al., 2023), it is possible that, in particular, a shift of cDC1s and/or Treg frequencies in obesity might affect tolerance to food. In this sense, one study showed that down-regulation of cDC1 in mLN in the lean host results in failure of oral tolerance (Fukaya et al., 2023).

One of the key factors influencing food tolerance in obesity could be the altered gut microbiome. Obesity and metabolic syndrome are often associated with an altered gut microbiota or dysbiosis (Turnbaugh et al., 2008). The already mentioned study conducted by Fukaya and colleagues found that antibiotic-induced intestinal dysbiosis results in the loss of tolerogenic cDC1 in the mLNs (Fukaya et al., 2023). Here, dysbiosis lead to an impaired cross-talk between ILC3s and cDC1s, which resulted in a reduction of cDC1s in the mLN. This finally abrogated the generation of Treg cells, and mice failed to establish oral tolerance. Another study by Hussain and colleagues found that obesity is associated with a shift in the composition and diversity of gut microbiota (Hussain et al., 2019). This dysbiosis promoted mast cell-mediated food allergy. Gu and colleagues showed that HFD and OVA irritation in an OVA and cholera toxin-induced food allergy model enhanced the levels of mast cell degranulation and Th2 humoral response, which was related to intestinal barrier destruction and inflammation through the PPAR γ/NFκB signalling pathway (Gu et al., 2022). However, IgE-mediated food allergy and food intolerance are not to be confused.

The mass of white adipose tissue (WAT) is increased in obesity. The discovery of leptin revolutionised our understanding of WAT, revealing its significant role as an endocrine organ (Hersoug and Linneberg, 2007; Zhang et al., 1994). Adipocytes produce various signalling proteins, collectively termed adipokines, including leptin. Additionally, adipose tissue secretes cytokines and chemokines

such as TNF-α, IL-6, IL-10, IL-1β, and MCP-1 (Hersoug and Linneberg, 2007). Some of these molecules can directly influence immune function and might have an impact on food tolerance. Obesity is characterised by elevated serum leptin levels in both humans and mice (Bryson et al., 1999; Considine and Caro, 1997). Leptin's immunomodulatory effects include the protection of T lymphocytes from apoptosis and the regulation of T-cell activity (Fantuzzi, 2005). Notably, leptin plays a crucial role in controlling Treg proliferation, which is essential for maintaining food tolerance (De Rosa et al., 2007). *In vitro* studies have shown that neutralising leptin enhances human Treg proliferation, and leptin and leptin receptor-deficient mice exhibit increased Treg proliferation (De Rosa et al., 2007). Consequently, the elevated leptin levels observed in obesity may potentially suppress Treg proliferation, eventually impacting food tolerance mechanisms.

In sum, the relationship between obesity and food tolerance is not well understood and might involve alterations in gut microbiota, immune cell crosstalk, particularly between cDC1s and Tregs, adipokine and cytokine imbalance and general chronic low-grade inflammation of the host. It is not even clear if obesity promotes or attenuates food tolerance. However, the worldwide rise in both food intolerance and obesity raises questions regarding the relationship between those phenomena.

2

Research Objective and Hypothesis

2.1 Significance of the study

The current global trend in obesity is alarming, with substantial increases in overweight and obesity observed across all age groups and regions in the world. Obesity is a complex health condition and is associated with a range of complications. The immune system is heavily influenced by the diet of the host, and itself influences the host's physiology. While studies have investigated adaptations of the small intestinal immune system to obesogenic diet, the regional specialisation of the gut was not taken into consideration. Each intestinal segment harbours a distinct immune landscape adapted to its physiologic role. As such, different segments might show distinctive adaptations to obesogenic diets. It is important to characterise the impact of diet on the enteric immune system and the resulting consequences for the obese host to understand the physiology of the obese human. In addition to obesity, food intolerances are on the rise and the relationship between obesity and food tolerance is not well understood.

2.2 Hypothesis

1. The composition of the enteric immune system is dependent on the diet of the host. Different intestinal segments might show distinct alterations to obesogenic diet.

2. A shifted enteric immune landscape in the obese host might influence the physiology of the host. cDC1s are able to induce Treg cells and are increased in the obese lamina propria. This might result in an increased tolerance to food antigens as oral tolerance relies on Tregs.

2.3 Objectives

1. Characterisation of the immune system in duodenum, jejunum and ileum of the obese host to understand its adaptation and the kinetics of the response to obesogenic diet.

2. Evaluation of similarities between diet-indued adaptations of the immune system in different small intestinal segments.

3. Identification of cell types that are significantly altered by obesogenic diet and examination of their phenotype in the obese host.

4. Investigation of the potential physiological consequences of these alterations for the host.

3

Materials and Methods

3.1 Experimental Animals

All mouse lines were bred on a full C57BL/6J background. Mice were housed and bred at Champalimaud Centre for the Unknown (CCU) under specific pathogen-free conditions. Mice were maintained on 12 h light-dark cycles, with access to food and water *ad libitum*, if not stated otherwise. All used mice were male and age-matched. All animal experiments were approved by national and local institutional review boards (IRBs), Direção Geral de Veterinária and CCU ethical committees. C57BL/6J mice were purchased from Charles River and maintained in our facilities. OT-II TCR-transgenic (Barnden et al. 1998) were originally purchased from Jackson Laboratories, crossed with C57BL/6 Ly5.1 and with Rag1-/- (Mombaerts et al. 1992), and maintained in our facilities.

Resource	Source	Identifier
Mouse: C57BL6/J (CD45.2)	Charles River	632C57BL/6J
Mouse : C57BL6/J (CD45.1)	Jackson Laboratories	002014
Mouse: C57BL6/J Rag1$^{-/-}$	Jackson Laboratories	002216
Mouse: OTII	Jackson Laboratories	004194

Table 1. Experimental animal models

3.2 Dietary Intervention

Mice were raised on a standard chow diet until they reached 8 weeks of age. Adult mice were randomly assigned to one of two dietary regimens: a high-fat, high-sugar diet (HFHSD) or a continuation of the normal chow as a control diet (CD). The HFHSD was designed to mimic the composition of a typical American fast-food diet (Asgharpour et al., 2016). This diet included 60% of calories from fat (lard), corresponding to the D12492 formulation. Drinking water was supplemented with a sugar solution comprising 23.1 g/l fructose (55% w/w) and 18.9 g/l glucose (45% w/w), resulting in a total sugar concentration of 42 g/l. The control group continued on the standard CD, which contained 12% calories from fat-origin. CD-fed animals were provided normal, non-supplemented drinking water. Mice had access to food and drinking *ad libitum*.

Reagent or Resource	Source	Identifier
CD	SDS	801030
HFHSD	Ssniff GMbH	E15742-347
Glucose	Fisher Scientific	10539380
Fructose	Sigma-Aldrich	F3510

Table 2. Reagents for dietary intervention.

3.3 Glucose Tolerance Test

Glucose tolerance in mice was evaluated 1 week prior to experiments. Following an overnight fast, mice were administered an intraperitoneal injection of glucose (2 mg/kg body weight) dissolved in PBS. Blood glucose levels were measured using an ACCU-CHEK Aviva glucometer immediately before glucose administration and at 15-minute intervals for 2.5 hours post-injection.

Reagent or Resource	Source	Identifier
Glucose	Fisher Scientific	10539380
PBS	Corning	46-013-CM
Glucometer	Roche	7819382037
Accu-Chek Aviva Test Stripes	Roche	6453970023

Table 3. Reagents and materials for glucose tolerance test.

3.4 Cell Isolation and purification

Reagent or Resource	Source	Identifier
Collagenase D	Roche	11088882001
Collagenase II	Gibco	LTI 17101-015
Collagenase VI	Worthington	LS004188
DMEM	StemCell	36254
DNase I	Roche	10104159001
DTT	Sigma-Aldrich	43816-0010
EDTA	Corning	46-034-CI
FBS	Corning	35-079-CV
HBSS	Corning	21-023-CV

(to be continued)

Reagent or Resource	Source	Identifier
Heparine	Braun	P8721
HEPES	Corning	25-060-CI
L-glutamine	Corning	10-040-CVR
Liberase TL	Roche	5401020001
Liberase TM	Roche	5401127001
PBS	Corning	46-013-CM
Penicillin/Streptomycin	Corning	30-002-CI
Percoll	Cytiva	GE17-0891-01
RBC lysis buffer	eBioscience	00-4300-54
RPMI	Corning	10-040-CVR
Sodium pyruvate	Corning	25-000-CIR
β-mercaptoethanol	Gibco	31350-010

Table 4. Reagents and materials for cell isolation and purification.

3.4.1 Small intestine

For the examination of the small intestinal immune system in obesity and steady-state conditions, mice were fasted overnight (8 hours) prior to euthanasia. All mice were sacrificed using CO_2. Small intestines were isolated and thoroughly rinsed with ice-cold PBS. Residual fat and Peyer's patches were removed. The intestines were opened longitudinally and divided into three sections: duodenum (proximal 25% of the intestine), jejunum (medial 50% of the intestine) and ileum (distal 25% of the intestine). Each section was cut into 1 cm long pieces and placed into individual 50 ml Falcon tubes containing 20 ml of complete RPMI medium (cRPMI) supplemented with 5 mM DTT to remove mucous. cRPMI was composed of RPMI supplemented with 5% FBS, 1% HEPES,

1% sodium pyruvate, 1% streptomycin and penicillin and 0.1% β-mercapto-ethanol. The intestinal pieces were incubated at 37°C in an orbital shaker at 700 x g for 20 minutes. Following incubation, the supernatant was removed, and 20 ml of cRPMI supplemented with 5 mM EDTA was added to each tube. The intestinal pieces were then agitated at 700 x g for 20 minutes at 37°C to remove epithelial cells. This washing step was repeated once.

For epithelial cell isolation, supernatants of both washing steps were collected, pooled, and filtered through 70 μm cell sieves. Mucous was removed from suspensions by sedimentation for 10 minutes at room temperature and discharge of sediment.

To isolate cells from the lamina propria, the intestinal pieces underwent further digestion after epithelial cell removal. Pieces were washed with 10 ml HBSS supplemented with 10 mM HEPES by shaking at 700 x g for 10 minutes at 37°C to remove residual FBS and EDTA. The intestinal pieces were then minced into smaller fragments using a blade. For digestion, the fragments were incubated in 7 ml HBSS supplemented with 10 mM HEPES, DNase I (20 U/ml) and Collagenase D (5 mg/ml) or, for cDC isolation, Liberase TM (25 μg/ml). Fragments were digested for 30 minutes at 37°C at 200 rpm. The reaction was halted by adding 10 ml of ice-cold cRPMI. The resulting cell suspension was passed three times through an 18G needle, filtered through a 100 μm cell strainer, and then through a 70 μm cell sieve.

For lymphocyte analysis, the digests were purified by centrifugation for 30 minutes at 1200 x g in a 40/80 Percoll gradient. When necessary, erythrocytes in the samples were lysed using RBC lysis buffer.

Single cell suspensions were filtered through a 40 μm strainer and centrifuged at 600 x g for 2 minutes at 4ºC. Cell pellets were transferred to 96-well plates for staining.

3.4.2 Liver

Mice were anaesthetised with an intraperitoneal injection of pentobarbital (100 μL per mouse). Cardiac perfusion was then performed using 20 mL of PBS administered through a 25G needle. Following perfusion, the liver was removed and placed into a 6-well plate containing 6 mL of HBSS. For the digestion process, 1 ml of HBSS containing Collagenase IV (0.5 mg/mL) and DNase I (20 U/ml) was injected into the liver using a 25G needle, after which the tissue was cut into small pieces. The digestion was allowed to proceed at room temperature for 20 minutes and was subsequently terminated by the addition of 1 mL of FBS. The resulting cell suspension was filtered through a 70 μm strainer and washed with 10 mL FACS buffer (2.5% FCS and 2.5 mM EDTA in PBS). To remove hepatocytes, the suspension underwent a series of low-speed centrifugations. Specifically, it was centrifuged at 50 x g for 2 minutes at 4ºC, and the supernatant was collected. This process was repeated twice to ensure thorough hepatocyte removal. The final supernatant was then centrifuged at 300 x g for 10 minutes at 4ºC to pellet the remaining cells. Erythrocytes were lysed using RBC lysis buffer. Cells were washed with 5 ml of FACS buffer, filtered through a 40 μm strainer, and pelleted by centrifugation at 600 x g for 2 minutes at 4ºC. Cell pellets were transferred to 96-well plates for staining.

3.4.3 Pancreas

Mice were anaesthetised with an intraperitoneal injection of pentobarbital (100 μL per mouse). Cardiac perfusion was then performed using 20mL of PBS

administered through a 25G needle. Following perfusion, the pancreas tissue was minced into small pieces. Enzymatic digestion was performed by incubating the minced tissue in 1 mL of HBSS supplemented with Collagenase D (5 mg/ml) and DNase I (20 U/ml). The digestion mixture was incubated at 37°C for 30 minutes with constant agitation at 1100 rpm using an Eppendorf shaker. After digestion, the cell suspension was filtered through a 40 μm strainer. The resulting filtrate was then washed with 10 mL of FACS buffer and centrifuged at 600 x g for 2 minutes at 4ºC. Cell pellets were transferred to 96-well plates for staining.

3.4.4 Spleen

Mice were sacrificed using CO_2, and the spleen was extracted and placed in 1 mL of HBSS in a 24-well plate. A digestion solution containing 2 ml HBSS supplemented with Liberase TL (5 mg/ml) and DNase I (20 U/ml) was injected into the spleen with a 25G needle, after which the organ was cut into small pieces. Digestion was carried out in two 15 minutes incubations at 37°C, with additional mechanical disruption between incubations. The reaction was terminated by adding 100 μL of FBS. The resulting cell suspension was filtered through a 70 μm strainer and washed with 5 mL of FACS buffer. Cells were then pelleted by centrifugation at 600 x g for 5 minutes at 4ºC. Erythrocyte lysis was performed using RBC lysis buffer. Cells were washed with 5 ml of FACS buffer, filtered through a 30 μm syringe filter, and pelleted by centrifugation at 600 x g for 2 minutes at 4ºC. Cell pellets were transferred to 96-well plates for staining.

3.4.5 Colon

Mice were sacrificed using CO_2, and the colon was excised and cut longitudinally into 1 cm pieces. The tissue was then incubated in 50 ml falcons containing 15 ml PBS supplemented with 5% FBS, 2 mM EDTA, and 1 mM DTT

at 37°C in an orbital shaker at 700 x g for 15 minutes at 37ºC. The tissue was washed thoroughly with PBS. For enzymatic digestion, the tissue was incubated in 10 mL of HBSS supplemented with 1% FBS, Collagenase IV (0.5 mg/mL), and DNase I (20 U/ml) for 45 minutes at 700 x g at 37ºC. The reaction was stopped by adding 10 mL of cRPMI. The resulting cell suspension was filtered sequentially through 70 µm and 30 µm strainers, washing each with 5 mL of FACS buffer. Cells were then centrifuged at 600 x g for 2 minutes at 4°C and transferred to 96-well plates for staining.

3.4.6 Fat

Mice were sacrificed using CO_2, and epididymal white adipose tissue (eWAT) was excised. The tissue was mechanically disaggregated using scissors and then placed in 3 mL (5 mL for obese mice) of HBSS supplemented with 0.5% BSA and type II collagenase (3 mg/ml). Enzymatic digestion was performed at 37°C for 20 minutes. The reaction was terminated by adding 300 µL of FBS. The resulting cell suspension was filtered through a 100 µm cell strainer and washed with 5 mL FACS buffer. Cells were then pelleted by centrifugation at 600 x g for 10 minutes at 4ºC. The cell pellet was resuspended in FACS buffer and filtered through a pre-wetted 35 µm cell strainer, followed by an additional wash with 5 mL of FACS buffer. Cells were again collected by centrifugation at 600 x g for 2 minutes at 4ºC and transferred to 96-well plates for staining.

3.4.7 Lymph nodes

Mice were sacrificed using CO_2, and lymph nodes were carefully dissected and placed in 1 ml cold cRPMI. The tissue was then mechanically disaggregated to increase surface area for enzymatic digestion. Collagenase D (400 U/ml) was added, and the mixture was incubated for 25 minutes at 37°C in a 5% CO_2

atmosphere. To obtain a single-cell suspension, the digested tissue was further mechanically disrupted by passing it 3 times through an 18G syringe needle. Cells were washed with FACS buffer and filtered through a 40 um cell strainer. The cell suspension was then centrifuged at 600 x g for 2 minutes at 4°C and transferred to 96-well plates for staining.

3.4.8 Bone marrow

Mice were sacrificed using CO_2, and femurs and tibia were removed and cleaned carefully. Bones were collected in 8 ml of ice-cold DMEM supplemented with 2% FCS, 1% L-glutamine and 1% Penicillin/Streptomycin. Bones were cut open and placed into an Eppendorf tube. Bones were centrifuged for 30 seconds at 10,000 x g at 4°C to extract bone marrow. Erythrocytes were used using an RBC lysis buffer, and the cell suspension was washed with 2 ml FACS buffer, filtered through a 40 μm sieve, then centrifuged at 600 x g for 2 minutes at 4°C and transferred to 96-well plates for staining.

3.4.9 Blood

Mice were sacrificed using CO_2, and blood was collected by cardiac puncture and with syringes that were pre-coated with heparin. Blood samples were centrifuged at 350 x g for 10 minutes at room temperature. Erythrocytes were lysed using RBC lysis buffer, and cell suspension was washed with 2 ml FACS buffer. The cell suspension was filtered through a 40 μm cell sieve, then centrifuged at 600 x g for 5 minutes at 4°C and transferred to 96-well plates for staining.

3.5 Flow cytometry

3.5.1 Cell stimulation and staining

For cytokine analysis of cDCs, mice were injected i.p. with Brefeldin A (125 μg/mouse) in 100 μl PBS 12 hours prior to analysis.

Permeabilisation and fixation of cells for intracellular staining were performed using an IC fixation/permeabilisation kit for cytokine and transcription factor analysis. For blocking nonspecific antibody binding, cells were pre-incubated with anti-CD16/32 (1:200) in PBS for 10 minutes at 4°C. Afterwards, samples were stained for 20 minutes at 4°C with an antibody cocktail and LIVE/DEAD Fixable Aqua Dead Cell Stain Kit in PBS. When necessary, Brilliant Stain Buffer was added to the staining medium. For intracellular staining, cells were incubated overnight with intracellular antibodies and acquired on the next day.

Reagent or Resource	**Source**	**Identifier**
Brefeldin A	BD Horizon	420601
anti-CD16/32 (2.4G2)	Invitrogen	14-0161-86
Brilliant Stain Buffer	BD Horizon	566385
LIVE/DEAD Fixable Aqua Dead Cell Stain Kit	eBioscience	00-8222-49
IC fixation/permeabilization kit	Invitrogen	00-8222-49

Table 5. Reagents for cell stimulation and staining.

3.5.2 Cell markers and gating strategy

Cell suspensions were stained with antibodies shown in Table M6. Cell populations were gated on live cells and defined as ILC2: $CD45^+Lin^-Thy1.2^+$ $Sca\text{-}1^+KLRG1^+$; ILC3: $CD45^+Lin^-Thy1.2^{high}ROR\gamma t^+$ (lineage comprised CD3ε, CD8α, TCRβ, TCRγδ, CD19, GR1, CD11c, CD11b and TER119); NK: $CD45^+Lin^-$ $NK1.1^+NKp46^+CD27^+CD49b^+CD127^-$; ILC1: $CD45^+Lin^-NK1.1^+NKp46^+CD27^+$ $CD49b^-CD127^+$ (lineage without CD11b and NK1.1); B cell: $CD45^+TCR^-CD19^+$; $CD4^+$ $TCR\beta^+$ T cell: $CD45^+TCR\beta^+CD4^+$; $CD4^+$ $TCR\gamma\delta^+$ T cell: $CD45^+TCR\gamma\delta^+CD4^+$; $CD8\alpha^+TCR\beta^+$ T cell: $CD45^+TCR\beta^+CD8\alpha^+$; $CD8\alpha^+TCR\gamma\delta^+$ T cell: $CD45^+TCR\gamma\delta^+$ $CD8\alpha^+$; cDC: $CD45^+CD64^-MHCII^+CD11c^+$; cDC1: $CD45^+CD64^-MHCII^+CD11c^+$ $CD11b^-CD8\alpha^+$ or $CD45^+CD64^-MHCII^+CD11c^+Xcr1^+Sirp^{low}$ cDC2: $CD45^+CD64^-$ $MHCII^+CD11c^+CD11b^+CD8\alpha^-$ or $CD45^+CD64^-MHCII^+CD11c^+Xcr1^-Sirp^{high}$; neutrophil: $CD45^+CD3\varepsilon^-CD64^-Ly6G^+CD11b^-$; macrophage and transitional monocyte: $CD45^+CD11b^+CD11c^{low}MHCII^+$; monocyte: $CD45^+CD11b^+CD11c^{low}$ $MHCII^-$; $CD8\alpha\alpha^+TCR\beta+$ T cell: $CD45^+CD8\alpha^+CD8\beta^-TCR\gamma\delta^-TCR\beta^+CD4^-$; $CD8\alpha\alpha^+TCR\gamma\delta^+$ T cell: $CD45^+CD8\alpha^+CD8\beta^-TCR\gamma\delta+TCR\beta^-CD4^-$; dpIEL: $CD45^+$ $CD8\alpha^+CD8\beta^-TCR\gamma\delta^-TCR\beta^+CD4^+$; $CD8\alpha\beta^+$ T cell: $CD45^+CD8\alpha^+CD8\beta^+$; Treg: $CD45^+MHCII^-CD19^-CD8\alpha^-CD8\beta^-CD4^+TCR\beta^+CD25^+FoxP3^+$. Lineage for DC progenitors included: CD3ε, CD19, NK1.1, B220, Ly6D, Ly6G, SiglecF and Ter119 and cells were defines as MDP: $lin^-CD115^+CD135^+CD117^{hi}$; CDP: lin^-CD115^+ $CD135^+CD117^{low}$; pre-cDC: $CD45^+CD11c^+Lin^-MHCII^-CD11b^{low/int}Sirpa^{low}Flt3^+$ $CD43^+$; pre-cDC1: $CD45^+CD11c^+lin^-MHCII^-CD11b^{low/int}Sirpa^{low}Flt3^+CD43^+$ $SiglecH^-Ly6C^-$; pre-cDC2: $CD45^+CD11c^+lin^-MHCII^-CD11b^{low/int}Sirpa^{low}Flt3^+$ $CD43^+SiglecH^-Ly6C^+$; uncommitted pre-cDC: $CD45^+$ $CD11c^+lin^-MHCII^-CD11b^{low/int}Sirpa^{low}Flt3^+CD43^+SiglecH^+Ly6C^-$. OT-II T cells were defined as $CD45.1^+CD4^+$ $TCRV\alpha2^+$, encompassing naïve ($CD44^-$), Tregs ($FoxP3^+$), Tfh ($CXCR5^+$), Th1 ($Tbet^+$), Th17 ($Ror\gamma t^+$) and $FoxP3^+Ror\gamma t^+$ cells.

Reagent or Resource	Source	Identifier
Anti-mouse B220 FITC	BioLegend	103206
Anti-mouse c-Kit APC Cy-7	eBioscience	17-1171-82
Anti-mouse Ccr9 Pe Cy-7	BioLegend	128712
Anti-mouse c-Kit APC	eBioscience	17-1171-82
Anti-mouse CD103 BV605	BioLegend	121433
Anti-mouse CD115 BV605	BioLegend	135517
Anti-mouse CD11b BV785	BioLegend	101243
Anti-mouse CD11b FITC	BioLegend	101206
Anti-mouse CD11b BV785	BioLegend	101243
Anti-mouse CD11c PE	BioLegend	117308
Anti-mouse CD11c BV650	BioLegend	117339
Anti-mouse CD11c FITC	BioLegend	117306
Anti-mouse CD127 BV421	BioLegend	135024
Anti-mouse CD150 Pe Cy-7	eBioscience	25-1502-82
Anti-mouse CD172 AF700	BioLegend	144021
Anti-mouse CD19 FITC	BioLegend	115506
Anti-mouse CD19 BV605	BioLegend	115540
Anti-mouse CD25 PerCP Cy-5.5	Invitrogen	45-0251-82
Anti-mouse CD25 BV711	BioLegend	102049
Anti-mouse CD27 PE	Invitrogen	12-0271-82
Anti-mouse CD34 BV421	BioLegend	119321
Anti-mouse CD3ε FITC	BioLegend	100306
Anti-mouse CD3ε Biotin	BioLegend	100304
Anti-mouse CD4 BV785	BioLegend	100551
Anti-mouse CD4 BV421	BioLegend	100437
Anti-mouse CD4 BV650	BioLegend	100555

(to be continued)

Reagent or Resource	Source	Identifier
Anti-mouse CD40 PerCP Cy-5.5	BioLegend	124623
Anti-mouse CD43 Biotin	eBioscience	13-0341-82
Anti-mouse CD44 FITC	BioLegend	103006
Anti-mouse CD45.1 BV650	BioLegend	110735
Anti-mouse CD45.2 APC	BioLegend	109814
Anti-mouse CD45.2 BV711	BD Horizon	563685
Anti-mouse CD45.2 Pe Cy-7	BioLegend	109830
Anti-mouse CD45.2 AF700	Invitrogen	56-0454-82
Anti-mouse CD45.2 APC Cy-7	BioLegend	109824
Anti-mouse CD48 AF647	BioLegend	103416
Anti-mouse CD49b PE-ef610	Invitrogen	61-5971-82
Anti-mouse CD5 FITC	Invitrogen	11-0051-82
Anti-mouse CD64 BV711	BioLegend	139311
Anti-mouse CD64 FITC	BioLegend	139316
Anti-mouse CD80 FITC	BioLegend	104706
Anti-mouse CD86 PE Cy-5	BioLegend	105016
Anti-mouse CD8α Bv711	BioLegend	100747
Anti-mouse CD8α FITC	BioLegend	100706
Anti-mouse CD8α APC	BioLegend	100712
Anti-mouse CD8β PE	Invitrogen	12-0081-83
Anti-mouse CXCR5 APC/Fire750	BioLegend	126539
Anti-mouse Flt3 Pe	BioLegend	135306
Anti-mouse Flt3 Pe Cy-5	BioLegend	135312
Anti-mouse FoxP3 APC	Invitrogen	17-5773-80
Anti-mouse GM-CSF PerCP Cy-5.5	BioLegend	505410
Anti-mouse Gr1 FITC	BioLegend	108406

(to be continued)

Reagent or Resource	Source	Identifier
Anti-mouse Il-6 APC	BioLegend	505410
Anti-mouse Il-10 Pe Cy-7	BioLegend	505025
Anti-mouse Il-12 PE	BD Bioscience	554480
Anti-mouse KLRG1 BV421	BioLegend	138414
Anti-mouse Ly6C PE	Abcam	ab25572
Anti-mouse Ly6D FITC	BioLegend	138606
Anti-mouse Ly6G FITC	BioLegend	127606
Anti-mouse Ly6G PE	BioLegend	127607
Anti-mouse MHCII APC Cy-7	BioLegend	107628
Anti-mouse MHCII FITC	BD Bioscience	562009
Anti-mouse MHCII PerCP	BioLegend	107624
Anti-mouse MHCI eF450	Invitrogen	48-5958-82
Anti-mouse NK1.1 FITC	Invitrogen	11-5941-85
Anti-mouse NK1.1 Pe Cy-7	Invitrogen	25-5941-82
Anti-mouse Nkp46 PerCP ef710	Invitrogen	46-3351-82
Anti-mouse Rorγt PE	Invitrogen	12-6988-82
Anti-mouse Sca-1 BV785	BioLegend	108139
Anti-mouse Siglec-H PB	BioLegend	129610
Anti-mouse SiglecF FITC	BioLegend	155504
Anti-mouse T-bet PerCP Cy-5.5	Invitrogen	45-5825-82
Anti-mouse TCRVα2 ef450	Invitrogen	48-5812-82
Anti-mouse TCRβ PE Cy-5	BioLegend	109209
Anti-mouse TCRβ FITC	BioLegend	109206
Anti-mouse TCR$\gamma\delta$ PE Cy-7	Invitrogen	25-5711-82
Anti-mouse TCR$\gamma\delta$ FITC	BioLegend	118106
Anti-mouse Ter119 FITC	BioLegend	116206

(to be continued)

Reagent or Resource	Source	Identifier
Anti-mouse Thy1.2 Bv605	BioLegend	140318
Anti-mouse TNFα BV421	BioLegend	506328
Anti-mouse XCR1 af647	BioLegend	148213
Anti-mouse α4β7 APC	BioLegend	120607
Anti-biotin Streptavidin BV421	BD Horizon	563259
Anti-biotin Streptavidin BV605	BioLegend	405229

Table 6. Antibodies for cell staining.

3.5.3 Flow cytometry and cell sorting

Stained cells were acquired in FACS buffer with an LSRFortessa X-20 Flow cytometer (BD Biosciences), and cell sorting was performed using a FACSFusion (BD Biosciences). Sorted cell populations were >95% pure. Data were analysed using FlowJo 10.10.0 software (Tree Star). All cell populations were gated in live cells (Aqua-negative cells).

3.6 Quantitative PCR with reverse transcription (RT–PCR)

Reagent or Resource	Source	Identifier
RNeasy Plus Mini Kit	Qiagen	50974136
RNase-Free DNase Set	Qiagen	50979254
Ethanol	VWR Chemicals	VWRC20821.330
PreAmp Master Mix	Applied Biosystems	LTAB 4488593
TaqMan Gene Expression Master Mix	Applied Biosystems	LTAB 4370074

(to be continued)

Reagent or Resource	Source	Identifier
Aldh1a1	Thermo Scientific Fisher	Mm00657317_m1
Gapdh	Thermo Scientific Fisher	Mm99999915_g1
Hprt	Thermo Scientific Fisher	Mm03024075_m1
Itgb8	Thermo Scientific Fisher	Mm00623991_m1
Il10	Thermo Scientific Fisher	Mm00439614_m1
Il12a	Thermo Scientific Fisher	Mm00434169_m1
Il15	Thermo Scientific Fisher	Mm00434210_m1
Tnfa	Thermo Scientific Fisher	Mm00443260_g1
Tgfb1	Thermo Scientific Fisher	Mm01178820_m1

Table 7. Reagents for RNA extraction and RT-qPCR.

3.6.1 RNA extraction

Total RNA extraction in sorted cells was performed using the RNeasy mini kit, following the manufacturer's protocol. The concentration and purity of the isolated RNA were determined after the extraction using a Nanodrop Spectrophotometer (Nanodrop Technologies).

3.6.2 Real-time RT-PCR

Quantitative real-time RT-PCR was performed in a QuantStudio 5 real-time PCR system (Applied Biosystems) with Hprt and Gapdh as housekeeping genes. To retro-transcribe RNA, a High-Capacity RNA-to-cDNA Kit was used. Afterwards, pre-amplification PCR was performed using TaqMan PreAmp Master Mix. A TaqMan Gene Expression Master Mix was used for the real-time PCR. The following TaqMan Gene Expression Assays were used: *Tgfb1, Il10, Il12a, Il15,*

Itgb8, *Aldh1a1*, *Tnfa*, *Hprt*, *Gapdh*. Analysis was performed with the comparative CT method ($2^{\Delta CT}$), and comparison or fold change between samples was assessed with the comparative ΔCT method ($2^{\Delta\Delta CT}$) (Livak and Schmittgen, 2001).

3.6 Elisa

Reagent or Resource	Source	Identifier
2-Mercaptoethanol	Gibco	31350-010
BCA Protein Assay Kits	Pierce	23225
DuoSet ELISA Ancillary Reagent Kit 2	Biotechne	DY008B
EDTA	Corning	46-034-Cl
EGTA	Sigma-Aldrich	E3889
Mouse FLT3L DuoSet ELISA	Biotechne	DY427
NaF	Sigma-Aldrich	201154
PMSF	Sigma-Aldrich	P7626
Protease inhibitor cocktail	Roche	11836153001
Pyrophosphate	Sigma-Aldrich	221368
Sodium glycerophosphate	Sigma-Aldrich	61668
Sodium orthovanadate	Sigma-Aldrich	567540
Sucrose	Sigma-Aldrich	S9378
Tris-HCL	Fisher Scientific	J/4315/17
Triton X-100	Fisher Scientific	BP151-500

Table 8. Reagents for ELISA.

3.6.1 Tissue preparation

Tissues were weighed and collected in 1 ml Triton X-100 lysis buffer (50 mM Tris-HCl pH 7.5, 1 mM EGTA, 1 mM EDTA pH 8.0, 50 mM NaF, 1 mM sodium glycerophosphate, 5 mM pyrophosphate, 0.27 M sucrose 0.5% Triton X-100, 0.1 mM PMSF, 0.1% 2-mercaptoethanol, 1 mM sodium orthovanadate, and protease inhibitor cocktail (Roche)). Samples were frozen and stored at -80°C until further processing. One stainless steel bead was added per sample, and samples were disrupted with a tissue lyser at 30 Hz for 10 minutes. To pellet cellular debris, samples were centrifuged at 16,000 x g for 15 minutes at 4°C. The supernatant was collected for Elisa and BCA total protein assay.

To obtain serum, blood was collected as described above. Samples were centrifuged at room temperature at 200 x g for 10 minutes, and serum was collected in 1.5 ml Eppendorf tubes. Serum was stored at -80°C until further use.

3.6.2 Elisa

Elisa was conducted according to the manufacturer's protocol. Optical density was measured with a SPARK plate reader set to 450 nm. For correction of optical imperfections in the plate, readings at 540 nm were subtracted from the readings at 450 nm. Protein was normalised to total protein measured using a BCA protein assay kit.

3.7 Food tolerance

Reagent or Resource	Source	Identifier
Anti-mouse B220 Biotin	eBioscience	13-0452-82
Anti-mouse CD11b Biotin	Invitrogen	13-0112-85
Anti-mouse CD11c Biotin	Invitrogen	13-0114-85
Anti-mouse CD19 Biotin	BioLegend	115504
Anti-mouse CD25 BV711	BioLegend	102049
Anti-mouse CD45.1 Biotin	eBioscience	13-0453-82
Anti-mouse CD45.1 BV650	BioLegend	110735
Anti-mouse CD45.2 PE Cy-7	BioLegend	109830
Anti-mouse CD8α Biotin	Invitrogen	13-0081-85
Anti-mouse Gr-1 Biotin	Invitrogen	13-5931-85
Anti-mouse NK1.1 Biotin	Invitrogen	13-5941-85
Anti-mouse TCRVα2 ef450	Invitrogen	48-5812-82
Anti-mouse TCRVβ5 PE	BioLegend	139501
Anti-mouse Ter-119 Biotin	eBioscience	13-5921-85
MACS beads	Milteny Biotec	130-048-102
LS columns	Milteny Biotec	130-042-401
QuadroMAC Separator	Milteny Biotec	130-091-051
OVA (Grade V)	Sigma-Aldrich	A5503
Isoflurane	Zoetis	571329.8

Table 9. Reagents for food tolerance experiments.

3.7.1 Adoptive T cell transfer

Spleens and lymph nodes were extracted and processed as described above. To isolate naïve $CD4^+$ T cells, single-cell suspensions were negatively selected through LS columns on QuadroMACS Separators using biotinylated antibodies against CD8α, CD25, CD11c, CD11b, TER-119, NK1.1, and B220 and anti-biotin MACS beads. The purity of transgenic $CD4^+$ OT-II T cells was verified by flow cytometry (typically >80% of cells were $CD45.1^+V\alpha2^+V\beta5^+CD25^-$). Recipient mice were anaesthetised using isoflurane gas, and $1x10^6$ OT-II cells were transferred by retro-orbital injection.

3.7.2 Oral antigen administration

For antigen challenge, OVA was administered intragastrically (50 mg in 200 µl PBS) using plastic gavage needles. Mice were challenged twice, once 16-18 hours after adoptive OT-II cell transfer and the second time 24 hours after the first OVA administration. Animals were analysed 24 hours after the second OVA administration.

3.8 Dimensionality reduction

The Principal Component Analysis (PCA) was done following the Eigen-decomposition method. For that, first, the mean cell count ratio over all animals that underwent the same staining was computed. This mean would contain a value per cell type and per week, segment and diet. Since the data was already a ratio, no further normalisation was done besides centring the data. Centring was done by subtracting the overall mean value per cell type across all weeks, all

segments, and all diets from the previously obtained means, this gave us the matrix X, which was used for the PCA.

The PCA was computed using the sklearn python package, which computes the variance (V) of the centred data and does the eigenvalue decomposition of such variance.

$$V = WDW^T$$

PCA separates the data into two matrices: D and W. D is a diagonal matrix in which each of the diagonal entries is the variance explained by each of the principal components. Each column of the W matrix () corresponds to one component, in which each entry represents the weight of each of the cell types.

The projections of the principal component were then computed, projecting these weights back on the data (X).

$$PC_i = Xw_i$$

Each of these projections is a one-dimensional vector with a value for each of the weeks, the segments, and the diets. In the analysis, the first three projections were used to visualise a summary of the data. Colouring for diet type and week or diet and segment allowed us to visually inspect how separable the data is. The bigger the distance between points of different colours, the larger the separability.

3.9 Multi-linear regression

The multi-linear regression was done using the sklearn python package. For every computed regression, the dependent variable was the ratio of the number of cells for each staining and the predictor of the type of diet (1 for HFHSD and 0 for CD).

The data was separated into test (20% of the data) and train (80% of the data) sets. The train set was used to calculate the value of the regressors (the weight of each of the cell types). The higher the value of the regressor for a particular cell type, the more its value increased in the HFSHSD. At the same time, the more negative the value, the more it decreased its value in the HFHSD in comparison to the CD. Since the value of many cell types can be correlated, ridge regression was calculated. This type of multi-linear regression punishes high regressor values, therefore only leaving regressors that have the strongest relationship with the predictor. The value of the penalisation was 0.01.

Once the ridge regression was calculated on the train data set, the performance of this regression on the test set was calculated. This data was not seen by the model. Therefore, it provides insight into how good the obtained regressors are at predicting the diet type. Thus, the test data was used to calculate the accuracy of the model. This accuracy was calculated by the number of times the model inferred the diet correctly divided by the total number of data points. The whole procedure was repeated 100 times, and the average values of the accuracy and the regressors were shown in the figures.

3.10 Statistical analysis

The statistical analysis for the screen of the immune landscape in HFHSD and CD was conducted using ANOVA and post-hoc statistics. To obtain the statistical significance between the two diets and the weeks, a between-subject ANOVA was employed, as every animal could only be a data point for a given week and diet. In the case of the segment of the intestine, a mixed ANOVA was employed, as the same animal provided one value per segment, making the intestine

segment a within-subject comparison and only one treatment, a between-subject comparison.

In both cases, to correct for multiple comparisons, a Bonferroni correction was employed. Additionally, once the ANOVA was significant for either the diet, the week or the treatment, a post-hoc analysis was performed. This analysis showed the particular combination that was significantly different.

Results in graphs are shown as mean ± s.e.m. Statistical analysis for all other experiments was conducted with GraphPad Prism software (GraphPad Software, La Jolla, Calif). Student's t-test was performed on homoscedastic populations. The Mann–Whitney test was used when data were not normally distributed. Repeated-measures ANOVAs were performed on body weights and glucose tolerance test measurements with Sidak correction for multiple comparisons. Results were considered statistically significant at $P<0.05$.

3.11 Analysis of scRNA-seq dataset

Data was made publicly available by Wang et al., 2023. The output files of Cellranger were downloaded from GSE221006. Files were analysed in R using Seurat (v.5) following the guidelines. Seurat was developed in 2017 by the Satija lab (Butler et al., 2018). In detail, according to Wang et al., immune cells were filtered to have at least 100 unique molecular identifiers, at least 100 genes and maximal 10% mitochondrial gene expression. Gene counts for each cell were normalised with the Seurat function NormalizeData. This function first divides by the total gene counts for the cell and multiplied by a scale factor of 10,000, followed by natural logarithm transformation. The datasets were integrated using canonical correlation analysis through the Seurat IntegrateLayers methodology.

The analysis pipeline identified variable genes and corrected for sequencing depth bias using the standard Seurat functions FindVariableGenes and ScaleData.

Dimensionality was reduced using a PCA on previously identified variable genes, and the top 50 components were selected. These components served as input for UMAP visualisation, creating a two-dimensional representation. Cell clustering was performed by the Seurat function FindClusters on the same 50 principal components, and distinctive markers for each cluster were subsequently identified using the FindAllMarkers function.

Cell types and subtypes were annotated by analysing differentially expressed genes, which were compared against established cell type definitions from the Wang et al. study. DEG validation was performed using DESeq2 analysis.

Sample composition analysis involved calculating the proportion of each cell type by determining cell counts per type and then converting these counts to percentages of the total cell population.

CellChat version 2.1.0 was employed to analyse cellular communication patterns. The analysis merged cDC1 and $CD4^+$ T cell subtypes and conducted separate analyses for intestinal cells from both CD and HFHSD conditions. Subsequently, the differential interaction between conditions was evaluated. The results visualised intercellular communication patterns through heatmaps and circular interaction diagrams, highlighting both the frequency and intensity of cell-cell interactions.

