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Host Immunity-Microbiota-Virus Interactions at the Intestinal Mucosal surface in Health and Disease

KEDIR HUSSEN HAMZA

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Kedir Hussen Hamza



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DOCTORAL DISSERTATION

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Host Immunity-Microbiota-Virus Interactions at the Intestinal Mucosal surface in Health and Disease

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MADE IN SWEDEN 

Dedicated to

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

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

Kedir Hussen Hamza, Emma Dunér, Isabel Ulmert, Armando Arias, Daniel Sorobetea, and Katharina Lahl

Scientific Reports, 11(1), 13485 (2021).

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

Kedir Hussen Hamza, Konjit Getachew Muleta, and Katharina Lahl

Manuscript

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

Konjit Getachew Muleta, Isabel Ulmert, Kedir Hussen Hamza, Sharné van Dijl, Joy Nakawesi, and Katharina Lahl

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

Biyang Zhang, Elise Liu, Jake A. Gertie, Julie Joseph, Lan Xu, Elisha Y. Pinker, Daniel A. Waizman, Jason Catanzaro, Kedir Hussen Hamza, Katharina Lahl, Uthaman Gowthaman, and Stephanie C. Eisenbarth

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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- κ B nuclear factor- κ B
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

REGIII γ Regenerating Islet-derived protein III γ

RIG-I retinoic acid-inducible gene I

RNA ribonucleic acid

ROR γ t 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 ileum². In the large intestine, the caecum is the first segment followed by the colon and rectum before ending in the anus².

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 gut³. 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 stimuli²⁻⁴. 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 flat¹.

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 enzymes². 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 III γ (REGIII γ)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 secrete 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 infection¹¹. 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 outgrowth¹⁰.

Histologically, the intestine consists of four main structural layers: Mucosa, Submucosa, Muscularis externa (Muscle layer) and Serosa¹. 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 here².

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 nodes¹.

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 microbiome²¹. 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 response²¹. Failure to regulate the interaction between the host and microbiota and to defend against pathogenic invasion can lead to a multitude of complex diseases²¹⁻²³.

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 cells². In general, it is believed that Innate lymphoid cells (ILCs) have a role in GALT development, intestinal immunity and inflammation². 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 CD64². 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 response²⁸.

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 propria²⁸. Ly6C^{hi} monocytes continuously replenish intestinal macrophages in a CCR2-dependent manner in steady state and inflammatory conditions, except for CD11c^{neg} subpopulation²⁸.

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 intestine¹. They play an important role in maintaining immune homeostasis by suppressing immune responses to harmless antigens and by enforcing the integrity of the intestinal mucosa²⁹. 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 coreceptor³¹. Type A derived from the conventional gut mucosal T cells express TCR $\alpha\beta$ together with CD4 or CD8 $\alpha\beta$, while type B also called the non-conventional gut mucosal T cells express either TCR $\alpha\beta$ or TCR $\gamma\delta$ and CD8 $\alpha\alpha$ 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 γ t (ROR γ t), 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 region⁴²⁻⁴⁴. Unlike mice, which have a single IgA isotype, two isotypes, IgA1 and IgA2, exist in humans⁴⁵. 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 component³⁵. 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 IgA⁴⁷⁻⁴⁹.

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 mucosa⁵⁴, but the microbial diversity is 3-fold lower than in the adult gut and is dominated by Lactobacilli, Streptococci and Bifidobacteria⁵⁴. 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 cells⁵⁴.

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 neonates⁵⁸. 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 exposure⁵⁸. Through crosstalk between lymphoid tissue inducer (LTi) and stroma cells within the sterile womb, Peyer's patch anlagen are formed⁵⁴. 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 $\gamma\delta$ -T lymphocytes⁵⁴. Shortly after birth, B and $\alpha\beta$ -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 microbiota⁶⁵. 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 prevention⁶⁸.

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 asthma⁷¹. 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 intestine⁷⁰. 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 health⁵⁶.

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 microbiota⁷⁴⁻⁷⁶. Their collective genes are referred to as the microbiome⁷⁷. The gut microbiota provide a number of benefits to the host, including immune system development, metabolism, colonization resistance to pathogens and homeostasis⁷⁸⁻⁸⁰. On the other hand, they pose a threat of breaching the intestinal epithelium to cause pathologies⁸¹. Microbiota are inherited at birth, but the existence of bacterial DNA in the placenta and colonization of the foetus is still controversial⁸²⁻⁸⁴.

