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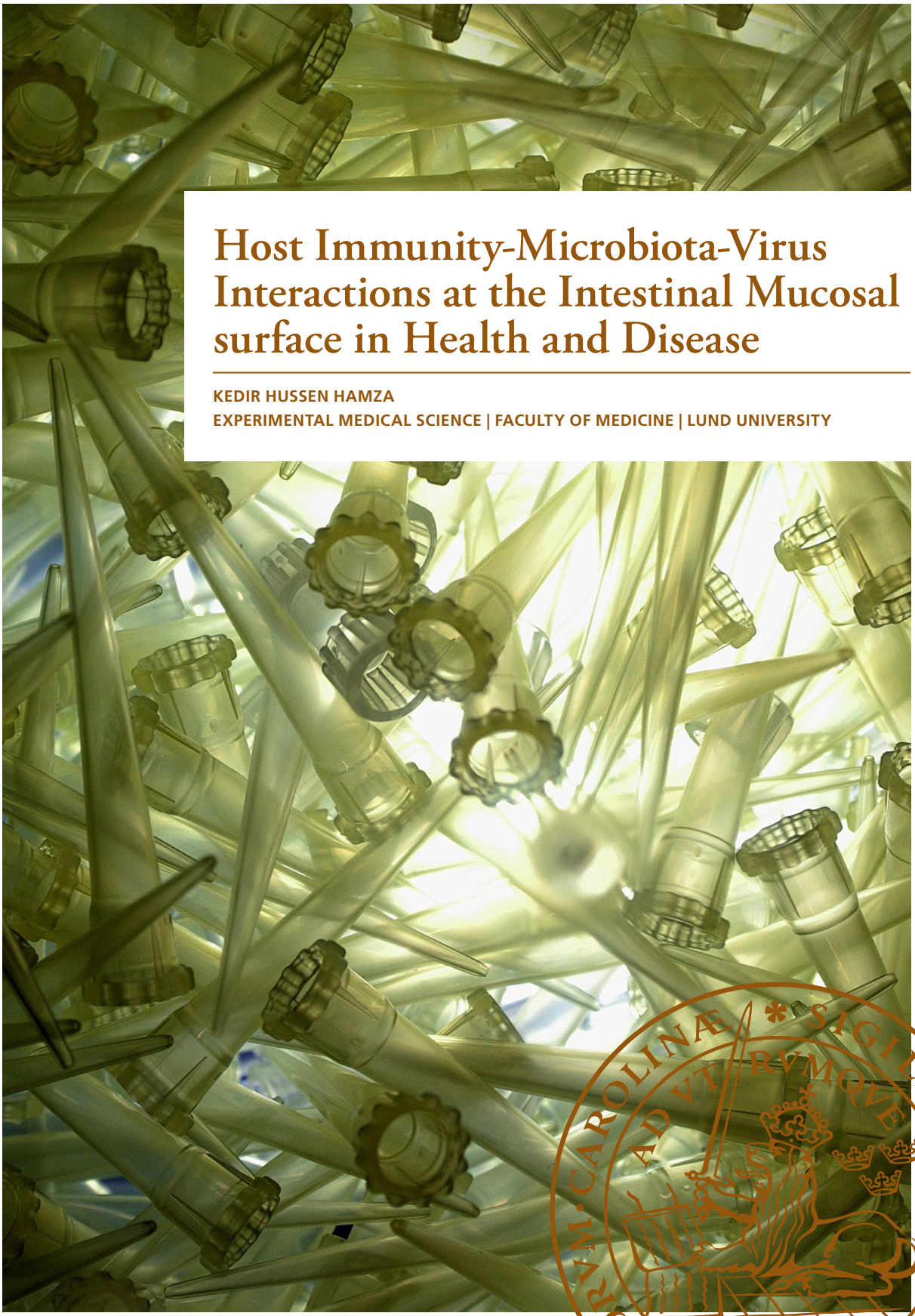

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LUND
UNIVERSITY

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Abstract

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The overall aim of this thesis was to understand the host immunity-virus-microbiota interaction at the intestinal mucosal surface in adults and neonates under homeostatic and inflammatory conditions. In the first paper, we showed that adult murine rotavirus (RV) infection did not induce significant long-lasting microbial community changes across the length of the intestine. Additionally, using acute Dextran Sodium Sulphate (DSS) colitis model, we demonstrated that prior infection with RV did not ameliorate inflammation of the colon. In the second paper, we demonstrated that the absence of maternal antibodies causes hyper-induction of IgA in neonates and this hyper-induction requires T cells help under homeostasis and RV infection conditions. We also discovered preferential IgA coating of colonic bacteria in neonates, as opposed to the stronger coating in the small intestine in adult mice, regardless of the antibody source. Additionally, we found that the increase in IgA^+ plasma cells during RV infection does not affect the level of IgA coating of bacteria in the neonatal gut. In the third paper, we showed that RV-induced expansion of antigen-specific $CD8^+$ T cells does not require signaling via TLR3, MyD88 or type I interferon receptor. In the fourth paper, we extended our studies to delineate when and how IgA against food antigens is induced and showed that induction of food-specific IgA in the gut requires adjuvant and T cells, but not T_{FH} cells.

Collectively, the work included in this thesis has broadened our understanding of intestinal homeostasis development and maintenance and of the complex interaction of host immunity, virus, and microbiota.

Key words: Rotavirus, Intestinal Homeostasis, Microbiota, IgA^+ bacteria, DSS colitis, Neonate, IgA^+ Plasma cells

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Dedicated to

My Parents,
My wife Yasmin,
My kids Nuha, Imran, and Mariam

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Abbreviations

AID activation-induced cytidine deaminase
APRIL A proliferation-inducing ligand
ASV amplicon sequence variant
ATG16L1 Autophagy Related 16 Like 1
BAFFR B-cell activating factor receptor
BCMA B cell maturation antigen
BCR B cell receptor
CCR Chemokine receptor
CD Cluster of differentiation
CD Crohn's disease
CPs Colonic patches
CRAMP cathelicidin-related antimicrobial peptide
CX3CR1 C-X3-C Motif Chemokine Receptor 1
DAI disease activity index
DC dendritic cells
DLP Double layer particle
DNA deoxyribonucleic acid
dsRNA double-stranded RNA
DSS Dextran Sodium Sulphate
FAE follicle-associated epithelium
GALT Gut-associated lymphoid tissue
GWAS Genome-wide association studies