4

RESULTS

4.1 Obesogenic diet reshapes the enteric immune system of the host

4.1.1 Mice on HFHS diet develop obesity

In the present study, we investigated the impact of a high-fat, high-sugar diet (HFHSD) on the enteric immune system of male C57BL/6J mice. Mice were initially fed a standard chow diet (CD) until they reached 8 weeks of age. Mice were then divided into two groups: one group continued on CD, while the other group was switched to HFHSD. The lamina propria and intra-epithelium immune compartments in the duodenum, jejunum, and ileum were analysed at 4, 8 and 16-week time points by flow cytometry (Figure 4a). To investigate the obesogenic effect of HFHSD, we followed body weight and glucose metabolism during the dietary intervention.

Bodyweight measurements indicated a significant increase in the weight of mice fed with HFHSD when compared to those on CD (Figure 4b). Weight gain was consistent across the duration of the study, with significant differences noted already at 4 weeks on HFHSD. Glucose tolerance tests (IGTT) were conducted at

the specified time points to evaluate the metabolic impact of the diets (Figure 4c). Mice on HFHSD exhibited impaired glucose tolerance compared to mice on CD already 4 weeks after dietary intervention. At this time point, mice showed primarily difficulties in efficient glucose uptake until 45 minutes after glucose administration. Significant differences in blood glucose levels were observed at all measured intervals throughout the 150-minute experiment at 8 weeks of dietary intervention, and those parameters were even more pronounced at 16 weeks.

Thus, HFHSD leads to obesity and impaired glucose tolerance in mice.

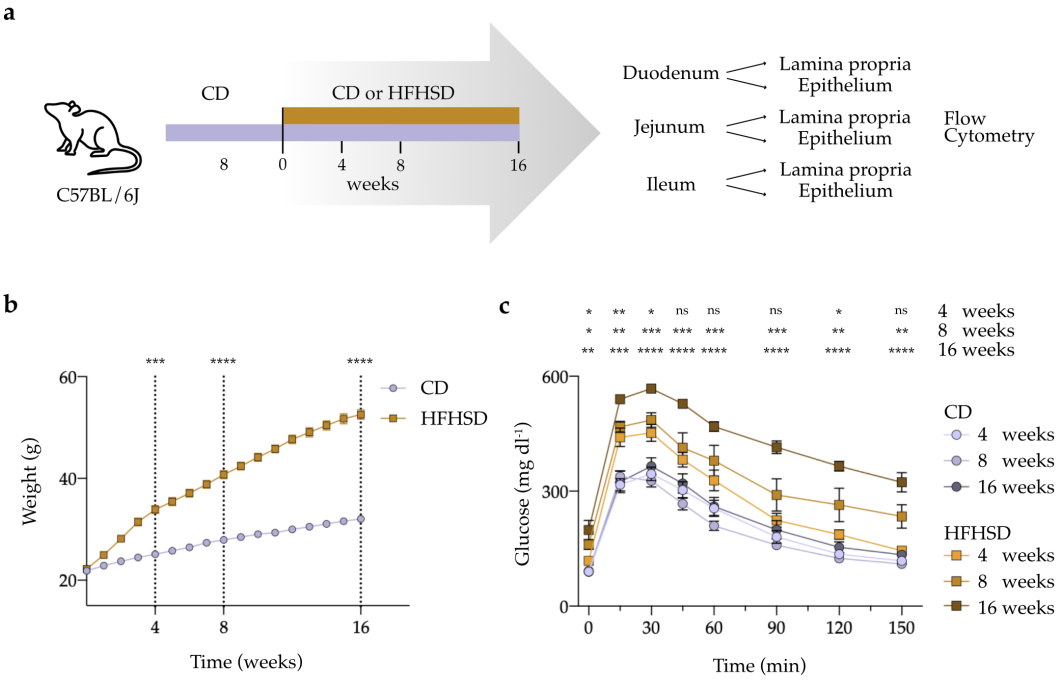


Figure 4. HFHSD leads to obesity. (a) Experimental setup. Male C57BL/6 mice were fed CD until 8 weeks of age. Mice were then either fed HFHSD or CD. Immune compartment of lamina propria and epithelium of duodenum, jejunum and ileum was analysed at indicated time points by flow cytometry. (b) Bodyweight of mice (n = 15). (c) Intraperitoneal glucose tolerance test (IGTT) at indicated time points (n = 15). Data are pooled from three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-sided two-way repeated measures ANOVA corrected for multiple comparisons (b, c) *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

4.1.2 The phenotype of the enteric immune system is dependent on intestinal segment, age and dietary choices of the host

To investigate the impact of obesogenic diet on the composition of the immune system in the small intestine, we analysed lamina propria and intraepithelial immune cells from mice fed either CD or HFHSD for varying durations. Using multi-parametric flow cytometry, the immune landscape was assessed by calculating the frequencies and numbers of immune cell types within the haematopoietic $CD45^+$ cell fraction in lamina propria and epithelium.

Taking into consideration the dimensionality of the data (time points, intestinal segments and cell types), we first assessed whether the diet affected the enteric immune phenotype. Furthermore, we asked whether the composition of the enteric immune system was more prone to influences by duration of dietary intervention, age of animal, or intestinal segment. Additionally, we asked whether diet would have a distinct effect on different intestinal segments. To this end, we employed Principal component analysis (PCA) (Pearson, 1901), a known dimensionality reduction technique that allows for the visualisation of dietary effects, time points and intestinal segments (Figure 4). The score plot for the first three PCs demonstrated distinct clustering patterns between HFHSD and CD groups, indicating substantial alterations in the composition of the immune system due to dietary intervention. When labelling for time points and diet, immune phenotypes did not cluster based on age or duration of dietary intervention, but all time points were evenly distributed throughout space (Figure 5a). In contrast, data were separated into clusters along the PC1 and PC2 axis dependent on intestinal segment (duodenum, jejunum or ileum), while the same enteric segments of different diets clustered together (Figure 5b). Data from ileum showed greater distance to data from duodenum and jejunum, indicating greater similarities between the immune composition of the proximal and middle

part of the small intestine and a clear distinction between those and the distal small intestine.

Thus, we conclude that, from 4 weeks of dietary intervention, variability in the enteric immune phenotype can be explained by diet. The greatest variability in the data can be explained by diet and intestinal segment, while duration of diet is less crucial for the definition of the immune landscape. While these initial findings identified primary drivers of immune composition, a detailed statistical analysis was warranted to understand the specific effects of factors on individual cell populations.

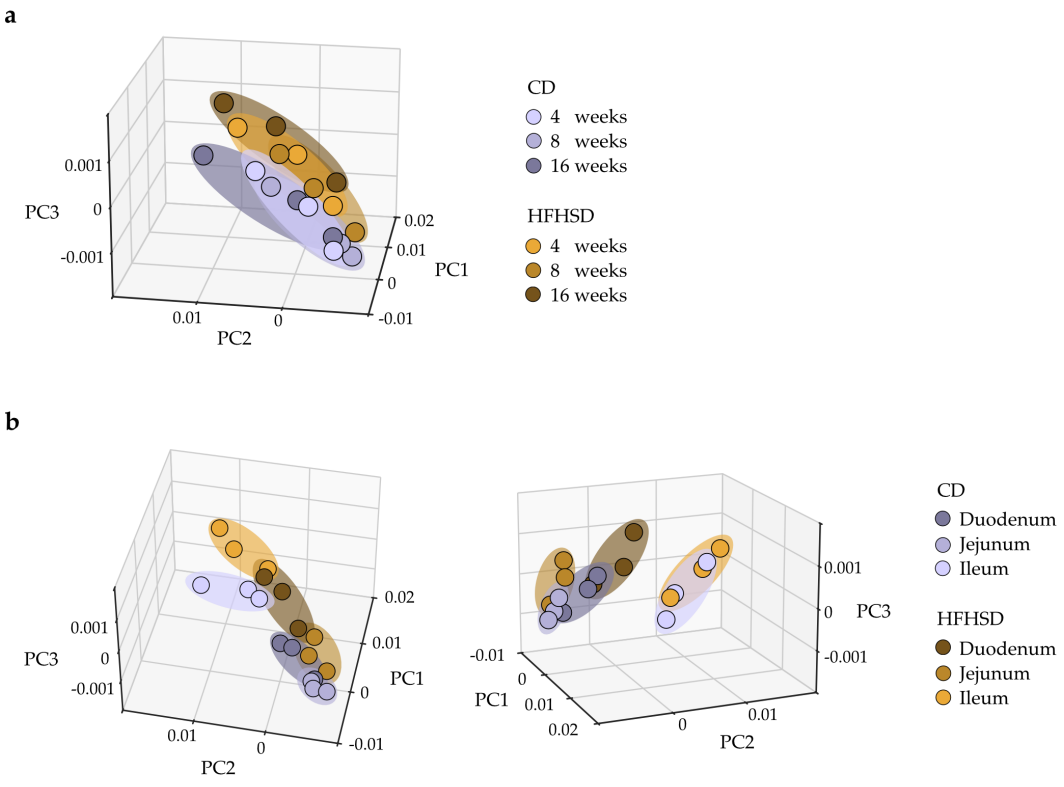


Figure 5. The adult enteric immunophenotype is dependent on diet of the host and intestinal segment. PCA representation of enteric immunophenotypes (n = 15). Each data point represents the cluster of the immune landscape of one intestinal segment at one time point in one condition, CD or HFHSD (Flow cytometry). Data points are coloured by (a) diet and experimental time point or (b) diet and intestinal segment. Data are pooled from three independent experiments. n represents biologically independent animals.

4.1.3 Effect of age, diet and duration of dietary intervention

Given the major impact of dietary regimen and intestinal segment in the enteric immune composition, sequentially, we interrogated the effect of diet, dietary intervention, age and intestinal segment on the immunophenotype of the gut. The appropriate test for this is ANOVA analysis. Different types of ANOVA tests account for whether comparisons are made between groups (diet and age) or within subjects (intestinal segments). In theory, it is possible to develop an ANOVA that could account for two between-subject comparisons between age and diet and one within-subject comparison for intestinal segment. However, we considered that testing these factor pairs in separate ANOVAs would be more valuable to interpreting variations in individual immune cell subsets. In these ANOVA comparisons, all cell types were examined as ratios in $CD45^+$ cells.

The effect of experimental time point, dietary intervention and duration of intervention on the composition of the intestinal immune system was assessed by two-way repeated measures ANOVA between individuals (Figure 6 & Table S1). The majority of immune cell types in the lamina propria was significantly affected by experimental time point alone, independently of dietary intervention, and age of the host. This was true for cDC1s, cDC2s, macrophages, transitional monocytes, Tregs, $CD8^+TCR\alpha\beta^+$, $CD4^+TCR\gamma\delta^+$, $CD4^+TCR\alpha\beta^+$ T cells, NKs, ILC2s, and ILC3s. Monocytes, dpDCs, B cells, neutrophils, $CD8^+TCR\gamma\delta^+$ T cells and ILC1s were not shown to be affected solely by experimental time points. In the intra-epithelial immune compartment, the ratio of $CD8\alpha\beta^+$, $CD8\alpha\alpha^+TCR\alpha\beta^+$ T cells, and dpIEL was significantly dependent on age alone, while the ratio of $CD8\alpha\alpha^+$ and $CD8\alpha\alpha^+TCR\gamma\delta^+$ T cells was not affected.

When focusing on the effect of dietary intervention on the composition of the immune system, regardless of duration of diet, only few cell types in the lamina

propria were shown to be significantly altered. Notably, total cDCs and all DC subtypes (cDC1, cDC2, dpDC) displayed higher frequencies in HFHSD when compared to CD conditions. CD8$^+$TCR$\gamma\delta^+$ and CD8$^+$TCR$\alpha\beta^+$ T cells were found in lower frequencies in HFHSD-fed animals. In contrast to the lamina propria, almost all cell types analysed in the epithelium were affected by diet alone. These were dpIELs, CD8$\alpha\alpha^+$, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ and CD8$\alpha\alpha^+$TCR$\gamma\delta^+$ T cells, which all displayed lower frequencies in HFHSD when compared to CD conditions.

Distinct diets in interaction with the duration of said dietary intervention impacted NKs, ILC2s and ILC3s in the lamina propria and intraepithelial CD8$\alpha\beta^+$ and CD8$\alpha\alpha^+$ TCR$\alpha\beta^+$ T cells and dpIELs. Notably, none of these cell types was affected only by diet alone, without also being affected by age alone. When comparing these cell types pairwise for each time point, NKs were only significantly altered by diet at the 4-week time point (increase of mean in HFHSD compared to CD condition), ILC2s at 4 and 8 weeks (lower and higher mean in HFHSD compared to CD condition, respectively) and ILC3s at 8 weeks (increase of mean in HFHSD compared to CD condition) (Table S2). In the intra-epithelial compartment, dpIELs at 4 and 16 weeks and CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells at 8 weeks were shown to be significantly lowered in their mean in HFHSD compared to CD animals. CD8$\alpha\beta^+$ T cells showed significance when analysing diet in combination with duration of dietary intervention. However, these cells were not shown to be significantly affected at any individual time point.

Frequencies of total cDCs, dpDCs and intraepithelial CD8$\alpha\alpha^+$ and CD8$\alpha\alpha^+$TCR$\gamma\delta^+$ T cells were demonstrated to be affected by diet, but neither by experimental time point nor by the interaction of diet and duration of dietary intervention. Thus, for these cell types, the factor of the age and duration of treatment is not of importance, which is in contrast to the major effect of the dietary intervention.

The effect of diet did not significantly change over time for cDC1s, cDC2s and $CD8^+TCR\alpha\beta^+$ T cells, which were shown to be influenced by diet and experimental time points but not by the interaction of diet and duration of dietary intervention. In this case, trends in the data depended on diet and age followed the same slope and did not significantly change over time. This was demonstrated by post-hoc analysis of the ANOVA (Table S2). Pairwise comparison of dietary treatment at individual time points revealed that means of cDC1s and cDC2s increased from CD to HFHSD, and at each time point. $CD8^+TCR\alpha\beta^+$ T cells showed a decreased frequency at all time points.

Macrophages, transitional monocytes, Tregs, and $CD4^+$ T cells were shown to be influenced by experimental time point alone, but neither diet alone nor interaction of diet and time point affected their frequencies. Thus, frequencies of these cell types were influenced by age of animals while diet, no matter how long the dietary intervention lasted, was shown not to influence their abundance.

In summary, the frequencies of enteric immune cell types were demonstrated to be influenced by the factors "diet" and "age" alone and by the interaction of diet and time points. Importantly, long-term exposure to HFHSD only significantly affected frequencies of cell types that were also susceptible to the factor "age" alone. Thus, age of the host impacts the enteric immune system, and this effect can be enhanced or attenuated by HFHSD. However, at least between 4 to 16 weeks of dietary intervention, the general effects that HFHSD has on the enteric immune system are not further enhanced or attenuated after exposure to obesogenic diet for longer than 4 weeks.

As most cell types were influenced by experimental time points, these data also revealed that the cell type frequencies are highly dependent on age, even in

adulthood, when changes in the environment are kept at a minimum, as is the case in a vivarium.

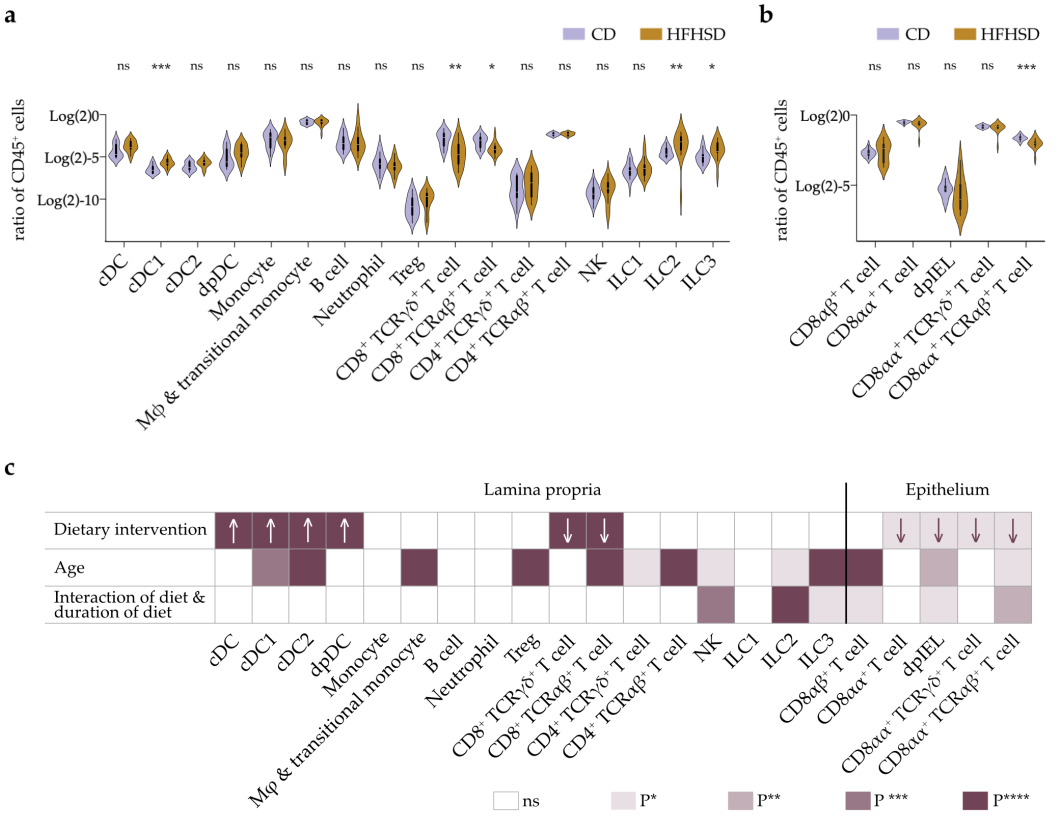


Figure 6. Diet, duration of diet and age shape the enteric immune landscape. (a) Lamina propria and (b) intraepithelial immune cells at 8 weeks of HFHSD or CD as ratios of CD45+ cells (n = 15). (c) Effect of diet, age and duration of dietary intervention on intestinal immune cell types (n = 15). Data are pooled of three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-way repeated measures ANOVA for tests between subjects (c). Post-hoc pairwise comparison (a-b). *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

4.1.4 Effect of diet and intestinal segments

The effect of dietary intervention and intestinal segment was assessed by two-way mixed repeated measures ANOVA that compares within and between individuals (Figure 7 & Table S3).

Regardless of the intestinal segment, dietary intervention alone was shown to have a significant impact on the ratio of total cDCs, B cells and $CD8^+$ T cells in the lamina propria. In the intra-epithelial compartment, only $CD8\alpha\beta^+$ T cells were demonstrated to be significantly affected by diet alone. It is notable that these results slightly differ from the aforementioned two-way repeated measures ANOVA. The p-value in ANOVA tests is sensitive to specific grouping of data and the design of the experiment. Different types of ANOVA tests account for whether comparisons are made between groups or within subjects. This distinction is crucial because it affects how variability is calculated, which in turn influences the resulting p-value. Generally and also here, within-subjects design detected smaller effects due to reduced error variance, which led to lower p-values compared to between-subjects design within the same dataset. When comparing both ANOVAs, all cDCs and $CD8^+$ T cells in the lamina propria and intraepithelial $CD8\alpha\beta^+$ T cells showed consistent results. These cell types are discussed in more detail in chapter 4.1.5.

The ratio of cell types in $CD45^+$ cells, the intestinal segment, regardless of the dietary intervention, was shown to be highly significant for all analysed cell types except lamina propria $CD4^+$ T cells. Thus, the composition of the enteric immune system shows high variability along the intestine, and specific composition is dependent on the intestinal segment. Every cell type that was shown to be significantly affected by diet alone was also significantly affected by intestinal segment alone.

When analysing the effect of diet in interaction with intestinal segment, total cDCs, cDC1s, dpDCs, monocytes, macrophages, transitional monocytes, B cells, neutrophils, $CD4^+TCR\alpha\beta^+$ T cells, and intraepithelial $CD8^+TCR\alpha\beta^+$ and $CD8^+TCR\gamma\delta^+$ T cells showed significance. Pairwise comparison demonstrated that the diet significantly affected the ratio of monocytes and intraepithelial $CD8^+TCR\gamma\delta^+$ T cells only in the duodenum, while macrophages and transitional monocytes were affected only in the ileum. B cells, neutrophils and $CD4^+TCR\beta^+$ T cells, as well as intraepithelial $CD8\alpha\beta^+$ T cells, were not significantly affected when comparing single intestinal segments between HFHSD and CD animals. In contrast, total cDCs and dpDCs showed significance in all individual segments between experimental conditions, and cDC1 were significantly affected in jejunum and ileum. Total cDCs and cDC1s, in particular, showed a consistent positive trend, while dpDC showed a consistent negative trend of their mean ratios in $CD45^+$ cells from CD to HFHSD conditions along all segments. Overall, pairwise comparison of cell types that showed significance for the interaction of diet and segments demonstrated that diet did have consistent effects on different segments (Table S4). Therefore, we decided that it is sufficient to concatenate the intestinal segments while representing the results of the screen of the immune system on an obesogenic diet (Figure 5a). All cell types that were significantly affected by the interaction of diet and segment were also significantly affected by segment alone, except for $CD4^+TCR\alpha\beta^+$ T cells.

Lamina propria $CD8^+$ T cells and cDC2s and intraepithelial $CD8\alpha\alpha^+$ T cells showed to be significantly altered depending on diet alone and segment alone, but not by the interaction of diet and segment. Pairwise comparison of dietary treatment at individual segments revealed a similar trend of increase (lamina propria $CD8^+$ T cells) or decrease ($CD8\alpha\alpha^+$ T cells and cDC2s) of the mean ratio in $CD45^+$ cells from proximal towards the distal intestine and increase from

HFHSD to CD in each intestinal segment (Table S4). The individual impact of diet and segment on these cells is not altered by their interference.

In sum, we could demonstrate that location determines the phenotype of the immune system, as almost all cell types are influenced by location. Diet is further affecting the diversity of the immune landscape along the small intestine as 45% of analysed cell types were affected by the interaction of diet and intestinal segment. Diet has consistent effects on the immune system of different intestinal segments.

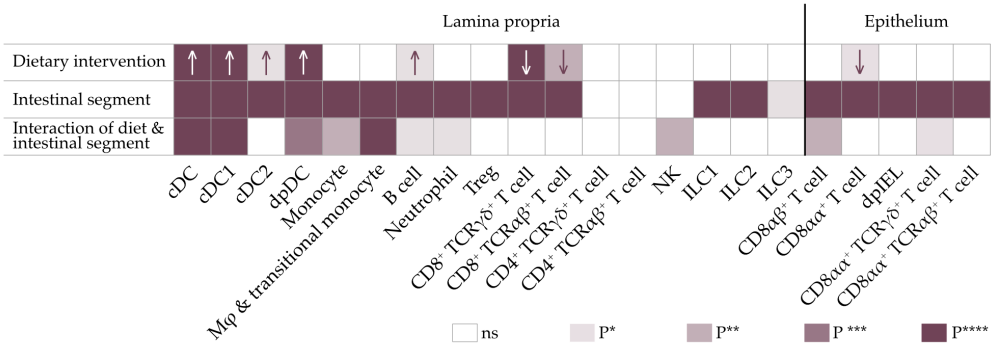


Figure 7. Diet, duration of diet and intestinal segment shape the enteric immune landscape. Effect of diet, intestinal segment and interaction of dietary intervention and intestinal segment on frequencies of lamina propria and intraepithelial immune cell types (n = 15). Data are pooled from three independent experiments. n represents biologically independent animals. Two-way mixed repeated measures ANOVA for tests within and between subjects. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

With temporal and spatial aspects of immune adaptation characterised, we next focused on cell types that showed consistent changes dependent on diet to understand diet-induced immune adaptation.

4.1.5 Enteric cDC1s are consistently increased throughout long-term exposure to HFHSD

As already mentioned, all cDCs and CD8$^+$ T cells in the lamina propria and intraepithelial CD8$\alpha\alpha^+$ T cells showed consistent results when comparing two-way repeated measures ANOVA and two-way mixed repeated measures ANOVA (Table S1 & S3). We conducted pairwise comparison of time points and intestinal segments in a post-hoc analysis to better describe the behaviour of these cell types dependent on time point and segment (Figure 8 & Table S2 & S4).

Pairwise comparison made evident that intraepithelial CD8$\alpha\alpha^+$ T cells had only been significantly affected by HFHSD in the duodenum, and even though treatment alone was determined to be overall significant, no individual time point showed significance when weeks were compared pairwise.

CD8$^+$TCR$\gamma\delta^+$ T cells were shown to significantly decrease in all intestinal segments upon HFHSD. CD8$^+$TCR$\alpha\beta^+$ T cells decreased in duodenum and jejunum in HFHSD when compared to CD-fed animals. When comparing segments independent of diet, CD8$^+$TCR$\alpha\beta^+$ T cells increased from proximal to distal intestinal segments, both in CD and HFHSD conditions. CD8$^+$TCR$\gamma\delta^+$ T cells showed the same behaviour in CD conditions, while in HFHSD, the ratio of these cells in CD45$^+$ cells increased from duodenum to jejunum. Analysis of age alone revealed that CD8$^+$TCR$\alpha\beta^+$ T cells increased with age, both in CD and HFHSD conditions. CD8$^+$TCR$\gamma\delta^+$ T cells also increased over time in CD-animals, while HFHSD-animals showed lowest mean ratio in total CD45$^+$ cells at 8 weeks, followed by 4 and 16 weeks time points. When comparing experimental conditions at individual time points, HFHSD was shown to be significantly impacting the ratio of both CD8$^+$ T cells in total CD45$^+$ cells at 16 weeks of treatment, while no significant effect was observed after 8 weeks of dietary

intervention and $CD8^+TCR\gamma\delta^+$ T cells were reduced at 4 weeks of HFHSD compared to CD feeding.

Notably, all cDCs were demonstrated to be significantly affected by HFHSD. Pairwise comparison demonstrated that the ratio of dpDCs in total $CD45^+$ cells was increased in all individual intestinal segments in HFHSD compared to CD condition, but treatment alone only showed a significant effect on these cells at the 16-week time point. cDC2s decreased along the small intestine, independent of diet, and increased in all segments in HFHSD compared to CD condition. Even though treatment alone significantly impacted frequency of cDC2, no single time point was shown to be significant when analysing the effect of diets at individual time points. In contrast, the ratio of cDC1s in total $CD45^+$ cells was highly increased in HFHSD compared to CD-fed animals at all time points. The effect of HFHSD at the 16-week time point was higher on cDC1 and dpDCs (p-value at 16 weeks for cDC1: 0.0042 and for dpDC: 0.01). The mean ratio of cDC1s in total $CD45^+$ cells was consistently decreasing along the small intestine from proximal to distal in CD condition. In HFHSD, animals showed highest ratio of cDC1s in $CD45^+$ cells in jejunum, followed by duodenum and ileum, and HFHSD resulted in a significantly higher ratio in jejunum and ileum when compared to CD intervention. Amongst all cDC subtypes, cDC1s were shown to be affected more strongly. When comparing the effect of HFHSD on cDC1s and $CD8^+TCR\beta^+$ T cells, cDC1s showed to be stronger affected by diet at each time point ($CD8^+TCR\alpha\beta^+$ T cells: p-value at 4 weeks 0.016, at 8 weeks: 0.029, at 16 weeks 0.076; cDC1s: p-value at 4 weeks 0.00003, at 8 weeks: 0.0024, at 16 weeks 0.0042).

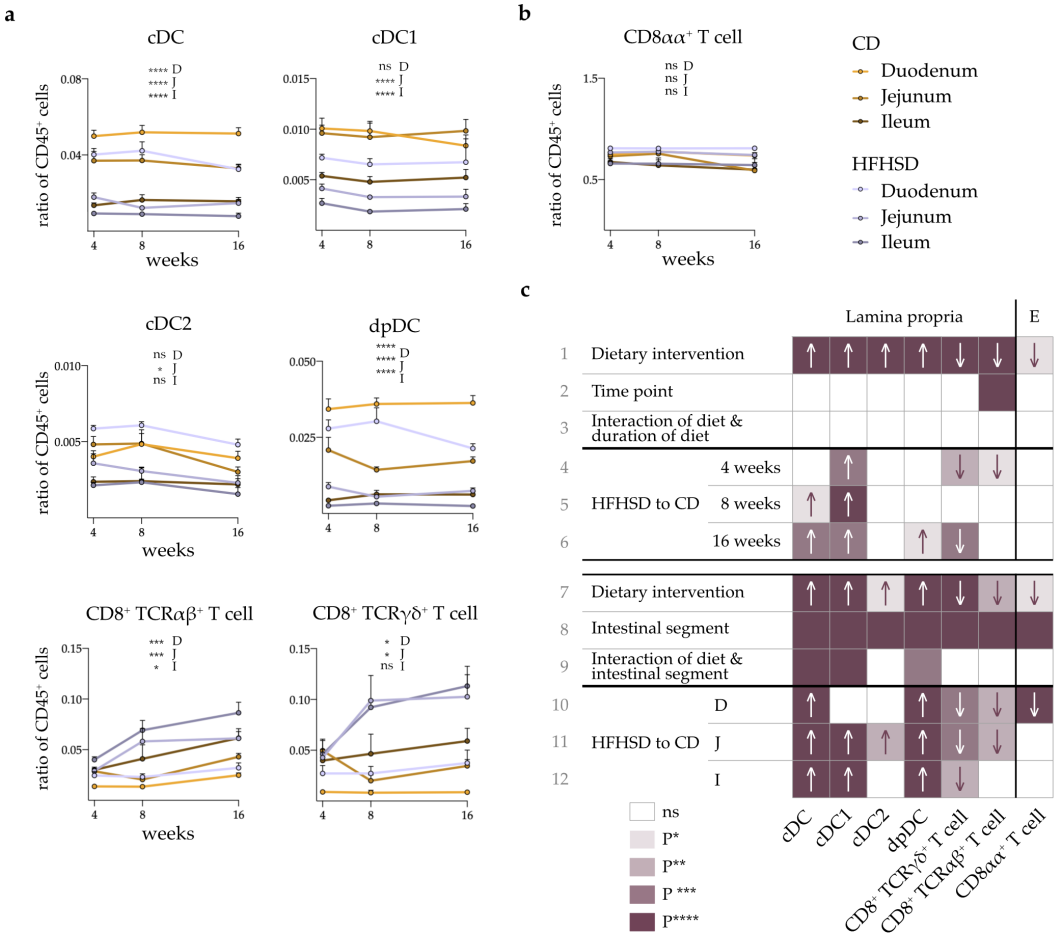


Figure 8. Kinetics of effect of diet and location on immunophenotype. (a) Ratios of selected lamina propria and (b) intraepithelial immune cell types in CD45⁺ cells at indicated time points and intestinal segments in HFHSD or CD conditions (n = 15). Data are pooled from three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m (a-b). (c) Effect of diet, time point, intestinal segment and interaction of these factors on frequencies of selected lamina propria and intraepithelial immune cell types.
Row 1-3: two-way repeated measures ANOVA for tests between subjects (diet and time point). Row 4-7: post-hoc pairwise comparison with Bonferroni correction. Row 7-9: mix-repeated measures ANOVA for tests between and within subjects (diet and intestinal segments). Row 10-12: post-hoc pairwise comparison with Bonferroni correction. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant. D: duodenum. J: jejunum. I: ileum. E: epithelium.

In sum, we demonstrated that obesogenic diet affects frequency of enteric cDC1 in total CD45$^+$ when compared to other cell types most consistently over time and intestinal segment. All cDC subtypes are affected, with cDC1 most consistently increased at 4, 8 and 16 weeks in the jejunum and ileum in HFHSD conditions. In contrast, CD8$^+$ T cells show to be decreased in HFHSD conditions. Importantly, while the role of CD8$^+$ T cells in obesity has been previously documented (Hung et al., 2024; Franco et al., 2017; Park et al., 2019; Tanaka et al., 2020;), the relevance of cDC1s and their functional consequences in obesity remain mostly unexplored.

Given these distinct patterns of immune cell adaptation to HFHSD, we next investigated whether these changes occurred randomly or followed predictable and reliable patterns.

4.1.6 Phenotype of the enteric immune landscape is not random

Given the strong impact of the dietary regimen on the enteric immune landscape, we investigated if the composition of the immune system could predict the type of diet of the host. To do so, we employed a multi-linear ridge regression to account for the correlation between the frequencies of cell types. Due to the nature of the analysis, we could only integrate cell types in one model that originated from the same flow cytometry staining. We therefore built one multi-linear regression using 80% of the data, including the lamina propria cell types cDC1, cDC2, monocyte, macrophage and transitional monocyte, B cell, neutrophil, CD8$^+$TCR$\alpha\beta^+$ and CD8$^+$TCR$\gamma\delta^+$ T cell CD4$^+$TCR$\alpha\beta^+$ and CD4$^+$TCR$\gamma\delta^+$ and one regression describing intraepithelial cell types, including CD8$\alpha\beta^+$ T cell, dpIEL, CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ and CD8$\alpha\alpha^+$TCR$\gamma\delta^+$ T cell. The regression models showed high accuracies in predicting the diet type in the remaining 20% of the data, which the model did not have access to, with 73.5 and 72.33, respectively,

indicating a predictable relationship between composition of the enteric immune system and dietary intervention (Figure 9). The models identified CD8$^+$TCR$\gamma\delta^+$ T cells and intraepithelial CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells as strongest predictors, and their weight was shown to be negative. Weight represents the estimated change in the frequency of a cell type for a change in diet from CD to HFHSD while holding all other regressors constant. This means that, according to this model, a reduction of frequencies of CD8$^+$TCR$\gamma\delta^+$ T cells in the lamina propria and CD8$\alpha\alpha^+$TCR$\alpha\beta^+$ T cells in the epithelium will most likely predict that the host is on an HFHSD.

Regressors that are less relevant in predicting the dietary choices of the host were shown to be cDC1, CD4$^+$TCR$\gamma\delta^+$ T cells in the lamina propria and dpIEL, CD8$\alpha\beta^+$ and CD8$\alpha\alpha^+$TCR$\gamma\delta^+$ T cells in the epithelial compartment. Thus, the models assume that frequencies of these cell types would not be affected by diet alone if all other regressors would stay constant.

In sum, the composition of the enteric immune system could reliably predict the host diet and cDC1 frequency might be indirectly affected by diet.

To validate the robustness and ensure the reproducibility of our findings about diet-induced immune adaptation, we next compared our results with an independent study that employed a different methodology but investigated similar questions.

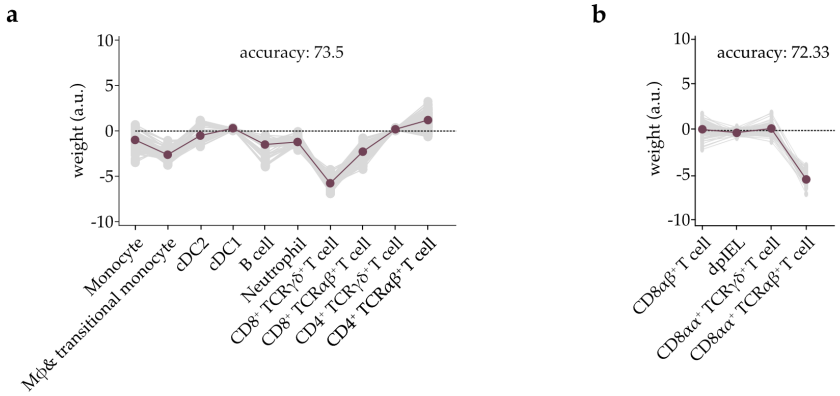


Figure 9. Relationship between enteric immunophenotype and diet of the host. Multi-linear ridge regression models for (a) lamina propria and (b) intraepithelial immune cell types show the relationship between regressors as frequencies of cell types in CD45$^+$ cells and dietary intervention (n = 15). Weight represents beta, the estimated change in the dependent variable for a change in the predictor variable from CD to HFHSD (x), while holding all other regressors constant. Data are pooled from three independent experiments. n represents biologically independent animals.

4.1.7 Diet-induced alterations in enteric immune compartments are reproducible

In a recently published paper, Wang et al. also investigated the enteric immune system in obesity (Wang et al., 2023). The authors employed single-cell RNA sequencing (scRNA-seq) to understand the adaptation of the immune system in the lamina propria and the epithelium and of structural cells (defined as CD45$^-$ cells in the epithelial layer) to an HFHSD. Methods and diets chosen in this study differ from our approach. We interrogated whether the adaptation of the enteric immune landscape to an obesogenic diet was comparable between the two independent studies.

We first compared the different diets used in both studies. Figure 10a illustrates the percentage of calories obtained from carbohydrates, fat, and proteins in the diets. The percentage of calories coming from specific

macronutrients seemed to be similar in both CDs as well as in both HFHSDs. We decided that it would not be appropriate to statistically evaluate the similarities of diets due to very few data points.

The dietary intervention in the experimental setup from Wang et al. lasted 8 weeks, wherefore we compared our 8 weeks time point to their results. Figure 10b presents a side-by-side comparison of lamina propria immune cell populations as a percentage of $CD45^+$ cells from both the scRNA-seq dataset generated by Wang et al. and our flow cytometry dataset.

When comparing both CD groups, Wang et al. identified higher frequencies of $CD4^+TCR\alpha\beta^+$ T cells, $CD8^+$ T cells and B cells, while our results showed higher ratios of cDCs, macrophages and transitional monocytes, ILC2s and ILC3s in total $CD45^+$ cells. The same trend was noticeable when comparing both HFHSD groups. However, it was evident that HFHSD altered the composition of immune cell populations in the lamina propria across studies. Furthermore, changes in immune subsets were consistent in both studies, with an increase of $CD4^+TCR\alpha\beta^+$ T cells, cDCs, ILC2s and ILC3s in HFHSD when compared to CD. Both studies showed a decrease in $CD8^+$ T cells and no or moderate changes in B cells, macrophages, and transitional monocytes. Only ILC1s and NK cells revealed different results between the two studies. The dataset of Wang et al. described an increase in their frequency, while we could not observe any change in these groups on HFHSD when compared to CD.

To assess the relationship between the dataset published by Wang et al. and our results, Pearson correlation analysis was conducted. We compared the fold changes of frequencies of specific cell types in $CD45^+$ cells from CD to HFHSD, and the analysis demonstrated strong correlations between the studies, with an R-value of 0.985 (Figure 10c).

Since adaptations of the enteric immune system published by Wang et al. were shown to be similar to our study, we decided to consult the published scRNA-seq dataset in further experiments.

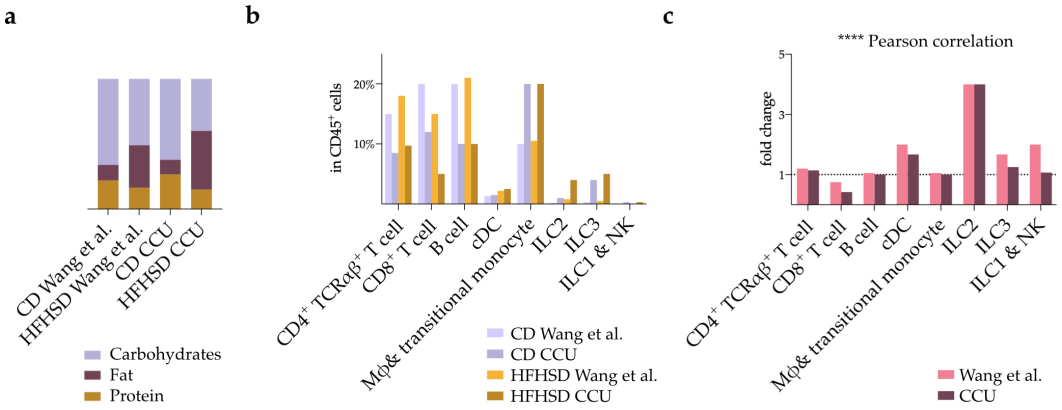


Figure 10. Comparison of the scRNA-seq dataset from Wang et al. and our flow cytometry dataset. (a) Percentage of calories obtained from carbohydrates, fat and proteins in different diets. (b) Side-by-side presentation of lamina propria immune cell populations as percentages in CD45$^+$ cells from mice that were fed 8 weeks HFHSD or CD. (c) Fold change of lamina propria immune cell populations from CD to HFHSD. Pearson correlation (c). CCU, Champalimaud Centre for the Unknown.

Overall, we could conclude that HFHSD leads to a significant and gradual increase in body weight of male wild-type mice and is accompanied by increased glucose intolerance. The factors "diet" and "intestinal segment" explain the largest variance in the composition of the enteric immune system in our study, while duration of diet alters the immune landscape only in the epithelium but is not crucial in the lamina propria. Diet strongly affects the enteric immunophenotype with the most pronounced effect on cDCs and CD8$^+$ T cells. Most consistently affected are cDC1 frequencies, which are significantly increased in HFHSD when compared to CD. Diet-dependent alterations in the intestinal immune landscape

are not random, and the effect of diet on cDC1s might occur indirectly or due to a combination of factors.

Given the consistent and pronounced increase in cDC1 frequency in HFHSD conditions, we next investigated the underlying mechanisms driving this accumulation and potential functional implications.