The intestinal microbiota coexists with the host in a homeostatic relationship⁸⁵. Until recently, it was thought that bacteria outnumbered host cells by about 10 times; now, it is estimated that there are about 3.8×10^{13} bacteria present in adult human which reflects a 1:1 ration with body cells⁸⁵⁻⁸⁷. 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 microbes⁸¹. 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 activity⁸⁸. 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 loss⁹⁴⁻⁹⁶. 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 perturbation⁹⁷⁻¹⁰⁰. Especially during early life, perturbations of the microbiome may be critical since many body systems, mainly the immune system, are under development¹⁰⁰⁻¹⁰³. 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 pathogenesis¹⁰⁴. The field is currently moving beyond correlation¹⁰⁴, 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 intestine³.

Through the production of antimicrobial substances and the recruitment of immune cells, the epithelium controls the composition and location of intestinal bacteria³. 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 pathogens¹¹⁰. 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 world¹¹⁷. 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 *Streptococcus 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, respectively¹¹⁴.

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 virus¹¹⁴. 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 virus¹²⁷.

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- κ B (NF- κ B) 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 intestine¹³¹. This type of RV recognition may reflect how RV enters the host cell by exploiting the endosomal vesicle transport system¹²⁷. Several other TLRs have been implicated in the detection of RVs, including TLR2, TLR5, and TLR7¹³².

RV employs several 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- κ B 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 alone¹⁴⁰. 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 mediators¹³⁷. 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 pathogens¹⁴³. Mutation of IL-10 in paediatric patients was shown to promote early disease onset¹⁴⁶. On the other hand, *Il10* KO mice develop colitis like adults¹³⁷.

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 colitis¹⁴⁷. 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 pathogenesis¹⁴⁷⁻¹⁵⁰. 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 sulphate¹⁴⁷. DSS is a negatively charged sulphated polysaccharide with a highly variable molecular weight ranging from 5 to 1400kDa with water-soluble properties¹⁵¹. Administration of 40-50kDa DSS in drinking water produces the desired inflammatory effect that resembles human UC¹⁵⁰. 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-induction¹⁶²⁻¹⁶⁴. 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 substantially¹⁶⁵.

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 components¹⁶⁴. 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) responses³⁶. 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 intestine³⁶. In adult mice, the anatomical location dictated the coating of commensal bacteria coating rather than the bacterial identity³⁶. 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 abundance 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 IFN γ 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 cholera toxin 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 cholera toxin 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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References

1. Agace, W. W. & McCoy, K. D. Regionalized Development and Maintenance of the Intestinal Adaptive Immune Landscape. *Immunity* (2017) doi:10.1016/j.immuni.2017.04.004.
2. Mowat, A. M. & Agace, W. W. Regional specialization within the intestinal immune system. *Nature Reviews Immunology* (2014) doi:10.1038/nri3738.
3. Yu, Y., Sitaraman, S. & Gewirtz, A. T. Intestinal epithelial cell regulation of mucosal inflammation. *Immunologic Research* (2004) doi:10.1385/ir:29:1-3:055.
4. Peloquin, J. M., Goel, G., Villablanca, E. J. & Xavier, R. J. Mechanisms of Pediatric Inflammatory Bowel Disease. *Annu. Rev. Immunol.* **34**, 31–64 (2016).
5. Yang, J.-Y. Y. *et al.* Enteric Viruses Ameliorate Gut Inflammation via Toll-like Receptor 3 and Toll-like Receptor 7-Mediated Interferon- β Production. *Immunity* **44**, 889–900 (2016).
6. Clevers, H. C. & Bevins, C. L. Paneth cells: Maestros of the small intestinal crypts. *Annu. Rev. Physiol.* **75**, 289–311 (2013).
7. Kaser, A. & Blumberg, R. S. Autophagy, microbial sensing, endoplasmic reticulum stress, and epithelial function in inflammatory bowel disease. *Gastroenterology* **140**, 1738-1747.e2 (2011).
8. Adolph, T. E. *et al.* Paneth cells as a site of origin for intestinal inflammation. *Nature* vol. 503 272–276 (2013).
9. Fu, J. *et al.* Loss of intestinal core 1–derived. *J. Clin. Invest.* **121**, 1657–1666 (2011).
10. Ramanan, D. & Cadwell, K. Intrinsic Defense Mechanisms of the Intestinal Epithelium. *Cell Host Microbe* **19**, 434–441 (2016).
11. Bergstrom, K. S. B. *et al.* Muc2 protects against lethal infectious colitis by disassociating pathogenic and commensal bacteria from the colonic mucosa. *PLoS Pathog.* **6**, (2010).