HET heterozygous

IBD Inflammatory bowel diseases

IEC Intestinal epithelial cells

IEL Intraepithelial lymphocytes

IFN interferon

IgA Immunoglobulin A

IL Interleukin

ILC Innate lymphoid cells

ILF Isolated lymphoid follicles

ISGs IFN stimulating genes

LI large intestine

LP Lamina propria

LTi lymphoid tissue inducer

Ly6C lymphocyte antigen 6 complexes

M cells microfold cells

MAVS mitochondrial antiviral-signaling protein

MDA-5 melanoma differentiation-associated protein 5

MERTK Mer tyrosine kinase

MHC Major Histocompatibility Complex

MLN mesenteric lymph node

MyD88 Myeloid differentiation primary response 88

NEC necrotizing enterocolitis

NF-kB nuclear factor-kB

NMDS Nonmetric multidimensional scaling

NOD2 nucleotide oligomerization domain 2

NSP non-structural proteins

OUT operational taxonomic unit

PAMPs Pathogen-associated molecular patterns

PCs Plasma cells

pIgR polymeric Immunoglobulin receptor

PPs Peyer's patches

PRRs Pattern recognition receptors

QIIME2 quantitative insights into microbial ecology 2

REGIIIγ Regenerating Islet-derived protein IIIγ

RIG-I retinoic acid-inducible gene I

RNA ribonucleic acid

RORγt Retinoic acid-related orphan receptor gamma t

RV Rotavirus

RVA Group A rotaviruses

RVV rotavirus vaccine

SI small intestine

SIgA secretory IgA

TACI Transmembrane activator and calcium-modulating cyclophilin ligand interactor

TCR T-cell receptor

TD T cell-dependent

TI T cell-independent

TLRs toll-like receptors

TRI Toll-IL-1 receptor

TRIF TRI domain-containing adaptor-inducing interferon-β

tTregs Thymus-derived regulatory T cells

UC Ulcerative colitis

WT wild type

Abstract

The presence of viral immune triggers at the intestinal mucosa can have multiple global effects on intestinal integrity, including relative protection from subsequent inflammatory bowel disease. During the last century, the western world has achieved a remarkable success in preventing infectious diseases, which increased the general life expectancy dramatically. However, the incidence and prevalence of immune mediated diseases have increased immensely. Especially, the lack of exposure to microbial products during early development is considered to lead to the increase of allergy and autoimmune disease incidence.

The overall aim of this thesis was to understand the host immunity-virus-microbiota interaction at the intestinal mucosal surface in adults and neonates under homeostatic and inflammatory conditions. In the first paper, we showed that adult murine rotavirus (RV) infection did not induce significant long-lasting microbial community changes across the length of the intestine. Additionally, using acute Dextran Sodium Sulphate (DSS) colitis model, we demonstrated that prior infection with RV did not ameliorate inflammation of the colon. In the second paper, we demonstrated that the absence of maternal antibodies causes hyper-induction of IgA in neonates and this hyper-induction requires T cells help under homeostasis and RV infection conditions. We also discovered preferential IgA coating of colonic bacteria in neonates, as opposed to the stronger coating in the small intestine in adult mice, regardless of the antibody source. Additionally, we found that the increase in IgA^+ plasma cells during RV infection does not affect the level of IgA coating of bacteria in the neonatal gut. In the third paper, we showed that RV-induced expansion of antigen-specific $CD8^+$ T cells does not require signaling via TLR3, MyD88 or type I interferon receptor. In the fourth paper, we extended our studies to delineate when and how IgA against food antigens is induced and showed that induction of food-specific IgA in the gut requires adjuvant and T cells, but not T_{FH} cells.

Collectively, the work included in this thesis has broadened our understanding of intestinal homeostasis development and maintenance and of the complex interaction of host immunity, virus, and microbiota.

Introduction

In the past decade, we have witnessed new and exciting developments in the research fields concerning the microbiome, mucosal immunology, and the crosstalk between them. Numerous studies have addressed the modulation of the microbiome and its consequences on host health.

During the last century, the western world has achieved a remarkable success in preventing infectious diseases, which increased the general life expectancy dramatically. However, the incidence and prevalence of immune mediated diseases have increased immensely. Especially the lack of exposure to microbial products during early development is considered to lead to the increase of allergy and autoimmune disease incidence. Therefore, the overarching questions are:

1. Do microbial triggers contribute to the prevention of immune mediated diseases?
2. What are the mechanistic links between microbial sensing and protection from immune mediated diseases?
3. How can we compensate for the lack of microbial triggering without reintroducing exposure to potentially infectious agents?

Unless otherwise mentioned the discussion below is based on findings derived from murine studies.

The Intestinal Mucosal Immune system and its Homeostasis

The intestinal structure and immune inductive sites

The intestinal tract is a continuous tube-like structure that stretches from the pylorus orifice to the anus. It is not a single homogeneous organ but consists of anatomically and physiologically distinct small and large intestinal regions that are further divided into different segments[1,2]. The small intestine is divided into three segments: the duodenum, located closest to the stomach, followed by the jejunum, and then the ileum[2]. In the large intestine, the caecum is the first segment followed by the colon and rectum before ending in the anus[2].

The intestinal epithelium is considered as one of the major interfaces with the external environment and is highly interconnected with the microbes residing in the gut[3]. It contains the largest amount of immune cells of any organ in the body and plays an important role in maintaining intestinal homeostasis as it is continuously exposed to a variety of foods and microbes that act as potential immune stimuli[2–4]. It also serves as a highly selective barrier that permits absorption of life-sustaining nutrients while regulating the tissue's interaction with the microbial communities and food antigens[3,4]. There is an increasing awareness of how the contents of the intestine, such as the commensal bacteria, enteric virome and dietary constituents, influence physiological and pathological processes throughout the body[2,5].

The small intestinal mucosal surface is characterized by long finger-like projections called villi. These projections become progressively shorter and broader towards the end of the small intestine. In colon and caecum, the villi are absent, so their surface is flat[1].

