博士論文 (要約)

Studies on Functions of Cells Involved in Immune Responses in the Intestinal Lymphoid Tissues

(腸管のリンパ組織における免疫応答に関わる細胞の機能についての研究)

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Abbreviations

Ab	antibody
Ag	antigen
AhR	aryl hydrocarbon receptor
AID	activation-induced cytidine deaminase
APRIL	a proliferation-inducing ligand
BAFF	B cell activating factor
BCR	B cell receptor
CaeP	caecal patch
CDR	complementarity-determining region
CFSE	5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester
CoP	colonic patch
CSR	class-switch recombination
DC	dendritic cell
DMSO	dimethyl sulfoxide
FcεR	Fcε receptor
FCS	fetal calf serum
FDC	follicular dendritic cell
GALT	gut-associated lymphoid tissue
GC	germinal center
GF	germ-free
GLT	germ line transcript
GM-CSF	granulocyte macrophage colony-stimulating factor
IDO	indoleamine 2,3-dioxygenase
IFN	interferon
Ig	immunoglobulin
IL	interleukin
ILC	innate lymphoid cell
ILC*N*	group *N* innate lymphoid cell (*N*: a natural number)
Int.	intermediate
Lin.	lineage markers
LN	lymph node
LP	lamina propria
LTi cell	lymphoid tissue inducer cell
MHC	major histocompatibility complex
MHC *X*	major histocompatibility complex class *X* (*X* = I, II)

MLN	mesenteric lymph node
NK cell	natural killer cell
OVA	ovalbumin
OVAp	ovalbumin peptide
PP	Peyer's patch
PST	post-switch transcript
pT_{reg} cell	peripherally derived regulatory T cell
RA	retinoic acid
RALDH	retinal dehydrogenase
SFB	segmented filamentous bacteria
SHM	somatic hyper-mutation
SPF	specific pathogen-free
SPL	spleen
TCR	T cell receptor
T_{FH} cell	T follicular helper cell
T_{FR} cell	follicular regulatory T cell
TGF	transforming growth factor
T_H*N* cell	T helper *N* cells (*N*: a natural number)
TLR	Toll-like receptor
TNF	tumor necrosis factor
T_{reg} cell	regulatory T cell
TSLP	thymic stromal lymphopoietin
tT_{reg} cell	thymus-derived regulatory T cell

Chapter 1

General Introduction

§1.1 Preface

The present studies focused on cells in the intestinal immune system (**Fig. 1-1**). The intestine is an important immune organ because it is exposed to many pathogens invading orally with food intake every day. The intestinal immune system should eliminate these pathogens. At the same time, the intestine needs to efficiently acquire nutrients from the foods. Further, the intestine harbors trillions of commensal microbes, which have beneficial functions to the host, e.g. digesting foods, eliminating pathogens, and even preventing diseases (*1*, *2*). The intestinal immune system should be regulated not to attack these beneficial substances and organisms. Deficiency in the regulation probably lead to detrimental abnormalities, such as food allergy and inflammatory bowel diseases. Thus, the intestinal immune system has to induce the opposite responses to different targets at the same place. Huge numbers of studies have intensely investigated into the intestinal immune system. However, we have not obtained the whole picture of the complex system.

The immune system is a complex and ingenious multi-cellular system. Various immune cells in the system function in a complex cell-cell network to satisfy the requirements for the system. In subsequent sections, the author will discuss about regulation of the immune responses and characteristics of the intestinal immune system. Such discussion is expected to present an overview of a cellular network in the immune regulation revealed by previous studies.

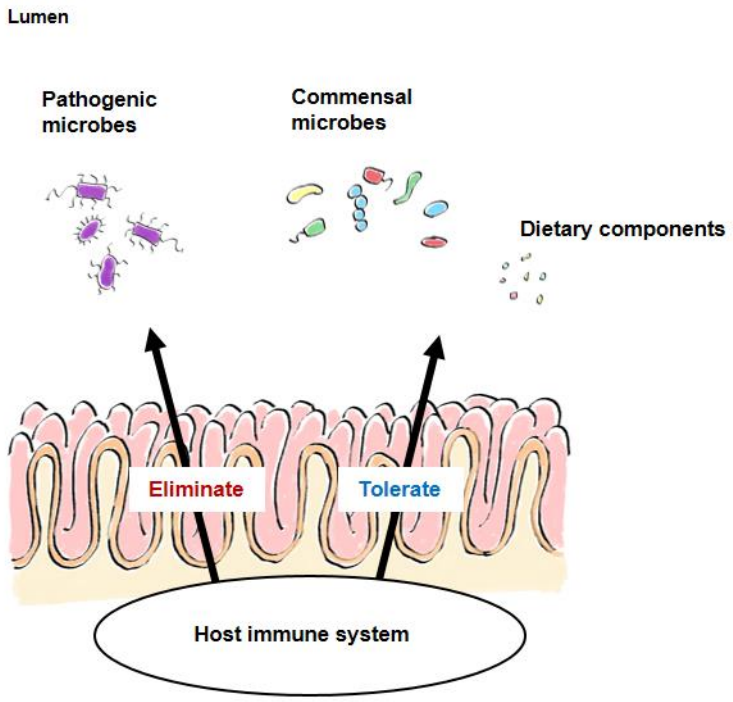

Fig. 1-1 The intestinal immune system

§1.2 Immune Responses to Various Agents

§1.2.1 The immune system distinguishes self and non-self.

We, living individuals, are separated physically from the external environment by epithelium at the skin and mucosal sites, which functions as a primary barrier to exogenous pathogens, such as bacteria and viruses. However, some pathogens could pass through this primal barrier taking advantage of physical injury or developing their own strategy to break down the barrier function. Such invading pathogens should be eliminated. For the elimination, mammals have evolved their immune system, which attacks the pathogens. To efficiently eliminate pathogens, the immune system should attack the pathogens specifically. Such specific elimination requires recognition of the exogenous pathogens. To accomplish this, the immune system utilizes self components to distinguish non-self pathogens, i.e. the system attacks non-self agents as pathogens.

§1.2.2 T cells play a pivotal role in recognizing specificity.

To distinguish self and non-self, the immune systems should be equipped with a mechanism to recognize specificity of various substances and organisms including exogenous non-self components and endogenous self components. This is accomplished by T cells. T cells express T cell receptors (TCRs) which recognize a specific sequence of peptides presented on major histocompatibility complex (MHC) molecules (**Fig. 1-2A**). Proteins which include such TCR-recognizable peptide sequences (epitopes) are called antigens (Ags). There are innumerable Ags derived from self and non-self.

T cells experience genomic diversification of genes encoding TCRα- and TCRβ-chains, called V(D)J recombination (*3*, *4*). This process produces diverse T cell clones, each of which express different TCRs recognizing different epitopes (**Fig. 1-2B**). Thus, T cells could distinguish diverse Ag-specificity as a whole. Theoretical study has estimated that the VDJ recombination process in a TCRβ gene can produce about 10^{14} different nucleotide sequences (*5*). In combination with TCRα, a diversity of TCRαβ repertoire is estimated to be about 10^{20} (*6*). Namely, each individual could contain 10^{20} different T cell clones potentially. The diversified T cells subsequently experience positive and negative selection in the thymus (*7*, *8*). Here, the author briefly describes a conceptual overview of the processes below (**Fig. 1-3A**).

The selection process is largely dependent on a reactivity of expressed TCRs on T cell precursors to self-peptide-MHC complex on Ag-presenting cells in the thymus. Each TCR chain contains three variable domains called complementarity-determining regions (CDRs), which are different among different T cell clones. The CDRs are involved in interaction of TCRs with not only Ag epitopes but also MHC molecules which have genetic polymorphism (*9*). Therefore, individuals require T cells expressing TCRs which can interact with their self MHC molecules. Such self-MHC-recognizing T cells are selected by positive selection. T cells require interaction of their TCRs with peptide-MHC for survival, and TCRs without any signals from peptide-MHC complex in the thymus lead the T cells to undergo apoptotic processes.

To prevent detrimental auto-immune responses, a negative selection process deletes T cell clones which

have TCRs strongly recognizing self-peptide on MHCs. This process is also known as clonal deletion. Among T cells having lower affinity to self-peptide-MHC complex, those with intermediate affinity to self-peptide-MHC probably undergo differentiation to immunosuppressive regulatory T (T_{reg}) cells. Indeed, it has been reported that TCR affinity strength correlates with T_{reg} cell generation in the thymus (*10*). The remaining T cells with low affinity to self-peptide-MHCs differentiated into naïve T cells, which survey all through the body to find invading foreign Ags. These T cells are composed of MHC class I (MHC I) - restricted $CD8^+$ cytotoxic T cells and MHC class II (MHC II) - restricted $CD4^+$ helper T cells. Cytotoxic T cells eliminate pathogens by induce death of infected cells whereas helper T cells help other immune cells, mainly through cytokine secretion, to eliminate pathogens.

Naïve T cells in individuals include various clones with diverse specificity. These T cells go through clonal selection in immune responses (*11*)(**Fig. 1-3B**). When foreign substances and organisms invade, naïve T cell clones expressing reactive TCRs to the Ags of the foreign agents are selected to respond to and eliminate the Ags.

Activated naïve T cells differentiate into effector T cells or long-lived memory T cells. Effector cytotoxic T cells secrete interferon (IFN) -γ, tumor necrosis factor (TNF) -α, perforin, and granzyme to kill infected cells (*12*). Although these effector cytotoxic T cells exhibit some heterogeneity in functional property and phenotype, such effector cells are principally directed to kill other cells (*13*). In contrast, effector helper T cells contain several subsets which have distinct functions. Well-known effector helper T cells include T helper (T_H) 1 cells, T_H2 cells, and T_H17 cells. These T_H cells express and secrete their characteristic transcription factors and cytokines: T_H1 cells express T-bet and secrete IFN-γ; T_H2 cells express GATA3 and secrete interleukin (IL) -4, IL-5, and IL-13; T_H17 cells express RORγt and secrete IL-17A and IL-22. In addition, T_{reg} cells, which express a transcription factor Foxp3, can be induced from naïve T cells in periphery. Such T_{reg} cells are distinguished from thymic T_{reg} cells mentioned above, and these T_{reg} cells are named as peripherally derived T_{reg} (pT_{reg}) cells (also called as induced T_{reg} cells) and thymus-derived T_{reg} (tT_{reg}) cells (also called as naturally occurring T_{reg} cells) (*14*). Differentiation of naïve $CD4^+$ T cells into these subsets is biased by environmental factors (*15*, *16*). For instance, differentiation into T_H1 cells, T_H2 cells, T_H17 cells, and pT_{reg} cells are induced by IL-12, IL-4, IL-6 with transforming growth factor (TGF) -β, and TGF-β, respectively. Such environmentally biased regulation enabled helper T cells to enhance a suitable immune response to each infection. In addition, it has been reported that TCR specificity itself could affect helper T cell differentiation fate (*17*).

§1.2.3 Lymphoid tissues function as a unit for immune responses.

Ag-presentation to naïve T cells mainly occurs in secondary lymphoid tissues, such as lymph nodes (LNs) and a spleen (SPL). The lymphoid tissues function as a site for naïve T cell priming. Naïve T cells, after positive and negative selection in the thymus, migrate blood, lymphoid tissue, and efferent lymph, and then recirculate into blood through thoracic duct (*18*, *19*)(**Fig. 1-4A**). LNs drain Ag-presenting cells from periphery (**Fig. 1-4B**). One peripheral region has its specific draining LN, i.e. Ags in the region is always carried to the region-specific LN (*18–20*). Thus, lymphoid tissues probably function as a unit for the immune system. Such region-specific immune unit enable the immune system to induce a suitable response to the region where Ags are derived from, i.e. immune responses to the intestinal Ags and to the skin Ags can be different, even though they are the same Ags.

To induce T cell responses in lymphoid tissues, Ags need to be carried into the lymphoid tissue. This is accomplished by Ag-presenting cells immigrating to the LN from periphery through afferent lymph. An important Ag-presenting cell type is dendritic cells (DCs; discussed later in ***§1.5***). DCs take up Ags in periphery, migrate into LNs, and present the Ag to naïve T cells inducing T cell subset differentiation. Lymphoid tissues have different mechanisms of Ag acquisition. In SPL, Ags in blood are obtained by marginal zone DCs and macrophages, i.e. Ag acquisition occurs in the lymphoid tissue (*21*). Another type of lymphoid tissues is mucosa-associated lymphoid tissues, which is located in mucosal sites. This type of lymphoid tissues has no afferent lymph and directly acquire exogenous Ags through epithelium on the tissues.

In the intestine, there are several lymphoid tissues which survey exogenous Ags (**Fig. 1-5**). These lymphoid tissues include both of LNs and mucosa-associated lymphoid tissue, the latter is called gut-associated lymphoid tissue (GALT) (*20, 22, 23*). GALTs include Peyer's patches (PPs), caecal patches (CaePs), and colonic patches (CoPs) in the small intestine, the caecum, and the large intestine, respectively. The GALT capture Ags from the intestinal lumen through epithelial cells on it and thus have no afferent lymph (*24*). The intestinal LNs include mesenteric LNs (MLNs) draining Ags from both of the small and large intestines and caudal LNs draining Ags from the large intestine. Therefore, these lymphoid tissues probably play a pivotal role in regulating Ag-specific immune responses in the intestine.

§1.2.4 Short summary

The immune responses to various Ags are regulated by T cells, which express Ag-specific TCRs. The TCR specificity has broad spectrum, resulted from V(D)J recombination process, and each T cells can respond to specific Ag inducing a suitable response specifically to the Ag. T cells recognize their Ags presented on MHC molecules of Ag-presenting cells in lymphoid tissues, which function as a unit of the immune system. In the intestine, MLNs, caudal LNs, and GALT including PP, CaeP, and CoP are probably important sites for immune regulation.