12. Johansson, M. E. V. *et al.* The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 15064–15069 (2008).
13. Schütte, A. *et al.* Microbial-induced meprin β cleavage in MUC2 mucin and a functional CFTR channel are required to release anchored small intestinal mucus. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 12396–12401 (2014).
14. Lycke, N. Y. & Bemark, M. The regulation of gut mucosal IgA B-cell responses: Recent developments. *Mucosal Immunol.* **10**, 1361–1374 (2017).
15. Rios, D. *et al.* Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. *Mucosal Immunol.* **9**, 907–916 (2016).
16. Bemark, M. & Angeletti, D. Know your enemy or find your friend?—Induction of IgA at mucosal surfaces. *Immunol. Rev.* **303**, 83–102 (2021).
17. Pabst, O. *et al.* Cryptopatches and isolated lymphoid follicles: Dynamic lymphoid tissues dispensable for the generation of intraepithelial lymphocytes. *Eur. J. Immunol.* **35**, 98–107 (2005).
18. MacPherson, A. J., McCoy, K. D., Johansen, F. E. & Brandtzaeg, P. The immune geography of IgA induction and function. *Mucosal Immunol.* **1**, 11–22 (2008).
19. Suzuki, K., Kawamoto, S., Maruya, M. & Fagarasan, S. *GALT. Organization and dynamics leading to IgA synthesis. Advances in Immunology* vol. 107 (Elsevier Inc., 2010).
20. Brandtzaeg, P. Function of mucosa-associated lymphoid tissue in antibody formation. *Immunol. Invest.* **39**, 303–355 (2010).
21. Thaiss, C. A., Zmora, N., Levy, M. & Elinav, E. The microbiome and innate immunity. *Nature* **535**, 65–74 (2016).
22. Thaiss, C. A., Levy, M., Suez, J. & Elinav, E. The interplay between the innate immune system and the microbiota. *Curr. Opin. Immunol.* **26**, 41–48 (2014).
23. Pott, J. & Hornef, M. Innate immune signalling at the intestinal epithelium in homeostasis and disease. *EMBO Rep.* **13**, 684–698 (2012).
24. Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. Recognition of commensal microflora by toll-like

- receptors is required for intestinal homeostasis. *Cell* **118**, 229–41 (2004).
25. Nenci, A. *et al.* Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* **446**, 557–561 (2007).
 26. Vaishnava, S. *et al.* The Antibacterial Lectin RegIII. *Science* (80-.). **334**, 255–258 (2011).
 27. Spits, H. *et al.* FOCUS ON the inbetweeners : innate-like PER lymphocytes S P E C T I V E S Innate lymphoid cells — a proposal for uniform nomenclature. **13**, 145–150 (2013).
 28. Joeris, T., Müller-Luda, K., Agace, W. W. & Mowat, A. M. I. Diversity and functions of intestinal mononuclear phagocytes. *Mucosal Immunol.* **10**, 845–864 (2017).
 29. Honda, K. & Littman, D. R. The microbiota in adaptive immune homeostasis and disease. *Nature* **535**, 75–84 (2016).
 30. Cheroutre, H. & Madakamutil, L. Acquired and natural memory T cells join forces at the mucosal front line. *Nat. Rev. Immunol.* **4**, 290–300 (2004).
 31. Van Wijk, F. & Cheroutre, H. Mucosal T cells in gut homeostasis and inflammation. *Expert Rev Clin Immunol* **6**, 559–566 (2010).
 32. BOLL, G., RUDOLPHI, A., SPIEB, S. & REIMANN, J. Regional Specialization of Intraepithelial T Cells in the Murine Small and Large Intestine. *Scand. J. Immunol.* **41**, 103–113 (1995).
 33. Ibraghimov, A. R. & Lynch, R. G. Heterogeneity and biased t cell receptor α/β repertoire of mucosal cd8⁺ cells from murine large intestine: Implications for functional state. *J. Exp. Med.* **180**, 433–444 (1994).
 34. Fagarasan, S., Kawamoto, S., Kanagawa, O. & Suzuki, K. Adaptive Immune Regulation in the Gut: T Cell–Dependent and T Cell–Independent IgA Synthesis. *Annu. Rev. Immunol.* **28**, 243–273 (2010).
 35. Bunker, J. J. & Bendelac, A. IgA Responses to Microbiota. *Immunity* **49**, 211–224 (2018).
 36. Bunker, J. J. *et al.* Innate and Adaptive Humoral Responses Coat Distinct Commensal Bacteria with Immunoglobulin A. *Immunity* **43**, 541–553 (2015).
 37. Macpherson, A. J. & Uhr, T. Induction of Protective IgA by Intestinal Dendritic Cells Carrying Commensal Bacteria. *Science* (80-.). **303**, 1662–1665 (2004).