The epithelial surface is composed of different kinds of specialized cell types. Most of these cells are enterocytes, but there are also Paneth cells, goblet cells, tuft cells, and neuroendocrine cells. The epithelial surface is

continuously renewed by multipotent stem cells called crypts. Newly formed epithelial cells move upwards from the bottom of the crypt to the tip of the villus except for Paneth cells, which move downwards to the base of the crypt. As the epithelial cells mature, they acquire several properties that are essential for their digestion and absorption function, such as the full range of enzymes[2]. Under normal conditions Paneth cells exist only in the small intestine and are long-lived. They are responsible for producing antimicrobial peptides like lysozyme, defensin and regenerating islet-derived protein IIIγ (REGIIIγ)[6]. In addition, these cells maintain the normal activity of crypt stem cells. Thus, their dysregulation makes the host more susceptible to microbiota-dependent intestinal inflammation[2,7,8]. The goblet cells secret heavily glycosylated mucins which oligomerize through disulphide bonds to form mucus. This in turn maintains the integrity of the epithelial barrier[9,10]. Mice deficient in Muc2, which is the most abundant intestinal mucin, lose the ability to contain microbiota within the lumen and are highly susceptible to infection[11]. Unlike the small intestinal mucus that lacks the inner dense layer, the colon has both inner dense and outer loose layers of mucus[12,13]. The inner dense layer helps to keep the area close to the colon epithelium free from bacteria while the outer layer serves as a habitat and nutrition source for many commensal bacteria. As a compensatory mechanism for the absence of an inner mucus layer in the small intestine, the presence of antimicrobial peptides and antibodies protects the villus area from bacterial outgrowth[10].

Histologically, the intestine consists of four main structural layers: Mucosa, Submucosa, Muscularis externa (Muscle layer) and Serosa[1]. The Mucosal layer where most of immunological process takes place is the innermost layer lining the intestine. It is composed of an epithelial single layer that lies above the lamina propria along with a thin muscle layer called muscularis mucosa directly beneath the lamina propria. The lamina propria supports the blood supply, lymph drainage, and nerve network required for a functional mucosa, in addition to providing structural support for the villus with loosely packed connective tissue. The submucosa is a connective tissue layer located immediately beneath the mucosa and above a thick external smooth muscle layer. In addition to ganglia and blood vessels, Peyer's patches, and colonic patches are also located here[2].

The gut is seen as a communicator between the outside environment and the host. In the gut there are specialized, organized structures together referred to as gut-associated lymphoid tissue (GALT), which is covered by a

specialized follicle-associated epithelium (FAE)[1,14]. The FAE contains the microfold cells (M cells) that originate from epithelial stem cells. They serve as the major entry point for pathogens and for the uptake of antigen from the lumen as GALTs lack afferent lymphatic vessels [1,2,14,15]. GALT comprises Peyer's patches (PPs), Colonic patches (CP) and Isolated lymphoid follicles (ILF)[16–20]. They are the major sites for priming adaptive immune cell in the intestine together with the intestinal draining lymph nodes, known as mesenteric lymph nodes[1].

Intestinal Immunity

Innate immune system in the gut

The intestinal innate immune system is comprised of haematopoietic and non-haematopoietic cells that are located at the interface between the host and the microbiome [21]. Together, these cells fulfil key roles in regulating the interaction of the host with its microbiota through their ability to sense microorganisms or their metabolic products, ultimately impacting on the ensuing physiological response[21]. Failure to regulate the interaction between the host and microbiota and to defend against pathogenic invasion can lead to a multitude of complex diseases[21–23].

Even though intestinal epithelial cells are not considered classical innate immune cells, they actively express different pattern recognition receptors (PRRs) such as TLRs and NOD-like receptors to maintain intestinal homeostasis[2,10,21,24]. There is evidence that the spatial separation of microbiota from the lamina propria of the intestine is compromised due to epithelial breaching occurring in the absence of PRR expression specifically on epithelial cells[21,24–26].

The lamina propria contains lymphoid and non-lymphoid innate immune cells [2]. In general, it is believed that Innate lymphoid cells (ILCs) have a role in GALT development, intestinal immunity and inflammation[2]. Different types of ILCs exist and differ in their function and localization within the intestine[2,27]. The non-lymphoid innate immune cells include dendritic cells (DCs), macrophages, neutrophils, eosinophils, and mast cells.

Intestinal DCs express CD11c and MHC class II on their surface, but lack expression of macrophage-associated markers F4/80 and CD64[2]. In mouse, based on the expression of CD103 and CD11b, DCs are classified into four subsets[2,28]. Each subset has the potential to initiate and regulate the adaptive immune response[28].

Intestinal macrophages are characterized by classical markers CD11b, CD64, F4/80, MERTK, MHCII, CX3CR1, and CD11c (not all) and are found abundantly in intestinal lamina propria[28]. Ly6C^{hi} monocytes continuously replenish intestinal macrophages in a CCR2-dependent manner in steady state and inflammatory conditions, except for CD11c^{neg} subpopulation [28].

Adaptive immune system in the gut

In the intestinal immune system, adaptive immune cells accumulate primarily within intestinal epithelium and the underlying LP of the intestine[1]. They play an important role in maintaining immune homeostasis by suppressing immune responses to harmless antigens and by enforcing the integrity of the intestinal mucosa[29]. CD8$^+$ intraepithelial lymphocytes (IELs) are the primary adaptive immune cells in the intestinal epithelium, whereas CD4$^+$ T cells and plasma cells are the main adaptive immune cells in the LP[1,30].

Intestinal T cell subsets

T cells in the intestinal mucosa are distinctly heterogeneous in phenotype and function, but they are grouped into type A and type B subsets based on the expression of the T-cell receptor (TCR) and coreceptor[31]. Type A derived from the conventional gut mucosal T cells express TCRαβ together with CD4 or CD8αβ, while type B also called the non-conventional gut mucosal T cells express either TCRαβ or TCRγδ and CD8αα homodimers[1,31,32]. Unlike type A mucosal CD4 T cells which are mainly present in the LP, almost all type B mucosal T cells are located in the epithelium and majority the of them are IELs[31,33].