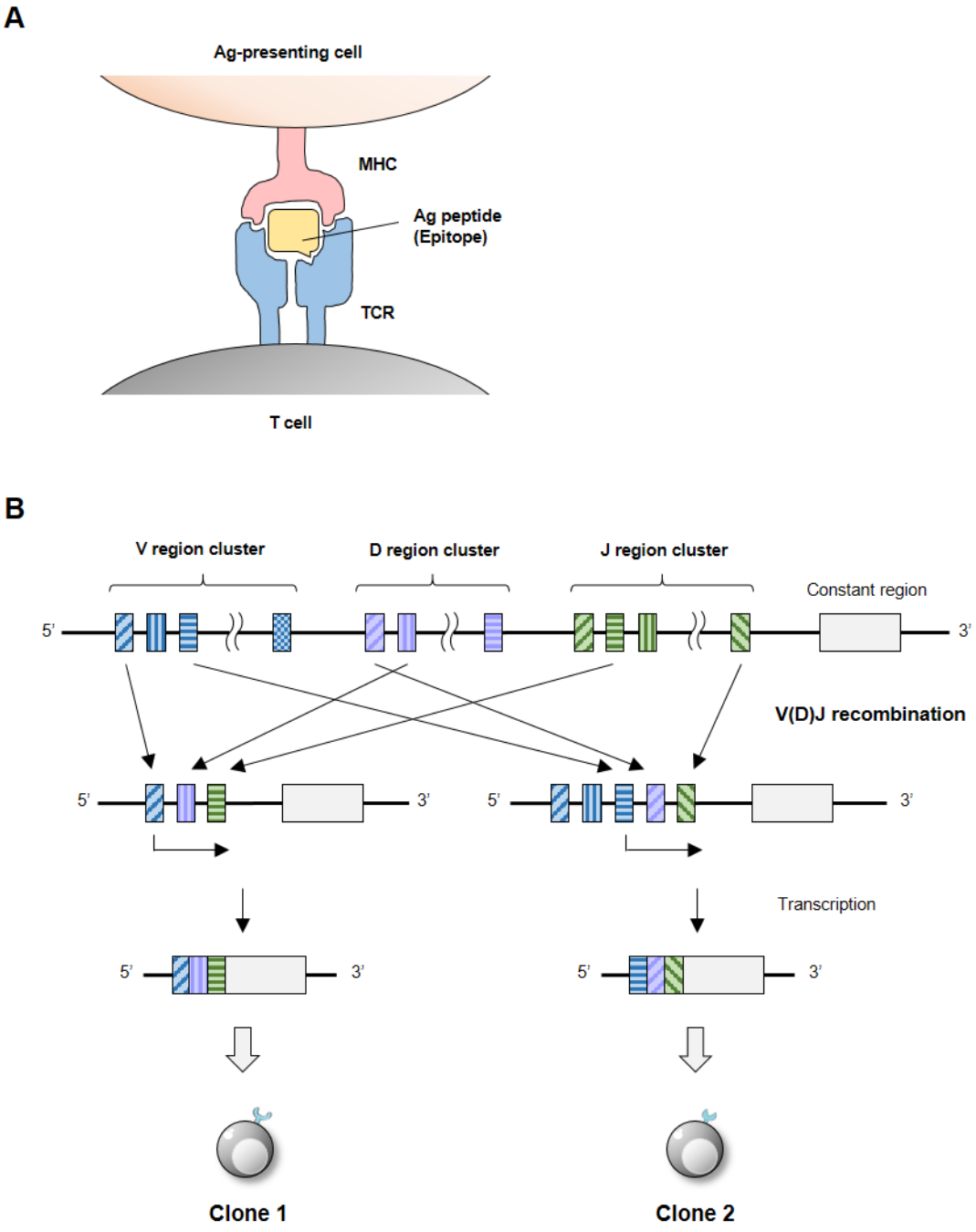

Fig. 1-2 Conceptual description of T cell specificity
A. TCRs recognize peptide-MHC complex.
B. Genes encoding TCRs are diversified by V(D)J recombination process.

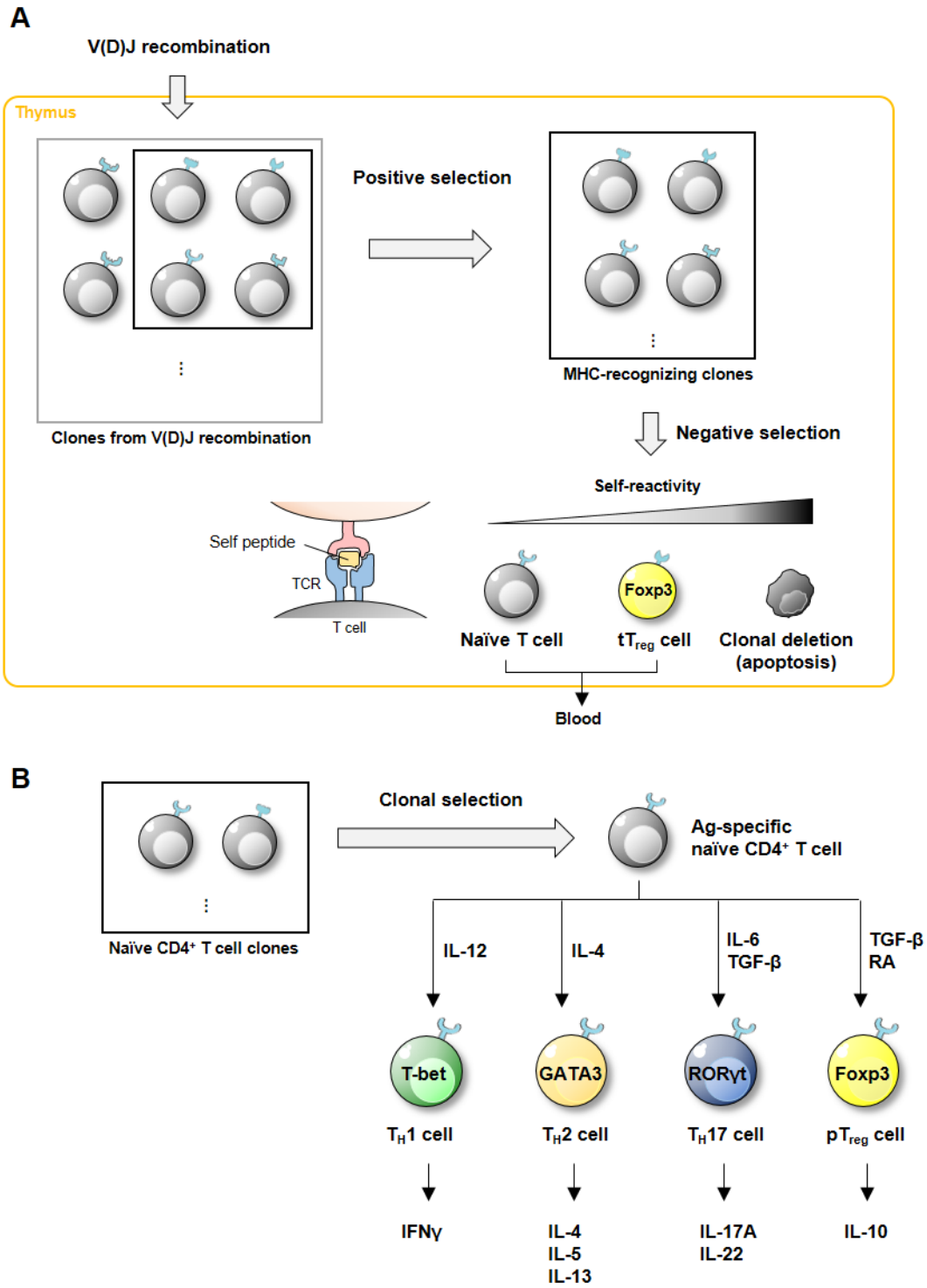

Fig. 1-3 Selection and responses of T cell

A. T cell clones with TCR recognizing self MHC and weakly responding to self peptides are selected in the thymus through positive and negative selection.

B. In response to foreign Ag, Ag-specific T cell clones differentiate into suitable subsets to an environmental que.

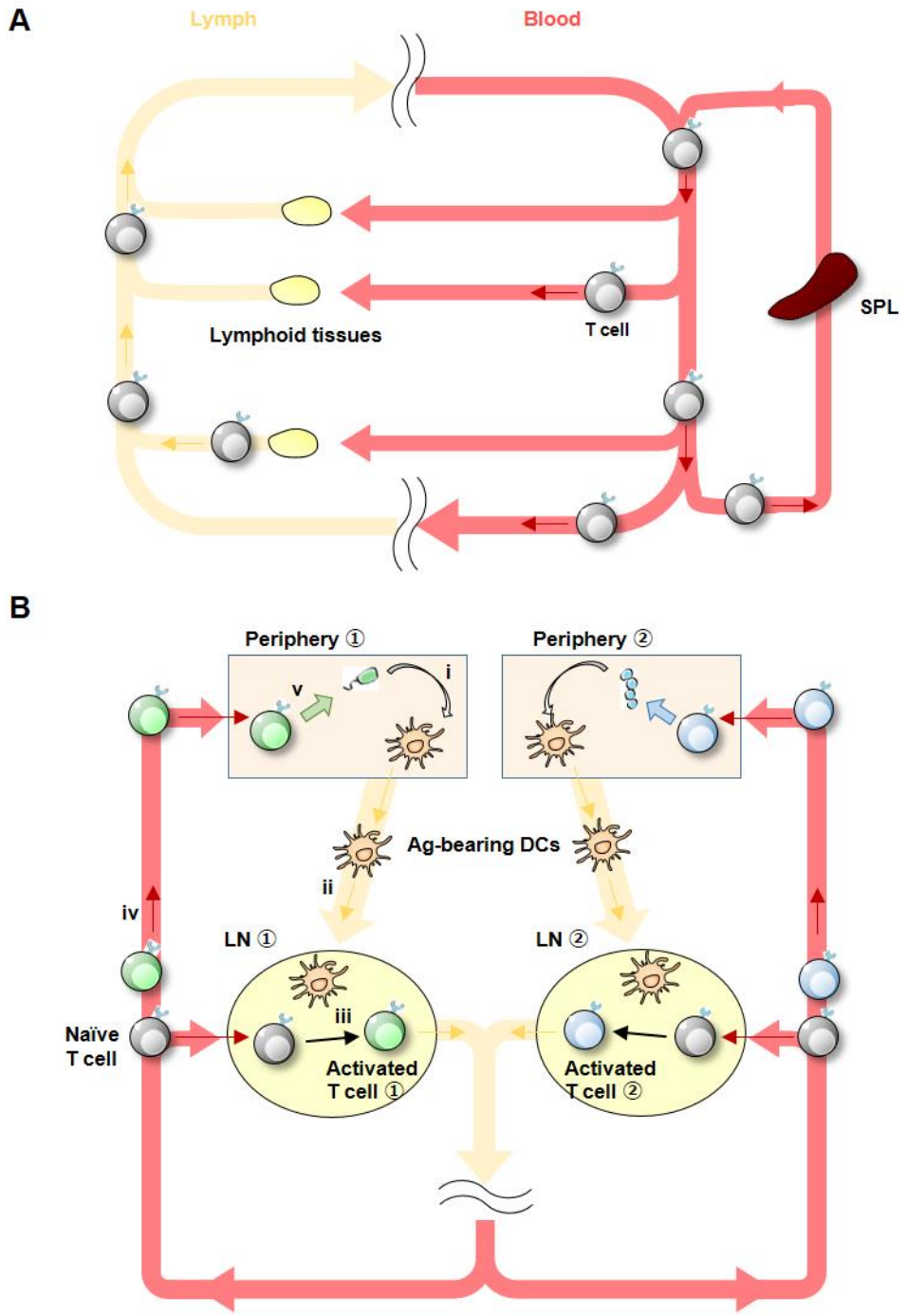

Fig. 1-4 T cell circulation

A. In the steady state, naïve T cells circulate lymphoid tissues through blood and lymph to survey their specific Ags.

B. In invasion of non-self, DCs capture Ags in the invaded periphery (i), migrate to LN (ii), and present the Ags to T cells (iii). Subsequently, activated T cells migrate to the periphery through lymph and blood (iv) and respond to the Ags in the periphery (v). DCs in each periphery (① and ② in the figure) migrate to the specific LNs (LN ① and LN ②, respectively).

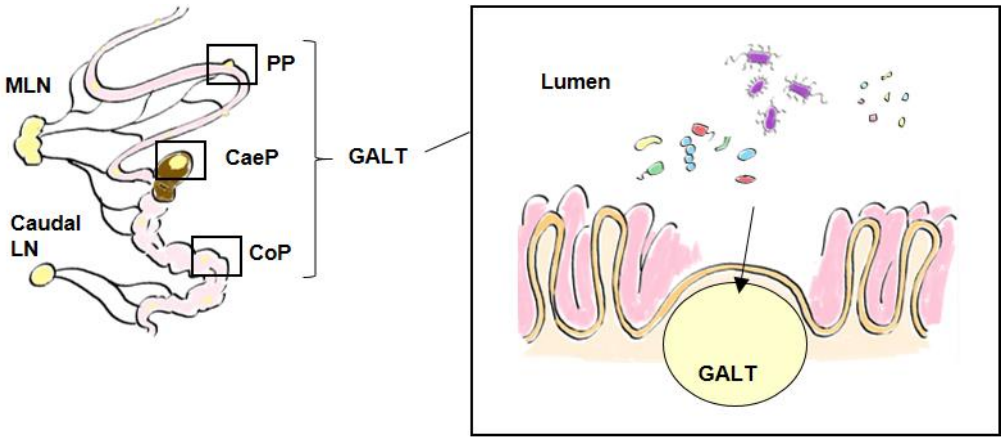

Fig. 1-5 Lymphoid tissues in the intestine

§1.3 Effector Functions of T Cells

§1.3.1 Helper T cell subsets induce various responses.

Naïve $CD4^+$ helper T cells are activated by their specific Ag-presenting MHC II and differentiate into several subsets, which have distinct functions as mentioned in *§1.2.2*. The fate of T cell differentiation is largely dependent on environmental factors at the activation. This enabled T cells to induce a suitable response to the circumstance around the Ag. The differentiated T_H cell subsets, including T_H1, T_H2, and T_H17 cells, secrete their specialized cytokines to induce subset-specific response (*25*, *26*)(**Fig. 1-6**).