4.2. cDC1s in the obese host

4.2.1 Ontogeny and homing of cDC1 are similar in the steady state and metabolically stressed host

cDCs originate from bone marrow precursors, which progress through a series of developmental stages to achieve full differentiation in peripheral tissues (Liu et al., 2009). We interrogated whether increased intestinal cDC1s in the obese host might be the result of an enhancement of cDC1 progenitors. Flow cytometry analysis of the bone marrow revealed no significant increase in the percentage of CDP and pre-cDCs within $CD45^+$ cells in the bone marrow in obesity when compared to steady-state (Figure 11a). Since only cDC1s frequencies and neither cDC2s nor dpDCs (frequency of dpDC was only elevated at 16 weeks) were modified in HFHSD, we tested whether the pre-cDCs might have been altered in obesity. We found that the frequencies of pre-cDC1s, pre-cDC2s and uncommitted pre-cDCs were unaffected (Figure 11a). This was also true when comparing total numbers of all progenitors or frequencies of pre-cDC subtypes in pre-cDCs (Figure S1a & b).

The process that drives pre-cDC egress from bone marrow to specific organs is complex and can be modulated by kinetics of turnover and release of precursors in and from the bone marrow (Cabeza-Cabrerizo et al. 2021). We interrogated

whether the increase of enteric cDC1s with unaffected numbers and frequencies of pre-cDCs in the bone marrow might be the result of elevated proliferation of pre-cDC1s in the bone marrow in combination with faster release of those into the bloodstream and potential homing to the small intestine. Therefore, we analysed the frequencies of cDC precursors in the blood. Flow cytometry analysis indicated a consistent percentage of pre-cDCs and pre-cDC1s in HFHSD-fed mice when compared to CD-fed animals (Figure 11b). Total number of pre-cDCs and pre-cDC1s and frequency of pre-cDC1s in total pre-cDCs in the blood did also not show any difference between experimental conditions (Figure S1c & d).

Sequentially, we asked whether homing of pre-cDC1s might have been affected in obesity, with more pre-cDC1s homing to the small intestine. Homing to the gut is induced by expression of the integrin $\alpha 4\beta 7$ and the chemokine receptor CCR9 on pre-cDCs (Zeng et al., 2012). Neither percentage of pre-cDC1 expressing $\alpha 4\beta 7$ or CCR9 in pre-cDC1 nor expression of these molecules per cell (MFI) was altered in mice fed an HFHSD, suggesting no enhanced gut homing capacity of these cells in the obese host (Figure 11c).

cDC development relies on the cytokine Flt3L in the bone marrow, and high Flt3L levels in tissues locally promote cDC cell division (Waskow et al., 2008). Thus, we interrogated if HFHSD would elevate local Flt3L levels in the small intestine, potentially promoting local proliferation of cDCs. ELISA measurements demonstrated no changes in Flt3L levels in the small intestine of obese individuals. Similarly, serum and bone marrow of HFHSD-fed mice showed no different Flt3L levels compared to CD-fed mice (Figure 11d). Additionally, enteric pre-cDCs and cDC1 did not show enhanced expression of the proliferation marker Ki67 (Figure S1e).

Since neither cDC1 precursors nor gut homing capacity of cDC1 were demonstrated to be elevated in HFHSD, but cDC1 frequency in the gut was

increased, we asked whether HFHSD could impact cDC1 in other organs. The proportion of cDC1 within the cDC population was significantly decreased in the liver of obese animals and showed a negative trend in all other analysed organs (Figure 11e). Only in the small intestine frequency of cDC1 in total $CD45^+$ cells was significantly altered, with an increase in HFHSD when compared to CD conditions as already mentioned (Figure S1f). These data indicate a weak systemic impact of the HFHSD on cDC1 distribution.

We next wondered if the increase in enteric cDC1 in HFHSD conditions might result from decreased migration out of the small intestine into mesenteric lymph nodes (mLN). We found that cDC1 frequencies significantly increased in mLNs from HFHSD animals compared to CD animals, indicating no decreased migration out of the small intestine (Figure 11f & S1g).

Overall, our data indicate that HFHSD does not substantially affect cDC1 progenitors, nor does it significantly alter gut-homing molecules of cDC1s. cDC1 are reduced in the liver, while cDC1s were increased in the small intestine and mLNs upon HFHSD.

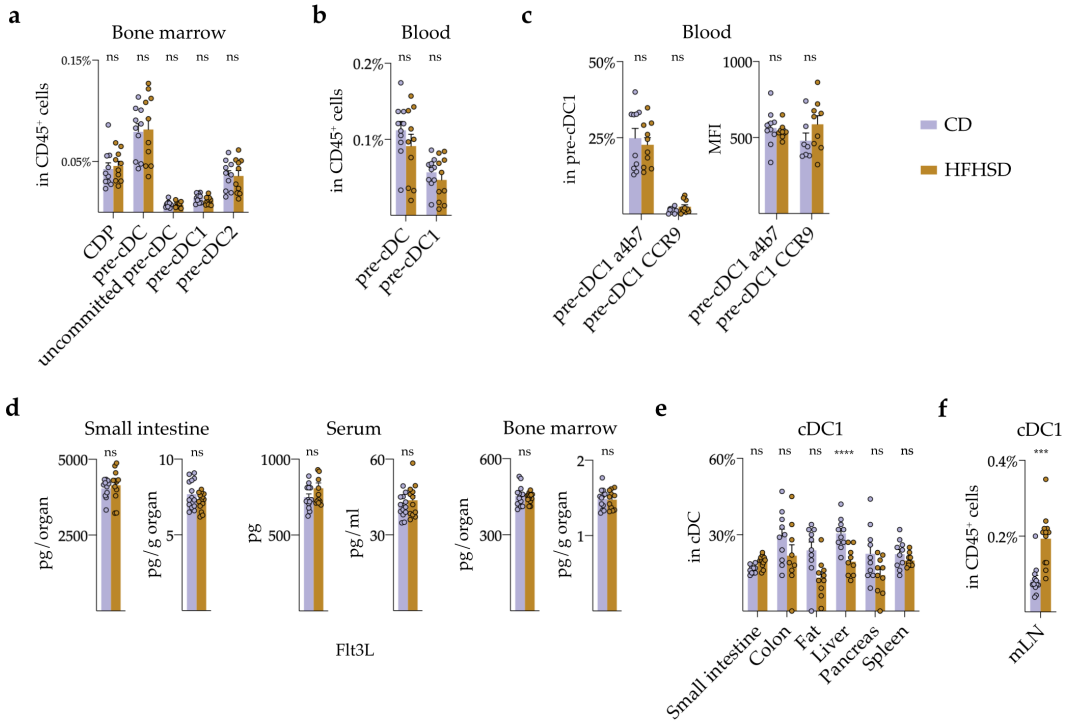


Figure 11. Similar ontogeny of cDC1s in the obese and stead-state host. Mice were fed for 8 weeks with HFHSD or CD. (a) cDC precursors in bone marrow as percentages in CD45$^+$ cells measured by flow cytometry (n = 10). (b) Pre-cDCs as percentages in CD45$^+$ cells in the blood and (c) their expression of gut homing molecules as percentages in pre-cDC1 and MFI (n = 10). (d) Flt3L levels in small intestine, serum and bone marrow in pg per organ and pg per g or ml organ measured by ELISA, normalised to total protein (n = 15). (e) cDC1s as percentages of cDCs in metabolically relevant organs and (f) as percentage in CD45$^+$ cells in mesenteric lymph nodes (n = 12). Flow cytometry (a-c, e, f) and ELISA (d). Data are pooled from three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-tailed unpaired Student's t-test (a-f). *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

Having established that increased enteric cDC1s in obesity did not result from altered development or expression of traditional gut-homing markers, we next investigated whether these cells maintained their functional properties in the obese state.

4.2.2 Enteric cDC1s exhibit functional similarities in the steady state and metabolically stressed host

To further understand the impact of HFHSD on cDC1s, we characterised this cell type in the small intestinal lamina propria of mice fed either an HFHSD or a CD. We measured the expression of co-stimulatory molecules on cDC1s using flow cytometry (Figure 12a). The expression levels of CD40, CD80, CD86, MHC II, and MHC I did not show any significant differences between conditions, indicating no differential status of cells upon HFHSD (Figure 12b). To evaluate cytokine expression, we performed RT-qPCR on sorted cDC1s (Figure 12c). HFHSD, compared to CD mice, exhibited significant downregulation of *Il10* mRNA in cDC1, suggesting a reduced anti-inflammatory phenotype of HFHSD cDC1s. However, the expression of Transforming growth factor beta (*Tgfb*), *Tnfα*, *Il12* and *Il15* mRNA did not significantly differ between groups. Similarly, no differences were observed in the expression of the integrin gene *Itgb8* and *Aldh1a*, two molecules that are essential for the induction of intestinal regulatory T cells. Results for Tnf-α and IL-12 were further validated at protein level by flow cytometry, and neither ratio of cells expressing cytokines nor amount of cytokines expressed per cell differed in obese mice (Figure 12d).

To interrogate cDC1 properties further, we analysed single-cell sequencing data. We identified two clusters inside the DC cluster and confirmed their identity as cDC1 and cDC2 by evaluating the expression of genes coding for *Itgam*, *Sirp1a*, *Clec9a* and *Xcr1*, distinctively expressed in cDC1 and cDC2 (Figure 12e & f). We employed DESeq2 analysis to compare gene expression in HFHSD and CD groups. cDC1 and cDC2 expression profiles were similar between groups, with no significantly different expressed genes (Figure 12g). Expression profiles of genes up-regulated in activated cDC1s were highlighted and are shown in Figure 12h.

In conclusion, our data revealed that enteric cDC1s in HFHSD do not exhibit a dramatically different activation status compared to cDC1s in CD conditions. However, cDC1 in HFHSD conditions might exhibit less anti-inflammatory properties compared to cDC1 coming from a host in steady-state as we could detect lower *Il10* mRNA expression levels in cDC1s of obese mice.

As cDC1s maintained their functional properties in obesity, the physiological consequences of their increased frequency remained to be determined. We hypothesised an effect of diet on processes that rely on cDC1/T cell interactions.

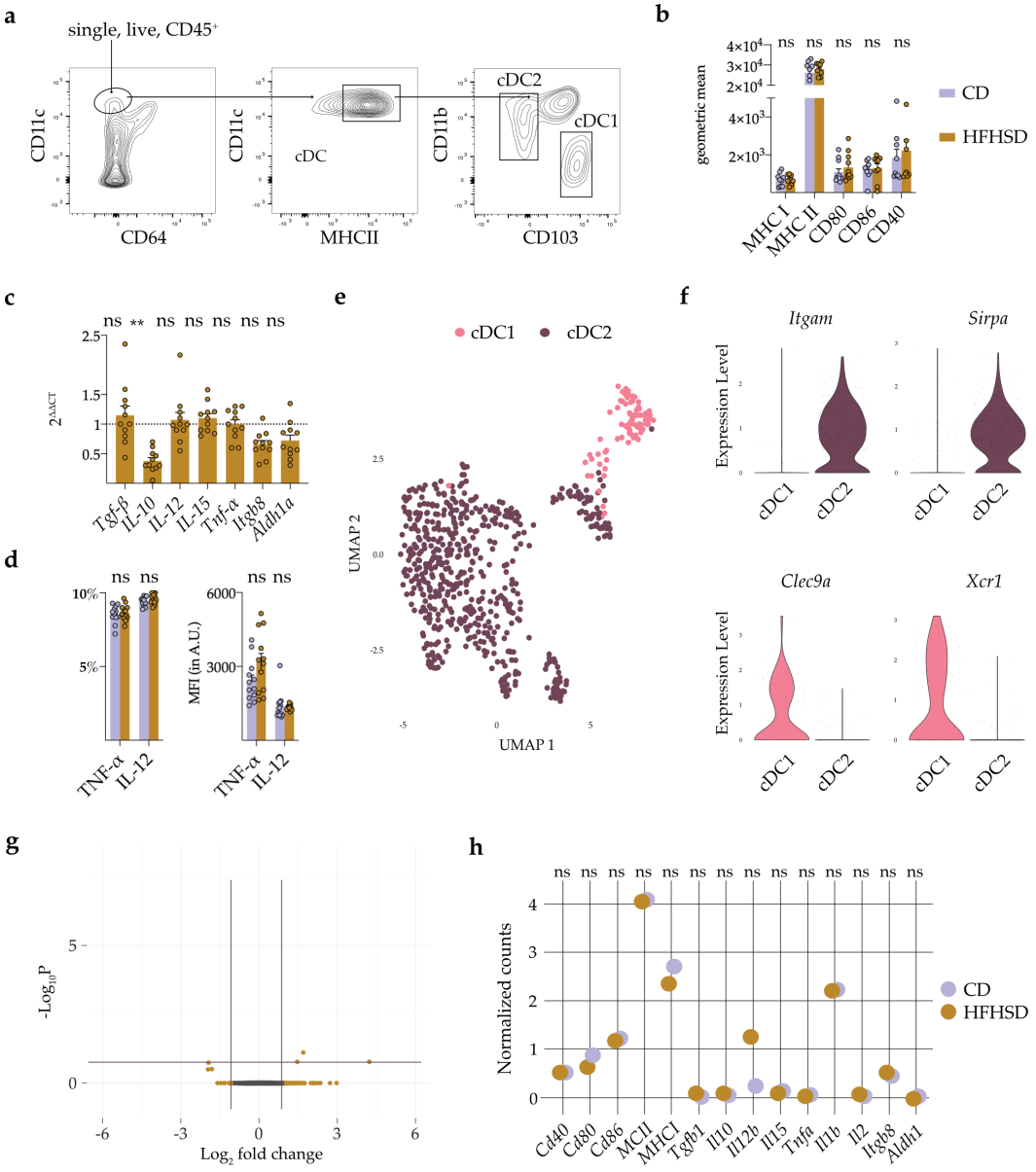


Figure 12. HFHSD does not alter status of cDC1 in the small intestine. (a) Flow cytometry gating strategy for cDC1. (b) Expression of co-stimulatory molecules on cDC1s measured by flow cytometry (n = 10). (c) Relative gene expression in sorted cDC1 from HFHSD-fed mice normalised to *Hprt* and CD-fed mice measured by RT-qPCR (n = 12). (d) Percentage of cDC1 expressing cytokines measured by flow cytometry and expression per cell as MFI (n = 12). (e) UMAP presentation of cDC subsets in scRNA-seq dataset from Wang et al. (f) Marker gene expression used for identification of cDC1 and cDC2 in scRNA-seq dataset. (g) Volcano plot of DESeq2 of cDC1 in scRNA-seq dataset. (h) Histogram showing normalised gene expression of selected genes in cDC1. Data are pooled from three independent experiments (b-d). n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-tailed unpaired Student's t-test (b-d). DESeq2 analysis (f-g). *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

4.3 Food tolerance in obesity

4.3.1 Interaction between intestinal cDC1 and CD4$^+$ T cells is altered in the obese host

Given the importance of cDC1 in interacting with T cells, we tested if the interaction between those cells could be altered in obesity. Analysis of single-cell data revealed specialised sub-populations within the CD4$^+$ T cell cluster. We could identify Th1, Th2, Th9, Th17, Th22, FoxP3$^+$Rorγt$^+$ Tregs and Tregs dependent on top differentially expressed genes (DEGs) (Figure 13a & b). Using CellChat, we conducted a systems-level analysis of cell-cell interactions between identified CD4$^+$ T cell subpopulations and cDC1s. The number and strength of interactions between HFHSD and CD-fed mice were compared, and CellChat predicted most pronounced differential interaction received by cDC1 and Th1 cells. All interactions coming from T cells towards cDC1s were up-regulated in obesity, and most pronounced were signals coming from memory-like CD4$^+$ T cells and FoxP3$^+$Rorγt$^+$ T cells. Vice versa, cDC1s were predicted to up-regulate their number of interactions towards all other T cells, except for signalling towards Th1 cells, which was predicted to be down-regulated, and towards Tregs, which was predicted to be unchanged. As for the predicted interaction strength, cDC1s showed stronger interactions towards all T cells except for memory-like CD4$^+$ T cells, with interaction towards Th1 cells being the most up-regulated one. All T cells also increased their strength of interaction with cDC1s, with Th1 cells being the most pronounced senders (Figure 13c & d).

Overall, scRNA-seq data suggested that HFHSD has an effect on interactions between cDC1 and CD4$^+$ T cells with an increase in number and strength of interactions towards cDC1. Vice versa, cDC1s were shown to send out a higher

number of interactions towards all T cells, except for Th1 and Treg cells, and interactions are stronger towards all T cells except for memory-like $CD4^+$ T cells.

To test whether the predicted alterations in cDC1/$CD4^+$ T cell interactions had functional consequences *in vivo*, we examined their impact on oral tolerance using the OVA/OT-II model.

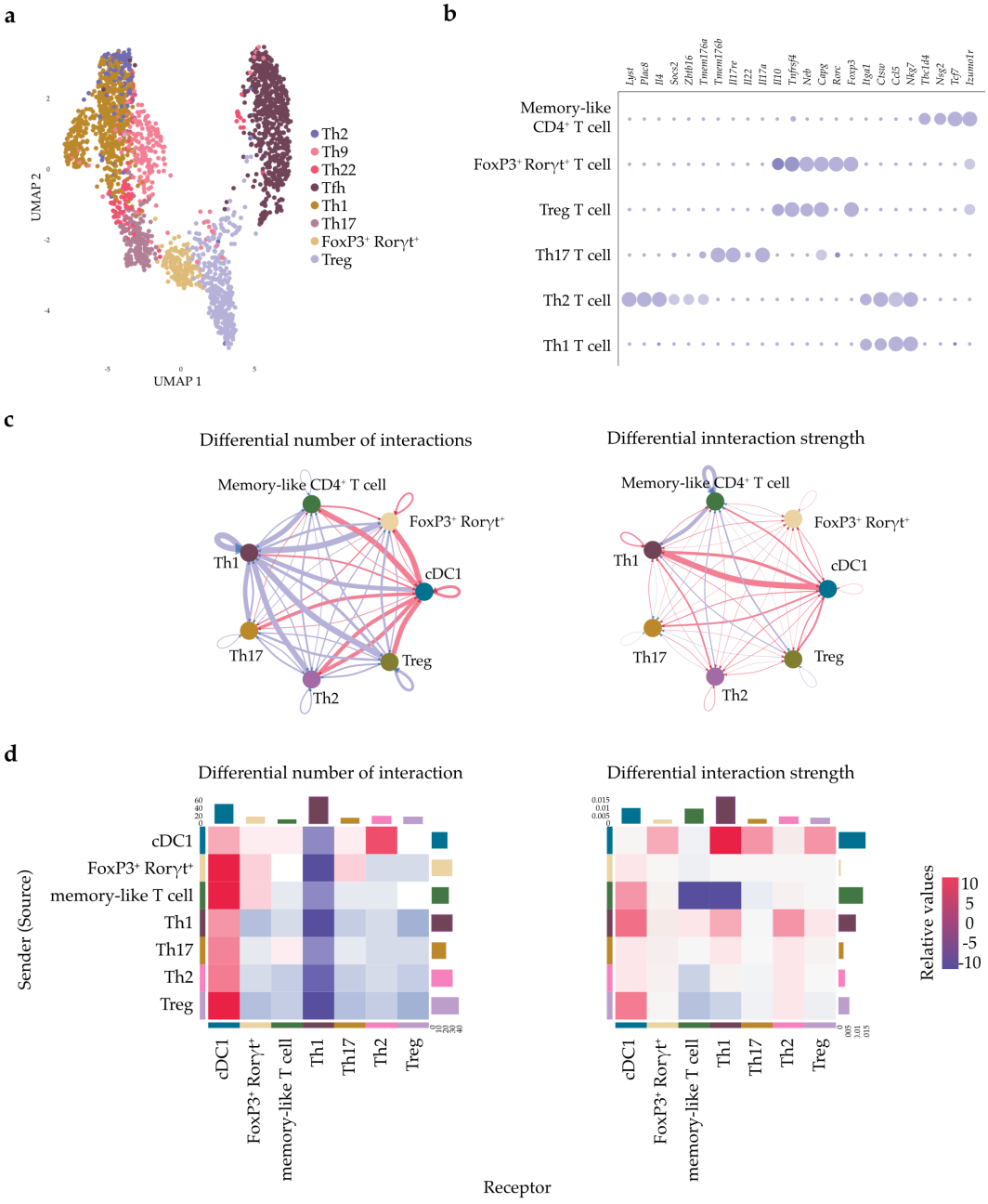


Figure 13. Functional impact of HFHSD on cDC1/T cell interaction. (a) UMAP presentation of CD4⁺ T cell cluster in scRNA-seq dataset from Wang et al. (b) Dot plot showing selected top DEG used for identification of CD4⁺ T cell populations. (c, d) Differential cell-cell interactions predicted by CellChat between CD4⁺ T cells and cDC1s in HFHSD compared to CD-fed mice. Violet colour indicates down-regulated interaction; pink colour indicates up-regulated interaction.

4.3.2 HFHSD increases food tolerance

Since there was a clear effect of diet on predicted interaction between cDC1 and $CD4^+$ T cells in the data generated by Wang et al., we next asked whether interaction between these cells might also be affected by diet in our setting. Anti-inflammatory cDC1 can induce Treg cells, which is important for the induction of oral tolerance (Esterházy et al., 2016; Hong et al., 2022). We speculated that an increase in cDC1 frequency in obesity might be relevant in the context of food tolerance, potentially leading to better food-antigen tolerance. We utilised the OVA/OT-II model to test our hypothesis. Obese and stead-state mice were adoptively transferred retro-orbital with 1 x 10^6 naïve OT-II T cells, followed by two doses of i.g. ovalbumin (OVA) 48 h and 24 h prior to analysis. Obese and control mice showed comparable frequencies of naïve T cells, Tfh, Th1, Th17 and $FoxP3^+Ror\gamma t^+$ Treg cells in the mLNs after OVA challenge. In large contrast, Tregs were significantly increased in obese mice, suggesting enhanced tolerogenic response to oral antigen (Figure 14).

To summarise, HFHSD reshapes the enteric immune landscape. Obese mice have a higher ratio of cDC1s, and interaction between these cells and $CD4^+$ T cells is predicted to be affected by diet. The obesogenic immunophenotype has physiologic relevance in the context of oral tolerance, with enhanced generation of Tregs upon food-antigene challenge.

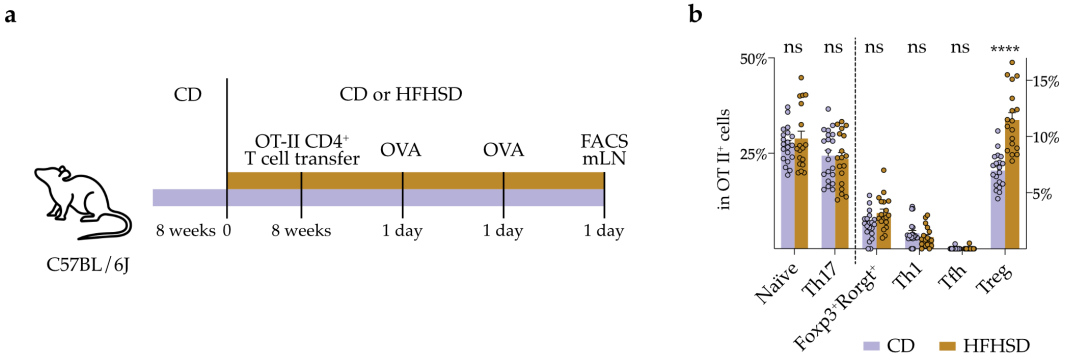


Figure 14. Functional impact of HFHSD shaped enteric immune landscape in food tolerance. (a) Male wild-type mice were fed HFHSD or CD for 8 weeks and adoptively transferred with 1 x 10^6 naïve CD45.1 $CD4^+$ OT-II T cells. Mice received two doses of intragastric OVA at 48 h and 24 h before analysis. (b) Percentage of cells in $OT\text{-}II^+$ cells from mesenteric lymph nodes (n= 15). Data are pooled from three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-tailed unpaired Student's t-test. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

5

DISCUSSION

5.1 Regional and temporal dynamics of diet-induced immune adaptation

We generated a comprehensive dataset to examine the intricacies of the adult small intestinal immune landscape and its modulation with increasing exposure time to an obesogenic diet. Utilising a dimensionality reduction, specifically PCA, enabled us to identify distinct immunophenotypes dependent on diet and intra-intestinal location. Leveraging the statistical framework of ANOVA analysis allowed for a detailed description of shifts in the enteric immune landscape in response to obesogenic diet, age, intestinal segment, and interactions among these factors.

5.1.1 Considerations in experimental design

5.1.1.1 The choice of age and sex

The investigation of obesity-induced immune adaptations required careful consideration of age and sex-specific variables that could influence our results. We chose to examine adult mice between 8 and 24 weeks of age, a period

representing mature adulthood while avoiding both developmental changes and age-related decline (Bresilla et al., 2022; Flurkey et al., 2007; Yanai and Endo, 2021). This timeframe corresponds to human ages between 20-30 years (Flurkey et al., 2007), characterised by complete physical development and stable metabolic function.

The decision to focus exclusively on male mice stemmed from the need to minimise hormonal variability in our experimental system. While female mice experience significant hormonal fluctuations due to their oestrus cycle beginning at 6-8 weeks of age (Ajayi and Akhigbe, 2020; Pantier et al., 2019), male mice maintain relatively stable hormone levels after reaching sexual maturity at 8 weeks (Bell, 2018; Nilsson et al., 2015). These hormonal differences substantially impact metabolic regulation, with oestrogen receptors playing crucial roles in insulin sensitivity, glucose metabolism, and energy homeostasis (Gao and Horvath, 2008; López and Tena-Sempere, 2015; Ropero et al., 2008; Smith et al., 2022). Generally, female mice experience a protective effect towards metabolic syndrome, especially insulin resistance, due to different fat-storage capacities resulting from oestrogen and genetic factors influencing glucose metabolism (Ali et al., 2023; Camilleri et al., 2021; White and Tchoukalova, 2014). They have also been shown to be obesity-resistant on an HFD and to modulate their oestrus cycle in response to HFD feeding, while males typically experience faster weight gain on an obesogenic diet (Hwang et al., 2010; Lainez et al., 2018; Lenert et al., 2021).

Beyond metabolic effects, sex hormones directly influence cells of the innate and adaptive immune system, which express the oestrogen receptor (Khan et al., 2012; Khan and Ansar, 2016; Kovats, 2015). For example, oestrogen has been demonstrated to enhance the maturation of DCs and regulate the expression of cytokines and chemokines like IL-6, IL-10, CCL2 and CXCL8 (Cunningham et al.,

2014; Liu et al., 2002; Kovats, 2012; Stojić-Vukanić et al., 2015; Papenfuss et al., 2011). Additionally, oestrogen modulates all subsets of T cells, including $CD4^+$ T cells (Th1, Th2, Th17, and Tregs) and $CD8^+$ T cells (Karpuzoglu-Sahin et al., 2001; Lélu et al., 2011; Priyanka et al., 2013; Robinson et al., 2014). These effects extend to the enteric immune system, where sex-specific differences in microbiota composition and immune regulation have been documented (Klein, 2000; Markle et al., 2013; Yurkovetskiy et al., 2013). The cyclical nature of female hormone levels introduces an additional layer of complexity to immune responses that could potentially mask diet-induced effects.

While our male-only approach allowed us to isolate diet-dependent effects from hormonal influences, we acknowledge this as a limitation of our study. The worldwide obesity epidemic affects both sexes, and our findings only reflect the physiology of half of the general population. This limitation reflects a broader issue in biomedical research, where male subjects are often overrepresented in experimental studies (Clayton and Collins, 2014; Justice, 2024). Male-biased research leads to a neglect of female physiology and a dangerous underestimation of health risks for women. With our choice, we aimed to add general knowledge to the understanding of enteric adaptation to an obesogenic diet, while studies focusing on hormonal effects are needed to form a more complete and appropriate representation of the enteric immune response to an obesogenic diet.

5.1.1.2 The choice of diet

Research in obesity employs various dietary models, each designed to mimic different aspects of human dietary patterns. For our study, we selected the model established by Asgharpour and colleagues that replicates key features of the

Western diet, characterised by both high fat content and sugar-enriched beverages (Asgharpour et al., 2016). By modifying both lipid content and adding fructose and glucose to drinking water, this model extends beyond simple caloric excess to capture the complexity of modern dietary habits. Consequently, the observed immune adaptations should be interpreted as responses to specific dietary components rather than merely increased caloric intake.

The inclusion of both glucose and fructose in our model holds particular significance, as these saccharides differentially affect metabolic and immune responses. Studies have shown that fructose, compared to glucose, leads to enhanced visceral fat accumulation and diminished insulin sensitivity in obese subjects (Stanhope et al., 2009). At the cellular level, fructose distinctly activates cDCs through accumulation of advanced glycation end products, triggering specific inflammatory pathways and metabolic adaptations (Jaiswal et al., 2019). Our choice to supplement drinking water with fructose reflects its prevalence in the Western diet. Fructose is sweeter than glucose or sucrose when compared in equal amounts and is, therefore, commonly used in the food industry as an economical bulk sweetener (Miller and Adeli, 2008). In our experiments, HFHSD mice consumed, on average, 0.3234 g of fructose more than CD mice per day.

The fatty acid composition in our HFD differs from the control diet primarily in levels of specific saturated and monounsaturated fatty acids, particularly C16:0, C18:0, C16:1, and C18:1, while maintaining similar levels of amino acids, vitamins, macro minerals and trace elements within an order of magnitude (SDS mouse diet RM3 (P); D12492). This specific nutrient profile allows us to examine the immune adaptation to dietary patterns rather than isolated macronutrient changes.

Importantly, our dietary model likely influences the intestinal microbiota, a crucial mediator of immune responses. Different dietary components distinctly

shape microbial communities, with both fats and sugars exerting dose-dependent effects on bacterial populations (David et al., 2014; Di Luccia et al., 2015; Do et al., 2018; Satokari, 2020; Wu et al., 2011). While glucose typically induces more pronounced alterations in microbial composition compared to fructose, both sugars contribute to microbiota-dependent immune modulation (Zhang et al., 2021). The type and proportion of fatty acids similarly influence microbial populations, creating complex interactions between diet, microbiota, and immune function (De Wit et al., 2012, 2011).

While we did not directly assess microbiota composition in our study, the established relationship between diet and microbial communities suggests that our observed immune adaptations may reflect both direct dietary effects and indirect microbiota-mediated changes (Daniel et al., 2014; Hildebrandt et al., 2009; Pessoa et al., 2023; Turnbaugh et al., 2009; Wang et al., 2020). This complexity mirrors the human condition, where dietary choices simultaneously influence multiple physiological systems. Future studies combining immune and microbiota analyses could help delineate these interconnected pathways and their relative contributions to shaping the enteric immune system.

5.1.1.3 Definition of immunophenotypes

A fundamental consideration in our experimental design was the choice to define immune landscapes through cell frequencies within the $CD45^+$ population rather than absolute cell numbers. This methodological decision was driven by both practical constraints and biological considerations inherent to obesity research.

Obesogenic diet induces substantial morphological changes in the small intestine, including alterations in length, thickness, and villous architecture.

Studies have documented intestinal shortening in obese animals, accompanied by decreased villus length and altered mucosal volume (Gruber et al., 2013; Mao et al., 2013; Xie et al., 2020). These structural changes create significant challenges for standardising cell count data across experimental groups. While absolute numbers could theoretically be normalised to intestinal length or weight, such adjustments would be confounded by diet-dependent variations in tissue composition, particularly mucus content, which differs markedly between lean and obese animals.

The separation of epithelial and lamina propria compartments in our analysis further complicated the use of absolute cell numbers. Traditional normalisation methods, such as cells per unit of tissue weight or length, become particularly problematic when examining these distinct compartments independently. The dynamic nature of the intestinal histology in obesity makes such normalisation potentially misleading.

Instead, we chose to normalise within the immune system itself by expressing cell frequencies as proportions of $CD45^+$ cells. This approach offers several advantages: it provides a stable reference point across experimental conditions, allows for direct comparison between different intestinal segments, better reflects the relative composition of the immune landscape and captures shifts in the balance between different immune populations.

5.1.2.1 General regional and temporal effects on enteric immune composition

Our comprehensive analysis of the enteric immune landscape revealed adaptation patterns to an obesogenic diet, with distinct temporal and regional signatures. Using dimensionality reduction and detailed statistical analyses, we uncovered that, while the diet profoundly influences immune composition, the nature and extent of these changes depend on complex interactions between time, intra-intestinal location, and cell type. This multifaceted response reflects the sophisticated ability of the immune system to adapt to environmental changes while maintaining tissue-specific functions. Noteworthy, many adaptations occurred in the first 4 weeks of HFHSD and remained stable over time, suggesting the establishment of a new homeostatic state rather than a progressive deterioration of immune function.

Age and duration of dietary intervention

Our dimensionality reduction analysis revealed that the overall immunophenotype was not primarily defined by age, as projections of immunophenotypes from lean mice did not cluster by age in the PC space. (Figure 5a). However, when examining individual cell populations through ANOVA, we found that most immune cell frequencies were significantly influenced by age alone (Figure 6c). This age-dependent variability in immune composition observed even during the relatively stable period of adulthood and under controlled environmental conditions, represents an under-appreciated aspect of intestinal immune regulation. This finding carries particular significance given our deliberate choice of male mice and the controlled vivarium environment — conditions typically assumed to minimise physiological variation.

The enteric immune system's response to obesogenic diet emerged as rapid and remarkably stable. Within 4 weeks of dietary intervention, a distinct immunophenotype was established that remained largely consistent throughout the 16-week study period. This was evident as projections of immunophenotypes into a PC space clustered dependent on diet, but immunophenotypes of HFHSD animals from the same experimental time point did not group (Figure 5a). This stability was particularly evident in the lamina propria, where duration of diet exposure did not significantly affect cell populations beyond those already influenced by age alone (Figure 6c). When dietary intervention did modulate age-related changes, it typically acted by enhancing or diminishing existing age-dependent trends rather than establishing novel patterns. The epithelium showed distinct temporal patterns compared to the lamina propria, with some cell populations demonstrating sensitivity to both diet alone and its interaction with time. This compartment-specific response pattern emphasises the importance of examining different intestinal immune niches independently and suggests distinct regulatory mechanisms in epithelial and lamina propria immune populations.

The relationship between metabolic state and immune function has been well documented (see chapter 1.2.3.2 The enteric immune system in obesity), prompting us to examine whether progressive metabolic dysfunction might drive continued changes in immune composition. Our experimental design deliberately included time points that capture distinct stages of metabolic decline. Body weight increased significantly between each time point (4, 8, and 16 weeks) in the HFHSD group (Table S5), and previous characterisation of this obesity model by Liu and colleagues demonstrated progressive worsening of metabolic syndrome, reaching nonalcoholic steatohepatitis at 16 weeks (Liu et al., 2018). If metabolic status were the primary driver of immune composition, we would expect to see distinct immunophenotype clusters corresponding to these

progressive stages of metabolic dysfunction in our dimensionality reduction analysis (Figure 5a). However, our data showed no such temporal clustering, suggesting that the immune landscape stabilises despite continuing metabolic deterioration. While this indicates that the severity of metabolic syndrome does not dictate ongoing immune adaptation, it remains possible that the transition from lean to obese status itself, and independent of other factors such as diet-induced shifts in microbiota, triggers the initial establishment of a new immune landscape to some degree. Testing this hypothesis would require examining earlier time points before overt metabolic dysfunction, coupled with comprehensive microbiota analysis and metabolic profiling. Nevertheless, our current data, spanning different severities of metabolic compromise, clearly demonstrate that the intestinal immunophenotype, once established, remains stable independent of progressive changes in body weight or metabolic parameters.

Importantly, the early establishment and subsequent stability of diet-induced changes suggest that the immune system quickly achieves a new equilibrium in response to altered dietary conditions. This observation carries profound implications for our understanding of obesity's impact on enteric immunity, indicating that many intestinal adaptations represent regulated responses rather than progressive dysfunction. The independence of these adaptations from increasing body weight or worsening metabolic parameters further supports this interpretation, as immunophenotypes remained stable even as metabolic disease progressed. It also suggests that the immune system establishes a new homeostatic set point in response to dietary changes, potentially representing an adaptive response to altered environmental and microbial conditions. This perspective aligns with emerging views of the immune system as a sophisticated environmental sensor capable of stable adaptation to ever-changing environmental conditions.

Segments and interaction of diet and segment

Regional differences in the intestinal immune composition have been reported and summarised previously (Mowat and Agace, 2014; Zwick et al. 2024), but a systematic comparison of all major immune cell types across intestinal segments in mice has been lacking until now. Our analysis provides this comprehensive comparison in steady-state mice (Figure S2) and reveals that nearly all major immune cell types, with the notable exception of $CD4^+$ T cells, are significantly influenced by their intestinal location (Figure 7). Through dimensionality reduction analysis, we identified distinct immunophenotypes associated with specific intestinal regions (Figure 5b). Notably, the immune landscapes of duodenum and jejunum showed greater similarity to each other than to the ileum, consistent with known anatomical and functional distinctions of the distal small intestine. This regional specialisation aligns with established knowledge about the ileum's unique features, including its higher density of Peyer patches, increased microbial load and diversity, and distinct immune cell composition compared to more proximal regions (Mowat and Agace, 2014; Zwick et al. 2024). Studies have shown that these distinctions persist under HFD conditions, where the duodenum and proximal jejunum exhibit hyperplasia and increased surface area, while the ileum shows hypoplasia and reduced surface area (West et al., 2019).

Intriguingly, our analysis revealed that immunophenotypes of specific intestinal segments remained more similar to their counterparts across dietary conditions than to other segments within the same diet. This observation suggests that the fundamental identity of each intestinal region, shaped by its specific physiological functions, remains preserved despite dietary perturbation. While previous studies have shown that HFD can alter gut motility and transit time (Gallagher et al., 2009; Wisén and Johansson, 1992), potentially shifting

nutrient availability along the intestine, adaptations in absorption capacity appear to maintain segment-specific functions. This is supported by observations of enhanced proximal nutrient absorption in obese subjects and low faecal energy excretion, indicating effective compensation for the increased nutrient load (Webb and Annis, 1983; Wisén and Johansson, 1992). Additionally, researchers found greater crypt density in duodenum and jejunum of HFD-fed mice as a result of an adaptive growth response by intestinal stem cells in response to nutrient overload (West et al., 2019). In our study, almost half of the analysed cell types showed diet-dependent responses that varied by intestinal location (Figure 7). However, these regional variations manifested as differences in magnitude rather than direction of response, suggesting coordinated but locally tuned adaptation. This pattern of graded responses likely enables fine-tuned regulation of intestinal homeostasis while maintaining segment-specific functions.

These findings challenge the traditional view of treating the intestinal immune system as a uniform entity. Our results strongly suggest that future research should distinguish between duodenal, jejunal, and ileal responses, particularly when studying intestinal pathologies that show anatomical preferences. For instance, celiac disease primarily affects the duodenum (Freeman, 2008), Crohn's disease often targets the terminal ileum (Farmer et al., 1975), and small intestinal bacterial overgrowth typically initiates in the ileum (Bures et al., 2010). This idea is supported by other studies that have already shown that intestinal immunity is regulated in a segment-specific manner (Esterházy et al., 2019; Mayer et al., 2020; Moreira et al., 2021). Understanding segment-specific immune responses could lead to more targeted therapeutic strategies for these and other intestinal diseases.

5.1.2.2 Cell type-specific responses to dietary intervention

A striking observation from our analysis is the greater spread of immune population data in CD conditions compared to HFHSD. The increased variance under CD conditions suggests greater immunological plasticity and flexibility in the healthy state. This observation aligns with the concept that a healthy immune system maintains a diverse repertoire of responses, likely supported by a more diverse microbiota (Belkaid and Hand, 2014). In contrast, the reduced variance in HFHSD conditions points to a more constrained, less flexible immune system, potentially reflecting the documented reduction in microbiota diversity associated with obesogenic diets (Andersen et al., 2016; Turnbaugh et al., 2008).

Our findings reveal particularly striking effects of dietary factors on cDCs and $CD8^+$ T cells. The most notable finding was the consistent and significant increase in cDC1 frequencies across intestinal segments in response to HFHSD. Interestingly, this finding is accompanied by a concurrent decrease in $CD8^+$ T cell populations, which we will discuss in more detail below.

Two-way repeated measures ANOVA revealed that T cell subtypes in the epithelium were more substantially affected by diet compared to those in the lamina propria. This compartment-specific effect likely reflects the unique position of the epithelium as the primary interface between the host immune system and luminal contents, including both dietary antigens and the microbiota. The heightened sensitivity of epithelial T cells to dietary changes suggests that direct exposure to food antigens and diet-induced alterations in the microbiota may be primary drivers of immune adaptation in obesity.

The reduction in $CD8^+$ T cells under HFHSD conditions reveals a complex interplay of mechanisms and physiological consequences. Recent research has identified both microbiota-dependent and independent pathways contributing to

this phenomenon. While Cervantes-Barragan and colleagues demonstrated the role of obesity-mediated changes in the microbiota (Cervantes-Barragan et al., 2017), Hung and colleagues uncovered a microbiota-independent mechanism involving phagocyte FXR-type I IFN signalling (Hung et al., 2024), suggesting the possibility of multiple parallel pathways regulating $CD8^+$ T cell populations in response to dietary changes.

The functional significance of reduced $CD8^+$ T cells, particularly $CD8^+$ IELs, presents an intriguing paradox. While the loss of IEL T cells typically results in severe intestinal inflammation (Muzaki et al., 2016), suggesting a potentially detrimental effect, emerging evidence points to an unexpected beneficial role in metabolic adaptation. Studies with integrin β7 knockout mice have revealed that reduced IEL density correlates with resistance to obesity and metabolic syndrome during HFD challenge. This protection appears to operate through the regulation of GLP-1 bioavailability, as IELs express the GLP-1 receptor, and their reduction leads to increased GLP-1 availability (He et al., 2019). Thus, the decrease in IELs may represent a host adaptation mechanism to mitigate the development of metabolic disorders associated with diet-induced obesity.