38. Fagarasan, S., Kinoshita, K., Muramatsu, M., Ikuta, K. & Honjo, T. In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* **413**, 639–643 (2001).
39. Macpherson, A. J. *et al.* A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science (80-. .)*. **288**, 2222–2226 (2000).
40. Kang, H. S. *et al.* Signaling via $l\text{t}\beta\text{r}$ on the lamina propria stromal cells of the gut is required for iga production. *Nat. Immunol.* **3**, 576–582 (2002).
41. Tsuji, M. *et al.* Requirement for Lymphoid Tissue-Inducer Cells in Isolated Follicle Formation and T Cell-Independent Immunoglobulin A Generation in the Gut. *Immunity* **29**, 261–271 (2008).
42. Pabst, O. & Slack, E. IgA and the intestinal microbiota: the importance of being specific. *Mucosal Immunol.* **13**, 12–21 (2020).
43. Brandtzaeg, P. Mucosal immunity: Induction, dissemination, and effector functions. *Scand. J. Immunol.* **70**, 505–515 (2009).
44. Stadtmueller, B. M. *et al.* The structure and dynamics of secretory component and its interactions with polymeric immunoglobulins. *Elife* **5**, 1–23 (2016).
45. Woof, J. M. & Russell, M. W. Structure and function relationships in IgA. *Mucosal Immunol.* **4**, 590–597 (2011).
46. Hamburger, A. E., Bjorkman, P. J. & Herr, A. B. Structural insights into antibody-mediated mucosal immunity. *Curr. Top. Microbiol. Immunol.* **308**, 173–204 (2006).
47. Van Itallie, C. M. & Anderson, J. M. Claudins and epithelial paracellular transport. *Annu. Rev. Physiol.* **68**, 403–429 (2006).
48. Rogier, E. W. *et al.* Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 3074–3079 (2014).
49. Leakiness, I. *et al.* Receptor / Secretory Component “deficient Mice. **190**, (1999).
50. Cerutti, A. The regulation of IgA class switching. *Nat. Rev. Immunol.* **8**, 421–434 (2008).
51. Hand, T. W. & Reboldi, A. Production and Function of Immunoglobulin A. *Annu. Rev. Immunol.* **39**, 695–718 (2021).

52. Fagarasan, S. *et al.* Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* (80-.). **298**, 1424–1427 (2002).
53. Litinskiy, M. B. *et al.* DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat. Immunol.* **3**, 822–829 (2002).
54. Torow, N., Marsland, B. J., Hornef, M. W. & Gollwitzer, E. S. Neonatal mucosal immunology. *Mucosal Immunol.* **10**, 5–17 (2017).
55. Cahenzli, J., Köller, Y., Wyss, M., Geuking, M. B. & McCoy, K. D. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* **14**, 559–570 (2013).
56. Al Nabhani, Z. *et al.* A Weaning Reaction to Microbiota Is Required for Resistance to Immunopathologies in the Adult. *Immunity* **50**, 1276–1288.e5 (2019).
57. Knoop, K. A. *et al.* Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. *Sci. Immunol.* **2**, 1–12 (2017).
58. Basha, S., Surendran, N. & Pichichero, M. Immune responses in neonates. *Expert Rev. Clin. Immunol.* **10**, 1171–1184 (2014).
59. Liu, L. *et al.* Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* **388**, 3027–3035 (2016).
60. Torow, N. & Hornef, M. W. The Neonatal Window of Opportunity: Setting the Stage for Life-Long Host-Microbial Interaction and Immune Homeostasis. *J. Immunol.* **198**, 557–563 (2017).
61. Garcia, A. M., Fadel, S. A., Cao, S. & Sarzotti, M. T cell immunity in neonates. *Immunol. Res.* **22**, 177–190 (2000).
62. Torow, N. *et al.* Active suppression of intestinal CD4 + TCR $\alpha\beta$ + T-lymphocyte maturation during the postnatal period. *Nat. Commun.* **6**, (2015).
63. Heninger, A. *et al.* Activation of Islet Autoreactive Naïve T Cells in Infants. (2013) doi:10.2337/db12-0942.
64. Mold, J. E. *et al.* Humans Linked references are available on JSTOR for this article : Fetal and Adult Hematopoietic Stem Cells Give Rise to Distinct T Cell Lineages in Humans. (2010).
65. Guo, J. *et al.* Role of IgA in the early-life establishment of the gut microbiota and immunity: Implications for constructing a healthy