Immunoglobulin A (IgA)

In both mice and men, the intestinal LP contains an estimate of 80% of all Plasma Cells with an overwhelming majority of them producing IgA[1,34]. The colonic LP harbors only a minor population of IgA^+ PCs, while the small intestine LP contains the largest population[35,36]. The primary sites for the induction of IgA are Peyer's patches but IgA class switch recombination can also occur in mesenteric lymph nodes, isolated lymphoid follicles, cecal patch and *in situ* in the LP[14,19,35,37,38]. These sites support both T cell-dependent (TD) and T cell-independent (TI) pathways of IgA production[36,39]. Mice lacking all GALT tissues due to, for instance, lack of lymphotoxin signaling or retinoic acid-related orphan receptor γt (RORγt), showed an incomplete but significant reduction in IgA^+ PCs[40,41].

IgA, as all mammalian antibody isotypes, is comprised of a Fab fragment and an Fc region[42–44]. Unlike mice, which have a single IgA isotype, two isotypes, IgA1 and IgA2, exist in humans[45]. In the gut, IgA is mostly produced in its dimeric form, where two monomers are linked by a J chain and secreted into the lumen using secretory component[44,46]. The term secretory IgA (SIgA) describes the complex formed by dimeric IgA, J chain, and secretory component[35]. The secretory component is formed from the cleavage of polymeric Ig receptor (pIgR) of the epithelial cells[42,44,46]. However, studies performed on pIgR-deficient mice indicate that alternative pathways might be available to compensate for the loss of pIgR and contribute to the steady-state secretion of IgA[47–49].

IgA-secreting plasma cells arise from naïve B cell precursors expressing IgM and IgD through a mechanism called class-switch recombination[35,50]. Studies on this process have demonstrated that this can occur both through T-dependent(TD) and independent(TI) mechanisms, though the specific signals involved are distinct in each case[14,50]. A TD response is driven by CD40-CD40L interactions with T cells, while the TI response is primarily driven by BAFF/APRIL interactions with receptors such as transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI), BAFF receptor (BAFFR) and B cell maturation antigen (BCMA). However, in both cases induction of activation-induced cytidine deaminase (AID) upon B cell receptor (BCR) stimulation is a necessary step[34,50–53].

Neonatal mucosal immunity

The event of birth marks the transition from life under sterile conditions to living under massive microbial and environmental pressure. Unlike the adult, the neonatal immune system is developing and characterized by little immunological memory. The murine intestinal tissue architecture is immature at birth and undergoes several developmental changes until it reaches adulthood state. The lack of crypts and crypt-residing paneth cells are the main anatomical characteristics of the neonatal intestinal mucosa. Instead of Paneth cell-derived microbial peptides, neonatal enterocytes express cathelicidin-related antimicrobial peptide (CRAMP)[54]. The microbial density of the neonate reaches a plateau quickly after birth due to the rapid colonization of the intestinal mucosa[54], but the microbial diversity is 3-fold lower than in the adult gut and is dominated by Lactobacilli, Streptococci and Bifidobacteria[54]. M cells, which are a major route for antigen uptake in the adult, do not mature until after the second week of life. The mucosal layer is thinner due to the reduced expression of mucins by goblet cells[54].

The infants' immune system was shown to be uniquely suited to deal with de novo antigen encounter by balancing host protection with immune tolerance establishment[1,55–57]. The neonatal immune systems can, however, be overwhelmed by infectious agents, such as rotavirus (RV). This leads to a general view of immaturity in the immune system during the infant period. It is estimated that around 40% of the annual neonatal mortality is caused by infectious agents[58,59]. Maternal antibodies transferred through the placenta and later through breast milk contribute to early life defence against pathogenic organisms in neonates[58]. Even though Immunoglobulin A (IgA)-driven humoral immunity in babies at steady state derives almost exclusively from mother's milk, neonatal natural infections and vaccinations can lead to powerful humoral immune memory, as exemplified by RV.

Development of cellular immunity in neonate has been studied extensively in the past few decades. Different factors initiate and facilitate the development of the intestinal mucosal immune system before and after birth. The development of the immune system starts at the embryonic stage. Its development is influenced by multiple factors including maternal factors and environmental exposure[58]. Through crosstalk between lymphoid tissue inducer (LTi) and stroma cells within the sterile womb, Peyer's patch anlagen are formed[54]. Microbiota play an important role in the development of

cryptopatches after birth and their maturation to isolated lymphoid follicles (ILFs)[54]. Both lymphocytes and polymorphonuclear cells - neutrophils, eosinophils and mast cells - initially originate from the fetal liver followed by hematopoietic stem cell-derived waves from the bone marrow. Before birth, the small intestinal mucosa is populated with a fetal wave of γδ-T lymphocytes[54]. Shortly after birth, B and αβ-T lymphocytes begin to populate the intestine[60,61]. These lymphocytes exhibit a distinct homing pattern to Peyer's patches, but remain mostly naïve until weaning under steady-state conditions[54,60,62]. The delay in lymphocyte maturation also occurs in human infants[60,63,64].

Despite our clear understanding of the importance of neonatal immunity for both protection of the new-born and setting up immune health throughout life, we know surprisingly little about the neonatal immune setup. The general view is that the young immune system is immature and less functional, to allow slow adaptation without a potentially harmful over-reaction of the immune system. When babies are infected however, they have the potential to mount very strong immune responses capable of inducing protection from reinfection throughout life.

Breastfeeding

Breast milk is not only a nutritional source but also plays a significant role in shaping the infant's immune system and gut microbiota[65]. Many infants, however, do not have access to breast milk and are instead formula fed. Formula milk differs from breast milk in that it is devoid of maternal microbiota and other bioactive factors such as cytokines, growth factors and secretory IgA[54,65,66]. Exclusive formula feeding is associated with a change in gut microbiota, allergies, infections, obesity and autoimmune diseases such as necrotizing enterocolitis (NEC) and diabetes[65,67,68]. For instance, NEC is a disease of preterm infants with intestinal inflammation driven by microbiota, causing high rates of morbidity and mortality. However, its incidence is substantially lower in infants fed with maternal milk. Using Immunoglobulin A (IgA) deficient dams, secretory IgA in the maternal milk was shown to be a critical factor for NEC prevention[68].