Naïve T cells differentiate into T_H1 cells, which highly produce IFN-γ to eliminate intracellular pathogens. This cytokine activates macrophages and cytotoxic T cells to kill the pathogens and infected cells, respectively. Reponses induced by T_H2 cells are toward helminth infection. These responses are mediated by IL-4, IL-5, and IL-13. Secreted IL-4 stimulates B cells to produce immunoglobulin (Ig) G1 and IgE antibodies (Abs; discussed later in ***§1.4***) and macrophages to promote tissue repair (*27*). Ab secretion is also enhanced by IL-5, which promotes differentiation of Ab-secreting cells from B cells (*28*). The other T_H2 cytokine, IL-13, promote mucin production by goblet cells, one of epithelial cells in mucosal sites. T_H17 cells secrete IL-17A, IL-17F, IL-22, granulocyte macrophage colony-stimulating factor (GM-CSF), and TNF-α. This T cell subset induces protective responses to extracellular pathogens through activating neutrophils (*29*). In addition, IL-17A, IL-17F, and IL-22 can promote antimicrobial peptides secretion from mucosal epithelium (*30*).

Although these helper T cell subsets promote protective responses to infection, they could have destructive effects on the host by excessive responses (*31*). Excess T_H1 and T_H17 cell responses could trigger autoimmune diseases whereas excess T_H2 cell responses could induce allergy and asthma. Therefore, the immune system should precisely regulate these responses. Such regulation is partly accomplished by T_{reg} cells, which suppress effector T cell responses (*32–36*). Suppressive effects of T_{reg} cells work on effector T cells, mediated by cell cycle arrest through suppressor cytokines including IL-10, IL-35, and TGF-β and apoptosis induction through IL-2 consumption and cytolysis, and on Ag-presenting cells, mediated by inhibiting Ag-presentation and co-stimulation molecules on the Ag-presenting cells (*37*, *38*). The immune suppressive ability is acquired by expression of a transcription factor Foxp3, which is considered as a master regulator for T_{reg} cells (*39*, *40*). Similarly, effector T cells are regulated by their master regulator transcription factors, namely T-bet, GATA3, and RORγt for T_H1, T_H2, and T_H17 cells, respectively. These transcription factors regulate genes involved in their functions and localization. Thus, T_{reg} cells also express these T_H cell master regulators to localize with the effector subsets and suppress their responses (*34*, *36*).

Effector T cells activated and differentiated in lymphoid tissues should migrate to the periphery where Ags are derived. The directional migration is accomplished by regionally specific homing receptors. The homing receptors expressed on activated T cells are regulated by environmental factors in lymphoid tissues. Homing to the small intestine requires expression of integrin $\alpha_4\beta_7$ and chemokine receptor CCR9 (*18*, *41*). Expression of these homing receptors is induced by retinoic acid (RA) in the intestinal lymphoid tissues (*42*). RA is a metabolite of vitamin A (retinol) via retinal. This metabolism process is largely dependent on retinal dehydrogenase (RALDH)

enzymes. In the intestinal lymphoid tissues, RALDHs are highly expressed on DCs and non-hematopoietic stromal cells (*42*, *43*).

§1.3.2 Regulation of intestinal T cells is affected by commensal microbiota.

Among T cell subsets, T_H17 cells and T_{reg} cells are abundant in the intestine (*44–46*). The abundance of these T cells is dependent on specific commensal microbiota. In the case of T_H17 cells, segmented filamentous bacteria (SFB) are required for induction of small intestinal T_H17 cells (*47*, *48*). These bacteria adhere to intestinal epithelial cells, and this adherence is critical for the T_H17 cell induction (*49*). Ags derived from the SFB are presented to T cells by DCs, and this Ag-presentation is also indispensable for SFB-dependent T_H17 cell induction (*50*). Indeed, T_H17 cells in the intestine express TCRs specific to SFB-derived Ags (*51*). In addition to the SFB-dependent mechanisms, T_H17 cells has been reported to be induced by ATP and DNA derived from commensal bacteria (*45*, *52*). Activated T_H17 cells are involved in autoimmune diseases, and SFB colonization has been reported to exacerbate the pathological score of autoimmune disease model of mice (*53*). Therefore, the intestinal immune regulation by microbiota could have effects on extra-intestinal homeostasis.

In the intestine, T_H17 cell population is regulated in balance with that of T_{reg} cells (*45*, *54*). The intestinal T_{reg} cells, especially in the large intestine, are also dependent on microbiota. A series of outstanding studies has revealed that the large intestinal T_{reg} cells are induced by colonization of *Clostridium* bacteria derived from mice and humans (*46*, *55*). This is mediated by the bacteria-derived butyrate (*56*, *57*). Another bacterial strain involved in T_{reg} cell induction is *Bacteroides fragilis* which express surface polysaccharide A (*58*). The induced T_{reg} cells suppress T_H17 cell differentiation through IL-10 production (*59*). Thus, the T_{reg} cells induced by commensal bacteria contribute to preventing excess T_H17 cell responses in the intestine. These T_{reg} cells have been reported to express commensal bacteria-specific TCRs and to be pT_{reg} cells, i.e. they are induced in the intestinal immune system rather than the thymus (*60*). Such commensal-specific T_{reg} cells probably tolerate immune responses to these commensal bacteria and contribute to their stable colonization.

Collectively, some species in commensal microbiota have a potential to induce T_H17 cells in Ag-specific and non-specific manners, which could be detrimental for the host when excess. Then, other species can induce T_{reg} cells in the intestinal immune system, which in turn induce immune tolerance toward the bacteria. Further, these T_{reg} cells suppress T_H17 cells probably contributing to an appropriate equilibrium between these T cell subsets in the intestine.

§1.3.3 Regulatory T cells are involved in oral tolerance.

In addition to commensal microbiota, the intestinal immune system also should regulate its responses to food components, which may also be beneficial to the host. Most of food-derived proteins are digested into di- or tri-peptides which TCRs cannot recognize (*61*). But some proteins are actively sampled by the intestinal immune system through several pathways (discussed later in *§1.5.4*). Immune responses to these Ags are set to be tolerogenic as default in the intestine (*61–63*). The intestine is rich in immunosuppressive factors, such as TGF-β and RA, which

are involved in T_{reg} cell differentiation. These T_{reg} cells could contribute to suppressing immune responses toward the Ags not only in the intestine but also in the systemic immune system. This phenomenon is called oral tolerance.

Oral tolerance is defined as a systemic hyporesponsiveness toward Ags administrated through the oral route (*61*, *62*, *64*). Dysregulation of oral tolerance could cause food allergy. Thus, to establish preventive and therapeutic strategies for food allergy, the mechanism underlying oral tolerance should be revealed. Furthermore, oral tolerance is probably a useful way as prevention or treatment for systemic diseases caused by immunological pathogenesis. Indeed, some studies have reported that autoimmune disease model is ameliorated by oral administration of the model Ag (*65*, *66*). Likewise, oral administration of transgenic rice expressing cedar pollen-derived epitope has been reported to induce tolerance for hay fever in mouse model (*67*). Further, therapeutic use of oral tolerance for food allergy has been started in clinical studies (*68–70*).

In oral tolerance, T_{reg} cells play a pivotal role (**Fig. 1-7**). Especially, pT_{reg} cells, but not tT_{reg} cells, are indispensable for oral tolerance (*71*). These T_{reg} cells are probably induced mainly in MLNs because surgical removal of MLNs abolished oral tolerance induction in mice (*72*). In contrast, oral tolerance has been induced in PP-depleted mice (*73–75*). These studies support the idea that a site for inducing oral tolerance is MLNs, although it should be noted that another group has reported that PP-depletion abolishes oral tolerance, which suggests that PPs may contribute to oral tolerance in some condition (*76*). Oral tolerance also requires expression of gut-homing receptors, CCR9 and $\alpha_4\beta_7$, on T_{reg} cells (*77*, *78*). These studies have suggested that induced T_{reg} cells migrate into the intestinal lamina propria (LP) and further differentiate to tolerogenic IL-10-producing T_{reg} cells in response to IL-10 secreted by LP CX_3CR1^+ macrophages. Collectively, the intestinal immune system is required to induce $CCR9^+\alpha_4\beta_7^+$ T_{reg} cells specific to food-derived Ags, and this induction probably occurs in MLNs.

§1.3.4 T follicular helper cells help B cell responses in germinal center.

As mentioned in *§1.3.1*, $CD4^+$ helper T cells differentiate into several types of effector subsets. Recently, a new subset, T follicular helper (T_{FH}) cells, has been established (*79*, *80*). This helper T cell subset functions in germinal center (GC) responses, which are involved in Ab production of B cells (discussed later in ***§1.4***). Although first studies on T_{FH} cells were reported in early 2000s, it had been barely regarded as an established helper T cell subset due to a lack of a lineage-defining transcription factor (*81–83*). In 2009, Bcl6 was identified as the transcription factor required for T_{FH} cell commitment, and then T_{FH} cells were broadly accepted as a helper T cell subset (*84–86*). This transcription factor functions reciprocally to Blimp1, i.e. T_{FH} cell commitment proceeds with Bcl6 up-regulation and Blimp1 down-regulation.

As well as other helper T cell subsets, T_{FH} cells have a signature phenotype. They secrete IL-4 and IL-21, and highly express co-stimulatory receptors, ICOS and PD-1, and a chemokine receptor, CXCR5. The cytokines, IL-4 and IL-21, are involved in class-switch recombination (CSR) and somatic hyper-mutation (SHM) of B cells (discussed later in ***§1.4***). Expression of ICOS on T_{FH} cells is required for development of T_{FH} cells and interaction between T_{FH} cells and B cells in GCs whereas PD-1 represses T_{FH} cell responses probably in order to prevent production of auto-reactive Abs (*87–90*). CXCR5 is a receptor for chemokine CXCL13, which is produced by GC stromal cells named follicular dendritic cells (FDCs). Thus, CXCR5-CXCL13 axis leads to T_{FH} cell localization

into GCs in corporation with down-regulation of CCR7, which mediates localization to T cell zone in lymphoid tissues (*91*).

In addition to T_{FH} cells, Foxp3$^+$ T_{reg} cells are also observed in GCs. These T_{reg} cells express T_{FH} cell-signature surface molecules such as CXCR5, PD-1, and ICOS, but not IL-21, and named as follicular regulatory T (T_{FR}) cells (*92*, *93*). Follicular regulatory T cells suppress T_{FH} cell function and GC reaction probably to prevent auto-reactive Ab production. Indeed, T_{FR} cells differentiate from tT_{reg} cells, which express TCRs specific to self-peptides (*92–94*).

In the intestine, properties of T_{FH} cells have been studied intensely in PPs (*95*). A notable characteristic of PP T_{FH} cells is their origin. PP T_{FH} cells could be generated not only from naïve T cells but also from other T cell subsets, Foxp3$^+$ T_{reg} cells and T_H17 cells (*96*, *97*). Germinal centers in PPs are largely dependent on microbiota, i.e. germ-free (GF) mice have no or very few GCs in PPs (*22*). Thus, PP GCs are probably important for microbiota-specific Ab production. As mentioned in *§1.3.2*, intestinal T_{reg} cells and T_H17 cells are induced by some specific bacteria. This raise a possibility that conversion of T_{reg} cells and T_H17 cells into T_{FH} cells in PP GCs helps production of Abs toward such commensal bacteria (*95*). In addition, it has been reported that T_{FR} cells also function to regulate microbiota (*98*).

§1.3.5 Short summary

Naïve T cells which recognize their specific Ags differentiate into a suitable subset for the environment. In the intestine, several characteristic subsets are involved in regulation of immune responses to foods and commensal bacteria, namely T_{reg} cells, T_H17 cells, and T_{FH} cells. T_{reg} cells and T_H 17 cells are regulated in the host-commensal interaction. Further, T_{reg} cells are mainly induced in MLNs and play a pivotal role in oral tolerance whereas T_{FH} cells are observed in PPs and control microbiota through regulation of Ab-producing GC reaction.