- start. *Gut Microbes* **13**, 1–21 (2021).
66. Mu, Q. *et al.* Regulation of neonatal IgA production by the maternal microbiota. *Proc. Natl. Acad. Sci. U. S. A.* **118**, 1–5 (2021).
 67. Humphrey, J. H. The risks of not breastfeeding. *J. Acquir. Immune Defic. Syndr.* **53**, 1–4 (2010).
 68. Gopalakrishna, K. P. *et al.* Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. *Nat. Med.* **25**, 1110–1115 (2019).
 69. Hornef, M. W. & Torow, N. ‘Layered immunity’ and the ‘neonatal window of opportunity’ – timed succession of non-redundant phases to establish mucosal host–microbial homeostasis after birth. *Immunology* **159**, 15–25 (2020).
 70. Al Nabhani, Z. & Eberl, G. Imprinting of the immune system by the microbiota early in life. *Mucosal Immunol.* **13**, 183–189 (2020).
 71. Russell, S. L. *et al.* scientific report enhance susceptibility to allergic asthma. **13**, (2012).
 72. Balzola, F., Cullen, G., Ho, G. T., Russell, R. K. & Wehkamp, J. Microbial exposure during early life has persistent effects on natural killer T cell function. *Inflamm. Bowel Dis. Monit.* **13**, 29–30 (2012).
 73. Scharschmidt, T. C. *et al.* Article A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes Article A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes. *Immunity* **43**, 1011–1021 (2015).
 74. Robertson, S. J., Goethel, A., Girardin, S. E. & Philpott, D. J. Innate Immune Influences on the Gut Microbiome: Lessons from Mouse Models. *Trends Immunol.* **39**, 992–1004 (2018).
 75. Li, Y., Handley, S. A. & Baldridge, M. T. The dark side of the gut: Virome-host interactions in intestinal homeostasis and disease. *J. Exp. Med.* **218**, 1–18 (2021).
 76. Shetty, S. A., Hugenholtz, F., Lahti, L., Smidt, H. & de Vos, W. M. Intestinal microbiome landscaping: Insight in community assemblage and implications for microbial modulation strategies. *FEMS Microbiol. Rev.* **41**, 182–199 (2017).
 77. Gilbert, J. A. *et al.* Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature* **535**, 94–103 (2016).
 78. Pickard, J. M., Zeng, M. Y., Caruso, R. & Núñez, G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory

- disease. *Immunol. Rev.* **279**, 70–89 (2017).
79. Weström, B., Arévalo Sureda, E., Pierzynowska, K., Pierzynowski, S. G. & Pérez-Cano, F. J. The Immature Gut Barrier and Its Importance in Establishing Immunity in Newborn Mammals. *Front. Immunol.* **11**, (2020).
 80. Gensollen, T., Iyer, S. S., Kasper, D. L. & Blumberg, R. S. How colonization by microbiota in early life shapes the immune system. *Science (80-.)*. **352**, 539–544 (2016).
 81. Macpherson, A. J., Hooper, L. V. & Littman, D. R. Interactions Between the Microbiota and the Immune System. *Science (80-.)*. **336**, 1268–1273 (2012).
 82. Kliman, H. J. Comment on ‘The placenta harbors a unique microbiome’. *Sci. Transl. Med.* **6**, (2014).
 83. Lauder, A. P. *et al.* Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome* **4**, 1–11 (2016).
 84. Walter, J. & Hornef, M. W. A philosophical perspective on the prenatal in utero microbiome debate. *Microbiome* **9**, 1–9 (2021).
 85. Wopereis, H., Oozeer, R., Knipping, K., Belzer, C. & Knol, J. The first thousand days – intestinal microbiology of early life : establishing a symbiosis. **25**, 428–438 (2014).
 86. Sender, R., Fuchs, S. & Milo, R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell* **164**, 337–340 (2016).
 87. Sender, R., Fuchs, S. & Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. 1–14 (2016) doi:10.1371/journal.pbio.1002533.
 88. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32 (2015).
 89. Huttenhower, C. *et al.* Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
 90. Falony, G. *et al.* Population-level analysis of gut microbiome variation. *Science (80-.)*. **352**, 560–564 (2016).
 91. Li, J. *et al.* An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014).
 92. Faith, J. J. *et al.* The Long-Term Stability of the Human Gut