In this context, the consistent increase in cDC1s, reciprocal to the spatio-temporal decrease of $CD8^+$ T cells along segments and duration of dietary intervention, might serve as a compensatory mechanism to maintain immune homeostasis while allowing for metabolic adaptation. The XCR1-XCL1 signalling axis plays a crucial role in this process, orchestrating the intricate balance between cDC1s and $CD8^+$ T cells. Recent studies by Wang et al. (2023) showed that blocking the XCR1-XCL1 signalling pathway through intraperitoneal injection of anti-XCL1 antibodies leads to an increase in intraepithelial $CD8\alpha\alpha^+$ T cells and lamina propria $CD4^+$ T cells. However, several genetic approaches have revealed seemingly contradictory results: mice lacking $XCR1^+$ cDC1s or

deficient in XCR1 show diminished intestinal T cell populations and ablation of XCR1 in *Xcr1*-DTA mice also results in a 20% decrease of *Xcl1* expression in LP T cells (Esterházy et al., 2016; Luda et al., 2016; Muzaki et al., 2016; Ohta et al., 2016). Mice deficient in the XCR1 ligand XCL1 show diminished intestinal T cell populations, along with an accumulation of $XCR1^+$ cDC1s in the gut. Additionally, studies in *Rag2*-deficient mice, which lack both B and T lymphocytes, showed increased LP cDC1s (Ohta et al., 2016), highlighting the interdependence of these immune cell populations.

When interpreting our findings in this context, two potential explanations emerge. The first possibility, supported by genetic knockout studies, suggests that the initial event in obesity is the reduction of $CD8^+$ T cells, which then leads to a compensatory increase in cDC1 numbers. This interpretation is strengthened by our regression analysis, which indicates that HFHSD feeding primarily affects $CD8^+$ T cell populations when all other regressors are assumed to stay constant, while changes in cDC1 numbers do not become apparent in the regression model but are only visible when cell type changes between conditions are assessed independently in the ANOVA analysis. The alternative explanation, and assuming a relationship between reciprocal changes in cDC1 and $CD8^+$ T cell frequencies, would suggest that increased cDC1 numbers drive the reduction in $CD8^+$ T cells. However, this scenario appears less likely given the well-documented role of $XCR1^+$ cDC1s in supporting T cell survival and function (Ohta et al., 2016). The discrepancy between antibody-mediated blockade and genetic knockout approaches suggests that acute interruption versus chronic absence of XCR1-XCL1 signalling may have fundamentally different effects on immune homeostasis or might simply display an artefact, warranting further investigation.

While considering the XCR1-XCL1 signalling axis, it is important to note that XCL1 expression is not exclusive to T cells. NK cells, for instance, also express this chemokine. However, several lines of evidence support the primary role of $CD8^+$ T cells in XCL1-mediated regulation during obesity. Wang and colleagues demonstrated that $CD8^+$ T cells are the predominant producers of XCL1 in obesity, and other studies have shown that LP T cells express similar levels of XCL1 when compared to NK cells under steady-state conditions (Ohta et al., 2016; Wang et al., 2023). Furthermore, our results and those of Wang et al. indicate that NK cells are present in very low numbers in the small intestine, suggesting that their contribution to local XCL1 production is likely minimal. This evidence strengthens the hypothesis that the observed changes in cDC1 numbers are primarily linked to alterations in $CD8^+$ T cell populations rather than other XCL1-producing cells.

Our multi-linear ridge regression analysis revealed an important insight into the nature of diet-induced immune adaptations: the changes in immune cell populations in response to HFHSD are not random but rather follow predictable patterns. This finding has significant implications for our understanding of how the immune system adapts to dietary challenges. The apparent discrepancies between our ANOVA and regression analyses highlight the complementary nature of different statistical approaches to understanding complex biological systems. While ANOVA examined individual cell populations in isolation, regression analysis provided a more holistic view by analysing cell types in concert, revealing relationships and effects that were not apparent when examining each population independently. This multivariate approach better reflects the biological reality of the immune system, where cell populations do not exist in isolation but function as part of interconnected networks. However, it is very important to acknowledge the limitations of our regression analysis. Due to experimental constraints, we were unable to include all cell types from our

screen in the regression models. This incomplete sampling of the immune landscape means that, while we can confidently conclude that immune adaptations to HFHSD are not random, we must be cautious about drawing any more specific conclusions about the nature of these adaptations. The primary value of this analysis lies in establishing the principle that immunophenotype can predict dietary status, suggesting that the immune system responds to dietary changes in a coordinated and predictable manner rather than through stochastic alterations. This finding of non-random adaptation has important implications for basic research and therapeutic approaches. From a research perspective, it suggests that there are underlying rules governing how the immune system responds to dietary perturbations, which could be discovered through more comprehensive analyses. From a therapeutic perspective, the predictable nature of these changes suggests that targeted interventions might be developed to modulate specific aspects of the immune response to diet.

In accordance with our observation of predictable immune changes due to dietary intervention was the reproducibility of our findings using the dataset published by Wang and colleagues (Wang et al. 2023). Such reproducibility is particularly noteworthy given the inherent challenges in replicating immunological findings across different laboratories, techniques, and dietary formulations. The consistency of major immunological adaptations to HFHSD across these varying conditions suggests that these represent conserved responses rather than technique- or context-specific artefacts.

However, our detailed re-analysis of Wang et al.'s data revealed important methodological considerations that affect the interpretation of their findings and led us to adapt their workflow according to our evaluation. Their study conducted RNA sequencing in two separate rounds, with the first round examining general immune landscape composition and the second aiming at

increasing cell numbers per cluster in order to properly evaluate functional adaptations. Our re-analysis identified substantial discrepancies between these sequencing rounds. While the first round showed good concordance with our results, the second round revealed anomalous findings, particularly an unphysiologically high proportion of B cells in both CD and HFHSD conditions. Based on these observations, we concluded that the second round of sequencing data likely did not represent steady-state conditions and should be excluded from analysis, especially not merged with data from the first round for differential gene expression and cell-cell interaction analyses. This methodological decision represents a significant departure from Wang et al.'s published analysis, where these concerns were not addressed.

Our attempts to reproduce Wang and colleagues' cell clustering analysis revealed additional complexities in single-cell data interpretation. Despite following their analytical protocol exactly, we were unable to reproduce their reported 17 distinct immune cell clusters (CD8$\alpha\alpha^+$TCR$\alpha\beta^+/\gamma\delta^+$ T cell, CD8$\alpha\beta^+$TCR$\alpha\beta^+/\gamma\delta^+$ T cell, CD4$^+$TCR$\alpha\beta^+$ T cell, CD4$^+$CD8$^+$TCR$\alpha\beta^+$ T cell, CD4$^-$CD8$^-$ TCR$\alpha\beta^+/\gamma\delta^+$ T cell, cDC, pDC, NK cell, ILC1, ILC2, ILC3, B cell, plasma cell, plasmablast cell, macrophage, eosinophil, and mast cell), particularly in separating NK cells from ILC1s. This discrepancy persisted even when we increased cluster resolution to 80 clusters and applied various batch integration and dimensionality reduction techniques. Instead of achieving separation between NK cells and ILC1s, increased resolution resulted mainly in further subdivision of B cells, T cells, and monocyte populations. Given our current understanding of immune cell transcriptional profiles, we consider it more likely that different T cell subsets would show greater transcriptional divergence than NK cells and ILC1s. This biological reasoning, combined with our rigorous analytical approach, led us to maintain confidence in our clustering results despite their deviation from the published analysis.

These methodological considerations highlight the importance of careful data analysis and validation in immunological studies, particularly when dealing with complex single-cell datasets. While the overall reproducibility of our main findings strengthens their biological significance, the discrepancies we identified in the published analysis underscore the need for transparent reporting of analytical decisions and potential limitations in high-dimensional data analysis.

5.2 cDC1 regulation and function in obesity

Our screening of the enteric immune landscape identified cDC1s as a key population showing consistent and significant changes in obesity, prompting us to investigate this cell type in greater detail. The distinct pattern of increased cDC1 frequency, coupled with their known importance in maintaining intestinal homeostasis and orchestrating tolerance, raised important questions about both the mechanisms driving their accumulation and the functional consequences of their enhanced presence. We, therefore, undertook a comprehensive analysis of cDC1 biology in the obese state, examining their development, distribution, and function to understand how obesity reshapes this crucial immune population and what the consequences are for the host.

5.2.1 Selective accumulation of cDC1s in the small intestine despite unaltered ontogeny

Our study revealed an intriguing pattern of cDC1 distribution in obesity, with selective accumulation in the small intestine despite no changes in bone marrow progenitor frequencies or gut-homing markers. This finding raises important questions about the regulation of cDC homeostasis in metabolic disease. The bone marrow undergoes substantial remodelling in obesity, with increased cellularity, expanded myelopoiesis, and altered stem cell function (Benova and Tencerova, 2020). However, our analysis revealed that frequencies and total numbers of CDPs and pre-cDCs remain unchanged in obesity. This is notable given previous findings of myeloid-biased haematopoiesis in obesity (Adler et al., 2014; Nagareddy et al., 2013). The stability of cDC progenitor populations suggests that obesity's effects on cDC homeostasis may be tissue-specific rather than developmental. Our result contrasts with findings by Deczkowska and colleagues, who observed increased cDC1s in the liver during NASH in mice on a

choline-deficient high-fat diet, driven by enhanced cycling of bone marrow progenitors (Deczkowska et al., 2021). The divergent findings may reflect differences in disease models — while their NASH induces liver inflammation that signals back to bone marrow, our HFHSD model may primarily affect peripheral tissue environments. While increased kinetics of both progenitor proliferation and bone marrow egress could theoretically explain elevated tissue cDC1s without changes in bone marrow frequencies, our observation of unchanged pre-cDC1 levels in blood argues against this mechanism. This is particularly notable given that the bone marrow can rapidly adjust dendritic cell production through emergency cDCpoiesis in response to inflammation, regulated by balancing retention and efflux signals (Pereira Da Costa et al., 2023).

Since developmental pathways appear unaltered, we explored other mechanisms that might explain the accumulation of cDC1s in the small intestine during obesity. The homing process of pre-cDCs remains poorly understood. It has been demonstrated that gut-homing markers $\alpha 4\beta 7$ and CCR9 guide cDCs to the small intestine (Zeng et al., 2013), whose levels are unchanged between our experimental conditions. However, studies have suggested that homing to healthy and inflamed tissues might involve context-specific pathways (Cabeza-Cabrerizo et al., 2021). The use of mixed bone marrow chimaeras could reveal if increased enteric cDC1s in obesity might solely arise from bone marrow precursors, potentially guided through alternative homing mechanisms.

In agreement with unchanged expression of homing molecules, we did find recruitment cues to the gut to be stable between lean and obese mice. cDC1s are known to be attracted to the small intestine by local vitamin A concentration, chemokines, and Flt3L. Vitamin A intake was equivalent between lean and obese mice (data not shown; calculated by vitamin A concentration in diet x food consumption). The chemokine axis presents a more complex picture — despite

observing fewer intestinal $CD8^+$ T cells in obesity (the main source of the cDC1-attracting chemokine XCL1 (Wang et al., 2023)), Wang and colleagues demonstrated that these cells up-regulate *Xcl1* expression (Wang et al., 2023). Our finding of stable total jejunal *Xcl1* mRNA levels could suggest a compensatory mechanism where reduced T cell numbers are offset by increased per-cell *Xcl1* production. In accordance with this, Ohta and colleagues suggested that high *Xcl1* expression in intestinal T cells is supported by the presence of $XCR1^+$ cDC1 (Ohta et al., 2016). The critical role of the XCR1–XCL1 signalling in intestinal cDC1 localisation is highlighted by the same study that showed both XCL1- and XCR1-deficient mice as having significantly larger cDC1 populations in the small intestine and significantly smaller cDC1 populations in mLNs. In both models, cDC1s showed insufficient *Ccr7* expression, explaining the impaired migration. In this context, our observation of elevated cDC1 frequencies in both intestine and mLNs in the obese state argues against defective migration out of the intestines, which was expected as *Xcl1* mRNA levels were demonstrated to be equal in obese and lean states (Figure 10 f & S1h). While Sikder and colleagues recently revealed how diet-dependent maternal microbiome shapes offspring DC development through Flt3L regulation (Sikder et al., 2023), we found no evidence for altered local or systemic Flt3L levels. High levels of local Flt3L could attract pre-cDC1 as well as promote division of otherwise post-mitotic cDC1 (Waskow et al., 2008). Our result goes in line with our finding of no enhanced local cDC1 proliferation in the obese intestine. In addition, studies also showed that pre-cDCs retain proliferative capacity even after seeding to the small intestine (Cabeza-Cabrerizo et al., 2019), but we found pre-cDC1 levels and pre-cDC proliferation to be stable between lean and obese mice. The possibility that differential survival rates contribute to cDC1 accumulation remains unexplored in our study, and future studies examining cell survival will be crucial for understanding how obesity drives compartment-specific changes in dendritic cell

populations — enhanced survival in the obese state could lead to cDC1 accumulation even with normal production and trafficking.

While cDCs can move from tissues through lymphatics to draining lymph nodes (Randolph et al., 2005), reverse trafficking from lymph nodes to tissues or direct migration between peripheral organs is not documented to our knowledge. According to the canonical view, cDCs are constantly replenished from precursors arriving through the blood (Ginhoux et al., 2009; Liu et al., 2009) and migrate unidirectionally from tissues to lymph nodes, utilising chemokine signals like the CCR7-CCL19/21 axis (Förster et al., 1999; Gunn et al., 1999). In our study, the reciprocal changes in cDC1 frequencies between liver and small intestine during obesity raise intriguing questions about potential inter-tissue trafficking. Resolving these questions would require experimental investigation, for example, selective blockade of potential exit pathways or the usage of photo-convertible cells for tissue-specific labelling (Victora et al., 2010). Tracking of their potential movement to other tissues could enable precise spatiotemporal tracking of cDC movement between organs, providing crucial insights into their migratory capabilities.

5.2.2 Preserved cDC1 function in obesity

Our comprehensive analysis of small intestinal cDC1s in diet-induced obesity revealed an intriguing dissociation between cellular abundance and functional state. Despite their increased frequency in the small intestine of HFHSD-fed mice, cDC1s maintained largely normal functional characteristics, with only subtle alterations in their regulatory profile. This finding is particularly noteworthy given the established role of cDC1s in metabolic regulation and intestinal homeostasis beyond their classical immunological functions (see chapter 1.2.3.1 cDC1 in obesity).

The preserved activation state of small intestinal cDC1s in obesity, evidenced by unchanged expression of canonical co-stimulatory and MHC molecules, contrasts with current paradigms of cDC behaviour in metabolic disease. Recent studies have demonstrated that lipid and cholesterol exposure typically triggers homeostatic cDC maturation, resulting in a distinct tolerogenic phenotype (Bosteels et al., 2023; Bosteels and Janssens, 2024; Gainullina et al., 2023; Plebanek et al., 2024). This discrepancy suggests that intestinal cDC1s may be resistant to obesity-induced functional alterations, potentially reflecting tissue-specific regulatory mechanisms.

Our transcriptional analysis revealed only modest functional changes, with *Il10* mRNA down-regulation being the most notable alteration in obese conditions. Gene expression analysis provides valuable insights but requires validation at the protein level due to the complex relationship between transcripts and their protein products. Several biological factors can cause discrepancies between RNA and protein abundance. First, not all transcripts are efficiently translated into proteins. Second, RNA and protein molecules can have vastly different degradation rates and temporal dynamics. Third, protein function and abundance can be regulated through various post-transcriptional mechanisms independent of mRNA levels. Additionally, transcriptomic analysis cannot account for already existing protein pools, changes in translation efficiency, or factors affecting protein stability. However, we could not validate the complete cytokine profile on protein level due to experimental limitations. Flow cytometry analysis of cytokines is deducted after stimulating cells and incubation with transport inhibitors Monensin or Brefeldin A. Monensin inhibits trans-Golgi function, while Brefeldin A is an inhibitor of protein transport between the endoplasmic reticulum and the Golgi apparatus. Each transport inhibitor is specific for a range of cytokines, e.g. IL-10 can exclusively be trapped inside the cell via Monensin while Brefeldin A inhibits transportation of IL-12

(Nylander and Kalies, 1999). Transport of other cytokines like IL-6 and TNF-α can be inhibited with either reagent. As *ex vivo* stimulation of cDCs and incubation with transport inhibitors do not result in sufficient cell survival for flow cytometry analysis, mice are usually injected *in vivo* with Brefeldin A and subsequently analysed on the following day. However, to our knowledge, *in vivo* treatment with Monensin is not feasible. This said, the actual functional distinctions between cDC1s in different experimental conditions may only become apparent through protein-level studies, and a comprehensive understanding of cDC1 biology in the obese state requires complementary analysis of both transcripts and their protein products (Worah et al., 2016).

Nevertheless, our findings suggest that core cDC1 functions are maintained, including the expression of regulatory T cell-inducing machinery (αvβ8 integrin and ALDH1A1). The preservation of these pathways is particularly significant given their critical role in maintaining intestinal tolerance, involved in the metabolism of RA and TGF-β activation, respectively (Iwata et al., 2004; Shiokawa et al., 2017). The stability of the cDC1 transcriptional program in obesity confirmed through comparison with analysis of a previously published dataset (Wang et al., 2023), indicates remarkable functional resilience. This observation raises important questions about tissue-specific mechanisms protecting intestinal cDC1s from obesity-induced dysfunction. While systemic metabolism and inflammation are profoundly altered in obesity, local tissue factors in the small intestine may buffer cDC1s from these perturbations.

The selective increase in cDC1 frequency without major functional alterations suggests a quantitative rather than qualitative shift in intestinal immune regulation during obesity. This pattern may represent an adaptive response aimed at maintaining tissue homeostasis under metabolic stress, though the reduction in *Il10* mRNA expression hints at partial erosion of regulatory capacity.

Understanding if this expression profile is indeed present at protein level warrants further investigation, e.g. by proteome comparison. Our findings may have important implications for therapeutic strategies targeting intestinal immunity in metabolic disease. The preservation of cDC1 function suggests these cells might remain relevant therapeutic targets in obesity.

We reasoned that the increased frequency of cDC1s in the small intestine during obesity could influence T cell responses independent of their activation state through numerical effects on antigen presentation and cellular interactions. First of all, the frequency of cDC1 might affect the frequency of other cell types, as discussed before (see chapters 5.1.2.2 Cell type-specific responses to dietary intervention & 5.2.1 Selective accumulation of cDC1s in the small intestine despite unaltered ontogeny). Additionally, cDC1s are specialised in cross-presentation to $CD8^+$ T cells and also support $CD4^+$ T cell responses through direct MHC II presentation (Merad et al., 2013). The probability and frequency of productive T cell/DC interactions in lymphoid tissues can be described as law of mass action, where higher numbers of antigen-presenting cells increase the likelihood of T cells encountering their cognate antigen (Bousso and Robey, 2003, 2004; Miller et al., 2004; Thakur et al., 2023). Indeed, studies manipulating DC numbers through selective depletion or expansion have demonstrated that DC frequency directly impacts T cell priming efficiency and the magnitude of immune responses (Jung et al., 2002; Meredith et al., 2012). In the context of our findings, elevated cDC1 numbers in both the small intestine and mLNs of obese mice could enhance T cell activation through increased opportunity for antigen uptake and cellular contact, even without changes in per-cell stimulatory capacity. This numerical effect might be particularly relevant for rare antigen-specific T cells or the acquisition of tolerance to novel antigens, where the probability of encountering their cognate antigen presented by a cDC is normally very low. Additionally, increased cDC1 frequency could affect the competitive

balance with other antigen-presenting cells, potentially shifting the quality of T cell responses through preferential engagement with cross-presenting cDC1s rather than other DC subsets or macrophages (Eisenbarth, 2019). Thus, the observed quantitative changes in cDC1 populations could have functional consequences for intestinal T cell responses in obesity, even in the absence of altered activation markers or cytokine production.

5.2.3 cDC1/T cell interaction in obesity

Our investigation into cDC1/T cell interactions in obesity was initially guided by a systematic evaluation of T cell-dependent physiological mechanisms and pathologies that show altered prevalence in obesity. This comprehensive approach revealed several potential areas where cDC1/T cell interactions might be modified in obesity.

Notably, several obesity-associated intestinal pathologies are shown to be $CD4^+$ T cell and CTL-dependent, including Inflammatory Bowel Disease (IBD) and Celiac disease (Garduño and Däbritz, 2021; Müller et al., 1998). Obesity promotes accumulation of cDC1 cells in the intestine, where they can stimulate T cells, and increased activation of $CD4^+$ T cells drives these pathologies (Gomez-Bris et al., 2023; Schreiber et al., 1991). Additionally, the unique expression of XCR1 on cDC1s among haematopoietic cells facilitates interactions with previously activated, XCL1-expressing $CD8^+$ T cells, enhancing CTL differentiation independently of their cross-presentation capacity (Dudziak et al., 2007) which may influence those diseases. Obesity is also an established risk factor for multiple gastrointestinal cancers, including oesophagal adenocarcinoma, gastric cardia and colorectal cancer (Loomans-Kropp and Umar, 2023). Alterations in cDC1/T cell interactions might influence cancer susceptibility through modified immune surveillance in these organs.

Of particular interest was the observation that obesity does not increase the risk of cancer in the small intestine and, independently of the metabolic state of the host, cDC1 levels are positively associated with cancer patient survival (Böttcher and Reis e Sousa, 2018). cDC1 mediated cancer immunity is possibly acting through uptake of dying cancer cells through DNRG-1, a receptor for dead cells. DNGR-1 can signal via Syk in response to engagement with dead cell ligands and promote cross-presentation by converting endosomes into the MHC-I presentation pathway, a mechanism not fully understood to date (Brown, 2012). This way, cancer cells are thought to be presented to the T cell compartment in a cDC1-dependent manner. Similar to the small intestine's apparent resistance to obesity-induced cancer, this organ also exhibits remarkably mild inflammatory responses during obesity compared to organs like the liver, suggesting the presence of regulatory mechanisms that limit excessive inflammation (Luck et al., 2015).

We concluded that both $CD8^+$ T cell and $CD4^+$ T cell interaction with cDC1s seemed to be altered in obesity. However, several key observations led us to focus specifically on cDC1/$CD4^+$ T cell interactions. First, we found $CD8^+$ T cells to be diminished in obesity, making $CD4^+$ T cells a more practical target for experimental investigation. Second, Wang and colleagues (Wang et al., 2023) showed that HFHSD feeding was also associated with significant $CD4^+$ T cell infiltration in the lamina propria and, more importantly, that cDC1s were the major receiver and the sender of immune-regulatory signals to those cells, suggesting that cDC1s may play a regulatory role in modulation of the $CD4^+$ T cells (however, we had concerns regarding this analysis). Third, our research interest focuses on understanding evolutionary adaptations of the enteric immune system to current environmental conditions, given this emphasis on physiological adaptation (e.g. relatively controlled inflammatory state compared to other organs) rather than pathological responses (e.g. cancer).

To validate our approach, we analysed the scRNA-seq dataset from Wang et al. using CellChat, which predicted highly altered cell-cell interactions between cDC1 and $CD4^+$ T cells in obesity. While this theoretical analysis suggested the viability of our research direction, we deliberately avoided drawing strong conclusions from computational predictions alone — despite the availability of additional analysis tools. Instead, we prioritised testing cDC1/$CD4^+$ T cell interactions through direct experimental investigation, believing this would provide more definitive evidence.

The decision to study cDC1/$CD4^+$ T cell interactions specifically in the context of food tolerance rather than in infection models was driven by several factors. There is limited knowledge about the correlation between increased food tolerance and obesity worldwide, despite parallel increases in both obesity and food allergies and intolerances (see chapter 1.2.3.3 Food tolerance in obesity). Current epidemiological data show inconsistent relationships between obesity and food allergy risk, especially in children, with some studies suggesting reduced risk while others indicate the opposite. The mechanisms underlying these relationships remain unclear, and research in this area is very limited. Additionally, this focus on oral tolerance rather than infection models aligns, as already mentioned, with our broader research interest in understanding evolutionary adaptations of the physiological state of the enteric immune system under current environmental conditions, emphasising physiological rather than pathological processes.

The functional relevance of altered cDC1/$CD4^+$ T cell interactions became apparent in our OVA/OT-II model experiments. Despite the general low-grade inflammation associated with obesity, obese mice demonstrated a significantly enhanced tolerogenic response to oral antigens, characterised by increased Treg frequency. This observation suggests that altered $CD4^+$ T cell polarisation

through cDC1 antigen presentation and potential modifications in regulatory T cell homeostasis may be key mechanisms in obesity-associated immune adaptation. Our results build upon previous work demonstrating the essential role of cDC1s in intestinal immune homeostasis. As recapitulated by Sasaki and colleagues, cDC1s are critical for maintaining intestinal T cell populations and preventing excessive inflammation (Sasaki et al., 2022). Our findings extend this understanding by revealing how these cells adapt their function in the context of obesity, potentially representing an evolutionary adaptation to current environmental conditions. However, in order to directly associate cDC1s to increased food tolerance in obesity, conditional cDC1 know-out studies are necessary. For example, food tolerance experiments using the OVA/OT-II model could be conducted in DTR-XCR1 KO mice that allow ablation of cDC1s in adult mice, before OVA stimulation.

The potential implications of our findings extend beyond food tolerance. The relatively controlled inflammatory state of the small intestine compared to other organs of the gastrointestinal tract in obesity might be partially explained by our observation of enhanced tolerogenic responses. The observed alterations in cDC1/T cell interactions might also influence cancer susceptibility through modified immune surveillance.

Several critical questions emerge from our findings that warrant further investigation. First, oral tolerance is encompassed through multiple distinct mechanisms, including the death or functional inactivation of food-antigen-specific T cells and the generation of IL-10-producing Treg cells. While our approach focused on initial T cell responses and early tolerance mechanisms — examining direct antigen presentation, T cell anergy induction, and initial Treg activation — complementary long-term studies are needed to elucidate stable tolerance establishment, memory Treg development, and bystander suppression.

Moreover, the use of monoclonal TCR transgenic mice, while experimentally advantageous, may generate atypical immune responses compared to individuals with normal T cell repertoires, necessitating validation in diverse model systems (Hataye et al., 2006). Of particular interest is the seemingly paradoxical relationship between enhanced oral tolerance and the worldwide increase in food allergies concurrent with rising obesity rates (Renz et al., 2018). This contradiction might be explained by the distinct immunological impacts of adult-onset versus early-life obesity. Our experiments examined the first phenomenon, but statistics show that obesity tends to occur already during the infantile period, as approximately 20% of children and adolescents were affected by obesity in 2022, while 43% of the global adult population was classified as obese or overweight (WHO, 2024). Environmental and host conditions in prenatal and postnatal periods, including dietary habits and microbial factors, significantly influence the development of oral tolerance (Zhang et al., 2023). Recent evidence suggests that maternal dysbiotic gut microbiota can be directly transmitted to offspring, shaping its immune system (Sikder et al., 2023), with the early-life microbiome being particularly susceptible to modulation by factors such as feeding modality (Chichlowski et al., 2023; Pärnänen et al., 2022) and maternal health status (Jones et al., 2024; Ren et al., 2023). Future experiments are needed to understand the correlation between increased obesity and food intolerances, either employing experimental setups using infantile obesity models or obese mothers. Additionally, the enhanced tolerance we observed may have broader implications, particularly regarding immune responses to pathogens and the risk of obesity-associated cancers, representing crucial areas for future investigation.

5.3 Physiological implications and evolutionary perspective

Throughout history, the perspective on immune system function has evolved dramatically from a simple defence mechanism to a sophisticated environmental sensor. Our findings of enhanced oral tolerance in obesity, despite the general inflammatory state, align with this modern understanding of immune function as a complex regulatory system that extends far beyond protective immunity.

From an evolutionary perspective, human dietary patterns were intrinsically linked to seasonal variations in food availability. Our ancestors experienced substantial fluctuations in their diet throughout the year, shifting from protein-rich hunting yields in certain seasons to gathered fruits and vegetables in others. These seasonal patterns varied across geographical locations and were further influenced by migration patterns of prey animals and the natural growth cycles of plants. This cyclical nature of food availability required remarkable metabolic and immunological flexibility to maintain health and economise energy expenditure despite varying nutrient availability, food compositions and antigens.

Just as recent immune theories emphasise, the immune system can maintain homeostasis under varying conditions, and we can observe that the intestinal immune system exhibits remarkable plasticity in response to dietary fluctuations. This adaptation transcends simple nutrient processing, encompassing a sophisticated network of cellular and molecular mechanisms that maintain gut homeostasis. At its core, the system discriminates between harmful and beneficial antigens, establishing tolerance to food components while mounting appropriate responses against pathogens. Beyond this fundamental recognition process, dietary adaptation shapes barrier integrity and nutrient absorption pathways while simultaneously modulating the intricate relationship with the gut microbiota. The mucosal barrier fosters a symbiotic relationship between host

and microorganisms, which are profoundly influenced by dietary patterns and affect both local and systemic metabolic processes. The resulting metabolites serve as crucial mediators, influencing energy homeostasis and metabolic regulation throughout the body. Early life represents a critical window for this adaptive programming, with dietary exposures during breastfeeding and weaning establishing long-lasting immunological patterns. The development of oral tolerance exemplifies this adaptation, creating a framework for appropriate immune responses throughout life. This plasticity persists into adulthood, allowing continuous adjustment to both acute and chronic dietary modifications.

Seasonal fluctuations in food availability have largely disappeared in modern societies and are replaced by year-round access to diverse foods through global supply chains and advanced storage methods. Despite this unprecedented dietary consistency, our intestinal immune system continues to adapt to our dietary choices. Notably, the predominant dietary pattern has shifted towards a Western-style diet, characterised by high fat and sugar content. As it is evident that diet-dependent adaptations are relevant for a diversity of physiological functions, the present study presents insights into the relationship between the immune landscape during HFHSD-feeding and tolerance to food antigens. Our findings may help explain the apparent paradox of enhanced oral tolerance in obesity despite low-grade inflammation. The increased frequency of cDC1s, coupled with their modified interaction patterns with $CD4^+$ T cells, appears to create an environment favouring Treg generation in response to dietary antigens. This mechanism could represent an evolutionary adaptation of the enteric immune system to current environmental conditions, potentially serving as a compensatory response to the chronic low-grade inflammation characteristic of obesity and aimed at maintaining tissue homeostasis. This aligns with previous studies showing that cDC1s play crucial roles in preventing intestinal

inflammation through various mechanisms, including the maintenance of regulatory T cell populations (Muzaki et al., 2016).

6

CONCLUSION

The present thesis provides novel insights into the adaptation of the enteric immune system to an obesogenic diet, with particular emphasis on regional specialisation and its functional consequences. Our findings demonstrate that the enteric immune landscape shows distinct adaptations to HFHSD that are primarily determined by intestinal location and dietary intervention rather than by duration of exposure. This reveals a previously unappreciated stability in diet-induced immune adaptation, suggesting that the immune system reaches a new homeostatic state relatively quickly after dietary change.

We identified cDC1 as a key cell population that shows consistent and significant increases across intestinal segments in response to obesogenic diet. This increase occurs despite unaltered development and gut-homing capacity, suggesting complex tissue-specific regulatory mechanisms. Importantly, the elevated cDC1 frequency is accompanied by largely preserved functional characteristics, with only subtle alterations in their regulatory profile.

Our findings showed enhanced oral tolerance in obese mice, as indicated by increased Treg frequency during oral antigen challenge, though further studies are needed to fully characterise this response. This observation reveals an

unexpected adaptation of the immune system that may represent a protective mechanism against excessive inflammation in the context of chronic metabolic stress. The relationship between increased cDC1 frequency and enhanced tolerance suggests a coordinated adaptation of the immune system to metabolic stress.

These results significantly advance our understanding of how dietary choices shape immune function and suggest that some adaptations to an obesogenic diet may serve protective functions. For basic research, our work emphasises the importance of considering regional differences when studying intestinal immunity and suggests that the immune system may adapt to diet in a more nuanced manner than previously appreciated. Our findings also provide new insights into the relationship between obesity and immune function, particularly regarding oral tolerance, which may be relevant for understanding and treating food sensitivities and metabolic diseases.

Looking forward, this work opens new avenues for research into the mechanisms governing tissue-specific immune adaptation and the role of cDC1s in metabolic disease. Understanding the molecular mechanisms underlying the selective accumulation of cDC1s in the intestine during obesity and the long-term consequences of enhanced oral tolerance in the context of metabolic disease will be crucial next steps. Additionally, investigating whether similar adaptations occur in human obesity will be essential for translating these findings into clinical applications.

This work ultimately contributes to our evolving understanding of the immune system as not merely a defence mechanism but as a complex sensory and regulatory system that adapts to environmental changes to maintain organismal homeostasis. The observed adaptations suggest that the immune response to dietary challenges is sophisticated, potentially serving to protect the

organism from the inflammatory consequences of metabolic stress while maintaining essential functions like oral tolerance.

7

APPENDICES

7.1 Two-way repeated measures ANOVA between subjects comparing treatment and duration of treatment

cDC

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000136	2	0.000068	0.268645	**0.764813**	0.003878
1	Treatment	0.005559	1	0.005559	21.954471	**0.000007**	0.137255
2	Duration x Treatment	0.000137	2	0.000069	0.270780	**0.763189**	0.003909
3	Residual	0.034941	138	0.000253			

cDC1

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000112	2	0.000056	5.526170	**0.004912**	0.074151
1	Treatment	0.000415	1	0.000415	40.829044	**2.37E-09**	0.228313
2	Duration x Treatment	0.000004	2	0.000002	0.198404	**0.820272**	0.002867
3	Residual	0.001402	138	0.000010			

(to be continued)

cDC2

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000075	2	0.000038	15.429735	**0.000001**	0.182752
1	Treatment	0.000028	1	0.000028	11.538410	**0.000891**	0.077160
2	Duration x Treatment	0.000004	2	0.000002	0.908627	**0.405475**	0.012997
3	Residual	0.000337	138	0.000002			

dpDC

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000047	2	0.000024	0.139177	**0.870196**	0.002013
1	Treatment	0.002067	1	0.002067	12.172085	**0.000651**	0.081054
2	Duration x Treatment	0.000083	2	0.000041	0.243386	**0.784305**	0.003515
3	Residual	0.023429	138	0.000170			

Monocyte

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000112	2	0.000056	5.526170	**0.004912**	0.074151
1	Treatment	0.000415	1	0.000415	40.829044	**2.37E-09**	0.228313
2	Duration x Treatment	0.000004	2	0.000002	0.198404	**0.820272**	0.002867
3	Residual	0.001402	138	0.000010			

Macrophage and transitional Monocyte

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.190219	2	0.095109	16.231243	**0.000001**	0.221644
1	Treatment	0.001023	1	0.001023	0.174588	**0.676852**	0.001529
2	Duration x Treatment	0.002910	2	0.001455	0.248289	**0.780555**	0.004337
3	Residual	0.667999	114	0.005860			

(to be continued)

B cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.004923	2	0.002462	0.266533	**0.766507**	0.004654
1	Treatment	0.033891	1	0.033891	3.669507	**0.057921**	0.031185
2	Duration x Treatment	0.006589	2	0.003294	0.356689	**0.700768**	0.006219
3	Residual	1.052880	114	0.009236			

Neutrophil

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000052	2	0.000026	1.177942	**0.311631**	0.020247
1	Treatment	0.000044	1	0.000044	1.994605	**0.160584**	0.017196
2	Duration x Treatment	0.000060	2	0.000030	1.360512	**0.260662**	0.023312
3	Residual	0.002515	114	0.000022			

Treg

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.041188	2	0.020594	189.730313	**3.06E-44**	0.688101
1	Treatment	0.000221	1	0.000221	2.031584	**0.155872**	0.011674
2	Duration x Treatment	0.000116	2	0.000058	0.533319	**0.587621**	0.006163
3	Residual	0.018669	172	0.000109			

$CD8^+$ $TCR\gamma\delta^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.011233	2	0.005617	3.004672	**0.053496**	0.050074
1	Treatment	0.044555	1	0.044555	23.835023	**0.000003**	0.172924
2	Duration x Treatment	0.010743	2	0.005372	2.873580	**0.060598**	0.047994
3	Residual	0.213101	114	0.001869			

(to be continued)

$CD8^+$ $TCR\beta^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.012102	2	0.006051	10.340520	**0.000075**	0.153556
1	Treatment	0.008140	1	0.008140	13.910469	**0.000300**	0.108752
2	Duration x Treatment	0.001231	2	0.000616	1.051981	**0.352610**	0.018121
3	Residual	0.066711	114	0.000585			

$CD4^+$ $TCR\gamma\delta^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000002	2	0.000001	3.077772	**0.049909**	0.051230
1	Treatment	3.64E-07	1	3.64E-07	1.008599	**0.317366**	0.008770
2	Duration x Treatment	0.000001	2	0.000001	1.648255	**0.196937**	0.028104
3	Residual	0.000041	114	3.61E-07			

$CD4^+$ $TCR\beta^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.030861	2	0.015431	16.788795	**4.07E-07**	0.227525
1	Treatment	0.001783	1	0.001783	1.939680	**0.166414**	0.016730
2	Duration x Treatment	0.003124	2	0.001562	1.699754	**0.187326**	0.028957
3	Residual	0.104778	114	0.000919			

NK

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000001	2	2.69E-07	4.012589	**0.019816**	0.044827
1	Treatment	2.44E-07	1	2.44E-07	3.640050	**0.058081**	0.020843
2	Duration x Treatment	0.000001	2	4.41E-07	6.582390	**0.001762**	0.071484
3	Residual	0.000011	171	6.70E-08			

(to be continued)

ILC1

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000030	2	0.000015	0.837717	**0.434465**	0.009703
1	Treatment	0.000002	1	0.000002	0.100220	**0.751952**	0.000586
2	Duration x Treatment	0.000040	2	0.000020	1.119545	**0.328809**	0.012925
3	Residual	0.003019	171	0.000018			

ILC2

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.005248	2	0.002624	4.366648	**0.014884**	0.071157
1	Treatment	0.001446	1	0.001446	2.405616	**0.123672**	0.020666
2	Duration x Treatment	0.011633	2	0.005817	9.679064	**0.000131**	0.145159
3	Residual	0.068508	114	0.000601			

ILC3

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.008671	2	0.004336	8.456118	**0.000376**	0.129188
1	Treatment	0.000132	1	0.000132	0.257843	**0.612587**	0.002257
2	Duration x Treatment	0.004121	2	0.002061	4.018689	**0.020582**	0.065860
3	Residual	0.058451	114	0.000513			

IEL CD8$\alpha\beta^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.026379	2	0.013190	14.094110	**0.000003**	0.198246
1	Treatment	0.000937	1	0.000937	1.001039	**0.319178**	0.008705
2	Duration x Treatment	0.006020	2	0.003010	3.216596	**0.043757**	0.053417
3	Residual	0.106685	114	0.000936			

(to be continued)

IEL CD8$\alpha\alpha^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.047665	2	0.023833	2.416139	**0.093824**	0.040665
1	Treatment	0.052699	1	0.052699	5.342578	**0.022608**	0.044767
2	Duration x Treatment	0.023259	2	0.011630	1.179010	**0.311305**	0.020265
3	Residual	1.124488	114	0.009864			

dpIEL

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.000340	2	0.000170	5.385684	**0.005821**	0.086329
1	Treatment	0.000146	1	0.000146	4.643010	**0.033284**	0.039134
2	Duration x Treatment	0.000196	2	0.000098	3.100479	**0.048845**	0.051588
3	Residual	0.003596	114	0.000032			

IEL CD8$\alpha\alpha^+$ TCR$\gamma\delta^+$ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.012331	2	0.006165	1.005421	**0.369111**	0.017333
1	Treatment	0.033995	1	0.033995	5.543912	**0.020256**	0.046376
2	Duration x Treatment	0.005547	2	0.002774	0.452325	**0.637284**	0.007873
3	Residual	0.699049	114	0.006132			

IEL CD8$\alpha\alpha^+$ TCRβ^+ T cell

Row	Source	SS	DF	MS	F	**p-unc**	np2
0	Duration	0.014049	2	0.007025	3.592330	**0.030695**	0.059287
1	Treatment	0.012230	1	0.012230	6.254644	**0.013808**	0.052012
2	Duration x Treatment	0.019964	2	0.009982	5.104686	**0.007530**	0.082195
3	Residual	0.222918	114	0.001955			

Table S1. Effect of diet and duration of diet on ratios of cell types in CD45$^+$ cells. Two-way repeated measures ANOVA between individuals. Row 0 shows the effect of the duration of the experiment while not considering the treatment, row 1 shows the effect of the treatment while not considering the duration of the dietary intervention, row 2 shows the interaction effect of treatment and duration of treatment, and finally, row 3 shows the residuals that are explained by neither duration nor treatment.