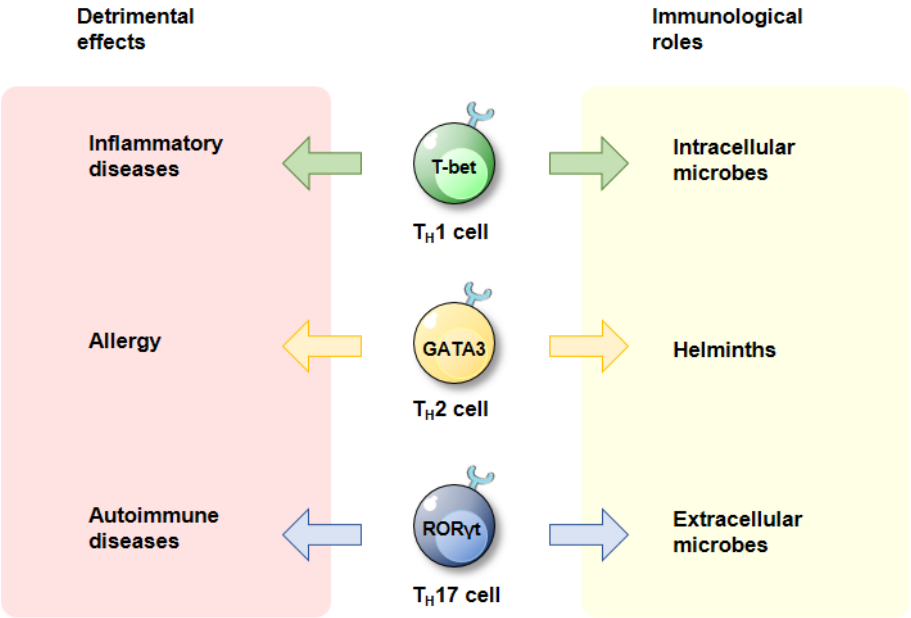

Fig. 1-6 Helper T cell subsets in health and disease

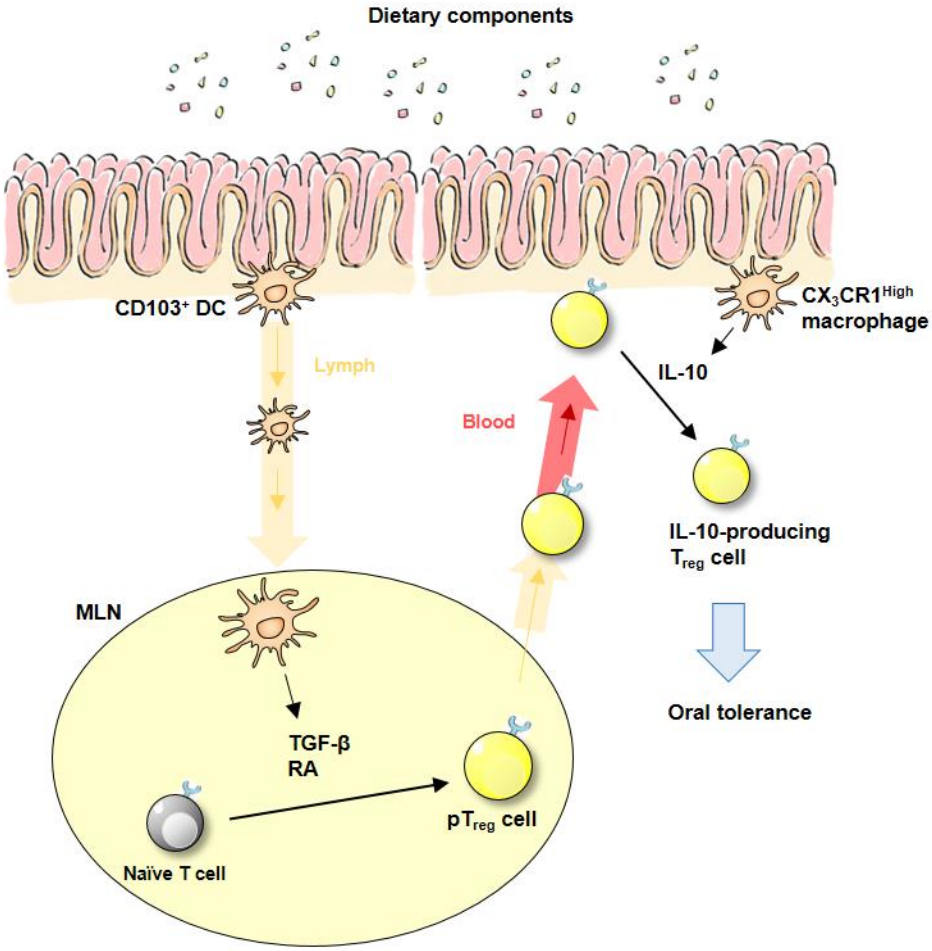

Fig. 1-7 Oral tolerance induction

§1.4 Antibody Production by B Cells

§1.4.1 Antibodies also have antigen-specificity through genetic diversification.

As well as T cells, B cells express Ag-specific receptors which are processed through somatic gene rearrangement, named B cell receptors (BCRs). BCRs are secreted as Abs from terminally differentiated B cells, named plasma cells. Abs are composed of two immunoglobulin (Ig) heavy chains and two Ig light chains connected by disulphide bonds (*99*, *100*). Both chains have variable and constant regions, and the variable regions of two chains recognize Ags. The variable region of the Ig chains are genetically diversified by VDJ recombination like TCRs (*101–104*). Thus, each Ab binds to its specific Ag. Further, BCR genes are processed by somatic manipulation, CSR and SHM, after the VDJ recombination. CSR occurs in constant regions of heavy chains and leads to a functional switching whereas SHM occurs in variable regions of both chains and increases affinity of the Ab to its Ag. These processes are induced by a common enzyme, activation-induced cytidine deaminase (AID). This enzyme mediates conversion of cytidine to uridine, which in turn leads to DNA repair process resulting in genetic recombination and mutation. Detailed molecular mechanism underlying AID activity is reviewed in elsewhere (*105*, *106*).

§1.4.2 Class-switch recombination results in distinct immunoglobulin-class antibodies with functional difference.

The constant regions of Ig heavy chain has several classes. Available classes are somewhat different between species. Humans have IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and IgM whereas mice have IgA, IgD, IgE, IgG1, IgG2a, IgG2b, IgG3, and IgM (*106*, *107*). These classes are encoded by Ig gene exons, such as Cα, Cδ, Cε, Cγ, and Cμ. The exons encoding Ig classes are sequentially ordered, starting with Cμ to downstream other exons (**Fig. 1-8**). Upstream of Cα, Cε, Cγ, and Cμ contains switch regions, namely Sα, Sε, Sγ, and Sμ respectively whereas Cδ exon exists in immediate downstream of Cμ without S region. Such ordered exons play a pivotal role in Ig class expression on B cells. Naïve B cells express IgD and IgM as BCRs, which are encoded by upstream Cδ and Cμ genes, respectively. These genes of Ig class is switched to other classes through recombination between Sμ and one of other S regions by AID-dependent mechanism. The recombination results in replacement of targeted C region to immediate downstream of variable region with depletion of any other upstream C regions including Cδ and Cμ genes. This leads to transcription of variable region followed by the switched C region, i.e. switched Ig class Ab with the same specificity as pre-CSR IgD and IgM. Target class of CSR is controlled by environmental factors around the B cell.

Class-switched Abs, such as IgA, IgE, and IgGs, have specific effector functions and localization properties (*108*). For example, IgE constant regions are recognized by Fcε receptors (FcεRs) on mast cells leading to secretion of inflammatory mediators in allergic pathology. Meanwhile, constant regions of IgGs highly activate the complementary system, which helps, e.g., phagocytosis of macrophages.

Whereas blood is rich in IgG, mucosal sites, including the intestine, contain large quantity of IgA. These IgA are produced by plasma cells in the LP and are secreted into the lumen across a epithelial layer through

transcytosis (*109*). Such secretory IgA are mainly in dimeric form. These properties of mucosal IgA are due to constant region property. In dimeric IgA produced by plasma cells, constant regions of two IgA monomers are linked to each other through joining protein called J chain (*110*). Such IgA-J chain-IgA complex is taken up by epithelial cells through poly Ig receptors expressed on basal side of the epithelial cells (*111*). The polymeric Ig receptors bind to IgA dimers through the J chain. Subsequently, IgA dimer is secreted into luminal side with an ectodomain fragment of polymeric Ig receptor called secretory component. Secreted IgA functions to neutralize pathogens and regulate commensal bacteria (discussed in *§1.4.4*).

Many factors in the intestine are involved in CSR to IgA. Early studies have revealed that TGF-β induce IgA production from B cells (*112*, *113*). This factor directly stimulate B cells through TGF-β receptor to express Iα-Sα-Cα mRNA, which is required for CSR to IgA (*114–116*). The effect of TGF-β is supported by IL-21 (*116–119*). In addition, TNF superfamily members, B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), are involved in IgA CSR (*120–124*).

The site for IgA CSR in the intestine are probably lymphoid tissues, such as GALT and MLNs, although it is still in controversy (*107*, *125*). Some groups have reported IgA CSR in LP as well as GALT (*126–128*). In contrast, other groups have reported that IgA CSR is limited in lymphoid tissues including PPs and isolated lymphoid follicles (*129–132*). This discrepancy may be due to difficulty in isolating LP cells without lymphoid tissue contamination. It has been reported that careful exclusion of lymphoid contamination resulted in no CSR detection in LP (*131*, *132*). Further, B cell proliferation, which is required for CSR, is not detected in the intestinal LP (*125*).

§1.4.3 Somatic hyper-mutation increase affinity of antibodies.

Whereas CSR occurs in constant regions of Abs, SHM induces mutation in gene loci encoding variable regions, which determine specificity of Abs (**Fig. 1-9A**). Somatic hyper-mutation is induced by AID-dependent conversion of cytidine to uridine and subsequent error-prone DNA repair (*105*, *106*). The gene mutation in variable regions occurs with frequency of 10^{-4}-10^{-3} per base (*133*). Accumulation of SHM-induced mutation is involved in improving the affinity of Ab to its Ag, called affinity maturation. This process takes place in GCs in B cell follicles of secondary lymphoid tissues. In the steady state, GC structure is not observed in the systemic immune system. Upon infection, the structure is formed dynamically in an Ag-dependent manner (*134*, *135*).

The process of the GC reaction is a miniature of Darwinian evolution, characterized by random mutation and subsequent selection of high affinity B cells (**Fig. 1-9B**). Structure of GCs includes two regions called light zone and dark zone. B cells in the GCs circulate these two regions in affinity maturation process. In the light zone, high affinity Ab-expressing B cells are selected and SHM is induced whereas the dark zone is a site for proliferation of the selected B cells (*136*). The proliferated B cells are processed by the selection, and some of the selected cells recirculate into the dark zone and the others emigrate from the GC to differentiate memory B cells or plasma cells.

Light zones contain two characteristic cell types involved in affinity maturation, i.e. T_{FH} cells and FDCs. These cells play critical roles in affinity-dependent B cell selection. B cells in the light zone capture Ags presented on FDCs in an affinity-dependent manner (*137*, *138*). The number of Ags on FDCs is limited, and thus B cells with

low affinity BCRs cannot capture the Ags. Subsequently, the Ag-bearing B cells present the Ags to T_{FH} cells. In response to the Ag-presentation, T_{FH} cells offer help signals to the B cells, such as CD40 ligand, IL-21, and IL-4, which induce SHM in BCR of the B cell. Therefore, B cells with Ags, which is due to their high affinity BCRs, can receive the help signals from T_{FH} cells and proliferate in a dark zone (*136*). B cells experience this dynamical process repeatedly, which results in generating and selecting B cells producing high affinity Abs.

§1.4.4 Host-microbe interaction through immunoglobulin A in the intestine.

The intestine is frequently exposed to harmful pathogenic microbes invading with foods. To prevent infection of such microbes, the intestinal immune system intensely secretes IgA Abs into the lumen. Indeed, polymeric Ig receptor-deficient mice is more susceptible to *Salmonella* infection in the intestine (*139*). Further, IgA-deficiency in human may causes gastroenteritis and *Helicobacter* infection (*140*).

In addition to pathogenic microbes, $>10^{15}$ non-harmful commensal bacteria, including $>10^{3}$ species, exist in the intestinal lumen (*141*, *142*). Further, some of them inhabit the inside of GALTs (*143*). Dysregulation of the intestinal microbiota, called dysbiosis, has harmful effects on the host, such as inflammatory bowel diseases and probably extra-intestinal diseases (*1*, *144*). Thus, the intestinal immune system should precisely regulate the microbiota. This regulation is largely dependent on IgA functions (*125*, *145*). Commensal microbes in the intestine are coated with IgA (*146*, *147*). In some case, IgA binding causes changes in gene expression of the bacteria (*148*). Further, deficiency in AID, which results in defect of CSR and SHM, causes dysbiosis (*149*, *150*). These findings suggest that IgA is required for appropriate regulation of the intestinal microbiota.

The intestinal IgA conceptually contains Ag-specific ‘classical high-affinity IgA’ and poly-reactive ‘natural low-affinity IgA’ (*125*). In parallel, IgA can be induced by two distinct pathways, namely T cell-dependent and -independent pathways (*107*). T cell-dependent pathway literally means IgA induction through T cell help, especially from T_{FH} cells in GCs, whereas T cell-independent pathway is IgA induction without T cells and GCs. Sequencing experiments have revealed that most of human intestinal plasma cells have highly mutated variable region gene probably processed by SHM, which means most IgA Abs in the intestine are induced through T cell-dependent pathway in GCs (*151*, *152*). The number of mutation in intestinal plasma cells in mice has been reported to be much lower (*153*). However, experiments usually use laboratory-reared mice in a strictly-controlled environment with specific pathogen-free (SPF) equipment, controlled and constant diets and drinking water. Such sanitary condition may effect on the homeostasis of intestinal immune system. Indeed, compared with laboratory-reared mice, wild-living mice have larger GCs in GALT, which suggests that, in wild-living mice, GC-dependent highly mutated IgA probably plays more dominant roles in the intestine like humans (*107*). In addition, AID^{G23S}-mutated mice, which lack SHM but not CSR activity of AID, spontaneously develop dysbiosis (*154*). Collectively, SHM-dependent highly-mutated IgA is important in regulation of the intestinal microbes.

As mentioned above, IgA is conceptually classified into Ag-specific ‘classical IgA’ and poly-reactive ‘natural IgA’. One plausible explanation for entities of these conceptual IgA groups is that classical IgA is T cell-dependent and natural one is T cell-independent. Considering a dominant role of T cell-dependent pathway as discussed above, classical IgA, recognizing specific Ags, is abundant in the intestine. However, this is against the

observation that most bacteria are coated by IgA even in the human intestine (*146*, *147*). Such discrepancy could be explained by Ag-binding through other than variable region and cross-reactivity of Abs (*125*). Secretory IgA can bind to many bacteria through secretory components mediated by modulated carbohydrate moiety (*155*, *156*). Meanwhile, the intestinal IgA contains poly-reactive Abs, which can recognize several Ags (*157*, *158*). Poly-reactive Abs probably recognize a common Ag among microbes. Such Abs can be selected and maturated through sequential exposure to several microbes in GCs (*159*). In the intestine, it has been reported that individual B cells circulate into several distinct GCs repeatedly (*160*). This process may contribute to the poly-reactive IgA through SHM-dependent pathway. These mechanisms enable the intestinal T cell-dependent IgA to recognize broad spectrum of bacteria.