- Microbiota. **340**, (2013).
93. Martínez, I., Muller, C. E. & Walter, J. Long-Term Temporal Analysis of the Human Fecal Microbiota Revealed a Stable Core of Dominant Bacterial Species. *PLoS One* **8**, (2013).
 94. Janzon, A., Goodrich, J. K., Koren, O., Waters, J. L. & Ley, R. E. Interactions between the Gut Microbiome and Mucosal Immunoglobulins A, M, and G in the Developing Infant Gut. *mSystems* **4**, (2019).
 95. Goodrich, J. K. *et al.* Human genetics shape the gut microbiome. *Cell* **159**, 789–799 (2014).
 96. Knights, D., Parfrey, L. W., Zaneveld, J., Lozupone, C. & Knight, R. Human-associated microbial signatures: Examining their predictive value. *Cell Host Microbe* **10**, 292–296 (2011).
 97. Halfvarson, J. *et al.* Dynamics of the human gut microbiome in inflammatory bowel disease. *Nat. Microbiol.* **2**, 1–7 (2017).
 98. Uusitalo, U. *et al.* Association of Early Exposure of Probiotics and Islet Autoimmunity in the TEDDY Study. *JAMA Pediatr.* **170**, 20–28 (2016).
 99. Knights, D. *et al.* Complex host genetics influence the microbiome in inflammatory bowel disease. *Genome Med.* **6**, 1–11 (2014).
 100. Gilbert, J. A. *et al.* Current understanding of the human microbiome. *Nat. Med.* **24**, 392–400 (2018).
 101. Arrieta, M. C. *et al.* Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Science Translational Medicine* vol. 7 (2015).
 102. Durack, J. *et al.* Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by *Lactobacillus* supplementation. *Nature Communications* vol. 9 (2018).
 103. Fujimura, K. E. *et al.* Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat. Med.* **22**, 1187–1191 (2016).
 104. Surana, N. K. & Kasper, D. L. Moving beyond microbiome-wide associations to causal microbe identification. *Nature* **552**, 244–247 (2017).
 105. Sterlin, D., Fadlallah, J., Slack, E. & Gorochov, G. The antibody/microbiota interface in health and disease. *Mucosal Immunol.* **13**, 3–11 (2020).

106. Donaldson, G. P. *et al.* Gut microbiota utilize immunoglobulin a for mucosal colonization. *Science (80-.)*. **360**, 795–800 (2018).
107. Atarashi, K. *et al.* Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. *Cell* **163**, 367–380 (2015).
108. Farkas, A. M. *et al.* Induction of Th17 cells by segmented filamentous bacteria in the murine intestine. *J. Immunol. Methods* **421**, 104–111 (2015).
109. Ivanov, I. I. *et al.* Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* **139**, 485–498 (2009).
110. Bunker, J. J. & Bendelac, A. IgA Responses to Microbiota. *Immunity* **49**, 211–224 (2018).
111. Bishop, R. F., Davidson, G. P., Holmes, I. H. & Ruck, B. J. Virus Particles in Epithelial Cells of Duodenal Mucosa From Children With Acute Non-Bacterial Gastroenteritis. *Lancet* **302**, 1281–1283 (1973).
112. Greenberg, H. B. & Estes, M. K. Rotaviruses: From Pathogenesis to Vaccination. *Gastroenterology* **136**, 1939–1951 (2009).
113. Tate, J. E. *et al.* Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000–2013. *Clin. Infect. Dis.* **62**, S96–S105 (2016).
114. Desselberger, U. Rotaviruses. *Virus Res.* **190**, 75–96 (2014).
115. Kim, A. H. J., Hogarty, M. P., Harris, V. C. & Baldrige, M. T. The Complex Interactions Between Rotavirus and the Gut Microbiota. *Front. Cell. Infect. Microbiol.* **10**, (2021).
116. Aliabadi, N. *et al.* Global impact of rotavirus vaccine introduction on rotavirus hospitalisations among children under 5 years of age, 2008–16: findings from the Global Rotavirus Surveillance Network. *Lancet Glob. Heal.* **7**, e893–e903 (2019).
117. Troeger, C. *et al.* Rotavirus Vaccination and the Global Burden of Rotavirus Diarrhea among Children Younger Than 5 Years. *JAMA Pediatr.* **172**, 958–965 (2018).
118. García-López, R., Pérez-Brocal, V., Diez-Domingo, J. & Moya, A. Gut microbiota in children vaccinated with rotavirus vaccine. *Pediatr. Infect. Dis. J.* **31**, 1300–1302 (2012).
119. Ang, L. *et al.* The establishment of the infant intestinal microbiome is not affected by rotavirus vaccination. *Sci. Rep.* **4**, 1–6 (2014).
120. Harris, V. C. *et al.* Effect of Antibiotic-Mediated Microbiome