Window of Opportunity and Weaning reaction

Setting up the immune system correctly during early life has long-lasting benefits protecting from infections and non-communicable diseases alike

throughout life. Recent advances in neonatal immunology point towards a critical non-redundant priming period of the innate and adaptive immune system after birth that significantly influences life-long immune homeostasis and host-microbial interaction[60,69]. This critical neonatal period is referred to as "neonatal window of opportunity". It involves the imprinting of the intestinal immune system by the microbiota and as such the colonizing microbiota plays a crucial role in the process[1,70]. The concept first came to light through epidemiological studies, which revealed this period as being particularly important to the susceptibility to immune-mediated diseases in humans[60,69]. It was experimentally demonstrated that mice treated with antibiotics during this period developed increased susceptibility to experimental allergic asthma[71]. This process affects all mucosal surfaces and skin[70,72,73].

At the transition from breast-feeding to the uptake of solid food, the surge in microbiota causes a vigorous immune response referred to as "weaning reaction"[56]. When mice start diversifying their food intake from milk only to solid food, the intestinal microbiota expands dramatically both in number and diversity. This change causes the host to develop a weaning reaction during which high levels of pro-inflammatory cytokines are present in the intestine[70]. Surprisingly, this vigorous immune response is not observed when adult germ-free mice are colonized by microbiota, demonstrating that the weaning reaction is restricted to a specific time window during development[56,70]. The weaning reaction has long-lasting protective effects and seems to be required for life long immune health[56].

Virus-Host-microbiota interaction

Intestinal Microbiota

The gastrointestinal tract of vertebrates is densely populated by a diverse microbial community of bacteria, viruses, fungi, archaea and eukaryotes collectively termed gut microbiota[74–76]. Their collective genes are referred to as the microbiome[77]. The gut microbiota provide a number of benefits to the host, including immune system development, metabolism, colonization resistance to pathogens and homeostasis[78–80]. On the other hand, they pose a threat of breaching the intestinal epithelium to cause pathologies[81]. Microbiota are inherited at birth, but the existence of bacterial DNA in the placenta and colonization of the foetus is still controversial[82–84].

The intestinal microbiota coexists with the host in a homeostatic relationship[85]. Until recently, it was thought that bacteria outnumbered host cells by about 10 times; now, it is estimated that there are about $3.8x10^{13}$ bacteria present in adult human which reflects a 1:1 ration with body cells[85–87]. Microbiota in the intestinal tracts of adults comprise hundreds to thousands of species[88,89]. The dominant bacterial phyla are Bacteroidetes and Firmicutes, while Actinobacteria, Proteobacteria, and Verrucomicrobia make up the minor phyla[76,88–91].

The intestinal epithelium regulates the microbial and host immunity interaction in its midst to contribute to the mutualistic interaction between the host immunity and resident microbes[81]. The localization and the composition of gut bacteria are influenced by several factors, including physiological variations along the length of the small intestine and colon that include chemical and nutrient gradients, as well as compartmentalized host immune activity[88]. According to recent studies, the majority of bacterial species in the gut can persist for years as a stable core of microbiota[92,93]. Because of their stability and ability to respond to physiological changes, gut microbiota can serve as valuable biomarkers and therapeutic targets[88,92]. The gut microbiome, for instance, can predict the classification of individuals into lean or obese with 90% accuracy, and the bacterium Christensenella

correlates specifically with weight loss[94–96]. Allergic and immune related diseases such as asthma, allergies, inflammatory bowel disease and type 1 diabetes have also increased in frequency globally. A growing number of evidence links these disease conditions with microbiome perturbation[97–100]. Especially during early life, perturbations of the microbiome may be critical since many body systems, mainly the immune system, are under development[100–103]. Many microbiome-wide association studies have linked diseases to changes in microbiota, which have typically generated a list of commensals implicated as biomarkers of disease without any apparent evidence to disease pathogenesis[104]. The field is currently moving beyond correlation[104], trying to address causation, even though a lot of work remains to be done. Research on which factors influence the microbiome of healthy individuals and how they reshape the microbiome during health and disease could be extremely helpful for preventing many diseases.

Intestinal Homeostasis

Intestinal homeostasis is influenced by a complex interplay between genetic factors, the immune system, and environmental influences. Under normal conditions, the immune system is in a constant dynamic equilibrium with environmental factors. When the dynamic equilibrium is beaks down, intestinal inflammation is likely to occur. Dysbiosis (referring to a microbial imbalance) and immune dysfunction can lead to chronic inflammation in the intestine[3].

Through the production of antimicrobial substances and the recruitment of immune cells, the epithelium controls the composition and location of intestinal bacteria[3]. Protection against pathogens on the one hand and maintenance of tolerance to innocuous antigens on the other hand is accomplished by the gut-associated lymphoid tissue (GALT)[17].

Throughout life, the immune system helps to maintain homeostasis with resident microbes and thus facilitates promoting mutualistic relationships between hosts and microbes[81,88,105,106]. In other words, non-pathogenic bacteria must be tolerated by the host immune system to survive in the gut. Similarly, resident bacteria shape mammalian immunity profoundly[81,107–109].

The classical mechanisms of tolerance are challenged by the presence of a complex and dynamic mixture of largely innocuous foreign antigens from commensal microbiota and diet and harmful pathogens[110]. Thymus-derived regulatory T cells (tTregs) mediate life-long immunity to self-antigens and contribute to the tolerance of the microbiota in the intestinal tract, as well as other parts of the body like lung and skin.

Rotavirus Infection

Rotavirus (RV) is a non-enveloped, double-stranded RNA virus that belongs to the family *Reoviridae*. It primarily infects mature enterocytes at the top of the villi in the small intestine and leads to gastroenteritis in children under 5 years of age[111–113]. In the global perspective, successful implementation of a vaccine against RV leads to a significant decrease in child mortality due to gastroenteritis[114–116]. However, RV is still recognized as the leading enteric pathogen associated with high childhood mortality in the developing world[117]. The differences in the efficacy of the RV vaccine (RVV) between the developed and developing world might be, among other reasons, attributed to a difference in the composition of microbiota between communities. Species-level research of the microbiota suggested that the gut microbiota may play a role in regulating RVV efficacy, in contrast to studies at the phylum level, which showed no difference in microbiota composition between RotaTeq vaccinated and unvaccinated groups[115,118,119]. Clinical studies with cohorts from different parts of the world showed an association of RVV efficacy with gut bacterial compositions. These studies attempted to associate specific bacterial taxa with the anti-RVV response and revealed both positive and negative correlations with RVV vaccine efficacy[120–122]. For instance, the presence of *Streptococus bovis* correlated positively with the RVV response while Bacteroidetes associated negatively[121,122]. Understanding the mechanism of how bacterial taxa regulate the RV immune response is important for developing efficacious vaccine.