To regulate the intestinal microbes, the immune system should constantly survey microbial contents in the intestine. Therefore, GALT and MLN take up Ags from the lumen and induce IgA (*22*). Especially, GALT constantly includes GC structure, which is dependent on microbiota (*107*, *149*, *161*). The microbial composition is gradually changing along the intestinal parts, i.e. from duodenum to colon, a diversity and the number of bacteria is explosively increasing (*162*). Further, dominant microbial species also differ among the regions. Corresponding to such microbial difference, the intestine changes some of its properties among regions. One of the differences is in mucus layer (*163*). The small intestine has thinner mono-layer whereas the large intestine has thick two mucus layers. An inner layer of the large intestinal mucosa excludes bacteria (*164*). Another difference is in IgA repertoire. The small and large intestines have different IgA repertoire (*153*). This may reflect the microbial difference. Recently, it has been reported that plasma cells in these sites could have different sources. B cells in PPs migrate to the small intestine, whereas CaeP B cells migrate to the large intestine (*165*).

§1.4.5 Short summary.

As well as T cells, B cells have a receptor, which is genetically rearranged, with diverse Ag-specificity. This BCR is finally secreted as Abs. B cells pass through specific processes, namely CSR and SHM. CSR manipulates effector function of Abs, and SHM improves Ab affinity to its Ags. These processes are probably located in lymphoid tissues and their GCs depending on help from T_{FH} cells. The intestine is rich in secretory IgA in the lumen, which not only prevents infection but also regulates the commensal microbiota. The intestinal IgA is probably induced by GC reactions located in GALTs.

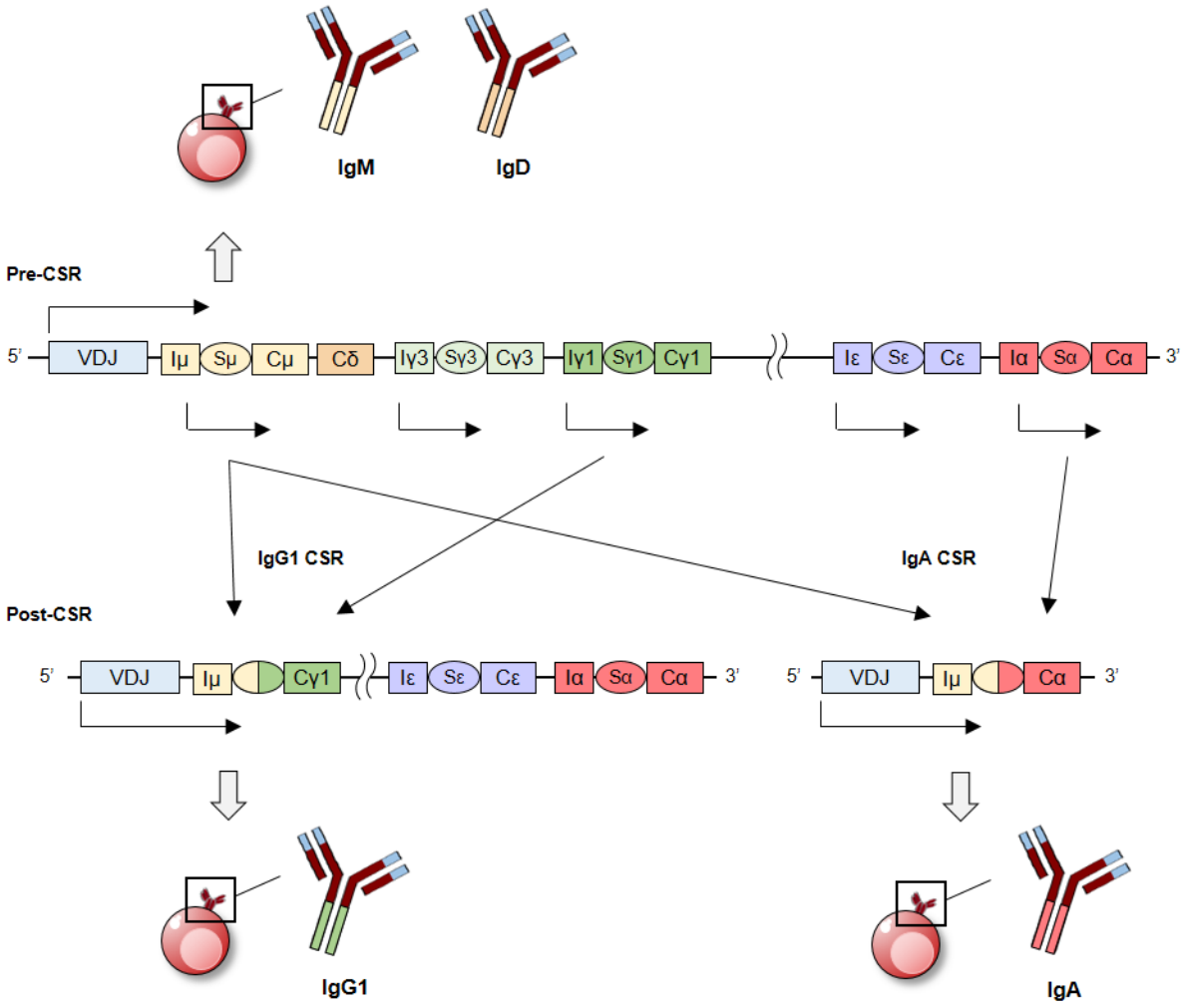

Fig. 1-8 Class-switch recombination

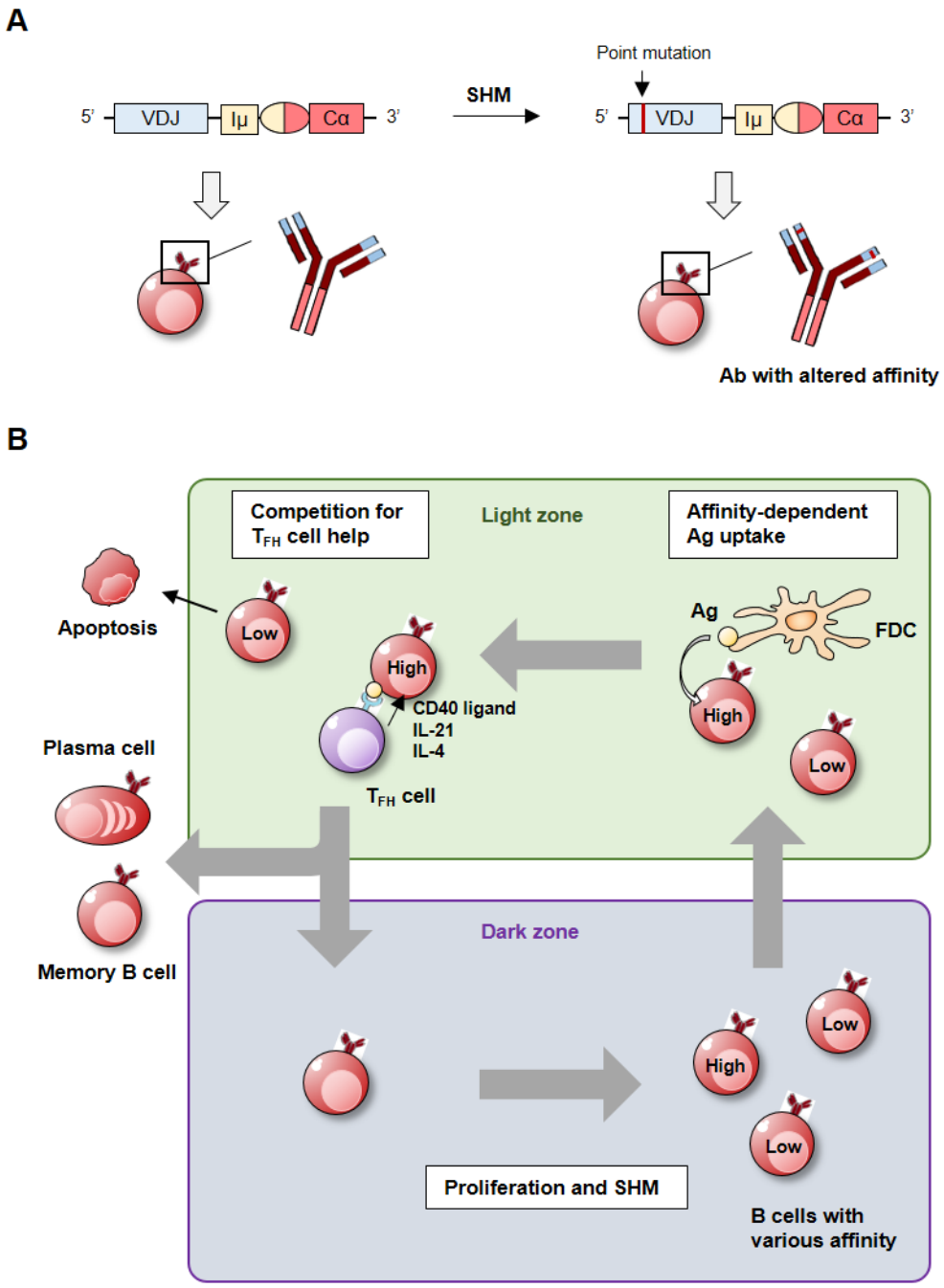

Fig. 1-9 Affinity maturation
A. SHM process induces a point mutation in VDJ region of Ab, leading to alteration of affinity.
B. Conceptual description of affinity maturation in GCs.

§1.5 Priming Immune Responses by Dendritic Cells

§1.5.1 Dendritic cells initiate antigen-specific immune responses.

As discussed above, T cells and B cells play pivotal roles in Ag-specific immune responses. These cells are activated in lymphoid tissues. Therefore, to prime immune responses, Ags should be carried into lymphoid tissues from periphery and be presented to T cells through peptide-MHC-TCR interaction. Such immune priming is dependent on Ag-presenting cells, especially DCs. A kind of DCs, Langerhans cells in skin, was firstly observed by Paul Langerhans in the 19th century (*166*). At the time, the cells were considered to have functions in the nerve system. In 1973, Ralph Steinmann identified DCs in lymphoid tissues (*167*, *168*). Later, Steinmann *et al.* have reported that DCs can highly prime immune responses (*169*). Then, other innumerable studies have revealed that DCs are professional Ag-presenting cells.

Generally, DCs capture Ags in periphery, migrate into LNs, and then present the Ags to T cells in the LNs (**Fig. 1-10**). To prime immune responses efficiently, DCs are equipped with several characteristic features. They highly express MHC II molecules on their surface, which may contribute to high potency to priming T cells. In addition, DCs can retain phagocytosed Ags for a long time because they have proteases of low activity (*170*). Further, DCs can present exogenous Ags on MHC I to CD8$^+$ T cells, called cross-presentation as reviewed in elsewhere (*171*).

Mononuclear phagocytes includes DCs and macrophages. These cells have marked heterogeneity and are classified into detailed subsets using expression of surface marker molecules (*172*). Although DCs and macrophages had been historically considered as distinctive cell types, it has been revealed that these cells have some overlaps in phenotypic and functional properties (*172–174*). There have been proposed a nomenclature based on the ontogeny (*174*). In the present studies, the author uses the nomenclature as much as possible.

The intestine harbors several types of DCs, usually classified based on expression of CD11b, CD103, CD8α, and other markers (*175*, *176*). Subset composition of DCs are locally different throughout the intestine. For example, small intestinal LP contains CD11b$^+$CD103$^+$CX$_3$CR1$^-$ and CD11b$^+$CD103$^-$CX$_3$CR1$^+$ subsets whereas PPs contain CD11b$^-$CD103$^+$ and CD11b$^+$CD103$^-$ subsets (*177*, *178*).

§1.5.2 Dendritic cells can initiate a suitable immune response sensing environmental factors.

To induce a suitable immune response to the environment, DCs can receive the environmental factors. Such environmental sensing is mediated, at least partly, by innate immune receptors, such as Toll-like receptors (TLRs), RIG-I-like receptors, and NOD-like receptors. These receptors recognize ligands conserved among many microbes, called microbe-associate molecular patterns. Among the receptors, TLRs, including several subtypes, are located on membranes and recognize ligands derived from microbes existing in topologically outer of the cells, *i.e.* ligands out of the plasma membrane and in the lysosomes. In contrast, RIG-I-like receptors and NOD-like receptors are in cytoplasm to recognize intracellular bacteria and viruses, and are reviewed in elsewhere (*179*, *180*).

Many studies have focused on TLRs from identification in the late 1990s (*181*). This type of innate

immune receptors are expressed on various cell types; DCs, macrophages, B cells, T cells, epithelial cells, and so on. As mentioned above, TLRs include several subtypes, TLR1 - TLR11 (*182*). Especially, TLR1 - TLR9 were identified earlier and thus have been well studied. These receptor molecules form homo- or hetero-dimers to sense their specific ligands (**Table 1-1**). Among TLRs, TLR2/1, 2/6, 4, and 5 are located in plasma membrane and recognize surface molecules of microbes whereas TLR3, 7, 8, and 9 are in endosome membrane and recognize nucleic acids. All subtypes consist of three domains, namely ligand-recognizing extracellular domain, transmembrane domain, and signal-transducing intracellular domain (*183*). The extracellular domains are different among the subtypes to recognize different ligands as shown in **Table 1-1** (*184–190*). In contrast to the extracellular domain, the intracellular domains of TLR family are relatively conserved to transduce signals to shared adaptor molecules, MyD88 and/or TRIF (*191–198*). These signals result in activation of NF-κB, MAP kinases, and IRFs.

In response to TLR stimulation, DCs are activated to prime T cell responses. Such activated DCs highly express MHC II and co-stimulatory molecules such as CD80 and CD86. Further, TLR stimulation has DCs secrete cytokines, such as IL-6, IL-12, and IL-23, to bias differentiation of T cell subsets (*199*). To control a suitable T cell differentiation, TLR-induced cytokines should change according to environmental cues. For example, IFN-γ and CD40 stimulation enhance TLR-induced IL-12 whereas IL-10, thymic stromal lymphopoietin (TSLP), and prostaglandin E2 have been reported to suppress TLR-induced IL-12 (*200–205*). In addition to the environmental factors, TLR subtypes could affect subsequent responses. It has been reported that specific response can be induced by specific TLR subtype, such as TLR2/1, but not other TLRs, induces RALDH2 gene expression whereas TLR9, but not others, induces IL-12 production (*206*, *207*). Collectively, in response to TLR stimulation, DCs efficiently present Ags to Ag-specific T cells under specific cytokine milieu resulting in a suitable T cell responses to the environment.