- Modulation on Rotavirus Vaccine Immunogenicity: A Human, Randomized-Control Proof-of-Concept Trial. *Cell Host Microbe* **24**, 197-207.e4 (2018).
121. Harris, V. C. *et al.* Significant Correlation Between the Infant Gut Microbiome and Rotavirus Vaccine Response in Rural Ghana. *J. Infect. Dis.* **215**, 34–41 (2017).
 122. Harris, V. *et al.* Rotavirus vaccine response correlates with the infant gut microbiota composition in Pakistan. *Gut Microbes* **9**, 93–101 (2018).
 123. Perrett, K. P., Jachno, K., Nolan, T. M. & Harrison, L. C. Association of Rotavirus Vaccination With the Incidence of Type 1 Diabetes in Children. *JAMA Pediatr.* **173**, 280 (2019).
 124. Rogers, M. A. M., Basu, T. & Kim, C. Lower Incidence Rate of Type 1 Diabetes after Receipt of the Rotavirus Vaccine in the United States, 2001–2017. *Sci. Rep.* **9**, 1–8 (2019).
 125. Desselberger, U. Differences of rotavirus vaccine effectiveness by country: Likely causes and contributing factors. *Pathogens* **6**, 1–13 (2017).
 126. Lin, J. Da *et al.* Distinct Roles of Type I and Type III Interferons in Intestinal Immunity to Homologous and Heterologous Rotavirus Infections. *PLoS Pathog.* **12**, 1–29 (2016).
 127. Sen, A., Ding, S. & Greenberg, H. B. *The role of innate immunity in regulating rotavirus replication, pathogenesis, and host range restriction and the implications for live rotaviral vaccine development. Mucosal Vaccines: Innovation for Preventing Infectious Diseases* (Elsevier Inc., 2019). doi:10.1016/B978-0-12-811924-2.00041-9.
 128. Broquet, A. H., Hirata, Y., McAllister, C. S. & Kagnoff, M. F. RIG-I/MDA5/MAVS Are Required To Signal a Protective IFN Response in Rotavirus-Infected Intestinal Epithelium. *J. Immunol.* **186**, 1618–1626 (2011).
 129. Hartmann, G. *Nucleic Acid Immunity. Advances in Immunology* vol. 133 (Elsevier Inc., 2017).
 130. Onoguchi, K. *et al.* Viral infections activate types I and III interferon genes through a common mechanism. *J. Biol. Chem.* **282**, 7576–7581 (2007).
 131. Pott, J. *et al.* Age-dependent TLR3 expression of the intestinal

- epithelium contributes to rotavirus susceptibility. *PLoS Pathog.* **8**, (2012).
132. Caddy, S., Papa, G., Borodavka, A. & Desselberger, U. Rotavirus research: 2014–2020. *Virus Res.* **304**, 198499 (2021).
 133. Iaconis, G. *et al.* Rotavirus nsp1 inhibits type I and type III interferon induction. *Viruses* **13**, 1–17 (2021).
 134. Kaser, A., Zeissig, S. & Blumberg, R. S. Inflammatory bowel disease. *Annu. Rev. Immunol.* **28**, 573–621 (2010).
 135. Aden, K. & Reindl, W. The Gut Microbiome in Inflammatory Bowel Diseases: Diagnostic and Therapeutic Implications. *Visc. Med.* **35**, 332–336 (2019).
 136. Yue, B. *et al.* Inflammatory Bowel Disease : A Potential Result from the Collusion between Gut Microbiota and Mucosal Immune System. (2019).
 137. Shan, Y., Lee, M. & Chang, E. B. The Gut Microbiome and Inflammatory Bowel Diseases. (2022).
 138. Liu, T. C. & Stappenbeck, T. S. Genetics and Pathogenesis of Inflammatory Bowel Disease. *Annu. Rev. Pathol. Mech. Dis.* **11**, 127–148 (2016).
 139. Fiocchi, C. Inflammatory bowel disease: Complexity and variability need integration. *Front. Med.* **5**, 5–8 (2018).
 140. Kaplan, G. G. The global burden of IBD: From 2015 to 2025. *Nat. Rev. Gastroenterol. Hepatol.* **12**, 720–727 (2015).
 141. Colombel, J. F. & Mahadevan, U. Inflammatory Bowel Disease 2017: Innovations and Changing Paradigms. *Gastroenterology* **152**, 309–312 (2017).
 142. Knox, N. C., Forbes, J. D., Van Domselaar, G. & Bernstein, C. N. The Gut Microbiome as a Target for IBD Treatment: Are We There Yet? *Curr. Treat. Options Gastroenterol.* **17**, 115–126 (2019).
 143. Abraham, C. & Cho, J. H. T 2066. 2066–2078 (2009).
 144. Xavier, R. J. & Podolsky, D. K. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427–34 (2007).
 145. Angeles, L. Genetics of Inflammatory Bowel Diseases. 1163–1176 (2015) doi:10.1053/j.gastro.2015.08.001.
 146. Article, R. Recent Advance in Very Early Onset Inflammatory Bowel. **22**, 41–49 (2019).