cDC1

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.006	0.004	0.007	0.003	-1.017	94.053	0.312	**0.935**	0.317	-0.193
1	Duration	-	16	8	0.006	0.004	0.004	0.002	2.141	81.988	0.035	**0.106**	1.671	0.415
2	Duration	-	4	8	0.007	0.003	0.004	0.002	3.883	71.310	2.28E-04	**0.001**	120.57	0.797
3	Treatment	-	CD	HFHSD	0.004	0.003	0.008	0.003	-6.228	142	5.03E-09		1946000	-1.033
4	Duration x Treatment	16	CD	HFHSD	0.004	0.005	0.008	0.004	-3.372	52	0.001	**0.004**	23.283	-0.904
5	Duration x Treatment	4	CD	HFHSD	0.005	0.002	0.008	0.003	-4.858	58	9.38E-06	**2.81E-05**	1784.42	-1.238
6	Duration x Treatment	8	CD	HFHSD	0.003	0.001	0.006	0.003	-3.756	28	0.001	**0.002**	36.827	-1.334

cDC2

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.003	0.001	0.003	0.002	-2.880	111.938	0.005	**0.014**	7.670	-0.533
1	Duration	-	16	8	0.003	0.001	0.005	0.002	-4.738	44.381	2.25E-05	**6.74E-05**	1876.03	-1.188
2	Duration	-	4	8	0.003	0.002	0.005	0.002	-2.765	47.114	0.008	**0.024**	5.997	-0.666
3	Treatment	-	CD	HFHSD	0.003	0.002	0.004	0.002	-3.098	142	0.002		13.298	-0.514
4	Duration x Treatment	16	CD	HFHSD	0.002	0.001	0.003	0.001	-2.256	52	0.028	**0.085**	2.150	-0.605
5	Duration x Treatment	4	CD	HFHSD	0.003	0.001	0.004	0.002	-1.513	58	0.136	**0.407**	0.682	-0.386
6	Duration x Treatment	8	CD	HFHSD	0.004	0.002	0.005	0.002	-2.218	28	0.035	**0.105**	2.048	-0.788

(to be continued)

CD8α^+ TCRβ^+ T cell

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.051	0.029	0.028	0.010	5.631	80.762	2.52E-07	**7.55E-07**	56560	0.951
1	Duration	-	16	8	0.051	0.029	0.038	0.028	2.207	61.676	0.031	**0.093**	1.875	0.478
2	Duration	-	4	8	0.028	0.010	0.038	0.028	-1.840	58	7.09E-02	**0.213**	1.068	-0.469
3	Treatment	-	CD	HFHSD	0.050	0.031	0.034	0.020	3.464	118	7.42E-04		37	0.628
4	Duration x Treatment	16	CD	HFHSD	0.060	0.035	0.043	0.020	2.293	58	0.025	**0.076**	2.278	0.584
5	Duration x Treatment	4	CD	HFHSD	0.031	0.009	0.024	0.010	2.026	28	5.23E-02	**1.57E-01**	1.545	0.720
6	Duration x Treatment	8	CD	HFHSD	0.050	0.028	0.025	0.021	2.773	28	0.010	**0.029**	5.182	0.985

NK

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.000	0.000	0.000	0.000	-2.406	119.719	0.018	**0.053**	2.510	-0.420
1	Duration	-	16	8	0.000	0.000	0.000	0.000	-2.010	59.457	0.049	**0.147**	1.225	-0.436
2	Duration	-	4	8	0.000	0.000	0.000	0.000	-0.357	68.379	7.22E-01	**1**	0.224	-0.076
3	Treatment	-	CD	HFHSD	0.000	0.000	0.000	0.000	-1.779	157	7.71E-02		1	-0.265
4	Duration x Treatment	16	CD	HFHSD	0.000	0.000	0.000	0.000	1.784	73	0.079	**0.236**	0.931	0.407
5	Duration x Treatment	4	CD	HFHSD	0.000	0.000	0.000	0.000	-3.262	58	1.86E-03	**5.57E-03**	18.475	-0.831
6	Duration x Treatment	8	CD	HFHSD	0.000	0.000	0.001	0.000	-1.830	40	0.075	**0.224**	1.129	-0.554

(to be continued)

ILC2

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.043	0.026	0.050	0.025	-1.096	59.196	0.277	**0.832**	0.391	-0.241
1	Duration	-	16	8	0.043	0.026	0.031	0.028	1.955	54.349	0.056	**0.167**	1.204	0.445
2	Duration	-	4	8	0.050	0.025	0.031	0.028	2.640	58	1.06E-02	**0.032**	4.486	0.673
3	Treatment	-	CD	HFHSD	0.038	0.030	0.045	0.023	-1.413	118	1.60E-01		0	-0.256
4	Duration x Treatment	16	CD	HFHSD	0.037	0.031	0.049	0.018	-1.797	58	0.077	**0.232**	1.003	-0.458
5	Duration x Treatment	4	CD	HFHSD	0.062	0.025	0.037	0.020	3.036	28	5.14E-03	**1.54E-02**	8.450	1.079
6	Duration x Treatment	8	CD	HFHSD	0.017	0.010	0.046	0.033	-3.270	28	0.003	**0.009**	13.361	-1.162

ILC3

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.024	0.015	0.041	0.038	-2.490	33.740	0.018	**0.054**	3.282	-0.708
1	Duration	-	16	8	0.024	0.015	0.019	0.017	1.190	51.823	0.239	**0.718**	0.429	0.276
2	Duration	-	4	8	0.041	0.038	0.019	0.017	2.936	58	4.76E-03	**0.014**	8.539	0.748
3	Treatment	-	CD	HFHSD	0.026	0.031	0.028	0.016	-0.468	118	6.41E-01		0	-0.085
4	Duration x Treatment	16	CD	HFHSD	0.021	0.015	0.026	0.014	-1.338	58	0.186	**0.558**	0.555	-0.341
5	Duration x Treatment	4	CD	HFHSD	0.050	0.051	0.033	0.014	1.250	28	2.22E-01	**6.65E-01**	0.619	0.444
6	Duration x Treatment	8	CD	HFHSD	0.012	0.010	0.027	0.020	-2.635	28	0.014	**0.041**	4.053	-0.936

(to be continued)

IEL CD8αβ+

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.062	0.025	0.029	0.021	6.681	67.736	5.39E-09	**1.62E-08**	4111000	1.399
1	Duration	-	16	8	0.062	0.025	0.065	0.047	-0.334	37.043	0.740	**1**	0.244	-0.090
2	Duration	-	4	8	0.029	0.021	0.065	0.047	-3.826	58	3.21E-04	**0.001**	81.166	-0.975
3	Treatment	-	CD	HFHSD	0.052	0.028	0.058	0.040	-0.892	118	3.74E-01		0	-0.162
4	Duration x Treatment	16	CD	HFHSD	0.060	0.029	0.065	0.019	-0.762	58	0.449	**1**	0.335	-0.194
5	Duration x Treatment	4	CD	HFHSD	0.036	0.027	0.022	0.008	1.884	28	7.00E-02	**2.10E-01**	1.269	0.669
6	Duration x Treatment	8	CD	HFHSD	0.052	0.018	0.079	0.063	-1.556	28	0.131	**0.393**	0.848	-0.553

dpIEL

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.005	0.007	0.001	0.001	4.339	60.956	5.48E-05	**1.64E-04**	516.089	0.683
1	Duration	-	16	8	0.005	0.007	0.003	0.005	1.054	77.378	0.295	**0.885**	0.376	0.209
2	Duration	-	4	8	0.001	0.001	0.003	0.005	-2.834	58	6.31E-03	**0.019**	6.801	-0.722
3	Treatment	-	CD	HFHSD	0.004	0.007	0.002	0.005	2.045	118	4.31E-02		1	0.371
4	Duration x Treatment	16	CD	HFHSD	0.007	0.008	0.002	0.005	2.608	58	0.012	**0.035**	4.203	0.665
5	Duration x Treatment	4	CD	HFHSD	0.001	0.001	0.000	0.000	2.685	28	1.21E-02	**3.62E-02**	4.423	0.954
6	Duration x Treatment	8	CD	HFHSD	0.003	0.002	0.004	0.007	-0.590	28	0.560	**1.000**	0.393	-0.210

(to be continued)

CD8αα^{+} TCRβ^{+} T cell

Row	Contrast	Duration	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Duration	-	16	4	0.180	0.043	0.165	0.047	1.458	53.333	0.151	**0.452**	0.582	0.334
1	Duration	-	16	8	0.180	0.043	0.154	0.054	2.279	47.771	0.027	**0.082**	2.149	0.546
2	Duration	-	4	8	0.165	0.047	0.154	0.054	0.827	58	4.12E-01	**1**	0.350	0.211
3	Treatment	-	CD	HFHSD	0.180	0.044	0.159	0.050	2.370	118	1.94E-02		2	0.430
4	Duration x Treatment	16	CD	HFHSD	0.180	0.043	0.180	0.043	0.004	58	0.997	**1**	0.262	0.001
5	Duration x Treatment	4	CD	HFHSD	0.174	0.049	0.156	0.045	1.024	28	3.14E-01	**9.43E-01**	0.512	0.364
6	Duration x Treatment	8	CD	HFHSD	0.186	0.041	0.122	0.047	3.937	28	0.000	**0.001**	54.719	1.399

Table S2. Pairwise comparison of effect of diet and duration of diet on ratio of cell types in CD45^{+} cells. Two-way repeated measures ANOVA. Rows 0-2 show pairwise comparison of duration, while not considering the treatment. Row 3 shows effect of treatment independent of duration. Rows 4-6 shows the effect of the treatment at each time point. Bonferoni correction

7.2 Mixed-repeated measures ANOVA between and within subjects comparing treatment and intestinal segment

cDC

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.005559	1	46	0.005559	57.997581	**0.000000**	0.557682	
1	Treatment	0.025735	2	92	0.012867	279.06	**8.64E-40**	0.858487	0.991902
2	Residual	0.000828	2	92	0.000414	8.981365	0.000273	0.163353	

cDC1

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000415	1	46	0.000415	28.585010	**0.000003**	0.383254	
1	Treatment	0.000352	2	92	0.000176	39.02	**5.36E-13**	0.458947	0.942985
2	Residual	0.000085	2	92	0.000042	9.377970	0.000196	0.169345	

cDC2

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000028	1	46	0.000028	6.814653	**0.012164**	0.129030	
1	Treatment	0.000094	2	92	0.000047	32.82	**1.74E-11**	0.416419	0.998359
2	Residual	0.000002	2	92	0.000001	0.575072	0.564674	0.012347	

dpDC

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.002067	1	46	0.002067	39.942597	**0.000000**	0.464759	
1	Treatment	0.017632	2	92	0.008816	259.58	**1.48E-38**	0.849467	0.942845
2	Residual	0.000422	2	92	0.000211	6.208291	0.002957	0.118914	

Monocyte

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.004228	1	38	0.004228	3.485859	**0.069624**	0.084025	
1	Treatment	0.072891	2	76	0.036445	120.36	**2.79E-24**	0.760040	0.763677
2	Residual	0.003396	2	76	0.001698	5.607865	0.005350	0.128598	

(to be continued)

Macrophage and transitional Monocyte

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.001023	1	38	0.001023	0.140279	**0.710086**	0.003678	
1	Treatment	0.419014	2	76	0.209507	162.26	**3.72E-28**	0.810249	0.777335
2	Residual	0.066860	2	76	0.033430	25.891406	0.000000	0.405241	

B cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.033891	1	38	0.033891	5.812487	**0.020856**	0.132667	
1	Treatment	0.381139	2	76	0.190570	35.35	**1.40E-11**	0.481910	0.547999
2	Residual	0.051933	2	76	0.025966	4.816167	0.010732	0.112485	

Neutrophil

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000044	1	38	0.000044	1.227243	**0.274907**	0.031285	
1	Treatment	0.000648	2	76	0.000324	43.96	**2.06E-13**	0.536361	0.741628
2	Residual	0.000057	2	76	0.000028	3.840924	0.025759	0.091798	

Treg

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000348	1	56	0.000348	0.397805	**0.530791**	0.007054	
1	Treatment	0.002413	2	112	0.001206	17.42	**2.58E-07**	0.237312	0.719176
2	Residual	0.000334	2	112	0.000167	2.413303	0.094161	0.041314	

$CD8^+$ $TCR\gamma\delta^+$ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.044555	1	38	0.044555	14.883985	**0.000429**	0.281446	
1	Treatment	0.057200	2	76	0.028600	36.19	**9.11E-12**	0.487776	0.990345
2	Residual	0.004058	2	76	0.002029	2.567075	0.083402	0.063280	

(to be continued)

CD8$^+$ TCRβ^+ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.008140	1	38	0.008140	8.542090	**0.005816**	0.183535	
1	Treatment	0.025955	2	76	0.012978	58.28	**4.54E-16**	0.605325	0.885839
2	Residual	0.000954	2	76	0.000477	2.142974	0.124341	0.053384	

CD4$^+$ TCR$\gamma\delta^+$ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000000	1	38	0.000000	0.400925	**0.530405**	0.010440	
1	Treatment	0.000000	2	76	0.000000	0.62	**5.41E-01**	0.016058	0.688344
2	Residual	0.000001	2	76	0.000000	2.467393	0.091575	0.060972	

CD4$^+$ TCRβ^+ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.001783	1	38	0.001783	0.815929	**0.372064**	0.021020	
1	Treatment	0.002391	2	76	0.001196	1.92	**1.53E-01**	0.048178	0.789361
2	Residual	0.006097	2	76	0.003049	4.903850	0.009929	0.114299	

NK

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000000	1	57	0.000000	1.782290	**0.187176**	0.030320	
1	Treatment	0.000001	2	114	0.000001	15.91	**8.05E-07**	0.218220	0.606219
2	Residual	0.000000	2	114	0.000000	0.112353	0.893827	0.001967	

ILC1

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000002	1	57	0.000002	0.062870	**0.802918**	0.001102	
1	Treatment	0.000563	2	114	0.000281	28.80	**7.53E-11**	0.335651	0.672745
2	Residual	0.000036	2	114	0.000018	1.827804	0.165446	0.031070	

(to be continued)

ILC2

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.001446	1	38	0.001446	0.939542	**0.338525**	0.024128	
1	Treatment	0.002949	2	76	0.001474	4.68	**1.21E-02**	0.109654	0.862806
2	Residual	0.000028	2	76	0.000014	0.043793	0.957176	0.001151	

ILC3

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000132	1	38	0.000132	0.123525	**0.727184**	0.003240	
1	Treatment	0.011944	2	76	0.005972	24.68	**5.50E-09**	0.393769	0.673636
2	Residual	0.000241	2	76	0.000121	0.498851	0.609202	0.012958	

IEL CD8$\alpha\beta^+$ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000937	1	38	0.000937	0.361604	**0.551187**	0.009426	
1	Treatment	0.014054	2	76	0.007027	22.82	**1.73E-08**	0.375241	0.566828
2	Residual	0.003186	2	76	0.001593	5.174323	0.007820	0.119847	

IEL CD8$\alpha\alpha^+$ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.052699	1	38	0.052699	4.775958	**0.035094**	0.111651	
1	Treatment	0.348372	2	76	0.174186	32.24	**7.28E-11**	0.458981	0.885660
2	Residual	0.017100	2	76	0.008550	1.582433	0.212169	0.039978	

dpIEL

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.000146	1	38	0.000146	2.549504	**0.118613**	0.062874	
1	Treatment	0.000453	2	76	0.000227	11.81	**3.41E-05**	0.237132	0.575049
2	Residual	0.000036	2	76	0.000018	0.950389	0.391136	0.024400	

(to be continued)

IEL CD8$\alpha\alpha^+$ TCR$\gamma\delta^+$ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.033995	1	38	0.033995	3.245852	**0.079543**	0.078695	
1	Treatment	0.057438	2	76	0.028719	9.38	**2.29E-04**	0.197947	0.916756
2	Residual	0.028766	2	76	0.014383	4.696858	0.011933	0.110005	

IEL CD8$\alpha\alpha^+$ TCRβ^+ T cell

Row	Source	SS	DF1	DF2	MS	F	**p-unc**	np2	eps
0	Duration	0.012230	1	38	0.012230	3.719887	**0.061263**	0.089163	
1	Treatment	0.087378	2	76	0.043689	79.14	**2.63E-19**	0.675615	0.963411
2	Residual	0.002660	2	76	0.001330	2.409634	0.096683	0.059630	

Table S3. Effect of diet and intestinal segment on ratios of cell types in CD45$^+$ cells. Mixed-repeated measures Anova within and between subjects. Row 0 shows the effect of the diet while not considering the intestinal segment; row 1 shows the effect of the segment while not considering the dietary intervention, row 2 shows the interaction effect of treatment and intestinal segment.

cDC

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.044	0.011	0.012	0.005	22.709	47	5.56E-27	**1.67E-26**	5.58E+23	3.647
1	Segment	-	D	J	0.044	0.011	0.024	0.013	12.877	47	5.02E-17	**1.51E-16**	1.17E+14	1.628
2	Segment	-	I	J	0.012	0.005	0.024	0.013	-8.299	47	9.16E-11	**2.75E-10**	1.10E+08	-1.282
3	Treatment	-	CD	HFHSD	0.021	0.005	0.033	0.006	-7.616	46	1.10E-09		6859000	-2.162
4	Segment x Treatment	D	CD	HFHSD	0.038	0.010	0.051	0.009	-4.879	46	1.32E-05	**3.96E-05**	1289.451	-1.385
5	Segment x Treatment	I	CD	HFHSD	0.009	0.003	0.015	0.005	-5.049	46	7.45E-06	**2.23E-05**	2138.71	-1.434
6	Segment x Treatment	J	CD	HFHSD	0.015	0.006	0.033	0.011	-6.808	46	1.77E-08	**5.31E-08**	517500	-1.933

cDC1

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.008	0.004	0.004	0.002	7.483	47	1.52E-09	**4.56E-09**	7554000	1.208
1	Segment	-	D	J	0.008	0.004	0.006	0.004	2.878	47	0.006	**0.018**	5.926	0.364
2	Segment	-	I	J	0.004	0.002	0.006	0.004	-5.806	47	5.28E-07	**1.59E-06**	30000	-0.778
3	Treatment	-	CD	HFHSD	0.004	0.002	0.008	0.002	-5.346	46	2.72E-06		5248.675	-1.518
4	Segment x Treatment	D	CD	HFHSD	0.006	0.004	0.009	0.003	-2.167	46	0.035	**0.106**	1.862	-0.615
5	Segment x Treatment	I	CD	HFHSD	0.003	0.002	0.005	0.002	-4.676	46	2.59E-05	**7.76E-05**	712.418	-1.328
6	Segment x Treatment	J	CD	HFHSD	0.003	0.002	0.009	0.003	-7.529	46	1.47E-09	**4.42E-09**	5204000	-2.138

(to be continued)

dpDC

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.031	0.010	0.004	0.002	21.113	47	1.26E-25	**3.79E-25**	2.65E+22	3.803
1	Segment	-	D	J	0.031	0.010	0.013	0.009	12.911	47	4.56E-17	**1.37E-16**	1.29E+14	1.948
2	Segment	-	I	J	0.004	0.002	0.013	0.009	-7.949	47	3.04E-10	**9.11E-10**	35050000	-1.385
3	Treatment	-	CD	HFHSD	0.012	0.004	0.020	0.005	-6.320	46	9.58E-08		109400	-1.795
4	Segment x Treatment	D	CD	HFHSD	0.026	0.008	0.035	0.008	-3.934	46	2.80E-04	**0.001**	90.923	-1.117
5	Segment x Treatment	I	CD	HFHSD	0.003	0.001	0.005	0.003	-4.777	46	1.85E-05	**5.56E-05**	955.381	-1.356
6	Segment x Treatment	J	CD	HFHSD	0.008	0.003	0.018	0.009	-5.339	46	2.79E-06	**8.38E-06**	5133.540	-1.516

Monocyte

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	3.814	2.722	3.208	2.277	1.776	39	0.084	**0.251**	0.713	0.239
1	Segment	-	D	J	3.814	2.722	6.834	4.003	-6.873	39	0	**0**	429100	-0.874
2	Segment	-	I	J	3.208	2.277	6.834	4.003	-9.600	39	0	**0**	1.13E+09	-1.103
3	Treatment	-	CD	HFHSD	5.826	2.951	3.411	1.918	3.069	38	0.004		10.254	0.951
4	Segment x Treatment	D	CD	HFHSD	4.891	3.261	2.736	1.460	2.697	38	0.010	**0.031**	4.824	0.836
5	Segment x Treatment	I	CD	HFHSD	3.913	2.169	2.504	2.211	2.035	38	0.049	**0.147**	1.532	0.631
6	Segment x Treatment	J	CD	HFHSD	8.674	4.426	4.993	2.484	3.244	38	0.002	**0.007**	14.984	1.005

(to be continued)

Macrophage & transitional monocyte

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	18.514	8.883	40.420	30.650	-5.274	39	5.25E-06	**1.58E-05**	3642.38	-0.961
1	Segment	-	D	J	18.514	8.883	25.839	15.791	-3.683	39	0.001	**0.002**	42.096	-0.566
2	Segment	-	I	J	40.420	30.650	25.839	15.791	5.116	39	8.68E-06	**2.60E-05**	2289.76	0.592
3	Treatment	-	CD	HFHSD	34.047	18.842	22.468	13.198	2.251	38	0.030		2.164	0.698
4	Segment x Treatment	D	CD	HFHSD	18.955	10.086	18.073	7.735	0.310	38	0.758	**1**	0.321	0.096
5	Segment x Treatment	I	CD	HFHSD	52.998	32.544	27.841	23.153	2.817	38	0.008	**0.023**	6.105	0.873
6	Segment x Treatment	J	CD	HFHSD	30.188	18.226	21.489	11.823	1.791	38	0.081	**0.244**	1.077	0.555

B cell

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.026	0.021	0.153	0.131	-6.044	39	4.50E-07	**1.35E-06**	35870	-1.348
1	Segment	-	D	J	0.026	0.021	0.043	0.030	-3.276	39	0.002	**0.007**	15.096	-0.660
2	Segment	-	I	J	0.153	0.131	0.043	0.030	5.386	39	3.68E-06	**1.10E-05**	5071.24	1.150
3	Treatment	-	CD	HFHSD	0.057	0.026	0.091	0.057	-2.411	38	0.021		2.846	-0.747
4	Segment x Treatment	D	CD	HFHSD	0.023	0.011	0.029	0.027	-0.903	38	0.372	**1**	0.427	-0.280
5	Segment x Treatment	I	CD	HFHSD	0.107	0.068	0.199	0.161	-2.366	38	0.023	**0.070**	2.631	-0.733
6	Segment x Treatment	J	CD	HFHSD	0.042	0.034	0.044	0.027	-0.260	38	0.797	**1**	0.317	-0.080

(to be continued)

Neutrophil

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.008	0.006	0.003	0.002	6.903	39.000	2.91E-08	**8.73E-08**	468900	1.309
1	Segment	-	D	J	0.008	0.006	0.007	0.004	2.426	39.000	0.020	**0**	2.268	0.271
2	Segment	-	I	J	0.003	0.002	0.007	0.004	-8.214	39	4.92E-10	**0.000**	22310000.(	-1.253
3	Treatment	-	CD	HFHSD	0.006	0.004	0.005	0.003	1.108	38	2.75E-01		1	0.343
4	Segment x Treatment	D	CD	HFHSD	0.010	0.006	0.007	0.004	1.828	38	0.075	**0**	1.135	0.567
5	Segment x Treatment	I	CD	HFHSD	0.003	0.002	0.003	0.002	-0.139	38	8.90E-01	**1**	0.311	-0.043
6	Segment x Treatment	J	CD	HFHSD	0.007	0.005	0.006	0.004	0.448	38	0.657	**1.000**	0.335	0.139

$CD4^+$ $TCR\beta^+$ T cell

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.121	0.041	0.110	0.027	1.526	39	0.135	**0.405**	0.496	0.310
1	Segment	-	D	J	0.121	0.041	0.116	0.033	1.137	39	0.263	**0.788**	0.311	0.141
2	Segment	-	I	J	0.110	0.027	0.116	0.033	-1.036	39	0.307	**0.920**	0.281	-0.183
3	Treatment	-	CD	HFHSD	0.119	0.030	0.112	0.024	0.903	38	0.372		0	0.280
4	Segment x Treatment	D	CD	HFHSD	0.115	0.043	0.127	0.039	-0.953	38	0.346	**1**	0.443	-0.295
5	Segment x Treatment	I	CD	HFHSD	0.118	0.031	0.102	0.021	2.011	38	0.051	**0.154**	1.479	0.623
6	Segment x Treatment	J	CD	HFHSD	0.125	0.037	0.106	0.027	1.845	38	0.073	**0.219**	1.160	0.572

(to be continued)

IEL CD8αβ+ T cell

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.045	0.026	0.070	0.043	-4.646	39	3.80E-05	**1.14E-04**	589	-0.702
1	Segment	-	D	J	0.045	0.026	0.050	0.026	-2.939	39	0.006	**0**	6.816	-0.220
2	Segment	-	I	J	0.070	0.043	0.050	0.026	4.646	39	3.80E-05	**1.139E-04**	589.08	0.540
3	Treatment	-	CD	HFHSD	0.052	0.019	0.058	0.037	-0.601	38	0.551		0	-0.186
4	Segment x Treatment	D	CD	HFHSD	0.036	0.016	0.053	0.031	-2.315	38	0.026	**0.078**	2.409	-0.717
5	Segment x Treatment	I	CD	HFHSD	0.074	0.033	0.066	0.052	0.532	38	0.598	**1**	0.346	0.165
6	Segment x Treatment	J	CD	HFHSD	0.047	0.017	0.053	0.033	-0.751	38	0.457	**1**	0.387	-0.233

CD8αα+ TCRγδ+ T cell

Row	Contrast	Segment	A	B	mean(A)	std(A)	mean(B)	std(B)	T	dof	p-unc	**p-corr**	BF10	hedges
0	Segment	-	D	I	0.519	0.052	0.471	0.082	4.020	39	2.58E-04	**7.75E-04**	102.86	0.693
1	Segment	-	D	J	0.519	0.052	0.515	0.091	0.323	39	0.748	**1**	0.179	0.051
2	Segment	-	I	J	0.471	0.082	0.515	0.091	-3.005	39	4.63E-03	**0.014**	7.920	-0.507
3	Treatment	-	CD	HFHSD	0.518	0.058	0.485	0.060	1.802	38	0.08		1	0.558
4	Segment x Treatment	D	CD	HFHSD	0.541	0.056	0.497	0.039	2.926	38	0.006	**0.017**	7.617	0.907
5	Segment x Treatment	I	CD	HFHSD	0.466	0.075	0.475	0.090	-0.320	38	0.75	**1**	0.322	-0.099
6	Segment x Treatment	J	CD	HFHSD	0.548	0.063	0.482	0.104	2.398	38	0.021	**0.064**	2.784	0.743

(legend next page)

Table S4. Pairwise comparison of effect of diet and intestinal segment on ratios of cell types in CD45⁺ cells. Mixed-repeated measures Anova within and between subjects. Rows 0-2 show pairwise comparison of intestinal segments while not considering the treatment. Row 3 shows effect of treatment independent of intestinal segment. Rows 4-6 show the effect of the treatment in each intestinal segment.

7.3 Ontogeny and distribution of cDC1 in obesity

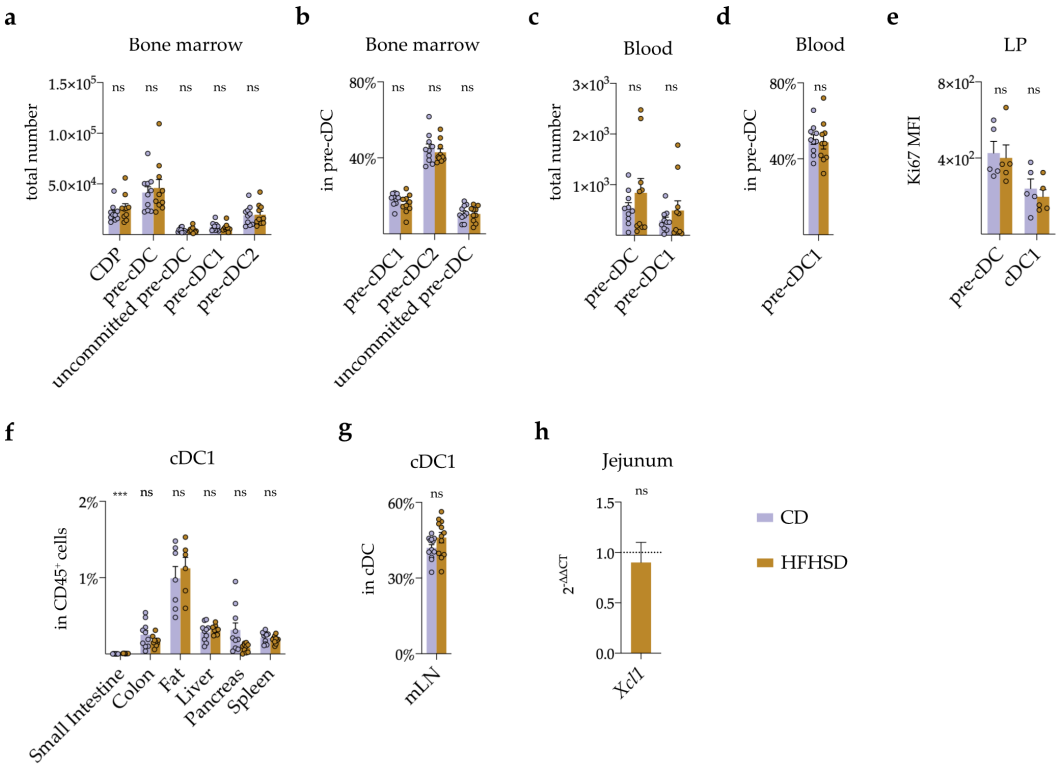


Figure S1. Ontogeny and distribution of cDC1s in the obese and stead-state host. Mice were fed for 8 weeks with HFHSD or CD. (a) Total numbers of cDC precursors in bone marrow and (b) as percentage in pre-cDCs measured by flow cytometry (n = 10). (c) Total numbers of pre-cDCs and pre-cDC1s and (d) as percentage of pre-cDCs in the blood. MFI of Ki67 of cells in the enteric lamina propria (n = 10). (f) cDC1 as percentage in CD45⁺ cells in metabolically relevant organs and (g) mesenteric lymph nodes (n = 12). (h) Xcl1 mRNA levels of total jejunal tissue of HFHSD mice compared to CD mice. Flow cytometry analysis (a-g). RT-qPCR analysis (h). Data are pooled of three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-tailed unpaired Student's t-test (a-g). *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant. LP: lamina propria.

7.4 Weight gain of mice on HFHSD

Body weight of obese animals

A	B	mean (A)	mean (B)	mean diff.	SE of diff.	T	dof	**p corrected**
4 weeks	8 weeks	34	41	-6.87	0.33	21.10	14	**<0.0001**
4 weeks	16 weeks	34	53	-18.65	0.68	27.63	14	**<0.0001**
8 weeks	16 weeks	41	53	-11.79	0.54	21.64	14	**<0.0001**

Table S5. Comparison of body weight of HFHSD-fed mice throughout time. Post-hoc pairwise comparison of repeated measures ANOVA (n = 15). Bonferoni correction. Data are pooled of three independent experiments. n represents biologically independent animals.

7.5 Spacial description of the enteric immune landscape

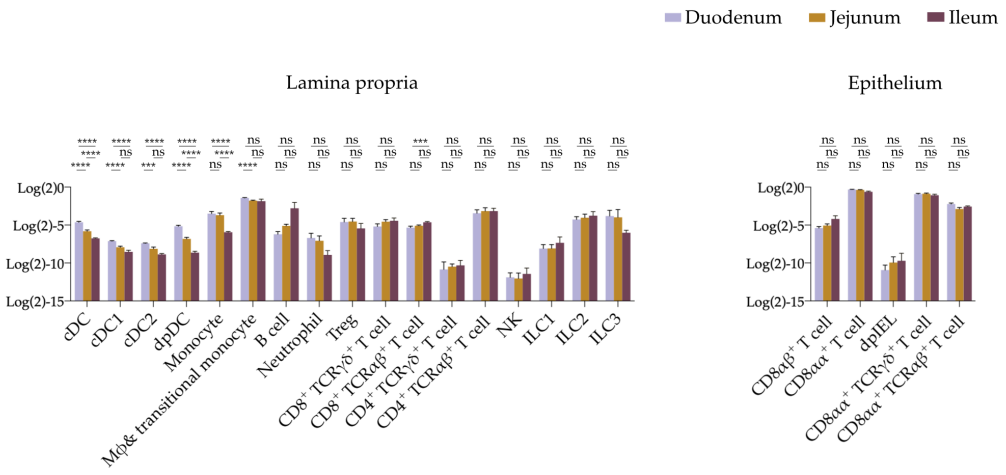


Figure S2. Regional comparison of immune cell types in steady-state. Lamina propria and intraepithelial immune cells of male wild-type mice at 8 weeks of age as ratios of CD45+ cells (n = 15). Data are pooled of three independent experiments. n represents biologically independent animals. Data are presented as mean ± s.e.m. Two-way repeated measures ANOVA for tests within subjects. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001; ns, not significant.

8

BIBLIOGRAPHY

Acuto, O., Fabbi, M., Smart, J., Poole, C.B., Protentis, J., Royer, H.D., Schlossman, S.F., Reinherz, E.L., 1984. Purification and NH2-terminal amino acid sequencing of the beta subunit of a human T-cell antigen receptor. Proc Natl Acad Sci U S A 81, 3851–3855. DOI: 10.1073/pnas.81.12.3851

Adler, B.J., Green, D.E., Pagnotti, G.M., Chan, M.E., Rubin, C.T., 2014. High fat diet rapidly suppresses B lymphopoiesis by disrupting the supportive capacity of the bone marrow niche. PLoS One 9, e90639. DOI: 10.1371/journal.pone.0090639

Ahonen, C.L., Doxsee, C.L., McGurran, S.M., Riter, T.R., Wade, W.F., Barth, R.J., Vasilakos, J.P., Noelle, R.J., Kedl, R.M., 2004. Combined TLR and CD40 triggering induces potent $CD8^+$ T cell expansion with variable dependence on Type I IFN. J Exp Med 199, 775–784. DOI: 10.1084/jem.20031591

Ajayi, A.F., Akhigbe, R.E., 2020. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res Pract 6, 5. DOI: 10.1186/s40738-020-00074-3

Akkaya, B., Oya, Y., Akkaya, M., Al Souz, J., Holstein, A.H., Kamenyeva, O., Kabat, J., Matsumura, R., Dorward, D.W., Glass, D.D., Shevach, E.M., 2019. Regulatory T cells mediate specific suppression by depleting peptide-MHC class II from dendritic cells. Nat Immunol 20, 218–231. DOI: 10.1038/s41590-018-0280-2

Albert, M.L., Pearce, S.F., Francisco, L.M., Sauter, B., Roy, P., Silverstein, R.L., Bhardwaj, N., 1998. Immature dendritic cells phagocytose apoptotic cells via

alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. J Exp Med 188, 1359–1368. DOI: 10.1084/jem.188.7.1359

Ali, J., Haider, S.M.S., Ali, S.M., Haider, T., Anwar, A., Hashmi, A.A., 2023. Overall clinical features of type 2 diabetes mellitus with respect to gender. Cureus 15, e35771. DOI: 10.7759/cureus.35771

Ali, N., Zirak, B., Rodriguez, R.S., Pauli, M.L., Truong, H.-A., Lai, K., Ahn, R., Corbin, K., Lowe, M.M., Scharschmidt, T.C., Taravati, K., Tan, M.R., Ricardo-Gonzalez, R.R., Nosbaum, A., Bertolini, M., Liao, W., Nestle, F.O., Paus, R., Cotsarelis, G., Abbas, A.K., Rosenblum, M.D., 2017. Regulatory T cells in skin facilitate epithelial stem cell differentiation. Cell 169, 1119-1129.e11. DOI: 10.1016/j.cell.2017.05.002

Andersen, C.J., Murphy, K.E., Fernandez, M.L., 2016. Impact of obesity and metabolic syndrome on immunity. Adv Nutr 7, 66–75. DOI: 10.3945/an.115.010207

Andrews, D.M., Scalzo, A.A., Yokoyama, W.M., Smyth, M.J., Degli-Esposti, M.A., 2003. Functional interactions between dendritic cells and NK cells during viral infection. Nat Immunol 4, 175–181. DOI: 10.1038/ni880

Apostolou, I., von Boehmer, H., 2004. In vivo instruction of suppressor commitment in naive T cells. J Exp Med 199, 1401–1408. DOI: 10.1084/jem.20040249

Ardouin, L., Luche, H., Chelbi, R., Carpentier, S., Shawket, A., Montanana Sanchis, F., Santa Maria, C., Grenot, P., Alexandre, Y., Grégoire, C., Fries, A., Vu Manh, T.-P., Tamoutounour, S., Crozat, K., Tomasello, E., Jorquera, A., Fossum, E., Bogen, B., Azukizawa, H., Bajenoff, M., Henri, S., Dalod, M., Malissen, B., 2016. Broad and largely concordant molecular changes characterize tolerogenic and immunogenic dendritic cell maturation in thymus and periphery. Immunity 45, 305–318. DOI: 10.1016/j.immuni.2016.07.019

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., Rudensky, A.Y., 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504, 451–455. DOI: 10.1038/nature12726

Artis, D., Spits, H., 2015. The biology of innate lymphoid cells. Nature 517, 293–301. DOI: 10.1038/nature14189

Asgharpour, A., Cazanave, S.C., Pacana, T., Seneshaw, M., Vincent, R., Banini, B.A., Kumar, D.P., Daita, K., Min, H.-K., Mirshahi, F., Bedossa, P., Sun, X., Hoshida, Y., Koduru, S.V., Contaifer, D., Warncke, U.O., Wijesinghe, D.S., Sanyal, A.J., 2016. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. Journal of Hepatology 65, 579–588. DOI: 10.1016/j.jhep.2016.05.005

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I.I., Umesaki, Y., Itoh, K., Honda, K., 2011. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331, 337–341. DOI: 10.1126/science.1198469

Austyn, J.M., 1987. Lymphoid dendritic cells. Immunology 62, 161–170.