Vaccination of infants with RV also correlates with a decrease in type I diabetes and celiac disease, suggesting that neonatal infections can have life-long consequences on host health[123,124].

Apart from clinical studies, different animal models are available to study the interaction between microbiota with RV infection. Murine RV models are

widely used as a model for intestinal viral infection, and multiple murine RV strains are available.

Rotavirus Structure and Classification

Rotaviruses comprise one of the 15 genera of the Reoviridae family. They are non-enveloped triple-layered particles containing 11 segments of genomic double-strand (ds) RNA. These 11 segments encode 6 structural proteins (VP1-VP4, VP6 and VP7) and 5-6 non-structural proteins (NSP1-NSP5/6). Except for RNA segment 11, which encodes NSP5 and NSP6, all RNA segments encode a single protein[112,125]. The core of the viral genome contains the replication enzyme complexes, which consists of VP1 (RNA-dependent RNA polymerase enzyme) and VP3 (the capping enzyme) and they are surrounded by inner protein layer, VP2. The second layer which is an intermediate layer formed by VP6. The third and outer layer consists of VP7 neutralization protein and VP4 spike-forming neutralization protein[112,114,125].

The traditional viral classification method, which is based on clinical, morphological and serological characteristics of different virus strains, is increasingly replaced by sequencing technology. The sequencing-based classification has been used to define the different species of viruses of the genus Rotavirus. The RVA species comprises of at least 27 G types and 37 P types according to the nucleotides sequence of VP7 and VP4, respectively[114].

Rotavirus Pathogenesis

RVs can infect immune and nonimmune cells, but the overwhelming viral replication occurs in the small intestinal enterocytes. Crypt hyperplasia, a histopathological observation preceded by vacuolization and epithelial loss, can occur when matured epithelial cells of the small intestine at the top of the villi are infected by the virus[114]. The pathogenesis of RV infection is mainly influenced by the age of the host and particular viral gene segment products in addition to homologous versus heterologous RV infections[112,114,126]. The properties of proteins encoded by the viral gene segments determine the virulence of RV. The virulence has been shown to be multigenic as it is linked with the involvement of multiple genes from the 11 RV gene segments. These genes control different aspects of the virus replication cycle as mentioned above.

In infants and young children malabsorptive diarrhea is the main clinical manifestation of rotavirus infection. Our understanding of the disease mechanism is mainly based on animal models. The virus primarily disrupts the absorptive enterocytes, while crypt cells are spared. This disruption affects the tight junctions between enterocytes leading to functional changes and paracellular leakage. The virus-induced down-regulation of expression of absorptive enzymes and the activation of the enteric nervous system also play dominant roles[112,114].

Innate Immune Response to Rotavirus Infection

The innate immune system employs diverse innate immune sensors to trigger an early non-specific anti-RV immune response[125,127]. Both membrane-associated and cytoplasmic sensors are activated during RV infection by recognition of the Pathogen-associated molecular patterns (PAMPs) encoded by the virus[127].

Several studies have shown that the cytosolic RIG-I/MDA-5-MAVS pathway is essential for the induction of IFNs and play an important role in determining the magnitude of RV replication in the intestinal epithelium[75,127,128]. Upon RV infection, RIG-I and MDA-5 receptors recognize the RNAs produced by DLPs (middle layer formed by V6) and trigger the activation of two principal transcription factors called nuclear factor-kB (NF-kB) and IFN regulatory factor 3 (IRF3) through the activation of mitochondrial antiviral-signaling protein (MAVS). These transcription factors are involved in stimulation of IFN stimulating genes (ISGs) and induction of different types of interferons (IFN)s[129,130].

Distinct from the cytosolic receptors, RV recognition also involves an immediate activation of membrane associated sensors that entails a class of viral receptors called the toll-like receptors (TLRs). TLR-dependent defense against RV plays an important role as apparent from an increase in RV susceptibility, viral shedding, and severity in the absence of MyD88 or TRIF[127,131]. TRIF-dependent signaling, for example, contributes to antiviral protection in adult mice and is linked to age dependent TLR3 expression in the intestine[131]. This type of RV recognition may reflect how RV enters the host cell by exploiting the endosomal vesicle transport system[127]. Several other TLRs have been implicated in the detection of RVs, including TLR2, TLR5, and TLR7[132].

RV employs serveral countermeasures to inhibit the host innate immune response, which is especially pronounced in a homologous infection. The RV non-structural gene product NSP1, for example, has been shown to block the IRF3/NF-κB pathway[114,133].

Inflammatory Bowel disease

Inflammatory bowel diseases (IBD) are intestinal disorders that comprise two types of chronic relapsing inflammatory conditions called Crohn's disease (CD) and Ulcerative colitis (UC)[134–137]. In the case of Crohn's disease, the inflammation is usually transmural showing a patchy pattern and can be found in any area of the gastrointestinal tract. Ulcerative colitis is restricted to the colon and rectum area[136,138,139]. The incidence of IBD is increasing worldwide with an estimation of over 1 million residents in the USA and 2.5 million in Europe alone[140]. The rise of IBD in newly industrialized countries is also noticeable as it follows the trend of the developed world[140,141].

Risk factors

Despite accumulating evidence suggesting that IBD results from an inappropriate inflammatory response to intestinal microbes in genetically susceptible hosts, its etiology is still unknown[137,142]. Genome-wide association studies (GWAS) of IBD identified more than 200 risk variants and many of the gene variants are associated with microbial sensing and clearance, T cell differentiation and maintenance, and regulation of inflammatory mediators[137]. Prominent findings among these risk variants are genomic regions containing nucleotide oligomerization domain 2 (NOD2), Autophagy genes (*ATG16L1*), and immunomodulatory IL-10[137,143–145]. The intracellular sensor protein NOD2 senses bacterial peptidoglycan while the autophagy gene helps cells to regulate and degrade diverse intracellular components, including pathogens[143]. Mutation of IL-10 in paediatric patients was shown to promote early disease onset[146]. On the other hand, *Il10* KO mice develop colitis like adults[137].