§1.5.3 The intestinal dendritic cells regulate the intestinal immune system through their characteristic properties.

To regulate the intestinal immune system, the intestinal DCs have several characteristics. Especially in T cell responses, the intestinal CD103$^+$ DCs tend to induce T_{reg} cells and T_H17 cells (*52*, *208–215*). This is largely dependent on TGF-β, which are produced and activated by the DCs. In addition, T_{reg} cells are also induced through RA production by DCs. The intestinal DCs, especially CD103$^+$ DCs, highly express RALDH2 (*208*, *216*). This also enhances T cell homing to the intestine through induction of CCR9 and $\alpha_4\beta_7$ expression on T cells (*208*, *212*, *217*, *218*). These properties can be obtained by the environmental factors. For example, stimulation on TLR2/1, which could occur frequently in the intestine from commensal bacteria, induces RALDH2 gene expression in DCs (*206*). Further, mucus components enhance TGF-β production and RALDH activity in the intestinal DCs (*219*). The intestinal DCs regulate T cell responses through these characteristic properties.

§1.5.4 The intestinal dendritic cells capture antigens from the lumen.

To prime T cell responses, DCs should take up Ags. At the infection, DCs can contact with invaded pathogens in

the tissue and blood. In contrast, luminal bacteria and foods are separated by epithelial layer in the steady state intestine. Thus, the intestinal DCs have some specialized pathways to obtain the luminal Ags (*24*).

One characteristic pathway is through GALT (see **Fig. 1-5**). These tissues are more accessible by luminal Ags than other sites in the intestine due to several properties of epithelial cells on them (*24*, *220*). The epithelium has thinner mucus, expresses less polymeric Ig receptors resulting in less secretory IgA around the tissues, secretes less anti-microbial peptides, and contains specialized cells transporting Ags from lumen, named M cells. M cells take up Ags through transcytosis. The transcytosed Ags are internalized by antigen-presenting cells under the M cells. Further, PP DCs have been reported to obtain Ags directly from the lumen extending their dendrites across M cells (*221*).

As well as in GALT, Ags are obtained by DCs in the intestinal LP through several pathways. Goblet cells, a kind of epithelial cells, hand over Ags to $CD103^+$ DCs (*222*). Further, $CD103^+$ DCs can obtain Ags directly from the lumen through their dendrites (*223*). CX_3CR1^+ macrophages can also internalize the luminal Ags through trans-epithelial dendrites (*224*). CX_3CR1^+ macrophages hand over obtained Ags to $CD103^+$ DCs (*225*). Through these pathways, DCs obtain Ags from the intestinal lumen and present the Ags to T cells in lymphoid tissues.

§1.5.5 Antigen-bearing dendritic cells migrate to lymphoid tissues from periphery.

To present Ags efficiently to naïve T cells, Ag-bearing DCs should migrate to lymphoid tissues. From the small and large intestinal LP, DCs mainly migrate to T cell zone of MLNs (*20*, *226*). Main migratory DCs have $CD103^+$ phenotype (*217*). But migration of $CD103^-$ subsets to MLNs are also detected (*227*, *228*). In PPs, DCs migrate to T cell zone in the PP from region under epithelium called sub-epithelium dome (*229*). In addition, PP DCs also migrate to MLNs (*230*). In all of these reports, such migration is dependent on CCR7 expression on the DCs. This chemokine receptor induces migration toward chemokines, CCL19 and CCL21, which are secreted from T cell zone stromal cells, fibroblastic reticular cells (*231*, *232*). Naïve T cells also express CCR7 and migrate toward such chemokines (*18*). Thus, both Ag-bearing DCs and naïve T cells migrate toward the same chemokines. This mediates co-localization of these cells for efficient Ag-presentation from DCs to T cells.

In T cell zone of lymphoid tissues, fibroblastic reticular cells organize a scaffold network, along which DCs and T cells can move (*233*, *234*). In the T cell zone DCs are in close contact with each other (*235*). In some case, migratory DCs hand over Ags obtained in periphery to LN resident subsets (*236*). Naïve T cells move to search on DCs for MHC presenting their specific Ags (*237*). Once T cells contact with the Ag-bearing DCs, they decrease their mobility and contact with the DCs for long time to receive signals. Then, activated T cells re-circulate into blood and migrate to peripheral tissues depending on expression of homing receptors.

§1.5.6 Short summary

Mononuclear phagocytes, including DCs and macrophages, can be classified into several subsets based on surface markers. In the intestine, $CD103^+$ DCs play a pivotal role in regulating the intestine-specific immune responses, such as T_{reg} cells and T_H17 cells induction. These properties are largely dependent on TGF-β and RA. The intestinal

DCs capture the luminal Ags through direct or indirect pathways and migrate to T cell zone in lymphoid tissues in a CCR7-dependent manner and present the Ags to naïve T cells there.

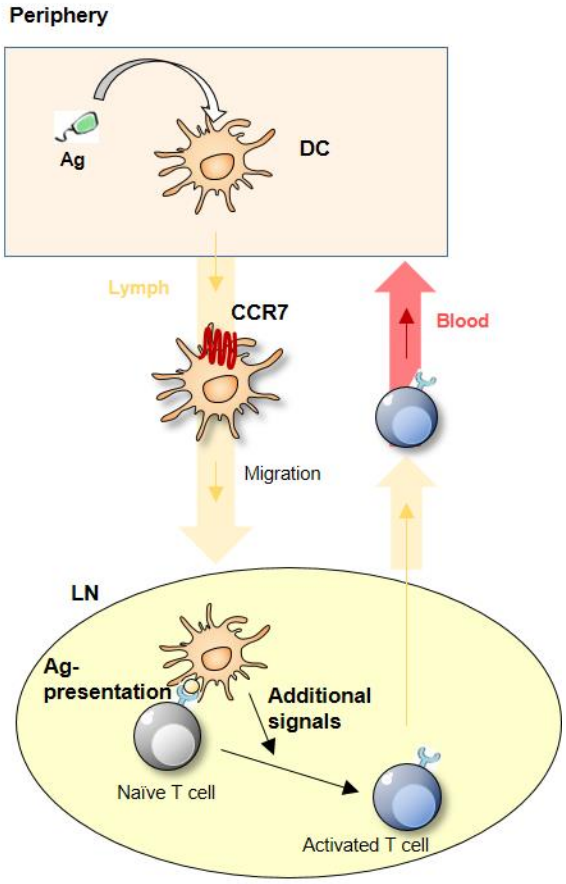

Fig. 1-10 DCs in the immune responses

Table 1-1 Toll-like receptors

TLR	Ligands
TLR2/1, TLR2/6	Lipopeptides
TLR3	Double-stranded RNA
TLR4	Lipopolysaccharides
TLR5	Flagellin
TLR7, TLR8	Single-stranded RNA
TLR9	Non-methylated CpG DNA

§1.6 Innate Lymphoid Cells in Mucosal Immune System

§1.6.1 Innate lymphoid cells are newly established immune cells.

One of the most remarkable current topics in immunology is establishment of a new lymphocyte lineage – innate lymphoid cells (ILCs). This cell type is now defined as lymphoid cells, i.e. derived from a common lymphoid progenitor, expressing no lineage markers of other immune cells including T cells, B cells, DCs, macrophages and so on (*238–240*)(**Table 1-2**). In 2010, three independent studies have reported that lineage markers$^-$ (Lin.$^-$) lymphoid cells in various tissues secrete T_H2 cytokines such as IL-5 and IL-13 (*241–243*). These discoveries were followed by many studies which have investigated in properties of Lin.$^-$ lymphoid cells, such as developmental processes and physiological functions. Then, a concept of ILCs has been generally accepted. In addition to newly identified cells, classically known Lin.$^-$ cells such as natural killer (NK) cells and lymphoid tissue inducer (LTi) cells are also categorized into ILCs.

ILCs are classified into several subsets based on transcription factor dependency, cytokine production, and effector function (**Fig. 1-11**). As mentioned below, ILCs have many similarities to T cells in transcription factors and cytokines, and classification of ILC subsets mirrors that of T cell subsets resulting in one cytotoxic subset and three groups of helper type subsets (*239*). The cytotoxic one is NK cells, which is a counterpart of $CD8^+$ T cells. The other three groups are named as group 1 ILC, group 2 ILC, and group 3 ILC (ILC1, ILC2, and ILC3). Roughly, ILC1s express T-bet and produce IFN-γ like T_H1 cells, ILC2s express GATA3 and produce IL-5 and IL-13 like T_H2 cells, and ILC3s express RORγt and produce IL-22 like T_H17 cells. Although such similarities, ILCs express no Ag-specific receptor like TCRs and BCRs. Instead, ILCs express cytokine receptors, such as receptors for IL-2, IL-7, IL-12, IL-23, IL-25, IL-33 and so on. Distinct ILC groups express different patterns of such receptors and regulate immune responses through cytokine secretion according to the environment. Further, some ILCs express MHC II and could directly regulate T cell responses (*50*, *244–246*).

§1.6.2 Functions of innate lymphoid cells in health and disease.

Although ILCs are very rare population, <1% of whole lymphocytes, they are largely involved in various physiological functions and pathological conditions. In response to viral infection, NK cells protect the host by killing the infected cells through granzyme and perforin and by secreting IFN-γ (*247*). Protective responses to infection are also regulated by other ILCs. ILC1s highly produce IFN-γ and TNF to provide protection from infection (*248*, *249*). Such ILC1s exist in intraepithelial region and LP in the intestine. ILC3s produce IL-22 in response to inflammatory cytokines such as IL-1β and IL-23 (*250–253*). IL-22 from ILC3s is in turn received by intestinal epithelial cells resulting in secretion of antimicrobial peptide and fucosylation of the epithelial cells to prevent infection of pathogens (*254–257*). Further, a subset of ILC3s can be converted to ILC1-like phenotype such as T-bet$^+$ and producing IFN-γ, which contributes to protecting the host from infection (*258–260*). These ILC1s and ILC3s also exacerbate inflammation in some contexts (*248*, *252*, *258*, *260–262*).

Whereas ILC1s and ILC3s protect against viral and bacterial infection, ILC2s contribute to expulsion of

helminths through T_H2 cytokines (*241–243*). ILC2s require GATA3 expression for their maintenance and function although this transcription factor is indispensable for development of all ILC subsets (*263–265*). Many studies have revealed that ILC2s can highly produce IL-5, IL-9, and IL-13 in response to IL-2, IL-4, IL-25, IL-33, TSLP, and TLA1, a TNF family member (*264*, *266–272*). These cytokines secreted from ILC2s induce mucus production and tissue repair in the lung and intestine, resulting in helminth expulsion. Meanwhile, ILC2s exacerbate allergic pathologies through T_H2 cytokine production in the lung and skin (*266*, *269*, *270*, *273–275*).

In addition to such immunological functions, ILCs are also involved in regulation of non-immunological homeostasis. LTi cells, a subset of ILC3s, are required for organization and remodeling of lymphoid tissues (*276–279*). ILC2s also restore epithelium after infection in the lung through amphiregulin, an epidermal growth factor, production (*280*). In addition, ILC2s have been reported to be involved in metabolic regulation in adipose tissues. IL-5 and IL-13 secreted from ILC2s promote eosinophil accumulation and subsequent macrophage activation in an adipose tissue resulting in protection from obesity (*281–283*). ILC2s also reduce obesity through production of a peptide which promotes adipose tissue beiging (*284*). Further, ILC2s can sense nutrient conditions, such as vitamin A deficiency and malnutrition (*285*, *286*). Vitamin A deficiency induces ILC2 increase and ILC3 decrease. Dietary components also affect ILC3 homeostasis through an aryl hydrocarbon receptor (AhR) (*287*).

§1.6.3 Innate lymphoid cells regulate the host-commensal interaction.

In the intestine, ILCs participate in homeostatic control of the interaction between the host and commensal microbiota. Especially, IL-22 production by ILC3s are largely involved in such regulation. As mentioned above, IL-22 enhance antimicrobial peptides secretion from intestinal epithelial cells (*254–256*). Such antimicrobial peptides probably suppresses overgrowth of commensal microbes. IL-22 from ILC3s also prevents PP-resident commensal bacteria from spreading systemically and confines the bacteria to the site (*288*). Further, ILC3-derived IL-22 promotes fucosylation on luminal side of intestinal epithelial cells, which protect the host from infection (*257*). Fucosylated moiety on intestinal epithelial cells also functions as an environmental niche for commensal bacteria (*289*). Thus, such ILC3-mediated fucosylation may promote commensal colonization. Conversely, ILC3s are regulated by the intestinal microbiota. The microbiota reduces IL-22 production from ILC3s indirectly through T cell responses (*290*, *291*). Further, aryl hydrocarbon receptor-deficient ILC3s have impaired IL-22 production, which suggests that food components could modulate commensal bacteria through ILC3 function (*287*, *292*).

ILC3s can regulate commensal bacteria also through Ag-presentation to T cells. ILC3s express MHC II and present commensal bacteria-derived Ags to T cells (*50*, *245*, *246*). ILC3s in the intestinal LP inhibits T cell responses through the Ag-presentation. Such suppression is due to the absence of co-stimulatory molecules on the ILCs, and may contribute to selection of commensal bacteria (*246*). The inhibitory state of ILC3s can be reversed in some context through IL-1β (*293*).

Some studies have revealed that ILCs regulate IgA production. ILC3s induce IgA production through lymphotoxins activating T cell-dependent and -independent pathways (*294*). In addition, SPL ILC3s enhance IgA production through BAFF, CD40 ligand, and Notch ligand (*295*). Further, IL-5, highly produced by ILC2s, promotes B cell differentiation to Ab-secreting cells (*28*). Thus, ILC2s could enhance IgA secretion (*241*).

§1.6.4 Short summary

Lin$^-$ lymphoid cells, ILCs, are a newly established cell type and have been intensely studied in recent years. These cells express no Ag-specific receptors and secrete various cytokines in response to environmental factors. ILCs are further classified into several subsets, i.e. NK cells, ILC1s, ILC2s, and ILC3s. Each subsets exhibit similarities to T cell subsets in transcription factor dependency and cytokine production. ILCs are involved in a broad spectrum of functions including immunological and non-immunological ones. In the intestine, ILCs regulate homeostasis in the host-commensal interaction through many pathways.