Awasthi, A., Carrier, Y., Peron, J.P.S., Bettelli, E., Kamanaka, M., Flavell, R.A., Kuchroo, V.K., Oukka, M., Weiner, H.L., 2007. A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 8, 1380–1389. DOI: 10.1038/ni1541

Bachem, A., Hartung, E., Güttler, S., Mora, A., Zhou, X., Hegemann, A., Plantinga, M., Mazzini, E., Stoitzner, P., Gurka, S., Henn, V., Mages, H.W., Kroczek, R.A., 2012. Expression of XCR1 Characterizes the Batf3-Dependent Lineage of Dendritic Cells Capable of Antigen Cross-Presentation. Front. Immunol. 3. DOI: 10.3389/fimmu.2012.00214

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252. DOI: 10.1038/32588

Bando, J.K., Gilfillan, S., Di Luccia, B., Fachi, J.L., Sécca, C., Cella, M., Colonna, M., 2020. ILC2s are the predominant source of intestinal ILC-derived IL-10. J Exp Med 217, e20191520. DOI: 10.1084/jem.20191520

Barnden, M.J., Allison, J., Heath, W.R., Carbone, F.R., 1998. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. Immunol Cell Biol 76, 34–40. DOI: 10.1046/j.1440-1711.1998.00709.x

Behring, N., Kitasato, N., 1890. Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. Dtsch Med Wochenschr 16, 1113–1114. DOI: 10.1055/s-0029-1207589

Belkaid, Y., Hand, T., 2014. Role of the Microbiota in immunity and inflammation. Cell 157, 121–141. DOI: 10.1016/j.cell.2014.03.011

Bell, M.R., 2018. Comparing postnatal development of gonadal hormones and associated social behaviors in rats, mice, and humans. Endocrinology 159, 2596–2613. DOI: 10.1210/en.2018-00220

Benova, A., Tencerova, M., 2020. Obesity-induced changes in bone marrow homeostasis. Front. Endocrinol. 11, 294. DOI: 10.3389/fendo.2020.00294

Berenbaum, F., Griffin, T.M., Liu-Bryan, R., 2017. Metabolic regulation of inflammation in osteoarthritis. Arthritis Rheumatol 69, 9–21. DOI: 10.1002/art.39842

Bonilla, F.A., Oettgen, H.C., 2010. Adaptive immunity. J Allergy Clin Immunol 125, S33-40. DOI: 10.1016/j.jaci.2009.09.017

Bordenstein, S.R., Theis, K.R., 2015. Host Biology in Light of the Microbiome: Ten principles of holobionts and hologenomes. PLoS Biol 13, e1002226. DOI: 10.1371/journal.pbio.1002226

Bosteels, C., Neyt, K., Vanheerswynghels, M., van Helden, M.J., Sichien, D., Debeuf, N., De Prijck, S., Bosteels, V., Vandamme, N., Martens, L., Saeys, Y., Louagie, E., Lesage, M., Williams, D.L., Tang, S.-C., Mayer, J.U., Ronchese, F., Scott, C.L., Hammad, H., Guilliams, M., Lambrecht, B.N., 2020. Inflammatory type 2 cDCs acquire features of cDC1s and macrophages to orchestrate immunity to respiratory virus infection. Immunity 52, 1039-1056.e9. DOI: 10.1016/j.immuni.2020.04.005

Bosteels, V., Janssens, S., 2024. Striking a balance: new perspectives on homeostatic dendritic cell maturation. Nat Rev Immunol 1–16. DOI: 10.1038/s41577-024-01079-5

Bosteels, V., Maréchal, S., De Nolf, C., Rennen, S., Maelfait, J., Tavernier, S.J., Vetters, J., Van De Velde, E., Fayazpour, F., Deswarte, K., Lamoot, A., Van Duyse, J., Martens, L., Bosteels, C., Roelandt, R., Emmaneel, A., Van Gassen, S., Boon, L., Van Isterdael, G., Guillas, I., Vandamme, N., Höglinger, D., De Geest, B.G., Le Goff, W., Saeys, Y., Ravichandran, K.S., Lambrecht, B.N., Janssens, S., 2023. LXR signaling controls homeostatic dendritic cell maturation. Sci Immunol 8, eadd3955. DOI: 10.1126/sciimmunol.add3955

Böttcher J.P., Reis E Sousa C., 2018. The Role of Type 1 Conventional Dendritic Cells in Cancer Immunity. Trends Cancer 11,784-792. DOI: 10.1016/j.trecan.2018.09.001

Boucard-Jourdin M., Kugler D., Endale Ahanda M., This S., De Calisto J., Zhang A., Mora J., Stuart L., Savill J., Lacy-Hulbert A., Paidassi H, 2016. . β8 Integrin expression and activation of TGF-β by intestinal dendritic cells are determined by both tissue microenvironment and cell lineage. J Immunol. Sep 1;197(5):1968-78. DOI: 10.4049/jimmunol.1600244

Bousso, P., Robey, E., 2003. Dynamics of $CD8^+$ T cell priming by dendritic cells in intact lymph nodes. Nat Immunol 4, 579–585. DOI: 10.1038/ni928

Brandtzaeg, P., 1998. Development and basic mechanisms of human gut immunity. Nutr Rev 56, S5-18. DOI: 10.1111/j.1753-4887.1998.tb01645.x

Brenner, M.B., McLean, J., Dialynas, D.P., Strominger, J.L., Smith, J.A., Owen, F.L., Seidman, J.G., Ip, S., Rosen, F., Krangel, M.S., 1986. Identification of a putative second T-cell receptor. Nature 322, 145–149. DOI: 10.1038/322145a0

Bresilla, D., Habisch, H., Pritišanac, I., Zarse, K., Parichatikanond, W., Ristow, M., Madl, T., Madreiter-Sokolowski, C.T., 2022. The sex-specific metabolic signature of C57BL/6NRj mice during aging. Sci Rep 12, 21050. DOI: 10.1038/s41598-022-25396-8

Brown, C.C., Gudjonson, H., Pritykin, Y., Deep, D., Lavallée, V.-P., Mendoza, A., Fromme, R., Mazutis, L., Ariyan, C., Leslie, C., Pe'er, D., Rudensky, A.Y., 2019. Transcriptional basis of mouse and human dendritic cell heterogeneity. Cell 179, 846-863.e24. DOI: 10.1016/j.cell.2019.09.035

Brown, G., 2012. Actin' dangerously. Nature 485, 589–590. DOI: 10.1038/485589a

Brown, H., Esterházy, D., 2021. Intestinal immune compartmentalization: implications of tissue specific determinants in health and disease. Mucosal Immunology 14, 1259–1270. DOI: 10.1038/s41385-021-00420-8

Bruce, M.G., Strobel, S., Hanson, D.G., Ferguson, A., 1987. Irradiated mice lose the capacity to "process" fed antigen for systemic tolerance of delayed-type hypersensitivity. Clin Exp Immunol 70, 611–618.

Bryson, J.M., Phuyal, J.L., Proctor, D.R., Blair, S.C., Caterson, I.D., Cooney, G.J., 1999. Plasma insulin rise precedes rise in ob mRNA expression and plasma

leptin in gold thioglucose-obese mice. Am J Physiol 276, E358-364. DOI: 10.1152/ajpendo.1999.276.2.E358

Bures, J., Cyrany, J., Kohoutova, D., Förstl, M., Rejchrt, S., Kvetina, J., Vorisek, V., Kopacova, M., 2010. Small intestinal bacterial overgrowth syndrome. World Journal of Gastroenterology : WJG 16, 2978. DOI: 10.3748/wjg.v16.i24.2978

Burnet, F.M., 1957. A modification of Jerne's theory of antibody production using the concept of clonal selection. CA: A Cancer Journal for Clinicians 26, 119–121. DOI: 10.3322/canjclin.26.2.119

Burnet, F.M., 1959. The clonal selection theory of acquired immunity. Vanderbilt University Press, Nashville. DOI: 10.5962/bhl.title.8281

Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J.L., Cerletti, M., Jang, Y., Sefik, E., Tan, T.G., Wagers, A.J., Benoist, C., Mathis, D., 2013. A special population of regulatory T cells potentiates muscle repair. Cell 155, 1282–1295. DOI: 10.1016/j.cell.2013.10.054

Butler, A., Hoffman, P., Smibert, P., Papalexi, E., Satija, R., 2018. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat Biotechnol 36, 411–420. DOI: 10.1038/nbt.4096

Buzzetti, E., Pinzani, M., Tsochatzis, E.A., 2016. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism 65, 1038–1048. DOI: 10.1016/j.metabol.2015.12.012

Cabeza-Cabrerizo, M., Cardoso, A., Minutti, C.M., Pereira da Costa, M., Reis e Sousa, C., 2021a. Dendritic cells revisited. Annu. Rev. Immunol. 39, 131–166. DOI: 10.1146/annurev-immunol-061020-053707

Cabeza-Cabrerizo, M., Minutti, C.M., Da Costa, M.P., Cardoso, A., Jenkins, R.P., Kulikauskaite, J., Buck, M.D., Piot, C., Rogers, N., Crotta, S., Whittaker, L., Encabo, H.H., McCauley, J.W., Allen, J.E., Pasparakis, M., Wack, A., Sahai, E., Reis E Sousa, C., 2021b. Recruitment of dendritic cell progenitors to foci of influenza A virus infection sustains immunity. Sci. Immunol. 6, eabi9331. DOI: 10.1126/sciimmunol.abi9331

Cabeza-Cabrerizo, M., van Blijswijk, J., Wienert, S., Heim, D., Jenkins, R.P., Chakravarty, P., Rogers, N., Frederico, B., Acton, S., Beerling, E., van Rheenen, J., Clevers, H., Schraml, B.U., Bajénoff, M., Gerner, M., Germain, R.N., Sahai, E., Klauschen, F., Reis e Sousa, C., 2019. Tissue clonality of dendritic cell

subsets and emergency DCpoiesis revealed by multicolor fate mapping of DC progenitors. Sci. Immunol. 4, eaaw1941. DOI: 10.1126/sciimmunol.aaw1941

Camilleri, G., Kiani, A.K., Herbst, K.L., Kaftalli, J., Bernini, A., Dhuli, K., Manara, E., Bonetti, G., Stuppia, L., Paolacci, S., Dautaj, A., Bertelli, M., 2021. Genetics of fat deposition. Eur Rev Med Pharmacol Sci 25, 14–22. DOI: 10.26355/eurrev_202112_27329

Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C., Waget, A., Delmée, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrières, J., Tanti, J.-F., Gibson, G.R., Casteilla, L., Delzenne, N.M., Alessi, M.C., Burcelin, R., 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 1761–1772. DOI: 10.2337/db06-1491

Cardoso, F., Klein Wolterink, R.G.J., Godinho-Silva, C., Domingues R.G., Ribeiro H., da Silva J.A., Mahú I., Domingos A.I., Veiga-Fernandes H., 2021. Neuro-mesenchymal units control ILC2 and obesity via a brain–adipose circuit. Nature 597, 410–414. DOI: 10.1038/s41586-021-03830-7

Castoldi, A., Naffah de Souza, C., Câmara, N.O.S., Moraes-Vieira, P.M., 2015. The macrophage switch in obesity development. Front Immunol 6, 637. DOI: 10.3389/fimmu.2015.00637

Cella, M., Sallusto, F., Lanzavecchia, A., 1997. Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol 9, 10–16. DOI: 10.1016/s0952-7915(97)80153-7

Cervantes-Barragan, L., Chai, J.N., Tianero, M.D., Luccia, B.D., Ahern, P.P., Merriman, J., Cortez, V.S., Caparon, M.G., Donia, M.S., Gilfillan, S., Cella, M., Gordon, J.I., Hsieh, C.-S., Colonna, M., 2017. Lactobacillus reuteri induces gut intraepithelial $CD4^{+}CD8aa^{+}$ T cells.

Chalasani, N., Younossi, Z., Lavine, J.E., Charlton, M., Cusi, K., Rinella, M., Harrison, S.A., Brunt, E.M., Sanyal, A.J., 2018. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 67, 328–357. DOI: 10.1002/hep.29367

Chan, P.-C., Hsieh, P.-S., Chan, P.-C., Hsieh, P.-S., 2017. The role of adipocyte hypertrophy and hypoxia in the development of obesity-associated adipose tissue inflammation and insulin resistance, in: adiposity - omics and molecular understanding. IntechOpen. DOI: 10.5772/65458

Chen, W., Jin, W., Hardegen, N., Lei, K., Li, L., Marinos, N., McGrady, G., Wahl, S.M., 2003. Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. J Exp Med 198, 1875–1886. DOI: 10.1084/jem.20030152

Chen, W., Konkel, J.E., 2015. Development of thymic Foxp3(+) regulatory T cells: TGF-β matters. Eur J Immunol 45, 958–965. DOI: 10.1002/eji.201444999

Chen, Y., Inobe, J., Marks, R., Gonnella, P., Kuchroo, V.K., Weiner, H.L., 1995. Peripheral deletion of antigen-reactive T cells in oral tolerance. Nature 376, 177–180. DOI: 10.1038/376177a0

Chichlowski, M., van Diepen, J.A., Prodan, A., Olga, L., Ong, K.K., Kortman, G.A.M., Dunger, D.B., Gross, G., 2023. Early development of infant gut microbiota in relation to breastfeeding and human milk oligosaccharides. Front. Nutr. 10. DOI: 10.3389/fnut.2023.1003032

Chien, Y.H., Iwashima, M., Kaplan, K.B., Elliott, J.F., Davis, M.M., 1987. A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. Nature 327, 677–682. DOI: 10.1038/327677a0

Cho, K.W., Zamarron, B.F., Muir, L.A., Singer, K., Porsche, C.E., DelProposto, J.B., Geletka, L., Meyer, K.A., O'Rourke, R.W., Lumeng, C.N., 2016. Adipose tissue dendritic cells are independent contributors to obesity-induced inflammation and insulin resistance. J Immunol 197, 3650–3661. DOI: 10.4049/jimmunol.1600820

Choi Y.S., Kageyama R., Eto D., Escobar T.C., Johnston R.J., Monticelli L., Lao C., Crotty S., 2011. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. Immunity 24, 932-46. DOI: 10.1016/j.immuni.2011.03.023

Christ, A., Günther, P., Lauterbach, M.A.R., Duewell, P., Biswas, D., Pelka, K., Scholz, C.J., Oosting, M., Haendler, K., Baßler, K., Klee, K., Schulte-Schrepping, J., Ulas, T., Moorlag, S.J.C.F.M., Kumar, V., Park, M.H., Joosten, L.A.B., Groh, L.A., Riksen, N.P., Espevik, T., Schlitzer, A., Li, Y., Fitzgerald, M.L., Netea, M.G., Schultze, J.L., Latz, E., 2018. Western diet triggers NLRP3-dependent innate immune reprogramming. Cell 172, 162-175.e14. DOI: 10.1016/j.cell.2017.12.013

Clayton, J.A., Collins, F.S., 2014. Policy: NIH to balance sex in cell and animal studies. Nature 509, 282–283. DOI: 10.1038/509282a

Clevers, H.C., Bevins, C.L., 2013. Paneth cells: maestros of the small intestinal crypts. Annu Rev Physiol 75, 289–311. DOI: 10.1146/annurev-physiol-030212-183744

Collin, M., Bigley, V., 2018. Human dendritic cell subsets: an update. Immunology 154, 3–20. DOI: 10.1111/imm.12888

Colonna, M., Trinchieri, G., Liu, Y.-J., 2004. Plasmacytoid dendritic cells in immunity. Nat Immunol 5, 1219–1226. DOI: 10.1038/ni1141

Considine, R.V., Caro, J.F., 1997. Leptin and the regulation of body weight. Int J Biochem Cell Biol 29, 1255–1272. DOI: 10.1016/s1357-2725(97)00050-2

Coombes, J.L., Siddiqui, K.R.R., Arancibia-Cárcamo, C.V., Hall, J., Sun, C.-M., Belkaid, Y., Powrie, F., 2007. A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-β^- and retinoic acid–dependent mechanism. Journal of Experimental Medicine 204, 1757–1764. DOI: 10.1084/jem.20070590

Cooper, M.D., 2010. A life of adventure in immunobiology. Annual Review of Immunology 28, 1–19. DOI: 10.1146/annurev-immunol-030409-101248

Couper, K.N., Blount, D.G., Riley, E.M., 2008. IL-10: the master regulator of immunity to infection. J Immunol 180, 5771–5777. DOI: 10.4049/jimmunol.180.9.5771

Crewe, C., An, Y.A., Scherer, P.E., 2017. The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. J Clin Invest 127, 74–82. DOI: 10.1172/JCI88883

Crozat, K., Guiton, R., Contreras, V., Feuillet, V., Dutertre, C.-A., Ventre, E., Vu Manh, T.-P., Baranek, T., Storset, A.K., Marvel, J., Boudinot, P., Hosmalin, A., Schwartz-Cornil, I., Dalod, M., 2010. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse $CD8alpha^+$ dendritic cells. J Exp Med 207, 1283–1292. DOI: 10.1084/jem.20100223

Cueto, F.J., Sancho, D., 2021. The Flt3L/Flt3 Axis in dendritic cell biology and cancer immunotherapy. Cancers 13, 1525. DOI: 10.3390/cancers13071525

Cummings, R.J., Barbet, G., Bongers, G., Hartmann, B.M., Gettler, K., Muniz, L., Furtado, G.C., Cho, J., Lira, S.A., Blander, J.M., 2016. Different tissue

phagocytes sample apoptotic cells to direct distinct homeostasis programs. Nature 539, 565–569. DOI: 10.1038/nature20138

Cunningham, M.A., Wirth, J.R., Naga, O., Eudaly, J., Gilkeson, G.S., 2014. Estrogen receptor alpha binding to ERE is required for full Tlr7- and Tlr9-induced inflammation. SOJ Immunol 2, 07. DOI: 10.15226/soji.2014.00107

Curtsinger, J.M., Johnson, C.M., Mescher, M.F., 2003. CD8 T cell clonal expansion and development of effector function require prolonged exposure to antigen, costimulation, and signal 3 cytokine. J Immunol 171, 5165–5171. DOI: 10.4049/jimmunol.171.10.5165

Curtsinger, J.M., Valenzuela, J.O., Agarwal, P., Lins, D., Mescher, M.F., 2005. Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. J Immunol 174, 4465–4469. DOI: 10.4049/jimmunol.174.8.4465

D12492. (15.11.2024) https://www.ssniff.de/documents/03-03 Purified DIO & Controls_v.pdf

Daniel, H., Gholami, A.M., Berry, D., Desmarchelier, C., Hahne, H., Loh, G., Mondot, S., Lepage, P., Rothballer, M., Walker, A., Böhm, C., Wenning, M., Wagner, M., Blaut, M., Schmitt-Kopplin, P., Kuster, B., Haller, D., Clavel, T., 2014. High-fat diet alters gut microbiota physiology in mice. ISME J 8, 295–308. DOI: 10.1038/ismej.2013.155

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J., Turnbaugh, P.J., 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559–563. DOI: 10.1038/nature12820

Davis, M.M., Boniface, J.J., Reich, Z., Lyons, D., Hampl, J., Arden, B., Chien, Y., 1998. Ligand recognition by alpha beta T cell receptors. Annu Rev Immunol 16, 523–544. DOI: 10.1146/annurev.immunol.16.1.523

De Rosa, V., Procaccini, C., Calì, G., Pirozzi, G., Fontana, S., Zappacosta, S., La Cava, A., Matarese, G., 2007. A key role of leptin in the control of regulatory T cell proliferation. Immunity 26, 241–255. DOI: 10.1016/j.immuni.2007.01.011

De Wit, N., Derrien, M., Bosch-Vermeulen, H., Oosterink, E., Keshtkar, S., Duval, C., De Vogel-van Den Bosch, J., Kleerebezem, M., Müller, M., Van Der Meer, R., 2012. Saturated fat stimulates obesity and hepatic steatosis and affects gut microbiota composition by an enhanced overflow of dietary fat to the distal

intestine. American Journal of Physiology-Gastrointestinal and Liver Physiology 303, G589–G599. DOI: 10.1152/ajpgi.00488.2011

De Wit, N.J., Bosch-Vermeulen, H., De Groot, P.J., Hooiveld, G.J., Bromhaar, M.M.G., Jansen, J., Müller, M., Van Der Meer, R., 2008. The role of the small intestine in the development of dietary fat-induced obesity and insulin resistance in C57BL/6J mice. BMC Med Genomics 1, 14. DOI: 10.1186/1755-8794-1-14

De Wit, N.J.W., Boekschoten, M.V., Bachmair, E.-M., Hooiveld, G.J.E.J., De Groot, P.J., Rubio-Aliaga, I., Daniel, H., Müller, M., 2011. Dose-dependent effects of dietary fat on development of obesity in relation to intestinal differential gene expression in C57BL/6J mice. PLoS ONE 6, e19145. DOI: 10.1371/journal.pone.0019145

Deczkowska, A., David, E., Ramadori, P., Pfister, D., Safran, M., Li, B., Giladi, A., Jaitin, D.A., Barboy, O., Cohen, M., Yofe, I., Gur, C., Shlomi-Loubaton, S., Henri, S., Suhail, Y., Qiu, M., Kam, S., Hermon, H., Lahat, E., Ben Yakov, G., Cohen-Ezra, O., Davidov, Y., Likhter, M., Goitein, D., Roth, S., Weber, A., Malissen, B., Weiner, A., Ben-Ari, Z., Heikenwälder, M., Elinav, E., Amit, I., 2021. XCR1$^+$ type 1 conventional dendritic cells drive liver pathology in non-alcoholic steatohepatitis. Nat Med 27, 1043–1054. DOI: 10.1038/s41591-021-01344-3

Del Fresno, C., Saz-Leal, P., Enamorado, M., Wculek, S.K., Martínez-Cano, S., Blanco-Menéndez, N., Schulz, O., Gallizioli, M., Miró-Mur, F., Cano, E., Planas, A., Sancho, D., 2018. DNGR-1 in dendritic cells limits tissue damage by dampening neutrophil recruitment. Science 362, 351–356. DOI: 10.1126/science.aan8423

den Haan, J.M.M., Lehar, S.M., Bevan, M.J., 2000. CD8$^+$ but Not CD8$^-$ dendritic cells cross-prime cytotoxic T cells in vivo. J Exp Med 192, 1685–1696.

Desch, A.N., Gibbings, S.L., Clambey, E.T., Janssen, W.J., Slansky, J.E., Kedl, R.M., Henson, P.M., Jakubzick, C., 2014. Dendritic cell subsets require cis-activation for cytotoxic CD8 T-cell induction. Nat Commun 5, 4674. DOI: 10.1038/ncomms5674

Di Luccia, B., Crescenzo, R., Mazzoli, A., Cigliano, L., Venditti, P., Walser, J.-C., Widmer, A., Baccigalupi, L., Ricca, E., Iossa, S., 2015. Rescue of fructose-induced metabolic syndrome by antibiotics or faecal transplantation in a rat model of obesity. PLoS One 10, e0134893. DOI: 10.1371/journal.pone.0134893

Dikiy, S., Rudensky, A.Y., 2023. Principles of regulatory T cell function. Immunity 56, 240–255. DOI: 10.1016/j.immuni.2023.01.004

Do, M., Lee, E., Oh, M., Kim, Y., Park, H., 2018. High-glucose or -fructose diet cause changes of the gut microbiota and metabolic disorders in mice without body weight change. Nutrients 10. DOI: 10.3390/nu10060761

Donaldson, G.P., Lee, S.M., Mazmanian, S.K., 2016. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14, 20–32. DOI: 10.1038/nrmicro3552

Drager, L.F., Togeiro, S.M., Polotsky, V.Y., Lorenzi-Filho, G., 2013. Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome. J Am Coll Cardiol 62, 569–576. DOI: 10.1016/j.jacc.2013.05.045

Dress, R.J., Dutertre, C.-A., Giladi, A., Schlitzer, A., Low, I., Shadan, N.B., Tay, A., Lum, J., Kairi, M.F.B.M., Hwang, Y.Y., Becht, E., Cheng, Y., Chevrier, M., Larbi, A., Newell, E.W., Amit, I., Chen, J., Ginhoux, F., 2019. Plasmacytoid dendritic cells develop from Ly6D$^+$ lymphoid progenitors distinct from the myeloid lineage. Nat Immunol 20, 852–864. DOI: 10.1038/s41590-019-0420-3

Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumpfheller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H.-W., Park, C.G., Steinman, R.M., Nussenzweig, M.C., 2007. Differential antigen processing by dendritic cell subsets in vivo. Science 315, 107–111. DOI: 10.1126/science.1136080

Dutertre, C.-A., Becht, E., Irac, S.E., Khalilnezhad, A., Narang, V., Khalilnezhad, S., Ng, P.Y., van den Hoogen, L.L., Leong, J.Y., Lee, B., Chevrier, M., Zhang, X.M., Yong, P.J.A., Koh, G., Lum, J., Howland, S.W., Mok, E., Chen, J., Larbi, A., Tan, H.K.K., Lim, T.K.H., Karagianni, P., Tzioufas, A.G., Malleret, B., Brody, J., Albani, S., van Roon, J., Radstake, T., Newell, E.W., Ginhoux, F., 2019. Single-Cell analysis of human mononuclear phagocytes reveals subset-defining markers and identifies circulating inflammatory dendritic cells. Immunity 51, 573-589.e8. DOI: 10.1016/j.immuni.2019.08.008

Dutta, A., Zhao, B., Love, P.E., 2021. New insights into TCR β-selection. Trends Immunol 42, 735–750. DOI: 10.1016/j.it.2021.06.005

Edelson, B.T., Kc, W., Juang, R., Kohyama, M., Benoit, L.A., Klekotka, P.A., Moon, C., Albring, J.C., Ise, W., Michael, D.G., Bhattacharya, D., Stappenbeck, T.S., Holtzman, M.J., Sung, S.-S.J., Murphy, T.L., Hildner, K., Murphy, K.M., 2010. Peripheral CD103$^+$ dendritic cells form a unified subset developmentally related to CD8α^+ conventional dendritic cells. Journal of Experimental Medicine 207, 823–836. DOI: 10.1084/jem.20091627

Egawa, T., Littman, D.R., 2008. ThPOK acts late in specification of the helper T cell lineage and suppresses Runx-mediated commitment to the cytotoxic T cell lineage. Nat Immunol 9, 1131–1139. DOI: 10.1038/ni.1652

Egerton, M., Scollay, R., Shortman, K., 1990. Kinetics of mature T-cell development in the thymus. Proc Natl Acad Sci U S A 87, 2579–2582. DOI: 10.1073/pnas.87.7.2579

Ehrlich, P., 1997. Croonian lecture — On immunity with special reference to cell life. Proceedings of the Royal Society of London 66, 424–448. DOI: 10.1098/rspl.1899.0121

Ehrlich, P., 1901. Die Schutzstoffe des Blutes. Dtsch. Med. Wschr. 27, 865. DOI: 10.1055/s-0029-1187219

Ehrlich, P., 1901. Die Seitenkettentheorie und ihre Gegner. Münch. Med. Wschr. 18, 2123.

Eisenbarth, S.C., 2019. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol 19, 89–103. DOI: 10.1038/s41577-018-0088-1

Eknoyan, G., 2008. Adolphe Quetelet (1796-1874) — the average man and indices of obesity. Nephrol Dial Transplant 23, 47–51. DOI: 10.1093/ndt/gfm517

Esterházy, D., Canesso, M.C.C., Mesin, L., Muller, P.A., De Castro, T.B.R., Lockhart, A., ElJalby, M., Faria, A.M.C., Mucida, D., 2019. Compartmentalized gut lymph node drainage dictates adaptive immune responses. Nature 569, 126–130. DOI: 10.1038/s41586-019-1125-3

Esterházy, D., Loschko, J., London, M., Jove, V., Oliveira, T.Y., Mucida, D., 2016. Classical dendritic cells are required for dietary antigen-mediated induction of peripheral Treg cells and tolerance. Nat Immunol 17, 545–555. DOI: 10.1038/ni.3408

Fantuzzi, G., 2005. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 115, 911–919; quiz 920. DOI: 10.1016/j.jaci.2005.02.023

Faria, A.M.C., Maron, R., Ficker, S.M., Slavin, A.J., Spahn, T., Weiner, H.L., 2003. Oral tolerance induced by continuous feeding: enhanced up-regulation of transforming growth factor-beta/interleukin-10 and suppression of experimental autoimmune encephalomyelitis. J Autoimmun 20, 135–145. DOI: 10.1016/s0896-8411(02)00112-9

Farmer, R.G., Hawk, W.A., Turnbull, R.B., 1975. Clinical patterns in Crohn's disease: A Statistical Study of 615 Cases. Gastroenterology 68, 627–635. DOI: 10.1016/S0016-5085(75)80270-8

Farrand, K.J., Dickgreber, N., Stoitzner, P., Ronchese, F., Petersen, T.R., Hermans, I.F., 2009. Langerin$^+$ CD8alpha$^+$ dendritic cells are critical for cross-priming and IL-12 production in response to systemic antigens. J Immunol 183, 7732–7742. DOI: 10.4049/jimmunol.0902707

Flurkey, K., M. Currer, J., Harrison, D.E., 2007. Chapter 20 - Mouse Models in Aging Research, in: Fox, J.G., Davisson, M.T., Quimby, F.W., Barthold, S.W., Newcomer, C.E., Smith, A.L. (Eds.), The mouse in biomedical research (second edition), American College of Laboratory Animal Medicine. Academic Press, Burlington, pp. 637–672. DOI: 10.1016/B978-012369454-6/50074-1

Förster, R., Davalos-Misslitz, A.C., Rot, A., 2008. CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol 8, 362–371. DOI: 10.1038/nri2297

Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E., Lipp, M., 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99, 23–33. DOI: 10.1016/s0092-8674(00)80059-8

Franco, R.E., Perez, V.V., Ramírez, E.J., Gonzalez, A.R., López-Briones, S., 2016. High fat diet induces alterations to intraepithelial lymphocyte and cytokine mRNA in the small intestine of C57BL/6 mice. RSC Advances. DOI: 10.1039/C6RA24689C

Freeman, H.J., 2008. Pearls and pitfalls in the diagnosis of adult celiac disease. Canadian Journal of Gastroenterology 22, 273. DOI: 10.1155/2008/905325

Fujisaka, S., Usui, I., Ikutani, M., Aminuddin, A., Takikawa, A., Tsuneyama, K., Mahmood, A., Goda, N., Nagai, Y., Takatsu, K., Tobe, K., 2013. Adipose tissue hypoxia induces inflammatory M1 polarity of macrophages in an HIF-1α-dependent and HIF-1α-independent manner in obese mice. Diabetologia 56, 1403–1412. DOI: 10.1007/s00125-013-2885-1

Fukaya, T., Uto, T., Mitoma, S., Takagi, H., Nishikawa, Y., Tominaga, M., Choijookhuu, N., Hishikawa, Y., Sato, K., 2023. Gut dysbiosis promotes the breakdown of oral tolerance mediated through dysfunction of mucosal dendritic cells. Cell Reports 42, 112431. DOI: 10.1016/j.celrep.2023.112431

Furtado, G.C., Curotto de Lafaille, M.A., Kutchukhidze, N., Lafaille, J.J., 2002. Interleukin 2 signaling is required for CD4+ regulatory T cell function. J Exp Med 196, 851–857. DOI: 10.1084/jem.20020190

Gabert, J., Langlet, C., Zamoyska, R., Parnes, J.R., Schmitt-Verhulst, A.M., Malissen, B., 1987. Reconstitution of MHC class I specificity by transfer of the T cell receptor and Lyt-2 genes. Cell 50, 545–554. DOI: 10.1016/0092-8674(87)90027-4

Gainullina, A., Mogilenko, D.A., Huang, L.-H., Todorov, H., Narang, V., Kim, K.-W., Yng, L.S., Kent, A., Jia, B., Seddu, K., Krchma, K., Wu, J., Crozat, K., Tomasello, E., Dress, R., See, P., Scott, C., Gibbings, S., Bajpai, G., Desai, J.V., Maier, B., This, S., Wang, P., Aguilar, S.V., Poupel, L., Dussaud, S., Zhou, T.-A., Angeli, V., Blander, J.M., Choi, K., Dalod, M., Dzhagalov, I., Gautier, E.L., Jakubzick, C., Lavine, K., Lionakis, M.S., Paidassi, H., Sieweke, M.H., Ginhoux, F., Guilliams, M., Benoist, C., Merad, M., Randolph, G.J., Sergushichev, A., Artyomov, M.N., ImmGen Consortium, 2023. Network analysis of large-scale ImmGen and Tabula Muris datasets highlights metabolic diversity of tissue mononuclear phagocytes. Cell Rep 42, 112046. DOI: 10.1016/j.celrep.2023.112046

Gallagher, T.K., Baird, A.W., Winter, D.C., 2009. Constitutive basal and stimulated human small bowel contractility is enhanced in obesity. Ann Surg Innov Res 3, 4. DOI: 10.1186/1750-1164-3-4

Gao, Q., Horvath, T.L., 2008. Cross-talk between estrogen and leptin signaling in the hypothalamus. Am J Physiol Endocrinol Metab 294, E817-826. DOI: 10.1152/ajpendo.00733.2007

Garduño, R.C., Däbritz, J., 2021. New insights on CD8+ T cells in inflammatory bowel disease and therapeutic approaches. Frontiers in Immunology 12, 738762. DOI: 10.3389/fimmu.2021.738762

Garidou, L., Pomié, C., Klopp, P., Waget, A., Charpentier, J., Aloulou, M., Giry, A., Serino, M., Stenman, L., Lahtinen, S., Dray, C., Iacovoni, J.S., Courtney, M., Collet, X., Amar, J., Servant, F., Lelouvier, B., Valet, P., Eberl, G., Fazilleau, N., Douin-Echinard, V., Heymes, C., Burcelin, R., 2015. The gut microbiota regulates intestinal CD4 T cells expressing RORγt and controls metabolic disease. Cell Metabolism 22, 100–112. DOI: 10.1016/j.cmet.2015.06.001

Gay, D., Maddon, P., Sekaly, R., Talle, M.A., Godfrey, M., Long, E., Goldstein, G., Chess, L., Axel, R., Kappler, J., 1987. Functional interaction between human T-

cell protein CD4 and the major histocompatibility complex HLA-DR antigen. Nature 328, 626–629. DOI: 10.1038/328626a0

Gerbe, F., Sidot, E., Smyth, D.J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., Bruschi, M., Harcus, Y., Zimmermann, V.S., Taylor, N., Maizels, R.M., Jay, P., 2016. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 529, 226–230. DOI: 10.1038/nature16527

Gerner, M.Y., Kastenmuller, W., Ifrim, I., Kabat, J., Germain, R.N., 2012. Histo-cytometry: a method for highly multiplex quantitative tissue imaging analysis applied to dendritic cell subset microanatomy in lymph nodes. Immunity 37, 364–376. DOI: 10.1016/j.immuni.2012.07.011

Gerner, M.Y., Torabi-Parizi, P., Germain, R.N., 2015. Strategically localized dendritic cells promote rapid T cell responses to lymph-borne particulate antigens. Immunity 42, 172–185. DOI: 10.1016/j.immuni.2014.12.024

Gershon, R.K., Kondo, K., 1970. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 18, 723–737.

Ginhoux, F., Liu, K., Helft, J., Bogunovic, M., Greter, M., Hashimoto, D., Price, J., Yin, N., Bromberg, J., Lira, S.A., Stanley, E.R., Nussenzweig, M., Merad, M., 2009. The origin and development of nonlymphoid tissue $CD103^+$ DCs. J Exp Med 206, 3115–3130. DOI: 10.1084/jem.20091756

Goldfine, A.B., Shoelson, S.E., 2017. Therapeutic approaches targeting inflammation for diabetes and associated cardiovascular risk. J Clin Invest 127, 83–93. DOI: 10.1172/JCI88884

Gomez-Bris, R., Saez, A., Herrero-Fernandez, B., Rius, C., Sanchez-Martinez, H., Gonzalez-Granado, J.M., 2023. CD4 T-cell subsets and the pathophysiology of inflammatory bowel disease. International Journal of Molecular Sciences 24, 2696. DOI: 10.3390/ijms24032696

Gondek, D.C., Lu, L.-F., Quezada, S.A., Sakaguchi, S., Noelle, R.J., 2005. Cutting edge: contact-mediated suppression by $CD4^+CD25^+$ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol 174, 1783–1786. DOI: 10.4049/jimmunol.174.4.1783

Goverse, G., Labao-Almeida, C., Ferreira, M., Molenaar, R., Wahlen, S., Konijn, T., Koning, J., Veiga-Fernandes, H., Mebius, R.E., 2016. Vitamin A controls the presence of $ROR\gamma^+$ innate lymphoid cells and lymphoid tissue in the small

intestine. The Journal of Immunology 196, 5148–5155. DOI: 10.4049/jimmunol.1501106

Grajales-Reyes, G.E., Iwata, A., Albring, J., Wu, X., Tussiwand, R., Kc, W., Kretzer, N.M., Briseño, C.G., Durai, V., Bagadia, P., Haldar, M., Schönheit, J., Rosenbauer, F., Murphy, T.L., Murphy, K.M., 2015. Batf3 maintains autoactivation of Irf8 for commitment of a CD8α^+ conventional DC clonogenic progenitor. Nat Immunol 16, 708–717. DOI: 10.1038/ni.3197

Grakoui, A., Bromley, S.K., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., Dustin, M.L., 1999. The immunological synapse: a molecular machine controlling T cell activation 285.

Green, W.D., Beck, M.A., 2017. Obesity impairs the adaptive immune response to influenza iirus. Ann Am Thorac Soc 14, S406–S409. DOI: 10.1513/AnnalsATS.201706-447AW

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J.E., Roncarolo, M.G., 1997. A CD4$^+$ T cell subset inhibits antigen-specific T cell responses and prevents colitis. Nature 389, 737–742. DOI: 10.1038/39614

Gruber, L., Kisling, S., Lichti, P., Martin, F., May, S., Klingenspor, M., Lichtenegger, M., Rychlik, M., Haller, D., 2013. High fat diet accelerates pathogenesis of murine Crohn's disease-like ileitis independently of obesity. PloS one 8. DOI: 10.1371/journal.pone.0071661

Grundy, S.M., 2016. Metabolic syndrome update. Trends Cardiovasc Med 26, 364–373. DOI: 10.1016/j.tcm.2015.10.004

Gu, Y., Guo, X., Sun, S., Che, H., 2022. High-fat diet-induced obesity aggravates food allergy by intestinal barrier destruction and inflammation. Int Arch Allergy Immunol 183, 80–92. DOI: 10.1159/000517866

Guh, D.P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C.L., Anis, A.H., 2009. The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. BMC Public Health 9, 88. DOI: 10.1186/1471-2458-9-88

Guilliams, M., Dutertre, C.-A., Scott, C.L., McGovern, N., Sichien, D., Chakarov, S., Van Gassen, S., Chen, J., Poidinger, M., De Prijck, S., Tavernier, S.J., Low, I., Irac, S.E., Mattar, C.N., Sumatoh, H.R., Low, G.H.L., Chung, T.J.K., Chan, D.K.H., Tan, K.K., Hon, T.L.K., Fossum, E., Bogen, B., Choolani, M., Chan, J.K.Y., Larbi, A., Luche, H., Henri, S., Saeys, Y., Newell, E.W., Lambrecht, B.N.,

Malissen, B., Ginhoux, F., 2016. Unsupervised high-dimensional analysis aligns dendritic cells across tissues and species. Immunity 45, 669–684. DOI: 10.1016/j.immuni.2016.08.015

Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S.H., Onai, N., Schraml, B.U., Segura, E., Tussiwand, R., Yona, S., 2014. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol 14, 571–578. DOI: 10.1038/nri3712

Gunn, M.D., Kyuwa, S., Tam, C., Kakiuchi, T., Matsuzawa, A., Williams, L.T., Nakano, H., 1999. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. J Exp Med 189, 451–460. DOI: 10.1084/jem.189.3.451

Hall, J.E., do Carmo, J.M., da Silva, A.A., Wang, Z., Hall, M.E., 2015. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res 116, 991–1006. DOI: 10.1161/CIRCRESAHA.116.305697

Halliday, N., Williams, C., Kennedy, A., Waters, E., Pesenacker, A.M., Soskic, B., Hinze, C., Hou, T.Z., Rowshanravan, B., Janman, D., Walker, L.S.K., Sansom, D.M., 2020. CD86 is a selective CD28 ligand supporting FoxP3$^+$ regulatory T cell homeostasis in the presence of high levels of CTLA-4. Front Immunol 11, 600000. DOI: 10.3389/fimmu.2020.600000

Hamade, H., Stamps, J.T., Stamps, D.T., More, S.K., Thomas, L.S., Blackwood, A.Y., Lahcene, N.L., Castanon, S.L., Salumbides, B.C., Shimodaira, Y., Goodridge, H.S., Targan, S.R., Michelsen, K.S., 2022. BATF3 protects against metabolic syndrome and maintains intestinal epithelial homeostasis. Front. Immunol. 13, 841065. DOI: 10.3389/fimmu.2022.841065

Harding, F.A., McArthur, J.G., Gross, J.A., Raulet, D.H., Allison, J.P., 1992. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature 356, 607–609. DOI: 10.1038/356607a0

Haribhai, D., Williams, J.B., Jia, S., Nickerson, D., Schmitt, E.G., Edwards, B., Ziegelbauer, J., Yassai, M., Li, S.-H., Relland, L.M., Wise, P.M., Chen, A., Zheng, Y.-Q., Simpson, P.M., Gorski, J., Salzman, N.H., Hessner, M.J., Chatila, T.A., Williams, C.B., 2011. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. Immunity 35, 109–122. DOI: 10.1016/j.immuni.2011.03.029

Hataye, J., Moon, J.J., Khoruts, A., Reilly, C., Jenkins, M.K., 2006. Naive and memory CD4+ T cell survival controlled by clonal abundance. Science 312, 114–116. DOI: 10.1126/science.1124228

Hayashi, K., Tsujiguchi, H., Hori, D., Yamada, Y., Shimizu, Y., Nguyen, T.T.T., Hibino, Y., Kambayashi, Y., Hara, A., Nakamura, H., 2021. The association between overweight and prevalence of food allergy in Japanese children: a cross-sectional study. Environ Health Prev Med 26, 44. DOI: 10.1186/s12199-021-00960-2

He, S., Kahles, F., Rattik, S., Nairz, M., McAlpine, C.S., Anzai, A., Selgrade, D., Fenn, A.M., Chan, C.T., Mindur, J.E., Valet, C., Poller, W.C., Halle, L., Rotllan, N., Iwamoto, Y., Wojtkiewicz, G.R., Weissleder, R., Libby, P., Fernández-Hernando, C., Drucker, D.J., Nahrendorf, M., Swirski, F.K., 2019. Gut intraepithelial T cells calibrate metabolism and accelerate cardiovascular disease. Nature 566, 115–119. DOI: 10.1038/s41586-018-0849-9

Hedrick, S.M., Nielsen, E.A., Kavaler, J., Cohen, D.I., Davis, M.M., 1984. Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. Nature 308, 153–158. DOI: 10.1038/308153a0

Heier, E.-C., Meier, A., Julich-Haertel, H., Djudjaj, S., Rau, M., Tschernig, T., Geier, A., Boor, P., Lammert, F., Lukacs-Kornek, V., 2017. Murine CD103+ dendritic cells protect against steatosis progression towards steatohepatitis. Journal of Hepatology 66, 1241–1250. DOI: 10.1016/j.jhep.2017.01.008

Henri, S., Poulin, L.F., Tamoutounour, S., Ardouin, L., Guilliams, M., de Bovis, B., Devilard, E., Viret, C., Azukizawa, H., Kissenpfennig, A., Malissen, B., 2010. CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. J Exp Med 207, 189–206. DOI: 10.1084/jem.20091964

Hernández-García, E., Cueto, F.J., Cook, E.C.L., Redondo-Urzainqui, A., Charro-Zanca, S., Robles-Vera, I., Conde-Garrosa, R., Nikolić, I., Sabio, G., Sancho, D., Iborra, S., 2022. Conventional type 1 dendritic cells protect against age-related adipose tissue dysfunction and obesity. Cell Mol Immunol 19, 260–275. DOI: 10.1038/s41423-021-00812-7

Herrera, B.M., Lindgren, C.M., 2010. The genetics of obesity. Curr Diab Rep 10, 498–505. DOI: 10.1007/s11892-010-0153-z

Hersoug, L. -G., Linneberg, A., 2007. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? Allergy 62, 1205–1213. DOI: 10.1111/j.1398-9995.2007.01506.x

Heymsfield, S.B., Wadden, T.A., 2017. Mechanisms, pathophysiology, and management of obesity. N Engl J Med 376, 254–266. DOI: 10.1056/NEJMra1514009