Intestinal inflammation animal models

The use of experimental models to study IBD has made significant contributions to our understanding of the pathogenesis of these diseases. It also improved our ability to dissect the complex response of mice to various causes of colitis[147]. Though no single model captures the complexity of human IBD, the availability of different animal models provide a valuable insight into different aspects of the disease pathogenesis[147–150]. The most often used models include Dextran Sodium Sulphate (DSS) Colitis, Trinitrobenzene Sulfonic Acid Colitis, Oxazolone Colitis, Adoptive Transfer Colitis, and IL-10 Knockout mouse models[147,149,150].

Dextran Sodium Sulphate (DSS) Colitis murine model

DSS colitis model is one of the most used mouse models for colitis by employing an anticoagulant colitogenic chemical called dextran sodium sulphate[147]. DSS is a negatively charged sulphated polysaccharide with a highly variable molecular weight ranging from 5 to 1400kDa with water-soluble properties[151]. Administration of 40-50kDa DSS in drinking water produces the desired inflammatory effect that resembles human UC[150]. This model leads to acute, chronic and relapsing forms of intestinal inflammation by changing frequency of administration and using different concentration of DSS[148,152,153]. It is one of most broadly used models due to its simplicity, rapidity, reproducibility, and controllability[151,153].

Aims of the Thesis

The overall aim of my thesis is to understand the host immunity-virus-microbiota interaction at the intestinal mucosal surface in adults and neonates under normal and inflammatory conditions using mice as a model organism.

The specific aims were the following:

- To better understand the impact that adult enteric RV infection might have on the composition of the intestinal microbiome and on the prognosis of IBD.
- To better understand the cellular requirements and the microbiota binding pattern of secretory IgA across the length of the neonatal intestine.
- To investigate which PRR pathways lead to RV specific CD8 T cell priming.
- To investigate the involvement of IgA in oral tolerance and the triggers able to induce specific IgA to food antigens.

Synopses of the Original work

Paper 1

Minor alterations in the intestinal microbiota composition upon Rotavirus infection do not affect susceptibility to DSS colitis

Background and Aim:
The intestinal environment is exposed to many external factors including sporadic enteric viral infections. Though viruses are well known for their pathogenic effect, recent studies performed in pattern-recognition receptor gene deficient mouse models or mice treated with antiviral cocktails suggest that viruses can act as an important contributor to the maintenance of intestinal homeostasis. Viral triggers at the intestinal mucosa can cause an increase in intestinal barrier strength and relative protection from subsequent IBD. Viruses can interact with the intestinal immune system both directly and indirectly through commensal bacteria. It is unknown how the delicate balance between the host immunity, commensals and viral infection is maintained in the environment that is rich in commensal as well as pathogenic microorganisms. Our understanding of virus-mediated protection is currently very limited. Therefore, the aim of this study was to better understand the impact that adult RV infection might have on murine intestinal homeostasis.

Results

- Adult murine RV infection did not induce significant long-lasting microbial community changes across the length of the intestine.
- Using the acute DSS model of colitis, we showed that prior infection with RV did not ameliorate inflammation of the colon.
- Prior RV infection does not alter the enhanced neutrophil and monocyte infiltration into the colon induced by DSS.

Discussion
As the role of microbiota in physiology and pathology becomes more and more evident, interest in studying how they interact with different pathogenic

and non-pathogenic enteric virus increased tremendously. The interaction of commensal bacteria with external stimuli such as viral infections may play a key role in intestinal homeostasis and host immune responses. Studies on the interaction between RV and gut microbiota are just beginning to surface, and the nature of these interactions is still unknown. In this study, we showed that temporary and asymptomatic RV infection of adult mice neither induced significant long-lasting microbiota community shifts in the small and large intestine nor affected the severity of subsequent DSS colitis. Seemingly in contrast to our findings, previous studies in mice showed that triggering the immune system with UV inactivated virus or viral mimics is crucial for intestinal resilience through inducing type I interferon signaling. Accordingly, depletion of the enteric virome by antiviral drugs or the lack of major nucleic-acid sensing pathways aggravated DSS colitis[5,24,154–156]. Differences between our and previous studies may be related to differences in strain of the mouse, experimental setup, and mouse facility, which in turn affect the microbiota composition of mice.

We here showed that RV infection of adult mice does not generally impact on the intestinal homeostasis and hence does not change the disease course of acute DSS colitis.

Paper 2

Establishment of early life humoral immunity in the absence of maternal immune protection and in the context of an enteric virus infection

Background and Aim:
Secretory immunoglobulin A (SIgA) is the most abundant antibody isotype produced in the body. Most of it is produced by Plasma Cells located within mucosal membranes lining the GI tract and secreted into the intestinal lumen where it coats a fraction of intestinal microbiota[157,158]. Its production at the intestinal wall is crucial for maintaining intestinal homeostasis and barrier protection through immune exclusion and neutralization[16,51,159,160]. In the neonatal gut, humoral immunity towards commensals at steady state derives almost exclusively from mother's milk. Breastmilk-derived sIgA plays important roles in limiting epithelial penetration of colonizing bacteria, thus preventing offspring from infection and inflammation[161,162]. In general, pups begin to actively generate their own intestinal SIgA after weaning (at approximately 21 days of age)[157]. However, pups raised by B cell deficient mothers show a significantly earlier onset of IgA, referred to as hyper-induction[162–164]. Similarly, it is also known that neonatal natural RV infections and vaccinations lead to powerful humoral immune response already during the first week of age regardless of the genotype of the dam, preceding the natural IgA induction phase even more substantially[165].

In adult mice, complementary T cell-dependent (TD) and T cell-independent (TI) B cell activation pathways contribute to the induction of SIgA[34,166]. It was also shown that hyper-induction of neonatal IgA in the absence of the maternal IgA source includes both T cell dependent and independent components[164]. In adult mice, apart from atypical subsets of commensals including segmented filamentous bacteria and Mucispirillum that elicited T-dependent IgA, most commensals elicit strong T-independent (TI) responses[36]. IgA coating of bacteria in the intestinal lumen is essential for the maintenance of immune / microbiota symbiosis, and hence could be used to predict some intestinal disease occurrence[35,167].