Table 1-2 Lineage markers

Molecules	Lineages
B220	B cell
CD3	T cell
CD4	T cell
CD5	B cell, T cell
CD8	T cell
CD11b	Monocyte
CD11c	Monocyte
CD19	B cell
FcεRIα	Mast cell
GR1	Granulocyte
TER119	Erythrocyte

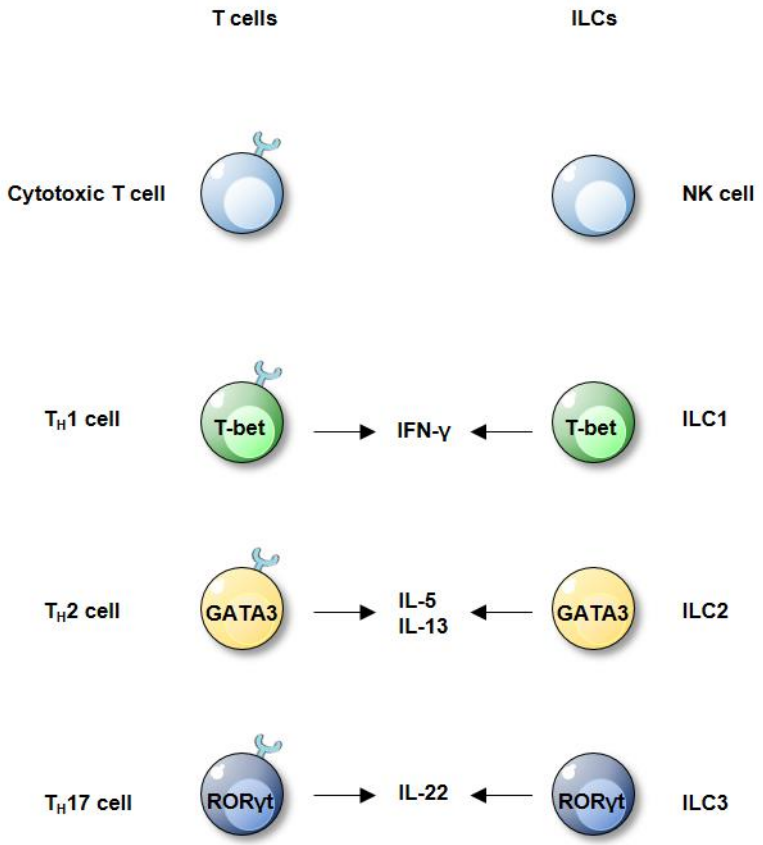

Fig. 1-11 ILC classification

§1.7 Summary

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Chapter 2

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Chapter 3

§3.1 Introduction

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§3.2 Materials and Methods

本項の内容は学術論文雑誌として出版する計画があるため公表できない。5 年以内に出版予定。

§3.3 Results

§3.3.1

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§3.3.2

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§3.3.3, 3.3.4, 3.3.5

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§3.4 Discussion

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Chapter 4

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Chapter 5

Overall Discussion

本章の内容は学術論文雑誌として出版する計画があるため公表できない。5 年以内に出版予定。

References

1. N. Kamada, S.-U. Seo, G. Y. Chen, G. Núñez, Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* **13**, 321–35 (2013).

2. Y. K. Lee, S. K. Mazmanian, Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science*. **330**, 1768–1773 (2010).

3. M. M. Davis, P. J. Bjorkman, T-cell antigen receptor genes and T-cell recognition. *Nature*. **334**, 395–402 (1988).

4. Y. Kurosawa *et al.*, Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes. *Nature*. **290**, 565–570 (1981).

5. A. Murugan, T. Mora, A. M. Walczak, C. G. Callan, Statistical inference of the generation probability of T-cell receptors from sequence repertoires. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 16161–16166 (2012).

6. V. I. Zarnitsyna, B. D. Evavold, L. N. Schoettle, J. N. Blattman, R. Antia, Estimating the diversity, completeness, and cross-reactivity of the T cell repertoire. *Front. Immunol.* **4**, 1–11 (2013).

7. L. Klein, B. Kyewski, P. M. Allen, K. A. Hogquist, Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* **14**, 377–91 (2014).

8. K. A. Hogquist, S. C. Jameson, The self-obsession of T cells: how TCR signaling thresholds affect fate "decisions" and effector function. *Nat. Immunol.* **15**, 815–23 (2014).

9. M. Attaf, E. Huseby, A. K. Sewell, αβ T cell receptors as predictors of health and disease. *Cell. Mol. Immunol.* **12**, 391–399 (2015).

10. H.-M. M. Lee, J. L. Bautista, J. Scott-Browne, J. F. Mohan, C.-S. S. Hsieh, A broad range of self-reactivity drives thymic regulatory T cell selection to limit responses to self. *Immunity*. **37**, 475–486 (2012).

11. F. M. Burnet, A modification of Jerne's theory of antibody production using the concept of clonal selection. *Aust. J. Sci.* **20**, 67–69 (1957).

12. N. Zhang, M. J. Bevan, CD8(+) T cells: foot soldiers of the immune system. *Immunity*. **35**, 161–8 (2011).

13. J. T. Chang, E. J. Wherry, A. W. Goldrath, Molecular regulation of effector and memory T cell differentiation. *Nat. Rev. Immunol.* **15** (2014).

14. A. K. Abbas *et al.*, Regulatory T cells: recommendations to simplify the nomenclature. *Nat. Immunol.* **14**, 307–308 (2013).

15. D. A. A. Vignali, V. K. Kuchroo, IL-12 family cytokines: immunological playmakers. *Nat. Immunol.* **13**, 722–728 (2012).

16. N. Schmitt, H. Ueno, Regulation of human helper T cell subset differentiation by cytokines. *Curr. Opin. Immunol.* **34**, 130–136 (2015).

17. N. J. Tubo *et al.*, Single naive $CD4^+$ T cells from a diverse repertoire produce different effector cell types during infection. *Cell*. **153**, 785–796 (2013).

18. D. Masopust, J. M. Schenkel, The integration of T cell migration, differentiation and function. *Nat. Rev. Immunol.* **13**, 309–20 (2013).

19. R. Förster, A. Braun, T. Worbs, Lymph node homing of T cells and dendritic cells via afferent lymphatics. *Trends Immunol.* **33**, 271–280 (2012).

20. A. M. Mowat, W. W. Agace, Regional specialization within the intestinal immune system. *Nat. Rev. Immunol.* **14,** 667-685 (2014).

21. V. Bronte, M. J. Pittet, The spleen in local and systemic regulation of immunity. *Immunity*. **39**, 806–818 (2013).

22. K. Suzuki, S. Kawamoto, M. Maruya, S. Fagarasan, GALT. Organization and dynamics leading to IgA synthesis. *Adv. Immunol.* **107,** 153-185 (2010).

23. P. Brandtzaeg, H. Kiyono, R. Pabst, M. W. Russell, Terminology: nomenclature of mucosa-associated lymphoid tissue. *Mucosal Immunol.* **1**, 31–37 (2008).

24. O. Schulz, O. Pabst, Antigen sampling in the small intestine. *Trends Immunol.* **34**, 155–161 (2013).

25. K. P. Walsh, K. H. G. Mills, Dendritic cells and other innate determinants of T helper cell polarisation. *Trends Immunol.* **34**, 521–530 (2013).

26. R. V. Luckheeram, R. Zhou, A. D. Verma, B. Xia, $CD4^+$T Cells: Differentiation and Functions. *Clin. Dev. Immunol.* **2012**, 925135 (2012).

27. F. Chen *et al.*, An essential role for T_H2-type responses in limiting acute tissue damage during experimental helminth infection. *Nat. Med.* **18**, 260–266 (2012).

28. J. Hasbold, L. M. Corcoran, D. M. Tarlinton, S. G. Tangye, P. D. Hodgkin, Evidence from the generation of immunoglobulin G-secreting cells that stochastic mechanisms regulate lymphocyte differentiation. *Nat. Immunol.* **5**, 55–63 (2004).

29. P. Kumar, K. Chen, J. K. Kolls, Th17 cell based vaccines in mucosal immunity. *Curr. Opin. Immunol.* **25**, 373–380 (2013).

30. J. K. Kolls, P. B. McCray, Y. R. Chan, Cytokine-mediated regulation of antimicrobial proteins. *Nat. Rev. Immunol.* **8**, 829–835 (2008).

31. E. Bettelli, M. Oukka, V. K. Kuchroo, T_H17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* **8**, 345–350 (2007).

32. S. Hori, Lineage stability and phenotypic plasticity of $Foxp3^+$ regulatory T cells. *Immunol. Rev.* **259**, 159–172 (2014).

33. H. Morikawa, S. Sakaguchi, Genetic and epigenetic basis of T_{reg} cell development and function: From a FoxP3-centered view to an epigenome-defined view of natural T_{reg} cells. *Immunol. Rev.* **259**, 192–205 (2014).

34. D. V. Sawant, D. A. A. Vignali, Once a T_{reg}, always a T_{reg}? *Immunol. Rev.* **259**, 173–191 (2014).

35. K. S. Smigiel, S. Srivastava, J. M. Stolley, D. J. Campbell, Regulatory T-cell homeostasis: Steady-state maintenance and modulation during inflammation. *Immunol. Rev.* **259**, 40–59 (2014).

36. X. Yuan, G. Cheng, T. R. Malek, The importance of regulatory T-cell heterogeneity in maintaining self-tolerance. *Immunol. Rev.* **259**, 103–114 (2014).

37. E. M. Shevach, Mechanisms of $Foxp3^+$ T Regulatory Cell-Mediated Suppression. *Immunity*. **30**, 636–645 (2009).

38. T. Yamaguchi, J. B. Wing, S. Sakaguchi, Two modes of immune suppression by $Foxp3^+$ regulatory T cells under inflammatory or non-inflammatory conditions. *Semin. Immunol.* **23**, 424–430 (2011).

39. S. Hori, T. Nomura, S. Sakaguchi, Control of regulatory T cell development by the transcription factor Foxp3. *Science.* **299**, 1057–1061 (2003).

40. L. M. Williams, A. Y. Rudensky, Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat. Immunol.* **8**, 277–284 (2007).

41. M. Salmi, S. Jalkanen, Lymphocyte homing to the gut: Attraction, adhesion, and commitment. *Immunol. Rev.* **206**, 100–113 (2005).

42. M. Iwata *et al.*, Retinoic acid imprints gut-homing specificity on T cells. *Immunity*. **21**, 527–538 (2004).

43. S. I. Hammerschmidt *et al.*, Stromal mesenteric lymph node cells are essential for the generation of gut-homing T cells in vivo. *J. Exp. Med.* **205**, 2483–2490 (2008).

44. I. I. Ivanov *et al.*, The Orphan Nuclear Receptor RORγt Directs the Differentiation Program of Proinflammatory IL-17^+ T Helper Cells. *Cell*. **126**, 1121–1133 (2006).

45. J. A. Hall *et al.*, Commensal DNA Limits Regulatory T Cell Conversion and Is a Natural Adjuvant of Intestinal Immune Responses. *Immunity*. **29**, 637–649 (2008).

46. K. Atarashi *et al.*, Induction of Colonic Regulatory T Cells by indigenous Clostridium species. *Science.* **331**, 337–341 (2011).

47. I. I. Ivanov *et al.*, Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell*. **139**, 485–498 (2009).

48. I. I. Ivanov *et al.*, Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine. *Cell Host Microbe*. **4**, 337–349 (2008).

49. K. Atarashi *et al.*, Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. *Cell*. **163**, 367–380 (2015).

50. Y. Goto *et al.*, Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity*. **40**, 594–607 (2014).

51. Y. Yang *et al.*, Focused specificity of intestinal T_H17 cells towards commensal bacterial antigens. *Nature*. **510**, 152–6 (2014).

52. K. Atarashi *et al.*, ATP drives lamina propria T_H17 cell differentiation. *Nature*. **455**, 808–812 (2008).

53. Y. K. Lee, J. S. Menezes, Y. Umesaki, S. K. Mazmanian, Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 4615–4622 (2011).

54. C. T. Weaver, R. D. Hatton, Interplay between the T_H17 and T_{Reg} cell lineages: a (co-)evolutionary perspective. *Nat. Rev. Immunol.* **9**, 883–889 (2009).

55. K. Atarashi *et al.*, Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. **500**, 232–236 (2013).

56. Y. Furusawa *et al.*, Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. **504**, 446–450 (2013).

57. N. Arpaia *et al.*, Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. **504**, 451–455 (2013).

58. June L. Round, S. K. Mazmanian, Inducible $Foxp3^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 12204–12209 (2010).

59. J. L. Round *et al.*, The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science.* **332**, 974–977 (2011).

60. S. K. Lathrop *et al.*, Peripheral education of the immune system by the colonic microbiota. *Nature*. **478**, 250–254 (2011).

61. L. Steele, L. Mayer, M. Cecilia Berin, Mucosal immunology of tolerance and allergy in the gastrointestinal tract. *Immunol. Res.* **54**, 75–82 (2012).

62. H. L. Weiner, H. Y. Wu, F. Quintana, H. Wu, Oral tolerance. *Immunol. Rev.* **241**, 241–259 (2011).

63. J. L. Coombes, K. J. Maloy, Control of intestinal homeostasis by regulatory T cells and dendritic cells. *Semin. Immunol.* **19**, 116–126 (2007).

64. O. Pabst, A. M. Mowat, Oral tolerance to food protein. *Mucosal Immunol.* **5**, 232–239 (2012).

65. P. J. Higgins, H. L. Weiner, Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. *J. Immunol.* **140**, 440–5 (1988).

66. C. Nagler-Anderson, L. A. Bober, M. E. Robinson, G. W. Siskind, G. J. Thorbecke, Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc. Natl. Acad. Sci. U. S. A.* **83**, 7443–7446 (1986).