Hickman, H.D., Takeda, K., Skon, C.N., Murray, F.R., Hensley, S.E., Loomis, J., Barber, G.N., Bennink, J.R., Yewdell, J.W., 2008. Direct priming of antiviral $CD8^+$ T cells in the peripheral interfollicular region of lymph nodes. Nat Immunol 9, 155–165. DOI: 10.1038/ni1557

Hildebrandt, M.A., Hoffmann, C., Sherrill–Mix, S.A., Keilbaugh, S.A., Hamady, M., Chen, Y., Knight, R., Ahima, R.S., Bushman, F., Wu, G.D., 2009. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 137, 1716-1724.e2. DOI: 10.1053/j.gastro.2009.08.042

Hildner, K., Edelson, B.T., Purtha, W.E., Diamond, M., Matsushita, H., Kohyama, M., Calderon, B., Schraml, B.U., Unanue, E.R., Diamond, M.S., Schreiber, R.D., Murphy, T.L., Murphy, K.M., 2008. Batf3 deficiency reveals a critical role for $CD8alpha^+$ dendritic cells in cytotoxic T cell immunity. Science 322, 1097–1100. DOI: 10.1126/science.1164206

Hildreth, A.D., Ma, F., Wong, Y.Y., Sun, R., Pellegrini, M., O'Sullivan, T.E., 2021. Single cell sequencing of human white adipose tissue identifies novel cell states in health and obesity. Nat Immunol 22, 639–653. DOI: 10.1038/s41590-021-00922-4

Hill, J.O., Wyatt, H.R., Peters, J.C., 2012. Energy balance and obesity. Circulation 126, 126–132. DOI: 10.1161/CIRCULATIONAHA.111.087213

Hochrein, H., Shortman, K., Vremec, D., Scott, B., Hertzog, P., O'Keeffe, M., 2001. Differential production of IL-12, IFN-alpha, and IFN-gamma by mouse dendritic cell subsets. J Immunol 166, 5448–5455. DOI: 10.4049/jimmunol.166.9.5448

Hong, C.-P., Park, A., Yang, B.-G., Yun, C.H., Kwak, M.-J., Lee, G.-W., Kim, J.-H., Jang, M.S., Lee, E.-J., Jeun, E.-J., You, G., Kim, K.S., Choi, Y., Park, J.-H., Hwang, D., Im, S.-H., Kim, J.F., Kim, Y.-K., Seoh, J.-Y., Surh, C.D., Kim, Y.-M., Jang, M.H., 2017. Gut-specific delivery of T-helper 17 cells reduces oesity and

insulin resistance in mice. Gastroenterology 152, 1998–2010. DOI: 10.1053/j.gastro.2017.02.016

Hong, S.W., Krueger, P.D., Osum, K.C., Dileepan, T., Herman, A., Mueller, D.L. and Jenkins, M.K., 2022. Immune tolerance of food is mediated by layers of $CD4^+$ T cell dysfunction. Nature, 607(7920), pp.762-768. DOI: 10.1038/s41586-022-04916-6

Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science 299, 1057–1061. DOI: 10.1126/science.1079490

Hotamisligil, G.S., 2010. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 140, 900–917. DOI: 10.1016/j.cell.2010.02.034

Hotamisligil, G.S., Shargill, N.S., Spiegelman, B.M., 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259, 87–91. DOI: 10.1126/science.7678183

Hruby, A., Hu, F.B., 2015. The epidemiology of obesity: a big picture. Pharmacoeconomics 33, 673–689. DOI: 10.1007/s40273-014-0243-x

Hsieh, C.-S., Zheng, Y., Liang, Y., Fontenot, J.D., Rudensky, A.Y., 2006. An intersection between the self-reactive regulatory and nonregulatory T cell receptor repertoires. Nat Immunol 7, 401–410. DOI: 10.1038/ni1318

Huehn, J., Siegmund, K., Lehmann, J.C.U., Siewert, C., Haubold, U., Feuerer, M., Debes, G.F., Lauber, J., Frey, O., Przybylski, G.K., Niesner, U., de la Rosa, M., Schmidt, C.A., Bräuer, R., Buer, J., Scheffold, A., Hamann, A., 2004. Developmental stage, phenotype, and migration distinguish naive- and effector/memory-like $CD4^+$ regulatory T cells. J Exp Med 199, 303–313. DOI: 10.1084/jem.20031562

Hui, E., Cheung, J., Zhu, J., Su, X., Taylor, M.J., Wallweber, H.A., Sasmal, D.K., Huang, J., Kim, J.M., Mellman, I., Vale, R.D., 2017. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science 355, 1428–1433. DOI: 10.1126/science.aaf1292

Hung, C.-T., Ma, C., Panda, S.K., Trsan, T., Hodel, M., Frein, J., Foster, A., Sun, S., Wu, H.-T., Kern, J., Mishra, R., Jain, U., Ho, Y.-C., Colonna, M., Stappenbeck, T.S., Liu, T.-C., 2024. Western diet reduces small intestinal intraepithelial lymphocytes via FXR-Interferon pathway. Mucosal Immunology 17, 1019–1028. DOI: 10.1016/j.mucimm.2024.07.001

Husby, S., Jensenius, J.C., Svehag, S.E., 1985. Passage of undegraded dietary antigen into the blood of healthy adults. Quantification, estimation of size distribution, and relation of uptake to levels of specific antibodies. Scand J Immunol 22, 83–92. DOI: 10.1111/j.1365-3083.1985.tb01862.x

Hussain, M., Bonilla-Rosso, G., Kwong Chung, C.K.C., Bäriswyl, L., Rodriguez, M.P., Kim, B.S., Engel, P., Noti, M., 2019. High dietary fat intake induces a microbiota signature that promotes food allergy. Journal of Allergy and Clinical Immunology 144, 157-170.e8. DOI: 10.1016/j.jaci.2019.01.043

Hwang, L.-L., Wang, C.-H., Li, T.-L., Chang, S.-D., Lin, L.-C., Chen, C.-P., Chen, C.-T., Liang, K.-C., Ho, I.-K., Yang, W.-S., Chiou, L.-C., 2010. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. Obesity (Silver Spring) 18, 463–469. DOI: 10.1038/oby.2009.273

Iborra, S., Izquierdo, H.M., Martínez-López, M., Blanco-Menéndez, N., Reis e Sousa, C., Sancho, D., 2012. The DC receptor DNGR-1 mediates cross-priming of CTLs during vaccinia virus infection in mice. J Clin Invest. 122(5):1628-43. DOI: 10.1172/JCI60660

Itano, A.A., McSorley, S.J., Reinhardt, R.L., Ehst, B.D., Ingulli, E., Rudensky, A.Y., Jenkins, M.K., 2003. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. Immunity 19, 47–57. DOI: 10.1016/s1074-7613(03)00175-4

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., Song, S.-Y., 2004. Retinoic acid imprints gut-homing specificity on T cells. Immunity 21, 527–538. DOI: 10.1016/j.immuni.2004.08.011

Iyengar, N.M., Gucalp, A., Dannenberg, A.J., Hudis, C.A., 2016. Obesity and cancer mechanisms: tumor microenvironment and inflammation. J Clin Oncol 34, 4270–4276. DOI: 10.1200/JCO.2016.67.4283

Jaiswal, N., Agrawal, S., Agrawal, A., 2019. High fructose-induced metabolic changes enhance inflammation in human dendritic cells. Clin Exp Immunol 197, 237–249. DOI: 10.1111/cei.13299

Janeway, C.A., 1989. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54 Pt 1, 1–13. DOI: 10.1101/sqb.1989.054.01.003

Johansson, M.E.V., Sjövall, H., Hansson, G.C., 2013. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol 10, 352–361. DOI: 10.1038/nrgastro.2013.35

Johnson, A.M.F., Costanzo, A., Gareau, M.G., Armando, A.M., Quehenberger, O., Jameson, J.M., Olefsky, J.M., 2015. High fat diet causes depletion of intestinal eosinophils associated with intestinal permeability. PLoS One 10, e0122195. DOI: 10.1371/journal.pone.0122195

Jones, J.M., Reinke, S.N., Mousavi-Derazmahalleh, M., Garssen, J., Jenmalm, M.C., Srinivasjois, R., Silva, D., Keelan, J., Prescott, S.L., Palmer, D.J., Christophersen, C.T., 2024. Maternal prebiotic supplementation during pregnancy and lactation modifies the microbiome and short chain fatty acid profile of both mother and infant. Clin Nutr 43, 969–980. DOI: 10.1016/j.clnu.2024.02.030

Josefowicz, S.Z., Lu, L.-F., Rudensky, A.Y., 2012. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 30, 531–564. DOI: 10.1146/annurev.immunol.25.022106.141623

June, C.H., Ledbetter, J.A., Gillespie, M.M., Lindsten, T., Thompson, C.B., 1987. T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. Mol Cell Biol 7, 4472–4481. DOI: 10.1128/mcb.7.12.4472-4481.1987

Jung, S., Unutmaz, D., Wong, P., Sano, G.-I., De los Santos, K., Sparwasser, T., Wu, S., Vuthoori, S., Ko, K., Zavala, F., Pamer, E.G., Littman, D.R., Lang, R.A., 2002. In vivo depletion of $CD11c^+$ dendritic cells abrogates priming of $CD8^+$ T cells by exogenous cell-associated antigens. Immunity 17, 211–220. DOI: 10.1016/s1074-7613(02)00365-5

Justice, M.J., 2024. Sex matters in preclinical research. Dis Model Mech 17, dmm050759. DOI: 10.1242/dmm.050759

Jutel, M., Akdis, C., 2008. T-cell regulatory mechanisms in specific immunotherapy. Chem Immunol Allergy 94, 158–177. DOI: 10.1159/000155000

Kaech, S.M., Wherry, E.J., 2007. Heterogeneity and cell-fate decisions in effector and memory $CD8^+$ T cell differentiation during viral infection. Immunity 27, 393–405. DOI: 10.1016/j.immuni.2007.08.007

Kahn, S.E., Hull, R.L., Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444, 840–846. DOI: 10.1038/nature05482

Kaliński, P., Hilkens, C.M., Wierenga, E.A., Kapsenberg, M.L., 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. Immunol Today 20, 561–567. DOI: 10.1016/s0167-5699(99)01547-9

Kanamori, M., Nakatsukasa, H., Okada, M., Lu, Q., Yoshimura, A., 2016. Induced regulatory T cells: their development, stability, and applications. Trends Immunol 37, 803–811. DOI: 10.1016/j.it.2016.08.012

Kapsenberg, M.L., 2003. Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol 3, 984–993. DOI: 10.1038/nri1246

Karpuzoglu-Sahin, E., Zhi-Jun, Y., Lengi, A., Sriranganathan, N., Ansar Ahmed, S., 2001. Effects of long-term estrogen treatment on IFN-gamma, IL-2 and IL-4 gene expression and protein synthesis in spleen and thymus of normal C57BL/6 mice. Cytokine 14, 208–217. DOI: 10.1006/cyto.2001.0876

Kastl, A.J., Terry, N.A., Wu, G.D., Albenberg, L.G., 2020. The structure and function of the human small intestinal microbiota: current understanding and future directions. Cell Mol Gastroenterol Hepatol 9, 33–45. DOI: 10.1016/j.jcmgh.2019.07.006

Kato, H., Sato, S., Yoneyama, M., Yamamoto, M., Uematsu, S., Matsui, K., Tsujimura, T., Takeda, K., Fujita, T., Takeuchi, O., Akira, S., 2005. Cell type-specific involvement of RIG-I in antiviral response. Immunity 23, 19–28. DOI: 10.1016/j.immuni.2005.04.010

Kawano, Y., Nakae, J., Watanabe, N., Kikuchi, T., Tateya, S., Tamori, Y., Kaneko, M., Abe, T., Onodera, M., Itoh, H., 2016. Colonic pro-inflammatory macrophages cause insulin resistance in an intestinal Ccl2/Ccr2-dependent manner. Cell Metab 24, 295–310. DOI: 10.1016/j.cmet.2016.07.009

Kessler, R.C., Berglund, P.A., Chiu, W.T., Deitz, A.C., Hudson, J.I., Shahly, V., Aguilar-Gaxiola, S., Alonso, J., Angermeyer, M.C., Benjet, C., Bruffaerts, R., de Girolamo, G., de Graaf, R., Haro, J.M., Kovess-Masfety, V., O'Neill, S., Posada-Villa, J., Sasu, C., Scott, K., Viana, M.C., Xavier, M., 2013. The prevalence and correlates of binge eating disorder in the WHO World Mental Health Surveys. Biol Psychiatry 73, 904–914. DOI: 10.1016/j.biopsych.2012.11.020

Khan, D., Ansar Ahmed, S., 2016. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. Front. Immunol. 6. DOI: 10.3389/fimmu.2015.00635

Khan, D., Cowan, C., Ahmed, S.A., 2012. Estrogen and ssignaling in the cells of immune system. Advances in Neuroimmune Biology 3, 73–93. DOI: 10.3233/NIB-2012-012039

Khan, S., Luck, H., Winer, S., Winer, D.A., 2021. Emerging concepts in intestinal immune control of obesity-related metabolic disease. Nat Commun 12, 2598. DOI: 10.1038/s41467-021-22727-7

Kim, Y.C., Bhairavabhotla, R., Yoon, J., Golding, A., Thornton, A.M., Tran, D.Q., Shevach, E.M., 2012. Oligodeoxynucleotides stabilize Helios-expressing Foxp3$^+$ human T regulatory cells during in vitro expansion. Blood 119, 2810–2818. DOI: 10.1182/blood-2011-09-377895

King, L.K., March, L., Anandacoomarasamy, A., 2013. Obesity & osteoarthritis. Indian J Med Res 138, 185–193.

Kingston, D., Schmid, M.A., Onai, N., Obata-Onai, A., Baumjohann, D., Manz, M.G., 2009. The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis. Blood 114, 835–843. DOI: 10.1182/blood-2009-02-206318

Kinnebrew, M.A., Buffie, C.G., Diehl, G.E., Zenewicz, L.A., Leiner, I., Hohl, T.M., Flavell, R.A., Littman, D.R., Pamer, E.G., 2012. Interleukin 23 production by intestinal CD103$^+$CD11b$^+$ dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. Immunity 36, 276–287. DOI: 10.1016/j.immuni.2011.12.011

Kisielow, P., Blüthmann, H., Staerz, U.D., Steinmetz, M., von Boehmer, H., 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes. Nature 333, 742–746. DOI: 10.1038/333742a0

Klein, L., Kyewski, B., Allen, P.M., Hogquist, K.A., 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). Nat Rev Immunol 14, 377–391. DOI: 10.1038/nri3667

Klein, S.L., 2000. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 24, 627–638. DOI: 10.1016/s0149-7634(00)00027-0

Klop, B., Elte, J.W.F., Cabezas, M.C., 2013. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients 5, 1218–1240. DOI: 10.3390/nu5041218

Knoop, K.A., Newberry, R.D., 2012. Isolated lymphoid follicles are dynamic reservoirs for the induction of intestinal IgA. Front. Immunol. 3. DOI: 10.3389/fimmu.2012.00084

Komano, H., Fujiura, Y., Kawaguchi, M., Matsumoto, S., Hashimoto, Y., Obana, S., Mombaerts, P., Tonegawa, S., Yamamoto, H., Itohara, S., 1995. Homeostatic regulation of intestinal epithelia by intraepithelial gamma delta T cells. Proc. Natl. Acad. Sci. U.S.A. 92, 6147–6151. DOI: 10.1073/pnas.92.13.6147

Kovats, S., 2015. Estrogen receptors regulate innate immune cells and signaling pathways. Cell Immunol 294, 63–69. DOI: 10.1016/j.cellimm.2015.01.018

Kovats, S., 2012. Estrogen receptors regulate an inflammatory pathway of dendritic cell differentiation: Mechanisms and implications for immunity. Hormones and Behavior, Special Issue: The Neuroendocrine-Immune Axis in Health and Disease 62, 254–262. DOI: 10.1016/j.yhbeh.2012.04.011

Kovesdy, C.P., Furth, S.L., Zoccali, C., World Kidney Day Steering Committee, 2017. Obesity and kidney disease: hidden consequences of the epidemic. J Nephrol 30, 1–10. DOI: 10.1007/s40620-017-0377-y

Kretschmer, K., Apostolou, I., Hawiger, D., Khazaie, K., Nussenzweig, M.C., von Boehmer, H., 2005. Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 6, 1219–1227. DOI: 10.1038/ni1265

Krishnaswamy, J.K., Alsén, S., Yrlid, U., Eisenbarth, S.C., Williams, A., 2018. Determination of T follicular helper cell fate by dendritic cells. Front Immunol 9, 2169. DOI: 10.3389/fimmu.2018.02169

Kurashima, Y., Kiyono, H., 2014. New era for mucosal mast cells: their roles in inflammation, allergic immune responses and adjuvant development. Exp Mol Med 46, e83–e83. DOI: 10.1038/emm.2014.7

Kurts, C., Cannarile, M., Klebba, I., Brocker, T., 2001. Dendritic cells are sufficient to cross-present self-antigens to CD8 T cells in vivo. J Immunol 166, 1439–1442. DOI: 10.4049/jimmunol.166.3.1439

Lack G., 2012. Update on risk factors for food allergy. J Allergy Clin Immunol. 129(5):1187-97. DOI: 10.1016/j.jaci.2012.02.036

Lainez, N.M., Jonak, C.R., Nair, M.G., Ethell, I.M., Wilson, E.H., Carson, M.J., Coss, D., 2018. Diet-induced obesity elicits macrophage infiltration and reduction in spine density in the hypothalami of male but not female mice. Front Immunol 9, 1992. DOI: 10.3389/fimmu.2018.01992

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Grosse, Y., Bianchini, F., Straif, K., International agency for research on ccancer handbook working group, 2016. Body fatness and cancer —Viewpoint of the IARC working group. N Engl J Med 375, 794–798. DOI: 10.1056/NEJMsr1606602

Laurenti, E., Göttgens, B., 2018. From haematopoietic stem cells to complex differentiation landscapes. Nature 553, 418–426. DOI: 10.1038/nature25022

Lécuyer, E., Le Roy, T., Gestin, A., Lacombe, A., Philippe, C., Ponnaiah, M., Huré, J.-B., Fradet, M., Ichou, F., Boudebbouze, S., Huby, T., Gautier, E., Rhimi, M., Maguin, E., Kapel, N., Gérard, P., Venteclef, N., Garlatti, M., Chassaing, B., Lesnik, P., 2021. Tolerogenic dendritic cells shape a transmissible gut microbiota that protects from metabolic diseases. Diabetes 70, 2067–2080. DOI: 10.2337/db20-1177

Lee, A.H., Dixit, V.D., 2020. Dietary regulation of immunity. Immunity 53, 510–523. DOI: 10.1016/j.immuni.2020.08.013

Lee, J.H., Kang, S.G., Kim, C.H., 2007. FoxP3$^+$ T cells undergo conventional first switch to lymphoid tissue homing receptors in thymus but accelerated second switch to nonlymphoid tissue homing receptors in secondary lymphoid tissues. J Immunol 178, 301–311. DOI: 10.4049/jimmunol.178.1.301

Lee, M., Gil, H., Cheon, E., Kim, S., Ryu, J., Khil, H., Kang, C., Park, S., Kang, S., Keum, N., Kwon, Y., 2021. Effect of food sensitivity on overweight assessed using food-specific serum immunoglobulin G Levels. BioChip J 15, 296–304. DOI: 10.1007/s13206-021-00028-x

Lélu, K., Laffont, S., Delpy, L., Paulet, P.-E., Périnat, T., Tschanz, S.A., Pelletier, L., Engelhardt, B., Guéry, J.-C., 2011. Estrogen receptor α signaling in T lymphocytes is required for estradiol-mediated inhibition of Th1 and Th17 cell differentiation and protection against experimental autoimmune encephalomyelitis. The Journal of Immunology 187, 2386–2393. DOI: 10.4049/jimmunol.1101578

Lenert, M.E., Chaparro, M.M., Burton, M.D., 2021. Homeostatic regulation of estrus cycle of young female mice on Western diet. J Endocr Soc 5, bvab010. DOI: 10.1210/jendso/bvab010

Lenz, M., Arts, I.C.W., Peeters, R.L.M., de Kok, T.M., Ertaylan, G., 2020. Adipose tissue in health and disease through the lens of its building blocks. Sci Rep 10, 10433. DOI: 10.1038/s41598-020-67177-1

Levings, M.K., Gregori, S., Tresoldi, E., Cazzaniga, S., Bonini, C., Roncarolo, M.G., 2005. Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not CD25+CD4+ Tr cells. Blood 105, 1162–1169. DOI: 10.1182/blood-2004-03-1211

Li, J., Zhou, J., Huang, H., Jiang, J., Zhang, T., Ni, C., 2023. Mature dendritic cells enriched in immunoregulatory molecules (mregDCs): A novel population in the tumour microenvironment and immunotherapy target. Clin Transl Med 13, e1199. DOI: 10.1002/ctm2.1199

Li, M.O., Wan, Y.Y., Sanjabi, S., Robertson, A.-K.L., Flavell, R.A., 2006. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 24, 99–146. DOI: 10.1146/annurev.immunol.24.021605.090737

Liu E., Yin X., Swaminathan A., Eisenbarth S., 2012. Antigen-presenting cells in food tolerance and allergy. Front Immunol. 8;11:616020. DOI: 10.3389/fimmu.2020.616020

Liu, H., Buenafe, A., Matejuk, A., Ito, A., Zamora, A., Dwyer, J., Vandenbark, A., Offner, H., 2002. Estrogen inhibition of EAE involves effects on dendritic cell function. Journal of neuroscience research 70. DOI: 10.1002/jnr.10409

Liu, K., Nussenzweig, M.C., 2010. Origin and development of dendritic cells. Immunological Reviews 234, 45–54. DOI: 10.1111/j.0105-2896.2009.00879.x

Liu, K., Victora, G.D., Schwickert, T.A., Guermonprez, P., Meredith, M.M., Yao, K., Chu, F.-F., Randolph, G.J., Rudensky, A.Y., Nussenzweig, M., 2009. In vivo analysis of dendritic cell development and homeostasis. Science 324, 392–397. DOI: 10.1126/science.1170540

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25, 402–408. DOI: 10.1006/meth.2001.1262

Loomans-Kropp, H.A., Umar, A., 2023. Analysis of body mass index in early and middle adulthood and estimated risk of gastrointestinal cancer. JAMA Network Open 6, e2310002. DOI: 10.1001/jamanetworkopen.2023.10002

López, M., Tena-SempereM, M., 2015. Estrogens and the control of energy homeostasis: a brain perspective. Trends in endocrinology and metabolism: TEM 26. DOI: 10.1016/j.tem.2015.06.003

Luck, H., Khan, S., Kim, J.H., Copeland, J.K., Revelo, X.S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M.K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., Gaisano, H., Allard, J.P., Guttman, D.S., Conner, M.E., Winer, S., Winer, D.A., 2019. Gut-associated IgA^+ immune cells regulate obesity-related insulin resistance. Nat Commun 10, 3650. DOI: 10.1038/s41467-019-11370-y

Luck, H., Tsai, S., Chung, J., Clemente-Casares, X., Ghazarian, M., Revelo, X.S., Lei, H., Luk, C.T., Shi, S.Y., Surendra, A., Copeland, J.K., Ahn, J., Prescott, D., Rasmussen, B.A., Chng, M.H.Y., Engleman, E.G., Girardin, S.E., Lam, T.K.T., Croitoru, K., Dunn, S., Philpott, D.J., Guttman, D.S., Woo, M., Winer, S., Winer, D.A., 2015. Regulation of obesity-related insulin resistance with gut anti-inflammatory agents. Cell Metabolism 21, 527–542. DOI: 10.1016/j.cmet.2015.03.001

Luckheeram, R.V., Zhou, R., Verma, A.D., Xia, B., 2012. $CD4^+$ T cells: differentiation and functions. Clinical and Developmental Immunology 2012, 1–12. DOI: 10.1155/2012/925135

Luda, K.M., Joeris, T., Persson, E.K., Rivollier, A., Demiri, M., Sitnik, K.M., Pool, L., Holm, J.B., Melo-Gonzalez, F., Richter, L., Lambrecht, B.N., Kristiansen, K., Travis, M.A., Svensson-Frej, M., Kotarsky, K., Agace, W.W., 2016. IRF8 transcription-factor-dependent classical dendritic cells are essential for intestinal T cell homeostasis. Immunity 44, 860–874. DOI: 10.1016/j.immuni.2016.02.008

Luppino, F.S., de Wit, L.M., Bouvy, P.F., Stijnen, T., Cuijpers, P., Penninx, B.W.J.H., Zitman, F.G., 2010. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry 67, 220–229. DOI: 10.1001/archgenpsychiatry.2010.2

Mabbott, N.A., Donaldson, D.S., Ohno, H., Williams, I.R., Mahajan, A., 2013. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. Mucosal Immunol 6, 666–677. DOI: 10.1038/mi.2013.30

Macpherson, A.J., McCoy, K.D., Johansen, F.-E., Brandtzaeg, P., 2008. The immune geography of IgA induction and function. Mucosal Immunol 1, 11–22. DOI: 10.1038/mi.2007.6

Mahnke, K., Johnson, T.S., Ring, S., Enk, A.H., 2007. Tolerogenic dendritic cells and regulatory T cells: a two-way relationship. J Dermatol Sci 46, 159–167. DOI: 10.1016/j.jdermsci.2007.03.002

Maier, B., Leader, A.M., Chen, S.T., Tung, N., Chang, C., LeBerichel, J., Chudnovskiy, A., Maskey, S., Walker, L., Finnigan, J.P., Kirkling, M.E., Reizis, B., Ghosh, S., D'Amore, N.R., Bhardwaj, N., Rothlin, C.V., Wolf, A., Flores, R., Marron, T., Rahman, A.H., Kenigsberg, E., Brown, B.D., Merad, M., 2020. A conserved dendritic-cell regulatory program limits antitumour immunity. Nature 580, 257–262. DOI: 10.1038/s41586-020-2134-y

Maldonado-López, R., De Smedt, T., Michel, P., Godfroid, J., Pajak, B., Heirman, C., Thielemans, K., Leo, O., Urbain, J., Moser, M., 1999. CD8alpha$^+$ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. J Exp Med 189, 587–592. DOI: 10.1084/jem.189.3.587

Mao, J., Hu, X., Xiao, Y., Yang, C., Ding, Y., Hou, N., Wang, J., Cheng, H., Zhang, X., 2013. Overnutrition stimulates intestinal epithelium proliferation through β-catenin signaling in obese mice. Diabetes 62, 3736–3746. DOI: 10.2337/db13-0035

Marghoub, S., Sanaie, S., Sullman, M.J.M., Nejadghaderi, S.A., Safiri, S., Mohammadinasab, R., 2023. Obesity from a sign of being rich to a disease of the new age: A historical review. Health Sci Rep 6, e1670. DOI: 10.1002/hsr2.1670

Marie, J.C., Letterio, J.J., Gavin, M., Rudensky, A.Y., 2005. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4$^+$CD25$^+$ regulatory T cells. J Exp Med 201, 1061–1067. DOI: 10.1084/jem.20042276

Markle, J.G.M., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., von Bergen, M., McCoy, K.D., Macpherson, A.J., Danska, J.S., 2013. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 339, 1084–1088. DOI: 10.1126/science.1233521

Martínez-López, M., Iborra, S., Conde-Garrosa, R., Sancho, D., 2015. Batf3-dependent CD103+ dendritic cells are major producers of IL-12 that drive local Th1 immunity against Leishmania major infection in mice. Eur J Immunol 45, 119–129. DOI: 10.1002/eji.201444651

Mathur, A.N., Zirak, B., Boothby, I.C., Tan, M., Cohen, J.N., Mauro, T.M., Mehta, P., Lowe, M.M., Abbas, A.K., Ali, N., Rosenblum, M.D., 2019. Treg-Cell Control

of a CXCL5-IL-17 Inflammatory axis promotes hair-follicle-stem-cell differentiation during skin-barrier repair. Immunity 50, 655-667.e4. DOI: 10.1016/j.immuni.2019.02.013

Mattei, F., Schiavoni, G., Belardelli, F., Tough, D.F., 2001. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. J Immunol 167, 1179–1187. DOI: 10.4049/jimmunol.167.3.1179

Matteoli, G., Mazzini, E., Iliev, I.D., Mileti, E., Fallarino, F., Puccetti, P., Chieppa, M. and Rescigno, M., 2010. Gut CD103+ dendritic cells express indoleamine 2, 3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. Gut, 59(5), pp.595-604. DOI: 10.1136/gut.2009.185108

Matzinger, P., 2012. The evolution of the danger theory. Expert Review of Clinical Immunology 8, 311–317. DOI: 10.1586/eci.12.21

Matzinger, P., 2002. The danger model: a renewed sense of self. Science 296, 301–305. DOI: 10.1126/science.1071059

Mayer, J.U., Brown, S.L., MacDonald, A.S., Milling, S.W., 2020. Defined intestinal regions are drained by specific lymph nodes that mount distinct Th1 and Th2 responses against schistosoma mansoni eggs. Front. Immunol. 11, 592325. DOI: 10.3389/fimmu.2020.592325

Mazzini E., Massimiliano L., Penna G., Rescigno M., 2014. Oral tolerance can be established via gap junction transfer of fed antigens from $CX3CR1^{+}$ macrophages to $CD103^{+}$ dendritic cells. Immunity 40(2):248–61. DOI: 10.1016/j.immuni.2013.12.012

McDole, J.R., Wheeler, L.W., McDonald, K.G., Wang, B., Konjufca, V., Knoop, K.A., Newberry, R.D., Miller, M.J., 2012. Goblet cells deliver luminal antigen to $CD103^{+}$ dendritic cells in the small intestine. Nature 483, 345–349. DOI: 10.1038/nature10863

McKenna, H.J., Stocking, K.L., Miller, R.E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C.R., Lynch, D.H., Smith, J., Pulendran, B., Roux, E.R., Teepe, M., Lyman, S.D., Peschon, J.J., 2000. Mice lacking Flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood 95, 3489–3497.

Medzhitov, R., Janeway, C., 2000. Innate immunity. N Engl J Med 343, 338–344. DOI: 10.1056/NEJM200008033430506

Medzhitov, R., Schneider, D.S., Soares, M.P., 2012. Disease tolerance as a defense strategy. Science 335, 936–941. DOI: 10.1126/science.1214935

Meier, K.H.U., Trouillon, J., Li, H., Lang, M., Fuhrer, T., Zamboni, N., Sunagawa, S., Macpherson, A.J., Sauer, U., 2023. Metabolic landscape of the male mouse gut identifies different niches determined by microbial activities. Nat Metab 5, 968–980. DOI: 10.1038/s42255-023-00802-1

Mellor, A.L., Munn, D.H., 2004. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 4, 762–774. DOI: 10.1038/nri1457

Mempel, T.R., Marangoni, F., 2019. Guidance factors orchestrating regulatory T cell positioning in tissues during development, homeostasis, and response. Immunol Rev 289, 129–141. DOI: 10.1111/imr.12761

Merad, M., Sathe, P., Helft, J., Miller, J., Mortha, A., 2013. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the ssteady state and the inflamed setting. Annu. Rev. Immunol. 31, 563–604. DOI: 10.1146/annurev-immunol-020711-074950

Meredith, M.M., Liu, K., Darrasse-Jeze, G., Kamphorst, A.O., Schreiber, H.A., Guermonprez, P., Idoyaga, J., Cheong, C., Yao, K.-H., Niec, R.E., Nussenzweig, M.C., 2012. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. The Journal of Experimental Medicine 209, 1153. DOI: 10.1084/jem.20112675

Miller, A., Adeli, K., 2008. Dietary fructose and the metabolic syndrome. Curr Opin Gastroenterol 24, 204–209. DOI: 10.1097/MOG.0b013e3282f3f4c4

Miller, J.F., 1961. Immunological function of the thymus. Lancet 2, 748–749. DOI: 10.1016/s0140-6736(61)90693-6

Miller, M.J., Hejazi, A.S., Wei, S.H., Cahalan, M.D., Parker, I., 2004. T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. Proceedings of the National Academy of Sciences 101, 998–1003. DOI: 10.1073/pnas.0306407101

Minutti, C. M., Piot, C., Pereira da Costa, M., Chakravarty, P., Rogers, N., Huerga Encabo, H., Cardoso, A., Loong, J., Bessou, G., Mionnet, C., Langhorne, J., Bonnet, D., Dalod, M., Tomasello, E., & Reis E Sousa, C., 2024. Distinct ontogenetic lineages dictate cDC2 heterogeneity. Nature immunology, 25(3), 448–461. DOI: 10.1038/s41590-024-01745-9

Mombaerts, P., Iacomini, J., Johnson, R.S., Herrup, K., Tonegawa, S., Papaioannou, V.E., 1992. RAG-1-deficient mice have no mature B and T lymphocytes. Cell 68, 869–877. DOI: 10.1016/0092-8674(92)90030-g

Monteiro-Sepulveda, M., Touch, S., Mendes-Sá, C., André, S., Poitou, C., Allatif, O., Cotillard, A., Fohrer-Ting, H., Hubert, E.-L., Remark, R., Genser, L., Tordjman, J., Garbin, K., Osinski, C., Sautès-Fridman, C., Leturque, A., Clément, K., Brot-Laroche, E., 2015. Jejunal T cell inflammation in human obesity correlates with decreased enterocyte insulin signaling. Cell Metab 22, 113–124. DOI: 10.1016/j.cmet.2015.05.020

Moog, F., 1981. The lining of the small intestine. Sci Am 245, 154–158, 160, 162 et passiom. DOI: 10.1038/scientificamerican1181-154

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., O'Garra, A., 2001. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19, 683–765. DOI: 10.1146/annurev.immunol.19.1.683

Moreira, T.G., Mangani, D., Cox, L.M., Leibowitz, J., Lobo, Eduardo.L.C., Oliveira, M.A., Gauthier, C.D., Nakagaki, B.N., Willocq, V., Song, A., Guo, L., Lima, D.C.A., Murugaiyan, G., Butovsky, O., Gabriely, G., Anderson, A.C., Rezende, R.M., Faria, A.M.C., Weiner, H.L., 2021. PD-L1+ and XCR1+ dendritic cells are region-specific regulators of gut homeostasis. Nat Commun 12, 4907. DOI: 10.1038/s41467-021-25115-3

Moseman, E.A., Liang, X., Dawson, A.J., Panoskaltsis-Mortari, A., Krieg, A.M., Liu, Y.-J., Blazar, B.R., Chen, W., 2004. Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of $CD4^+CD25^+$ regulatory T cells. J Immunol 173, 4433–4442. DOI: 10.4049/jimmunol.173.7.4433

Mowat, A.M., Agace, W.W., 2014. Regional specialization within the intestinal immune system. Nat Rev Immunol 14, 667–685. DOI: 10.1038/nri3738

Mowat, A.M., Strobel, S., Drummond, H.E., Ferguson, A., 1982. Immunological responses to fed protein antigens in mice. I. Reversal of oral tolerance to ovalbumin by cyclophosphamide. Immunology 45, 105–113.

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., Cheroutre, H., 2007. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 317, 256–260. DOI: 10.1126/science.1145697

Müller, S., Lory, J., Corazza, N., Griffiths, G.M., Z'graggen, K., Mazzucchelli, L., Kappeler, A., Mueller, C., 1998. Activated $CD4^+$ and $CD8^+$ cytotoxic cells are present in increased numbers in the intestinal mucosa from patients with active inflammatory bowel disease. The American Journal of Pathology 152, 261.

Muzaki, A.R.B.M., Tetlak, P., Sheng, J., Loh, S.C., Setiagani, Y.A., Poidinger, M., Zolezzi, F., Karjalainen, K., Ruedl, C., 2016. Intestinal $CD103^+CD11b^-$ dendritic cells restrain colitis via IFN-γ-induced anti-inflammatory response in epithelial cells. Mucosal Immunology 9, 336–351. DOI: 10.1038/mi.2015.64

Nagareddy, P.R., Murphy, A.J., Stirzaker, R.A., Hu, Y., Yu, S., Miller, R.G., Ramkhelawon, B., Distel, E., Westerterp, M., Huang, L.-S., Schmidt, A.M., Orchard, T.J., Fisher, E.A., Tall, A.R., Goldberg, I.J., 2013. Hyperglycemia promotes myelopoiesis and impairs the resolution of atherosclerosis. Cell Metab 17, 695–708. DOI: 10.1016/j.cmet.2013.04.001

Naik, S.H., Sathe, P., Park, H.-Y., Metcalf, D., Proietto, A.I., Dakic, A., Carotta, S., O'Keeffe, M., Bahlo, M., Papenfuss, A., Kwak, J.-Y., Wu, L., Shortman, K., 2007. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. Nat Immunol 8, 1217–1226. DOI: 10.1038/ni1522

Nemazee, D., 2006. Receptor editing in lymphocyte development and central tolerance. Nat Rev Immunol 6, 728–740. DOI: 10.1038/nri1939

Netea, M.G., Joosten, L.A.B., Latz, E., Mills, K.H.G., Natoli, G., Stunnenberg, H.G., O'Neill, L.A.J., Xavier, R.J., 2016. Trained immunity: a program of innate immune memory in health and disease. Science 352, aaf1098. DOI: 10.1126/science.aaf1098

Nicholson, L.B., 2016. The immune system. Essays in Biochemistry 60, 275–301. DOI: 10.1042/EBC20160017

Nilsson, M.E., Vandenput, L., Tivesten, Å., Norlén, A.-K., Lagerquist, M.K., Windahl, S.H., Börjesson, A.E., Farman, H.H., Poutanen, M., Benrick, A., Maliqueo, M., Stener-Victorin, E., Ryberg, H., Ohlsson, C., 2015. Measurement of a comprehensive sex steroid profile in rodent serum by high-sensitive gas chromatography-tandem mass spectrometry. Endocrinology 156, 2492–2502. DOI: 10.1210/en.2014-1890

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T., Nagai, R.,

2009. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15, 914–920. DOI: 10.1038/nm.1964

Nishio, J., Baba, M., Atarashi, K., Tanoue, T., Negishi, H., Yanai, H., Habu, S., Hori, S., Honda, K., Taniguchi, T., 2015. Requirement of full TCR repertoire for regulatory T cells to maintain intestinal homeostasis. Proceedings of the National Academy of Sciences 112, 12770–12775. DOI: 10.1073/pnas.1516617112

Nobel Media, 2011. The nobel prize in physiology or medicine 2011. NobelPrize.org. URL https://www.nobelprize.org/prizes/medicine/2011/summary/ (accessed 10.10.2024).