147. Kiesler, P., Fuss, I. J. & Strober, W. Experimental models of inflammatory bowel diseases. *Med. Hyg. (Geneve)*. **59**, 241–248 (2001).
148. Okayasu, I. *et al.* A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* **98**, 694–702 (1990).
149. Mizoguchi, A. *Animal models of inflammatory bowel disease. Progress in Molecular Biology and Translational Science* vol. 105 (Elsevier Inc., 2012).
150. Eichele, D. D. & Kharbanda, K. K. Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. *World J. Gastroenterol.* **23**, 6016–6029 (2017).
151. Perše, M. & Cerar, A. Dextran sodium sulphate colitis mouse model: Traps and tricks. *J. Biomed. Biotechnol.* **2012**, (2012).
152. Das, S., Batra, S. & Rachagani, S. Mouse Model of Dextran Sodium Sulfate (DSS)-induced Colitis. *Bio-Protocol* **7**, 3–7 (2017).
153. Chassaing, B., Aitken, J. D., Malleshappa, M. & Vijay-kumar, M. Dextran Sulfate Sodium (DSS) -Induced Colitis in Mice. 1–14 (2014) doi:10.1002/0471142735.im1525s104.
154. Wang, Y. *et al.* Rig-I/- mice develop colitis associated with downregulation of Gai2. *Cell Res.* **17**, 858–868 (2007).
155. Li, X. D. *et al.* Mitochondrial antiviral signaling protein (MAVS) monitors commensal bacteria and induces an immune response that prevents experimental colitis. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 17390–17395 (2011).
156. Fischer, J. C. *et al.* RIG-I/MAVS and STING signaling promote gut integrity during irradiation- and immune-mediated tissue injury. *Sci. Transl. Med.* **9**, 1–14 (2017).
157. Pietrzak, B., Tomela, K., Olejnik-Schmidt, A., Mackiewicz, A. & Schmidt, M. Secretory iga in intestinal mucosal secretions as an adaptive barrier against microbial cells. *Int. J. Mol. Sci.* **21**, 1–15 (2020).
158. Brandtzaeg, P. & Johansen, F. E. Mucosal B cells: Phenotypic characteristics, transcriptional regulation, and homing properties. *Immunol. Rev.* **206**, 32–63 (2005).
159. Brandtzaeg, P. The Mucosal Immune System and Its Integration with

- the Mammary Glands. *J. Pediatr.* **156**, S8 (2010).
160. Olin, A. *et al.* Stereotypic Immune System Development in Newborn Children. *Cell* **174**, 1277-1292.e14 (2018).
 161. Rogier, E. W. *et al.* Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 3074–3079 (2014).
 162. Harris, N. L. *et al.* Mechanisms of Neonatal Mucosal Antibody Protection. *J. Immunol.* **177**, 6256–6262 (2006).
 163. Kramer, D. R. & Cebra, J. J. Early appearance of ‘natural’ mucosal IgA responses and germinal centers in suckling mice developing in the absence of maternal antibodies. *J. Immunol.* **154**, 2051–62 (1995).
 164. Mu, Q. *et al.* Regulation of neonatal IgA production by the maternal microbiota. *Proc. Natl. Acad. Sci. U. S. A.* **118**, 1–8 (2021).
 165. Feng, N. *et al.* Role of Interferon in Homologous and Heterologous Rotavirus Infection in the Intestines and Extraintestinal Organs of Suckling Mice. *J. Virol.* **82**, 7578–7590 (2008).
 166. Grasset, E. K. *et al.* Gut T cell-independent IgA responses to commensal bacteria require engagement of the TACI receptor on B cells. *Sci. Immunol.* **5**, (2020).
 167. Ding, M. *et al.* Crosstalk between sIgA-Coated Bacteria in Infant Gut and Early-Life Health. *Trends Microbiol.* **29**, 725–735 (2021).