Despite the importance of humoral immunity and our understanding that early life imprinting of the immune system sets the stage for life long immune health, the cellular and molecular basis for the neonatal immune imprinting is not well understood. Thus, the aim of this manuscript was to better understand the cellular requirements of SIgA and its interaction with the commensal bacteria in both the small and the large intestine.

We here focused on the requirements for humoral immune induction, specifically on early IgA producing plasma cell development at the intestinal wall during the first stage of life in the context of homeostasis and Rotavirus infection.

Result

- Absence of maternal antibodies causes hyper-induction of IgA in pups, leading to preferential coating of large intestinal bacteria at early age. Preferential coating of colonic bacteria was equally found in pups raised by wildtype dams.
- Early life IgA hyper-induction in pups raised by RAG deficient dams requires T cells help under homeostasis and in the context of RV infection.
- Though early life IgA^+ PC numbers increased during RV infection, the level of bacterial IgA coating remained the same.

Discussion and Future direction

In this report, we showed data on IgA responses in suckling mice raised by immunodeficient dams during homeostasis and RV infection and their interaction with the host intestinal microbiota. In this study, we reported that IgA hyper-induction in pups leads to coating of considerable amounts of large intestinal bacteria at early age, but this regional coating pattern at early age is not a specific phenomenon to hyper-induced IgA, as we also observed this in a wildtype setup where the pups receive maternal IgA. In contrast to this, in adult mice, IgA coating of commensal bacteria is higher in the small intestine site than in the large intestine[36]. In adult mice, the anatomical location dictated the coating of commensal bacteria coating rather than the bacterial identity[36]. What guides the localization of commensals in neonatal mice needs to be addressed in the future.

The maternal microbiota and the fact that mothers transfer their immune experience to their offspring through placenta and breast milk has been well established. In this study, we expand on those findings by showing that RV-induced IgA^+ PC accumulation in a neonatal mouse is similarly T cell dependent as the hyper-induced IgA but does not play a role in the IgA-coating of intestinal bacteria.

Paper 3

Rotavirus-induced expansion of antigen-specific CD8 T cells does not require signaling via TLR3, MyD88 or the type I interferon receptor

Background and Aim

Rotavirus is a double-stranded RNA virus with high epithelial tropism that causes severe dehydrating diarrhea in children under the age of 5. While the innate immune signaling pathways leading to the control of the initial viral replication have been dissected in detail, the requirements for the induction of adaptive immunity to RV remain elusive. Previous studies on BATF3 deficient mice showed a delayed clearance of RV and accompanied by significantly blunted RV specific $CD8^+$ T cell response.

We here set out to assess the role of TLR3, MyD88 and the type I IFN receptor in the induction of RV specific $CD8^+$ T cell response in adult mice.

Results

- TLR3 is not required for the generation of RV specific $CD8^+$ T cell responses in adult mice
- RV specific $CD8^+$ T cell accumulation is unaltered in MyD88-deficient adult mice
- cDC1 can prime RV specific $CD8^+$ T cell responses in the absence of type I IFN sensing
- Global type I IFNAR deficiency does not affect RV specific $CD8^+$ T cell abundancy in adult mice, but has possible effects on their function

Discussion

Although we know that Batf3-dependent DCs are the major DC subset responsible for priming antiviral $CD8^+$ T cell response against RV, less is known about the signaling cascade by which the DCs interact with the virus to activate RV specific $CD8^+$ T cells.

We here show a surprising redundancy for key immune sensing pathways in the induction of RV-specific $CD8^+$ T cells. However, despite similar clearance of RV infection, $CD8^+$ T cells induced in the absence of type I IFN signaling show defects in IFNγ production, Cytotoxin A production and presentation of CD107 on their surface upon antigen-specific restimulation. We did not assess the functionality of primed $CD8^+$ T cells in TLR3- or MyD88-deficient mice. Further, we cannot currently explain the unaffected clearance of RV in mice harboring functionally impaired type I IFN-receptor deficient $CD8^+$ T cells.

Paper 4

Divergent T follicular helper cell requirement for IgA and IgE production to peanut during allergic sensitization

Background and Aim

Food allergy is a pathological immune response to food antigen most probably resulting from a combination of environmental triggers and genetic factors. IgA is the predominant antibody in the gut that accounts for more than 80% of total antibodies. IgA plays an important role in regulating commensal bacteria composition, promoting clearance of pathogens and neutralizes toxins. However, the role of IgA in protecting or promoting food allergy is relatively unclear. This study addresses the following questions:

- Is food-specific IgA induced as part of oral tolerance?
- Does a potent food allergen such as peanut induce IgA via an intrinsic adjuvant activity or are additional innate stimuli required?
- What are the cellular pathways that mediate food-specific IgA production?

Results

Induction of food-specific IgA in the gut requires adjuvant and T cells but not T_{FH} cell

Chronic exposure to peanut leads to minimal production of IgA, which is cross-reactive to multiple food antigens. This cross-reactive IgA can be produced in the absence of T cells. In contrast, peanut-specific IgA, IgG1, and IgE productions require T cells. In addition, the authors found that the production of peanut-specific IgA requires adjuvant such as choleratoxin A. We contributed by showing that RV infection was insufficient to act as an adjuvant for the induction of chicken ovalbumin-specific IgA (data not shown). Curiously, induction of peanut-specific IgA, despite the general need for T cells, can occur in the absence of T_{FH} and T_{FR} cells, but critically depends on CD40L expression. In contrast to IgA, peanut specific IgG1 and IgE require T_{FH} cells.

Discussion

In contrast to the pathological reaction caused by IgE, IgA is considered protective in the context of food allergy. The mechanisms for protection are unclear but blocking the allergen from passing from the gut into the bloodstream is one possibility. Healthy adults mount peanut specific IgA in the gut that is stable over time. Even though it is becoming clear that IgA maintains gut homeostasis, this paper significantly contributes to our understanding of how IgA is induced against food antigen, both at steady state and in the context of pathology.

This study clarifies the involvement of IgA in oral tolerance and the need for an adjuvant to induce specific IgA to food antigens. Adjuvant is only required during the first exposure in the case of a strong IgA inducing adjuvant, as shown using choleratoxin A. RV infection during food antigen exposure does not induce specific IgA to that antigen. Understanding which triggers can and cannot induce food-antigen-specific IgA is important to gain knowledge on food-allergy disease etiology and requires further investigation.

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