Nylander, S., Kalies, I., 1999. Brefeldin A, but not monensin, completely blocks CD69 expression on mouse lymphocytes: efficacy of inhibitors of protein secretion in protocols for intracellular cytokine staining by flow cytometry. J Immunol Methods 224, 69–76. DOI: 10.1016/s0022-1759(99)00010-1

Obar, J.J., Khanna, K.M., Lefrançois, L., 2008. Endogenous naive CD8+ T cell precursor frequency regulates primary and memory responses to infection. Immunity 28, 859–869. DOI: 10.1016/j.immuni.2008.04.010

Ohl, L., Mohaupt, M., Czeloth, N., Hintzen, G., Kiafard, Z., Zwirner, J., Blankenstein, T., Henning, G., Förster, R., 2004. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity 21, 279–288. DOI: 10.1016/j.immuni.2004.06.014

Ohta, T., Sugiyama, M., Hemmi, H., Yamazaki, C., Okura, S., Sasaki, I., Fukuda, Y., Orimo, T., Ishii, K.J., Hoshino, K., Ginhoux, F., Kaisho, T., 2016. Crucial roles of XCR1-expressing dendritic cells and the XCR1-XCL1 chemokine axis in intestinal immune homeostasis. Sci Rep 6, 23505. DOI: 10.1038/srep23505

Olefsky, J.M., Glass, C.K., 2010. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol 72, 219–246. DOI: 10.1146/annurev-physiol-021909-135846

Owen, R.D., 1945. Immunogenetic consequences of vascular anastomoses between bovine twins. Science 102, 400–401. DOI: 10.1126/science.102.2651.400

Pantier, L.K., Li, J., Christian, C.A., 2019. Estrous cycle monitoring in mice with rapid data visualization and analysis. Bio Protoc 9, e3354. DOI: 10.21769/BioProtoc.3354

Papenfuss, P., Powell, N., McClain, M., Bedarf, A., Singh, S., Gienapp, I., Shawler, T., Whitacre, C., 2011. Estriol generates tolerogenic dendritic cells in vivo that protect against autoimmunity. Journal of immunology (Baltimore, Md. : 1950) 186. DOI: 10.4049/jimmunol.1001322

Park, C., Cheung, K.P., Limon, N., Costanzo, A., Barba, C., Miranda, N., Gargas, S., Johnson, A.M.F., Olefsky, J.M., Jameson, J.M., 2019. Obesity modulates intestinal intraepithelial T cell persistence, CD103 and CCR9 expression, and outcome in dextran sulfate sodium–induced colitis. The Journal of Immunology 203, 3427–3435. DOI: 10.4049/jimmunol.1900082

Pärnänen, K.M., Hultman, J., Markkanen, M., Satokari, R., Rautava, S., Lamendella, R., Wright, J., McLimans, C.J., Kelleher, S.L., Virta, M.P., 2022. Early-life formula feeding is associated with infant gut microbiota alterations and an increased antibiotic resistance load. The American Journal of Clinical Nutrition 115, 407–421. DOI: 10.1093/ajcn/nqab353

Patterson, S.J., Pesenacker, A.M., Wang, A.Y., Gillies, J., Mojibian, M., Morishita, K., Tan, R., Kieffer, T.J., Verchere, C.B., Panagiotopoulos, C., Levings, M.K., 2016. T regulatory cell chemokine production mediates pathogenic T cell attraction and suppression. J Clin Invest 126, 1039–1051. DOI: 10.1172/JCI83987

Pearson, K., 1901. LIII. On lines and planes of closest fit to systems of points in space. The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science 2, 559–572. DOI: 10.1080/14786440109462720

Peppard, P.E., Young, T., Barnet, J.H., Palta, M., Hagen, E.W., Hla, K.M., 2013. Increased prevalence of sleep-disordered breathing in adults. Am J Epidemiol 177, 1006–1014. DOI: 10.1093/aje/kws342

Pereira Da Costa, M., Minutti, C.M., Piot, C., Giampazolias, E., Cardoso, A., Cabeza-Cabrerizo, M., Rogers, N.C., Lebrusant-Fernandez, M., Iliakis, C.S., Wack, A., Reis E Sousa, C., 2023. Interplay between CXCR4 and CCR2 regulates bone marrow exit of dendritic cell progenitors. Cell Reports 42, 112881. DOI: 10.1016/j.celrep.2023.112881

Perry, J.S.A., Russler-Germain, E.V., Zhou, Y.W., Purtha, W., Cooper, M.L., Choi, J., Schroeder, M.A., Salazar, V., Egawa, T., Lee, B.-C., Abumrad, N.A., Kim, B.S., Anderson, M.S., DiPersio, J.F., Hsieh, C.-S., 2018. Transfer of cell-surface antigens by scavenger receptor CD36 promotes thymic regulatory T cell receptor repertoire development and allo-tolerance. Immunity 48, 923-936.e4. DOI: 10.1016/j.immuni.2018.04.007

Persson, E.K., Scott, C.L., Mowat, A.McI., Agace, W.W., 2013a. Dendritic cell subsets in the intestinal lamina propria: Ontogeny and function: highlights. Eur. J. Immunol. 43, 3098–3107. DOI: 10.1002/eji.201343740

Persson, E.K., Uronen-Hansson, H., Semmrich, M., Rivollier, A., Hägerbrand, K., Marsal, J., Gudjonsson, S., Håkansson, U., Reizis, B., Kotarsky, K., Agace, W.W., 2013b. IRF4 transcription-factor-dependent $CD103^{+}CD11b^{+}$ dendritic cells drive mucosal T helper 17 cell differentiation. Immunity 38, 958–969. DOI: 10.1016/j.immuni.2013.03.009

Pessoa, J., Belew, G.D., Barroso, C., Egas, C., Jones, J.G., 2023. The gut microbiome responds progressively to fat and/or sugar-rich diets and is differentially modified by dietary fat and sugar. Nutrients 15, 2097. DOI: 10.3390/nu15092097

Peterson, L.W., Artis, D., 2014. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 14, 141–153. DOI: 10.1038/nri3608

Plebanek, M.P., Xue, Y., Nguyen, Y.-V., DeVito, N.C., Wang, X., Holtzhausen, A., Beasley, G.M., Theivanthiran, B., Hanks, B.A., 2024. A lactate-SREBP2 signaling axis drives tolerogenic dendritic cell maturation and promotes cancer progression. Sci Immunol 9, eadi4191. DOI: 10.1126/sciimmunol.adi4191

Plitas, G., Rudensky, A.Y., 2016. Regulatory T cells: differentiation and function. Cancer Immunol Res 4, 721–725. DOI: 10.1158/2326-6066.CIR-16-0193

Poirier, P., Giles, T.D., Bray, G.A., Hong, Y., Stern, J.S., Pi-Sunyer, F.X., Eckel, R.H., American heart association, Obesity Committee of the council on nutrition, Physical activity, and metabolism, 2006. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American heart association scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. Circulation 113, 898–918. DOI: 10.1161/CIRCULATIONAHA.106.171016

Polansky, J.K., Schreiber, L., Thelemann, C., Ludwig, L., Krüger, M., Baumgrass, R., Cording, S., Floess, S., Hamann, A., Huehn, J., 2010. Methylation matters: binding of Ets-1 to the demethylated Foxp3 gene contributes to the stabilization of Foxp3 expression in regulatory T cells. J Mol Med (Berl) 88, 1029–1040. DOI: 10.1007/s00109-010-0642-1

Pooley, J.L., Heath, W.R., Shortman, K., 2001. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by $CD8^-$ dendritic cells, but cross-presented to CD8 T cells by $CD8^+$ dendritic cells. J Immunol 166, 5327–5330. DOI: 10.4049/jimmunol.166.9.5327

Popkin, B.M., Du, S., Green, W.D., Beck, M.A., Algaith, T., Herbst, C.H., Alsukait, R.F., Alluhidan, M., Alazemi, N., Shekar, M., 2020. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. Obes Rev 21, e13128. DOI: 10.1111/obr.13128

Povoleri, G.A.M., Scottà, C., Nova-Lamperti, E.A., John, S., Lombardi, G., Afzali, B., 2013. Thymic versus induced regulatory T cells – Who regulates the regulators? Front Immunol 4, 169. DOI: 10.3389/fimmu.2013.00169

Pradeu, T., Jaeger, S., Vivier, E., 2013. The speed of change: towards a discontinuity theory of immunity? Nat Rev Immunol 13, 764–769. DOI: 10.1038/nri3521

Priyanka, H.P., Krishnan, H.C., Singh, R.V., Hima, L., Thyagarajan, S., 2013. Estrogen modulates in vitro T cell responses in a concentration- and receptor-dependent manner: effects on intracellular molecular targets and antioxidant enzymes. Mol Immunol 56, 328–339. DOI: 10.1016/j.molimm.2013.05.226

Puhl, R.M., Heuer, C.A., 2009. The stigma of obesity: A review and update. Obesity 17, 941–964. DOI: 10.1038/oby.2008.636

Pulendran, B., 2004. Immune activation: death, danger and dendritic cells. Curr Biol 14, R30-32. DOI: 10.1016/j.cub.2003.12.020

Pulendran, B., Ahmed, R., 2011. Immunological mechanisms of vaccination. Nat Immunol 12, 509–517. DOI: 10.1038/ni.2039

Pulendran, B., Smith, J.L., Caspary, G., Brasel, K., Pettit, D., Maraskovsky, E., Maliszewski, C.R., 1999. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. Proc Natl Acad Sci U S A 96, 1036–1041. DOI: 10.1073/pnas.96.3.1036

Qi, H., Egen, J.G., Huang, A.Y.C., Germain, R.N., 2006. Extrafollicular activation of lymph node B cells by antigen-bearing dendritic cells. Science 312, 1672–1676. DOI: 10.1126/science.1125703

Qureshi, O.S., Zheng, Y., Nakamura, K., Attridge, K., Manzotti, C., Schmidt, E.M., Baker, J., Jeffery, L.E., Kaur, S., Briggs, Z., Hou, T.Z., Futter, C.E., Anderson, G.,

Walker, L.S.K., Sansom, D.M., 2011. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell extrinsic function of CTLA-4. Science 332, 600–603. DOI: 10.1126/science.1202947

Randolph, G.J., Angeli, V., Swartz, M.A., 2005. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. Nat Rev Immunol 5, 617–628. DOI: 10.1038/nri1670

Rapp, M., Wintergerst, M.W.M., Kunz, W.G., Vetter, V.K., Knott, M.M.L., Lisowski, D., Haubner, S., Moder, S., Thaler, R., Eiber, S., Meyer, B., Röhrle, N., Piseddu, I., Grassmann, S., Layritz, P., Kühnemuth, B., Stutte, S., Bourquin, C., von Andrian, U.H., Endres, S., Anz, D., 2019. CCL22 controls immunity by promoting regulatory T cell communication with dendritic cells in lymph nodes. J Exp Med 216, 1170–1181. DOI: 10.1084/jem.20170277

Rasko, J.E., Metcalf, D., Rossner, M.T., Begley, C.G., Nicola, N.A., 1995. The flt3/flk-2 ligand: receptor distribution and action on murine haemopoietic cell survival and proliferation. Leukemia 9, 2058–2066.

Reis E Sousa, C., 2006. Dendritic cells in a mature age. Nat Rev Immunol 6, 476–483. DOI: 10.1038/nri1845

Reis E Sousa, C., 2004. Activation of dendritic cells: translating innate into adaptive immunity. Curr Opin Immunol 16, 21–25. DOI: 10.1016/j.coi.2003.11.007

Reis E Sousa, C., Hieny, S., Scharton-Kersten, T., Jankovic, D., Charest, H., Germain, R.N., Sher, A., 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. J Exp Med 186, 1819–1829. DOI: 10.1084/jem.186.11.1819

Reizis B., 2019. Plasmacytoid Dendritic Cells: Development, Regulation, and Function. Immunity. 15;50(1):37-50. DOI: 10.1016/j.immuni.2018.12.027

Ren, Y., Zeng, Y., Wu, Y., Yu, J., Zhang, Q., Xiao, X., 2023. The role of gut microbiota in gestational diabetes mellitus affecting intergenerational glucose metabolism: possible mechanisms and interventions. Nutrients 15, 4551. DOI: 10.3390/nu15214551

Renz, H., Allen, K.J., Sicherer, S.H., Sampson, H.A., Lack, G., Beyer, K., Oettgen, H.C., 2018. Food allergy. Nat Rev Dis Primers 4, 17098. DOI: 10.1038/nrdp.2017.98

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.P., Ricciardi-Castagnoli, P., 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2, 361–367. DOI: 10.1038/86373

Rezende, R.M., Weiner, H.L., 2022. Oral tolerance: an updated review. Immunology Letters 245, 29–37. DOI: 10.1016/j.imlet.2022.03.007

Robert Koch, 1982. Die Aetiologie der Tuberculose. Zentralbl Bakteriol Mikrobiol Hyg A Med Mikrobiol Infekt Parasitol 251, 287–296.

Robinson, D.P., Hall, O.J., Nilles, T.L., Bream, J.H., Klein, S.L., 2014. 17β-estradiol protects females against influenza by recruiting neutrophils and increasing virus-specific CD8 T cell responses in the lungs. J Virol 88, 4711–4720. DOI: 10.1128/JVI.02081-13

Ropero, A.B., Alonso-Magdalena, P., Quesada, I., Nadal, A., 2008. The role of estrogen receptors in the control of energy and glucose homeostasis. Steroids, Rapid Responses to Steroid Hormones 73, 874–879. DOI: 10.1016/j.steroids.2007.12.018

Ross, R., Neeland, I.J., Yamashita, S., Shai, I., Seidell, J., Magni, P., Santos, R.D., Arsenault, B., Cuevas, A., Hu, F.B., Griffin, B.A., Zambon, A., Barter, P., Fruchart, J.-C., Eckel, R.H., Matsuzawa, Y., Després, J.-P., 2020. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol 16, 177–189. DOI: 10.1038/s41574-019-0310-7

Ruterbusch, M., Pruner, K.B., Shehata, L., Pepper, M., 2020. In vivo $CD4^+$ T cell differentiation and function: revisiting the Th1/Th2 paradigm. Annu Rev Immunol 38, 705–725. DOI: 10.1146/annurev-immunol-103019-085803

Saito, H., Kranz, D.M., Takagaki, Y., Hayday, A.C., Eisen, H.N., Tonegawa, S., 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. Nature 309, 757–762. DOI: 10.1038/309757a0

Sakaguchi, S., Yamaguchi, T., Nomura, T., Ono, M., 2008. Regulatory T cells and immune tolerance. Cell 133, 775–787. DOI: 10.1016/j.cell.2008.05.009

Sancho, D., Joffre, O.P., Keller, A.M., Rogers, N.C., Martínez, D., Hernanz-Falcón, P., Rosewell, I., Reis e Sousa, C., 2009. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature 458, 899–903. DOI: 10.1038/nature07750

Sasaki, I., Kato, T., Hemmi, H., Fukuda-Ohta, Y., Wakaki-Nishiyama, N., Yamamoto, A., Kaisho, T., 2022. Conventional type 1 dendritic cells in intestinal immune homeostasis. Front. Immunol. 13, 857954. DOI: 10.3389/fimmu.2022.857954

Sasaki, T., Moro, K., Kubota, T., Kubota, N., Kato, T., Ohno, H., Nakae, S., Saito, H., Koyasu, S., 2019. Innate lymphoid cells in the induction of obesity. Cell Reports 28, 202-217.e7. DOI: 10.1016/j.celrep.2019.06.016

Satokari, R., 2020. High intake of sugar and the balance between pro- and anti-inflammatory gut bacteria. Nutrients 12, 1348. DOI: 10.3390/nu12051348

Schatz, D.G., Ji, Y., 2011. Recombination centres and the orchestration of V(D)J recombination. Nat Rev Immunol 11, 251–263. DOI: 10.1038/nri2941

Schlitzer, A., McGovern, N., Teo, P., Zelante, T., Atarashi, K., Low, D., Ho, A.W.S., See, P., Shin, A., Wasan, P.S., Hoeffel, G., Malleret, B., Heiseke, A., Chew, S., Jardine, L., Purvis, H.A., Hilkens, C.M.U., Tam, J., Poidinger, M., Stanley, E.R., Krug, A.B., Renia, L., Sivasankar, B., Ng, L.G., Collin, M., Ricciardi-Castagnoli, P., Honda, K., Haniffa, M., Ginhoux, F., 2013. IRF4 transcription factor-dependent $CD11b^+$ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity 38, 970–983. DOI: 10.1016/j.immuni.2013.04.011

Schlitzer, A., Sivakamasundari, V., Chen, J., Sumatoh, H.R.B., Schreuder, J., Lum, J., Malleret, B., Zhang, S., Larbi, A., Zolezzi, F., Renia, L., Poidinger, M., Naik, S., Newell, E.W., Robson, P., Ginhoux, F., 2015. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol 16, 718–728. DOI: 10.1038/ni.3200

Schreiber, S., MacDermott, R.P., Raedler, A., Pinnau, R., Bertovich, M.J., Nash, G.S., 1991. Increased activation of isolated intestinal lamina propria mononuclear cells in inflammatory bowel disease. Gastroenterology 101, 1020–1030. DOI: 10.1016/0016-5085(91)90729-5

Schulz, O., Diebold, S.S., Chen, M., Näslund, T.I., Nolte, M.A., Alexopoulou, L., Azuma, Y.-T., Flavell, R.A., Liljeström, P., Reis e Sousa, C., 2005. Toll-like receptor 3 promotes cross-priming to virus-infected cells. Nature 433, 887–892. DOI: 10.1038/nature03326

Schwartz, R.H., 2003. T cell anergy. Annu Rev Immunol 21, 305–334. DOI: 10.1146/annurev.immunol.21.120601.141110

Scott, C.L., Aumeunier, A.M., Mowat, A.McI., 2011. Intestinal CD103$^+$ dendritic cells: master regulators of tolerance? Trends in Immunology 32, 412–419. DOI: 10.1016/j.it.2011.06.003

Scott, C.L., Bain, C.C., Wright, P.B., Sichien, D., Kotarsky, K., Persson, E.K., Luda, K., Guilliams, M., Lambrecht, B.N., Agace, W.W., Milling, S.W., Mowat, A.M., 2015. CCR2$^+$CD103$^-$ intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. Mucosal Immunology 8, 327–339. DOI: 10.1038/mi.2014.70

SDS mouse diet RM3 (P), (15.11.2024) https://www.sds-diets.com/sds-wAssets/docs/sds_rm3-p-_ds.pdf

See, P., Dutertre, C.-A., Chen, J., Günther, P., McGovern, N., Irac, S.E., Gunawan, M., Beyer, M., Händler, K., Duan, K., Sumatoh, H.R.B., Ruffin, N., Jouve, M., Gea-Mallorquí, E., Hennekam, R.C.M., Lim, T., Yip, C.C., Wen, M., Malleret, B., Low, I., Shadan, N.B., Fen, C.F.S., Tay, A., Lum, J., Zolezzi, F., Larbi, A., Poidinger, M., Chan, J.K.Y., Chen, Q., Rénia, L., Haniffa, M., Benaroch, P., Schlitzer, A., Schultze, J.L., Newell, E.W., Ginhoux, F., 2017. Mapping the human DC lineage through the integration of high-dimensional techniques. Science 356, eaag3009. DOI: 10.1126/science.aag3009

Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A.M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., Ghosh, S., Earl, A., Snapper, S.B., Jupp, R., Kasper, D., Mathis, D., Benoist, C., 2015. Individual intestinal symbionts induce a distinct population of RORγ^+ regulatory T cells. Science 349, 993–997. DOI: 10.1126/science.aaa9420

Sempowski, G.D., Cross, S.J., Heinly, C.S., Scearce, R.M., Haynes, B.F., 2004. CD7 and CD28 are required for murine CD4$^+$CD25$^+$ regulatory T cell homeostasis and prevention of thyroiditis. J Immunol 172, 787–794. DOI: 10.4049/jimmunol.172.2.787

Sender, R., Fuchs, S., Milo, R., 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 14, e1002533. DOI: 10.1371/journal.pbio.1002533

Sharpe, A.H., Pauken, K.E., 2018. The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol 18, 153–167. DOI: 10.1038/nri.2017.108

Sheridan, P.A., Paich, H.A., Handy, J., Karlsson, E.A., Hudgens, M.G., Sammon, A.B., Holland, L.A., Weir, S., Noah, T.L., Beck, M.A., 2012. Obesity is

associated with impaired immune response to influenza vaccination in humans. Int J Obes (Lond) 36, 1072–1077. DOI: 10.1038/ijo.2011.208

Shiokawa, A., Kotaki, R., Takano, T., Nakajima-Adachi, H., Hachimura, S., 2017. Mesenteric lymph node CD11b^{-} CD103^{+} PD-L1 High dendritic cells highly induce regulatory T cells. Immunology 152, 52–64. DOI: 10.1111/imm.12747

Shortman, K., Heath, W.R., 2010. The CD8^{+} dendritic cell subset. Immunol Rev 234, 18–31. DOI: 10.1111/j.0105-2896.2009.00870.x

Sikder, Md.A.A., Rashid, R.B., Ahmed, T., Sebina, I., Howard, D., Ullah, Md.A., Rahman, M.M., Lynch, J.P., Curren, B., Werder, R.B., Simpson, J., Bissell, A., Morrison, M., Walpole, C., Radford, K.J., Kumar, V., Woodruff, T.M., HuiYing, T., Ali, A., Kaiko, G.E., Upham, J.W., Hoelzle, R.D., Cuív, P.Ó., Holt, P.G., Dennis, P.G., Phipps, S., 2023. The maternal microbiome regulates infant respiratory disease susceptibility via intestinal Flt3L expression and plasmacytoid dendritic cell hematopoiesis (preprint). Immunology. DOI: 10.1101/2023.01.05.522516

Smith, A., Woodside, B., Abizaid, A., 2022. Ghrelin and the control of energy balance in females. Front Endocrinol (Lausanne) 13, 904754. DOI: 10.3389/fendo.2022.904754

Sojka, D.K., Huang, Y.-H., Fowell, D.J., 2008. Mechanisms of regulatory T-cell suppression — a diverse arsenal for a moving target. Immunology 124, 13–22. DOI: 10.1111/j.1365-2567.2008.02813.x

Spohn, S.N., Young, V.B., 2018. Chapter 32 - Gastrointestinal Microbial Ecology With Perspectives on Health and Disease, in: Said, H.M. (Ed.), Physiology of the gastrointestinal tract (Sixth Edition). Academic Press, pp. 737–753. DOI: 10.1016/B978-0-12-809954-4.00032-3

Spörri, R., Reis e Sousa, C., 2005. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4^{+} T cell populations lacking helper function. Nat Immunol 6, 163–170. DOI: 10.1038/ni1162

Stanhope, K.L., Schwarz, J.M., Keim, N.L., Griffen, S.C., Bremer, A.A., Graham, J.L., Hatcher, B., Cox, C.L., Dyachenko, A., Zhang, W., McGahan, J.P., Seibert, A., Krauss, R.M., Chiu, S., Schaefer, E.J., Ai, M., Otokozawa, S., Nakajima, K., Nakano, T., Beysen, C., Hellerstein, M.K., Berglund, L., Havel, P.J., 2009. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 119, 1322–1334. DOI: 10.1172/JCI37385

Starr, T.K., Jameson, S.C., Hogquist, K.A., 2003. Positive and negative selection of T cells. Annu Rev Immunol 21, 139–176. DOI: 10.1146/annurev.immunol.21.120601.141107

Stefanovic-Racic M., Yang X., Turner M.S., Mantell B.S., Stolz D.B., Sumpter T.L., Sipula I.J., Dedousis N., Scott D.K., Morel P.A., Thomson A.W., O'Doherty R.M., 2012. Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c$^+$ cells in adipose tissue and liver. Diabetes 61(9):2330-9. DOI: 10.2337/db11-1523

Steinbrink, K., Graulich, E., Kubsch, S., Knop, J., Enk, A.H., 2002. CD4$^+$ and CD8$^+$ anergic T cells induced by interleukin-10-treated human dendritic cells display antigen-specific suppressor activity. Blood 99, 2468–2476. DOI: 10.1182/blood.v99.7.2468

Steinman, R.M., Cohn, Z.A., 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 137, 1142–1162. DOI: 10.1084/jem.137.5.1142

Steinman, R.M., Hawiger, D., Nussenzweig, M.C., 2003. Tolerogenic dendritic cells. Annu Rev Immunol 21, 685–711. DOI: 10.1146/annurev.immunol.21.120601.141040

Stojić-Vukanić, Z., Nacka-Aleksić, M., Bufan, B., Pilipović, I., Arsenović-Ranin, N., Djikić, J., Kosec, D., Leposavić, G., 2015. 17β-Estradiol influences in vitro response of aged rat splenic conventional dendritic cells to TLR4 and TLR7/8 agonists in an agonist specific manner. International Immunopharmacology 24, 24–35. DOI: 10.1016/j.intimp.2014.11.008

Streich, K., Klein, M., Siebert, A., Bleich, A., Buettner, M., 2022. Diet-induced obesity results in impaired oral tolerance induction. Immunity Inflam & Disease 10, e720. DOI: 10.1002/iid3.720

Sullivan, Z.A., Khoury-Hanold, W., Lim, J., Smillie, C., Biton, M., Reis, B.S., Zwick, R.K., Pope, S.D., Israni-Winger, K., Parsa, R., Philip, N.H., Rashed, S., Palm, N., Wang, A., Mucida, D., Regev, A., Medzhitov, R., 2021. γδ T cells regulate the intestinal response to nutrient sensing. Science 371, eaba8310. DOI: 10.1126/science.aba8310

Sun, C.-M., Hall, J.A., Blank, R.B., Bouladoux, N., Oukka, M., Mora, J.R., Belkaid, Y., 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med 204, 1775–1785. DOI: 10.1084/jem.20070602

Sun, L., Su, Y., Jiao, A., Wang, X., Zhang, B., 2023. T cells in health and disease. Sig Transduct Target Ther 8, 235. DOI: 10.1038/s41392-023-01471-y

Swiecki, M., Colonna, M., 2015. The multifaceted biology of plasmacytoid dendritic cells. Nat Rev Immunol 15, 471–485. DOI: 10.1038/nri3865

Swinburn, B.A., Sacks, G., Hall, K.D., McPherson, K., Finegood, D.T., Moodie, M.L., Gortmaker, S.L., 2011. The global obesity pandemic: shaped by global drivers and local environments. Lancet 378, 804–814. DOI: 10.1016/S0140-6736(11)60813-1

Takeuchi, O., Akira, S., 2010. Pattern recognition receptors and inflammation. Cell 140, 805–820. DOI: 10.1016/j.cell.2010.01.022

Tall, A.R., Yvan-Charvet, L., 2015. Cholesterol, inflammation and innate immunity. Nat Rev Immunol 15, 104–116. DOI: 10.1038/nri3793

Tanaka, S., Nemoto, Y., Takei, Y., Morikawa, R., Oshima, S., Nagaishi, T., Okamoto, R., Tsuchiya, K., Nakamura, T., Stutte, S., Watanabe, M., 2020. High-fat diet-derived free fatty acids impair the intestinal immune system and increase sensitivity to intestinal epithelial damage. Biochemical and Biophysical Research Communications 522, 971–977. DOI: 10.1016/j.bbrc.2019.11.158

Tang, Q., Bluestone, J.A., 2008. The Foxp3^{+} regulatory T cell: a jack of all trades, master of regulation. Nat Immunol 9, 239–244. DOI: 10.1038/ni1572

Taniuchi, I., 2018. CD4 helper and CD8 cytotoxic T cell differentiation. Annu Rev Immunol 36, 579–601. DOI: 10.1146/annurev-immunol-042617-053411

Taniuchi, I., Osato, M., Egawa, T., Sunshine, M.J., Bae, S.C., Komori, T., Ito, Y., Littman, D.R., 2002. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. Cell 111, 621–633. DOI: 10.1016/s0092-8674(02)01111-x

Teh, H.S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Blüthmann, H., von Boehmer, H., 1988. Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. Nature 335, 229–233. DOI: 10.1038/335229a0

Thakur, J.S., Thakur, A., Lum, L.G., 2023. Mathematical model to predict polyclonal T-cell-dependent antibody synthesis responses. Mathematics 11, 4017. DOI: 10.3390/math11184017

Thornton, A.M., Korty, P.E., Tran, D.Q., Wohlfert, E.A., Murray, P.E., Belkaid, Y., Shevach, E.M., 2010. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3$^+$ T regulatory cells. J Immunol 184, 3433–3441. DOI: 10.4049/jimmunol.0904028

Thornton, A.M., Shevach, E.M., 2019. Helios: still behind the clouds. Immunology 158, 161–170. DOI: 10.1111/imm.13115

Tomiyama, A.J., 2019. Stress and obesity. Annu Rev Psychol 70, 703–718. DOI: 10.1146/annurev-psych-010418-102936

Turnbaugh, P.J., Bäckhed, F., Fulton, L., Gordon, J.I., 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3, 213–223. DOI: 10.1016/j.chom.2008.02.015

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Heath, A.C., Knight, R., Gordon, J.I., 2009. A core gut microbiome in obese and lean twins. Nature 457, 480–484. DOI: 10.1038/nature07540

Tussiwand, R., Everts, B., Grajales-Reyes, G.E., Kretzer, N.M., Iwata, A., Bagaitkar, J., Wu, X., Wong, R., Anderson, D.A., Murphy, T.L., Pearce, E.J., Murphy, K.M., 2015. Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. Immunity 42, 916–928. DOI: 10.1016/j.immuni.2015.04.017

Uematsu, S., Jang, M.H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., Kato, H., Sougawa, N., Matsui, H., Kuwata, H., Hemmi, H., Coban, C., Kawai, T., Ishii, K.J., Takeuchi, O., Miyasaka, M., Takeda, K., Akira, S., 2006. Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c$^+$ lamina propria cells. Nat Immunol 7, 868–874. DOI: 10.1038/ni1362

Ussar, S., Griffin, N.W., Bezy, O., Fujisaka, S., Vienberg, S., Softic, S., Deng, L., Bry, L., Gordon, J.I., Kahn, C.R., 2015. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. Cell Metab 22, 516–530. DOI: 10.1016/j.cmet.2015.07.007

van de Pavert, S., Ferreira, M., Domingues, R., Ribeiro H., Molenaar R., Moreira-Santos L., Almeida F.F., Ibiza S., Barbosa I., Goverse G., Labão-Almeida C., Godinho-Silva C., Konijn T., Schooneman D., O'Toole T., Mizee M.R., Habani Y., Haak E., Santori F.R., Littman D.R., Schulte-Merker S., Dzierzak E., Simas J.P., Mebius R.E., Veiga-Fernandes H., 2014. Maternal retinoids control type 3

innate lymphoid cells and set the offspring immunity. Nature 508, 123–127. DOI: 10.1038/nature13158

Veiga-Fernandes, H., Freitas, A.A., 2017. The S(c)ensory immune system theory. Trends in Immunology 38, 777–788. DOI: 10.1016/j.it.2017.02.007

Verginis, P., Li, H.S., Carayanniotis, G., 2005. Tolerogenic semimature dendritic cells suppress experimental autoimmune thyroiditis by activation of thyroglobulin-specific $CD4^+CD25^+$ T cells. J Immunol 174, 7433–7439. DOI: 10.4049/jimmunol.174.11.7433

Victora, G.D., Schwickert, T.A., Fooksman, D.R., Kamphorst, A.O., Meyer-Hermann, M., Dustin, M.L., Nussenzweig, M.C., 2010. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. Cell 143, 592–605. DOI: 10.1016/j.cell.2010.10.032

Vignali, D.A.A., Collison, L.W., Workman, C.J., 2008. How regulatory T cells work. Nat Rev Immunol 8, 523–532. DOI: 10.1038/nri2343

Villani, A.-C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., Griesbeck, M., Butler, A., Zheng, S., Lazo, S., Jardine, L., Dixon, D., Stephenson, E., Nilsson, E., Grundberg, I., McDonald, D., Filby, A., Li, W., De Jager, P.L., Rozenblatt-Rosen, O., Lane, A.A., Haniffa, M., Regev, A., Hacohen, N., 2017. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science 356, eaah4573. DOI: 10.1126/science.aah4573

Wang, B., Kong, Q., Li, X., Zhao, J., Zhang, H., Chen, W., Wang, G., 2020. A high-fat diet increases gut microbiota biodiversity and energy expenditure due to nutrient difference. Nutrients 12, 3197. DOI: 10.3390/nu12103197

Wang, X., Ota, N., Manzanillo, P., Kates, L., Zavala-Solorio, J., Eidenschenk, C., Zhang, J., Lesch, J., Lee, W.P., Ross, J., Diehl, L., van Bruggen, N., Kolumam, G., Ouyang, W., 2014. Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. Nature 514, 237–241. DOI: 10.1038/nature13564

Wang, Y.-C., Cao, Y., Pan, C., Zhou, Z., Yang, L., Lusis, A.J., 2023. Intestinal cell type-specific communication networks underlie homeostasis and response to Western diet. Journal of Experimental Medicine 220, e20221437. DOI: 10.1084/jem.20221437

Waskow, C., Liu, K., Darrasse-Jèze, G., Guermonprez, P., Ginhoux, F., Merad, M., Shengelia, T., Yao, K., Nussenzweig, M., 2008. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. Nat Immunol 9, 676–683. DOI: 10.1038/ni.1615

Webb, P., Annis, J.F., 1983. Adaptation to overeating in lean and overweight men and women. Hum Nutr Clin Nutr 37, 117–131.

Weiner, H.L., Friedman, A., Miller, A., Khoury, S.J., al-Sabbagh, A., Santos, L., Sayegh, M., Nussenblatt, R.B., Trentham, D.E., Hafler, D.A., 1994. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu Rev Immunol 12, 809–837. DOI: 10.1146/annurev.iy.12.040194.004113

Weissman, I.L., 2000. Stem cells: units of development, units of regeneration, and units in evolution. Cell 100, 157–168. DOI: 10.1016/s0092-8674(00)81692-x

Wells, H.G., Osborne, T.B., 1911. The biological reactions of the vegetable proteins I. Anaphylaxis. Journal of Infectious Diseases 8, 66–124. DOI: 10.1093/infdis/8.1.66

West, J.A., Tsakmaki, A., Huang, J.H.S., Ghosh, S.S., Parkes, D.G., Wismann, P., Rigbolt, K.T.G., Pedersen, P.J., Pavlidis, P., Maggs, D., Lopez-Talavera, J.C., Bewick, G.A., Rajagopalan, H., 2019. Proximal and distal gut mucosa adapt differently to westernized diet, promoting an insulin-resistant dysmetabolic state. DOI: 10.1101/822122

White, U., Tchoukalova, Y., 2014. Sex dimorphism and depot differences in adipose tissue function. Biochimica et biophysica acta 1842. DOI: 10.1016/j.bbadis.2013.05.006

WHO, 2024. Obesity and overweight. URL https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed 9.29.24).

Wilson, N.S., Young, L.J., Kupresanin, F., Naik, S.H., Vremec, D., Heath, W.R., Akira, S., Shortman, K., Boyle, J., Maraskovsky, E., Belz, G.T., Villadangos, J.A., 2008. Normal proportion and expression of maturation markers in migratory dendritic cells in the absence of germs or Toll-like receptor signaling. Immunol Cell Biol 86, 200–205. DOI: 10.1038/sj.icb.7100125

Winer, D.A., Winer, S., Shen, L., Wadia, P.P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M.G., Alonso, M.N., Leong, H.X., Glassford, A., Caimol, M., Kenkel, J.A., Tedder, T.F., McLaughlin, T., Miklos, D.B., Dosch, H.-M., Engleman, E.G.,

2011. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 17, 610–617. DOI: 10.1038/nm.2353

Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., Nomura, T., Sakaguchi, S., 2008. CTLA-4 control over Foxp3$^+$ regulatory T cell function. Science 322, 271–275. DOI: 10.1126/science.1160062

Wisén, O., Johansson, C., 1992. Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. Metabolism 41, 390–395. DOI: 10.1016/0026-0495(92)90073-j

Wohlfert, E.A., Grainger, J.R., Bouladoux, N., Konkel, J.E., Oldenhove, G., Ribeiro, C.H., Hall, J.A., Yagi, R., Naik, S., Bhairavabhotla, R., Paul, W.E., Bosselut, R., Wei, G., Zhao, K., Oukka, M., Zhu, J., Belkaid, Y., 2011. GATA3 controls Foxp3$^+$ regulatory T cell fate during inflammation in mice. J Clin Invest 121, 4503–4515. DOI: 10.1172/JCI57456

Worah, K., Mathan, T.S., Vu Manh, T.P., Keerthikumar, S., Schreibelt, G., Tel, J., Duiveman-de Boer, T., Sköld, A.E., van Spriel, A.B., de Vries, I.J.M., Huynen, M.A., Wessels, H.J., Gloerich, J., Dalod, M., Lasonder, E., Figdor, C.G., Buschow, S.I., 2016. Proteomics of human dendritic cell subsets reveals subset-specific surface markers and differential inflammasome function. Cell Reports 16, 2953. DOI: 10.1016/j.celrep.2016.08.023

Workman, C.J., Szymczak-Workman, A.L., Collison, L.W., Pillai, M.R., Vignali, D.A.A., 2009. The development and function of regulatory T cells. Cell. Mol. Life Sci. 66, 2603–2622. DOI: 10.1007/s00018-009-0026-2

World Obesity Federation, 2023. World of obesity atlas 2023. World Obesity Federation. URL https://www.worldobesity.org/news/economic-impact-of-overweight-and-obesity-to-surpass-4-trillion-by-2035 (accessed 9.29.24).

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F.D., Lewis, J.D., 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334, 105–108. DOI: 10.1126/science.1208344

Wyss, L., Stadinski, B.D., King, C.G., Schallenberg, S., McCarthy, N.I., Lee, J.Y., Kretschmer, K., Terracciano, L.M., Anderson, G., Surh, C.D., Huseby, E.S., Palmer, E., 2016. Affinity for self antigen selects Treg cells with distinct functional properties. Nat Immunol 17, 1093–1101. DOI: 10.1038/ni.3522

Xiao, Z., Casey, K.A., Jameson, S.C., Curtsinger, J.M., Mescher, M.F., 2009. Programming for CD8 T cell memory development requires IL-12 or Type I IFN. J Immunol 182, 2786–2794. DOI: 10.4049/jimmunol.0803484

Xie, Y., Ding, F., Di, W., Lv, Y., Xia, F., Sheng, Y., Yu, J., Ding, G., 2020. Impact of a high-fat diet on intestinal stem cells and epithelial barrier function in middle-aged female mice. Mol Med Rep 21, 1133–1144. DOI: 10.3892/mmr.2020.10932

Xu, H., 2013. Obesity and metabolic inflammation. Drug Discov Today Dis Mech 10, 21–25. DOI: 10.1016/j.ddmec.2013.03.006

Xu, J., Zanvit, P., Hu, L., Tseng, P.-Y., Liu, N., Wang, F., Liu, O., Zhang, D., Jin, W., Guo, N., Han, Y., Yin, J., Cain, A., Hoon, M.A., Wang, S., Chen, W., 2020. The cytokine TGF-β induces interleukin-31 expression from dermal dendritic cells to activate sensory neurons and stimulate wound itching. Immunity 53, 371-383.e5. DOI: 10.1016/j.immuni.2020.06.023

Yanai, S., Endo, S., 2021. Functional aging in Male C57BL/6J mice across the life-span: a systematic behavioral analysis of motor, emotional, and memory function to define an aging phenotype. Front. Aging Neurosci. 13. DOI: 10.3389/fnagi.2021.697621

Yarovinsky, F., Zhang, D., Andersen, J.F., Bannenberg, G.L., Serhan, C.N., Hayden, M.S., Hieny, S., Sutterwala, F.S., Flavell, R.A., Ghosh, S., Sher, A., 2005. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science 308, 1626–1629. DOI: 10.1126/science.1109893

Younossi, Z.M., Koenig, A.B., Abdelatif, D., Fazel, Y., Henry, L., Wymer, M., 2016. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–84. DOI: 10.1002/hep.28431

Yudanin, N.A., Schmitz, F., Flamar, A.-L., Thome, J.J.C., Tait Wojno, E., Moeller, J.B., Schirmer, M., Latorre, I.J., Xavier, R.J., Farber, D.L., Monticelli, L.A., Artis, D., 2019. Spatial and temporal mapping of human innate lymphoid cells reveals elements of tissue specificity. Immunity 50, 505-519.e4. DOI: 10.1016/j.immuni.2019.01.012

Yurkovetskiy, L., Burrows, M., Khan, A.A., Graham, L., Volchkov, P., Becker, L., Antonopoulos, D., Umesaki, Y., Chervonsky, A.V., 2013. Gender bias in autoimmunity is influenced by microbiota. Immunity 39, 10.1016/j.immuni.2013.08.013. DOI: 10.1016/j.immuni.2013.08.013

Zeng, R., Oderup, C., Yuan, R., Lee, M., Habtezion, A., Hadeiba, H., Butcher, E.C., 2013. Retinoic acid regulates the development of a gut-homing precursor for intestinal dendritic cells. Mucosal Immunology 6, 847–856. DOI: 10.1038/mi.2012.123

Zhang, Q., Zhang, C., Zhang, Y., Liu, Y., Wang, J., Gao, Z., Sun, Jinlyu, Li, Q., Sun, Jiachen, Cui, X., Wang, Y., Fu, L., 2023. Early-life risk factors for food allergy: Dietary and environmental factors revisited. Compr Rev Food Sci Food Saf 22, 4355–4377. DOI: 10.1111/1541-4337.13226

Zhang, X., Monnoye, M., Mariadassou, M., Beguet-Crespel, F., Lapaque, N., Heberden, C., Douard, V., 2021. Glucose but not fructose alters the intestinal paracellular permeability in association with gut inflammation and dysbiosis in mice. Front Immunol 12, 742584. DOI: 10.3389/fimmu.2021.742584

Zhang, Yifan, Gao, S., Xia, J., Liu, F., 2018. Hematopoietic hierarchy – an updated roadmap. Trends in Cell Biology 28, 976–986. DOI: 10.1016/j.tcb.2018.06.001

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432. DOI: 10.1038/372425a0

Zhang, Yuan, Wang, Y., Li, M.-Q., Duan, J., Fan, D.-X., Jin, L.-P., 2018. IL-25 promotes Th2 bias by upregulating IL-4 and IL-10 expression of decidual γδT cells in early pregnancy. Exp Ther Med 15, 1855–1862. DOI: 10.3892/etm.2017.5638

Zheng, S.G., Wang, J.H., Koss, M.N., Quismorio, F., Gray, J.D., Horwitz, D.A., 2004. $CD4^+$ and $CD8^+$ regulatory T cells generated ex vivo with IL-2 and TGF-beta suppress a stimulatory graft-versus-host disease with a lupus-like syndrome. J Immunol 172, 1531–1539. DOI: 10.4049/jimmunol.172.3.1531

Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., de Sauvage, F.J., Ouyang, W., 2008. Interleukin-22 mediates early host defence against attaching and effacing bacterial pathogens. Nat Med 14, 282–289. DOI: 10.1038/nm1720

Zhu, J., Yamane, H., Paul, W.E., 2010. Differentiation of effector CD4 T cell populations. Annu Rev Immunol 28, 445–489. DOI: 10.1146/annurev-immunol-030409-101212

Zmora, N., Suez, J., Elinav, E., 2019. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol 16, 35–56. DOI: 10.1038/s41575-018-0061-2

Zwick, R.K., Kasparek, P., Palikuqi, B., Viragova S., Weichselbaum L., McGinnis C.S., McKinley K.L., Rathnayake A., Vaka D., Nguyen V., Trentesaux C., Reyes E., Gupta A.R., Gartner Z.J., Locksley R.M., Gardner J.M., Itzkovitz S., Boffelli D., Klein O.D., 2024. Epithelial zonation along the mouse and human small intestine defines five discrete metabolic domains. Nat Cell Biol 26, 250–262. DOI: 10.1038/s41556-023-01337-z

ITQB NOVA, Avenida da República, 2780-157 Oeiras, Portugal | Tel +351 214 469 100 | itqb.unl.pt