67. H. Takagi *et al.*, A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 17525–17530 (2005).

68. I. Dello Iacono *et al.*, Specific oral tolerance induction with raw hen's egg in children with very severe egg allergy: a randomized controlled trial. *Pediatr. Allergy Immunol.* **24**, 66–74 (2013).

69. P. Varshney *et al.*, A randomized controlled study of peanut oral immunotherapy: Clinical desensitization and modulation of the allergic response. *J. Allergy Clin. Immunol.* **127**, 654–660 (2011).

70. C. A. Keet *et al.*, The safety and efficacy of sublingual and oral immunotherapy for milk allergy. *J. Allergy Clin. Immunol.* **129**, 448–455.e5 (2012).

71. M. A. Curotto de Lafaille *et al.*, Adaptive Foxp3$^+$ Regulatory T Cell-Dependent and -Independent Control of Allergic Inflammation. *Immunity*. **29**, 114–126 (2008).

72. T. Worbs *et al.*, Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J. Exp. Med.* **203**, 519–527 (2006).

73. T. W. Spahn *et al.*, Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. *Eur. J. Immunol.* **32**, 1109–1113 (2002).

74. T. W. Spahn *et al.*, Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches. *Eur. J. Immunol.* **31**, 1278–1287 (2001).

75. T. A. Kraus *et al.*, Induction of mucosal tolerance in Peyer's patch – deficient, ligated small bowel loops. *J. Clin. Invest.* **115**, 2234–2243 (2005).

76. K. Fujihashi *et al.*, Peyer's patches are required for oral tolerance to proteins. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 3310–3315 (2001).

77. U. Hadis *et al.*, Intestinal Tolerance Requires Gut Homing and Expansion of FoxP3$^+$ Regulatory T Cells in the Lamina Propria. *Immunity*. **34**, 237–246 (2011).

78. B. Cassani *et al.*, Gut-Tropic T Cells That Express Integrin $\alpha_4\beta_7$ and CCR9 Are Required for Induction of Oral Immune Tolerance in Mice. *Gastroenterology*. **141**, 2109–2118 (2011).

79. S. Crotty, T Follicular Helper Cell Differentiation , Function , and Roles in Disease. *Immunity*. **41**, 529–542 (2014).

80. S. Crotty, A brief history of T cell help to B cells. *Nat. Rev. Immunol.* **15,** 185-189 (2015).

81. D. Breitfeld *et al.*, Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* **192**, 1545–1552 (2000).

82. P. Schaerli *et al.*, CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* **192**, 1553–1562 (2000).

83. C. H. Kim *et al.*, Subspecialization of CXCR5$^+$ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5$^+$ T cells. *J. Exp. Med.* **193**, 1373–1381 (2001).

84. R. I. Nurieva *et al.*, Bcl6 mediates the development of T follicular helper cells. *Science*. **325**, 1001–1005 (2009).

85. R. J. Johnston *et al.*, Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science*. **325**, 1006–1010 (2009).

86. D. Yu *et al.*, The Transcriptional Repressor Bcl-6 Directs T Follicular Helper Cell Lineage Commitment. *Immunity*.

31, 457–468 (2009).

87. Y. S. Choi *et al.*, ICOS Receptor Instructs T Follicular Helper Cell versus Effector Cell Differentiation via Induction of the Transcriptional Repressor Bcl6. *Immunity*. **34**, 932–946 (2011).

88. D. Liu *et al.*, T–B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. *Nature*. **517**, 214–218 (2014).

89. R. A. Cubas *et al.*, Inadequate T follicular cell help impairs B cell immunity during HIV infection. *Nat. Med.* **19**, 494–499 (2013).

90. S. Kawamoto *et al.*, The Inhibitory Receptor PD-1 Regulates IgA Selection and Bacterial Composition in the Gut. *Science*. **336**, 485–489 (2012).

91. N. M. Haynes *et al.*, Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1^{high} germinal center-associated subpopulation. *J. Immunol.* **179**, 5099–5108 (2007).

92. M. A. Linterman *et al.*, Foxp3^{+} follicular regulatory T cells control the germinal center response. *Nat. Med.* **17**, 975–982 (2011).

93. Y. Chung *et al.*, Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat. Med.* **17**, 983–988 (2011).

94. I. Wollenberg *et al.*, Regulation of the germinal center reaction by Foxp3^{+} follicular regulatory T cells. *J. Immunol.* **187**, 4553–4560 (2011).

95. L. M. Kato, S. Kawamoto, M. Maruya, S. Fagarasan, Gut T_{FH} and IgA: key players for regulation of bacterial communities and immune homeostasis. *Immunol. Cell Biol.* **92**, 49–56 (2014).

96. M. Tsuji *et al.*, Preferential generation of follicular B helper T cells from Foxp3^{+} T cells in gut Peyer's patches. *Science*. **323**, 1488–1492 (2009).

97. K. Hirota *et al.*, Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. *Nat. Immunol.* **14**, 372–379 (2013).

98. S. Kawamoto *et al.*, Foxp3^{+} T Cells Regulate Immunoglobulin A Selection and Facilitate Diversification of Bacterial Species Responsible for Immune Homeostasis. *Immunity*. **41**, 152–165 (2014).

99. M. D. Cooper, The early history of B cells. *Nat. Rev. Immunol.* **15**, 191–197 (2015).

100. G. M. Edelman, Dissociation of γ-Globulin. *J. Am. Chem. Soc.* **184**, 3155–3156 (1959).

101. N. Hozumi, S. Tonegawa, Evidence for somatic rearrangement of immunoglobulin genes coding for variable and constant regions. *Proc. Natl. Acad. Sci. U. S. A.* **73**, 3628–3632 (1976).

102. C. Brack, M. Hirama, R. Lenhard-Schuller, S. Tonegawa, A complete immunoglobulin gene is created by somatic recombination. *Cell.* **15**, 1–14 (1978).

103. O. Bernard, N. Hozumi, S. Tonegawa, Sequences of mouse immunoglobulin light chain genes before and after

somatic changes. *Cell*. **15**, 1133–1144 (1978).

104. P. Early, H. Huang, M. Davis, K. Calame, L. Hood, An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: V_H, D and J_H. *Cell*. **19**, 981–992 (1980).

105. J. A. Daniel, A. Nussenzweig, The AID-induced DNA damage response in chromatin. *Mol. Cell*. **50**, 309–321 (2013).

106. D. J. Fear, Mechanisms regulating the targeting and activity of activation induced cytidine deaminase. *Curr. Opin. Immunol.* **25**, 619–628 (2013).

107. M. Bemark, P. Boysen, N. Y. Lycke, Induction of gut IgA production through T cell-dependent and T cell-independent pathways. *Ann. N. Y. Acad. Sci.* **1247**, 97–116 (2012).

108. K. M. Murphy, P. Travers, M. Walport, *Janeway's Immunobiology* (Garland Science, ed. 7th, 2007).

109. P. Brandtzaeg, Secretory IgA: Designed for anti-microbial defense. *Front. Immunol.* **4**, 222 (2013).

110. M. S. Halpern, R. L. Coffman, Polymer Formation and J Chain Synthesis in Mouse Plasmacytomas. *J. Immunol.* **109**, 674–680 (1972).

111. P. Brandtzaeg, H. Prydz, Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature*. **311**, 71–73 (1984).

112. R. L. Coffman, D. A. Lebman, B. Shrader, Transforming growth factor beta specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. *J. Exp. Med.* **170**, 1039–1044 (1989).

113. E. Sonoda *et al.*, Transforming growth factor beta induces IgA production and acts additively with interleukin 5 for IgA production. *J. Exp. Med.* **170**, 1415–1420 (1989).

114. B. B. Cazac, J. Roes, TGF-beta receptor controls B cell responsiveness and induction of IgA in vivo. *Immunity*. **13**, 443–451 (2000).

115. S. Borsutzky, B. B. Cazac, J. Roes, C. A. Guzmán, TGF-beta receptor signaling is critical for mucosal IgA responses. *J. Immunol.* **173**, 3305–3309 (2004).

116. G.-Y. Seo, J. Youn, P.-H. Kim, IL-21 ensures TGF-beta 1-induced IgA isotype expression in mouse Peyer's patches. *J. Leukoc. Biol.* **85**, 744–750 (2009).

117. M. Dullaers *et al.*, A T Cell-Dependent Mechanism for the Induction of Human Mucosal Homing Immunoglobulin A-Secreting Plasmablasts. *Immunity*. **30**, 120–129 (2009).

118. D. T. Avery, V. L. Bryant, C. S. Ma, R. D. W. Malefyt, S. G. Tangye, IL-21-Induced Isotype Switching to IgG and IgA by Human Naive B Cells Is Differentially Regulated by IL-4. *J. Immunol.* **181**, 1767–1779 (2008).

119. A. T. Cao *et al.*, Interleukin (IL)-21 promotes intestinal IgA response to microbiota. *Mucosal Immunol.* **8**, 1072–1082 (2015).

120. G. U. Von Bülow, J. M. Van Deursen, R. J. Bram, Regulation of the T-independent humoral response by TACI. *Immunity*. **14**, 573–582 (2001).

121. M. Yan *et al.*, Activation and accumulation of B cells in TACI-deficient mice. *Nat. Immunol.* **2**, 638–43 (2001).

122. M. B. Litinskiy *et al.*, DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat. Immunol.* **3**, 822–829 (2002).

123. E. Castigli *et al.*, Impaired IgA class switching in APRIL-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 3903–3908 (2004).

124. E. Castigli *et al.*, TACI and BAFF-R mediate isotype switching in B cells. *J. Exp. Med.* **201**, 35–39 (2005).

125. O. Pabst, New concepts in the generation and functions of IgA. *Nat. Rev. Immunol.* **12**, 821–832 (2012).

126. S. Fagarasan, K. Kinoshita, M. Muramatsu, K. Ikuta, T. Honjo, In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature*. **413**, 639–643 (2001).

127. E. E. Crouch *et al.*, Regulation of AID expression in the immune response. *J. Exp. Med.* **204**, 1145–1156 (2007).

128. B. He *et al.*, Intestinal Bacteria Trigger T Cell-Independent Immunoglobulin A2 Class Switching by Inducing Epithelial-Cell Secretion of the Cytokine APRIL. *Immunity*. **26**, 812–826 (2007).

129. T. Shikina *et al.*, IgA Class Switch Occurs in the Organized Nasopharynx- and Gut-Associated Lymphoid Tissue, but Not in the Diffuse Lamina Propria of Airways and Gut. *J. Immunol.* **172**, 6259–6264 (2004).

130. L. Boursier, J. N. Gordon, S. Thiagamoorthy, J. D. Edgeworth, J. Spencer, Human intestinal IgA response is generated in the organized gut-associated lymphoid tissue but not in the lamina propria. *Gastroenterology*. **128**, 1879–1889 (2005).

131. P. Bergqvist, E. Gärdby, A. Stensson, M. Bemark, N. Y. Lycke, Gut IgA class switch recombination in the absence of CD40 does not occur in the lamina propria and is independent of germinal centers. *J. Immunol.* **177**, 7772–7783 (2006).

132. P. Bergqvist, A. Stensson, N. Y. Lycke, M. Bemark, T cell-independent IgA class switch recombination is restricted to the GALT and occurs prior to manifest germinal center formation. *J. Immunol.* **184**, 3545–3553 (2010).

133. M. Liu *et al.*, Two levels of protection for the B cell genome during somatic hypermutation. *Nature*. **451**, 841–845 (2008).

134. G. D. Victora, M. C. Nussenzweig, Germinal Centers. *Annu. Rev. Immunol.* **30**, 429–457 (2012).

135. N. S. De Silva, U. Klein, Dynamics of B cells in germinal centres. *Nat. Rev. Immunol.* **15,** 137–148 (2015).

136. G. D. Victora *et al.*, Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell*. **143**, 592–605 (2010).

137. F. D. Batista, N. E. Harwood, The who, how and where of antigen presentation to B cells. *Nat. Rev. Immunol.* **9**, 15–27 (2009).

138. E. Natkanski *et al.*, B cells use mechanical energy to discriminate antigen affinities. *Science*. **340**, 1587–1590 (2013).

139. O. L. C. Wijburg *et al.*, Innate secretory antibodies protect against natural Salmonella typhimurium infection. *J. Exp. Med.* **203**, 21–26 (2006).

140. C. Aytekin, N. Tuygun, S. Gokce, F. Dogu, A. Ikinciogullari, Selective IgA deficiency: Clinical and laboratory features of 118 children in Turkey. *J. Clin. Immunol.* **32**, 961–966 (2012).

141. S. K. Mazmanian, H. L. Cui, A. O. Tzianabos, D. L. Kasper, An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*. **122**, 107–118 (2005).

142. F. Bäckhed, R. E. Ley, J. L. Sonnenburg, D. A. Peterson, J. I. Gordon, Host-bacterial mutualism in the human intestine. *Science*. **307**, 1915–1920 (2005).

143. T. Obata *et al.*, Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 7419–7424 (2010).

144. C. L. Maynard, C. O. Elson, R. D. Hatton, C. T. Weaver, Reciprocal interactions of the intestinal microbiota and immune system. *Nature*. **489**, 231–241 (2012).

145. M. Maruya, S. Kawamoto, L. M. Kato, S. Fagarasan, Impaired selection of IgA and intestinal dysbiosis associated with PD-1-deficiency. *Gut Microbes*. **4**, 165–171 (2013).

146. L. A. van der Waaij, P. C. Limburg, G. Mesander, D. van der Waaij, In vivo IgA coating of anaerobic bacteria in human faeces. *Gut*. **38**, 348–354 (1996).

147. G. De Palma *et al.*, Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. *BMC Microbiol.* **10**, 63 (2010).

148. D. A. Peterson, N. P. McNulty, J. L. Guruge, J. I. Gordon, IgA Response to Symbiotic Bacteria as a Mediator of Gut Homeostasis. *Cell Host Microbe*. **2**, 328–339 (2007).

149. S. Fagarasan, M. Muramatsu, K. Suzuki, Critical Roles of Activation-Induced Cytidine Deaminase in the Homeostasis of Gut Flora. *Science*. **298**, 1424–1427 (2002).

150. K. Suzuki *et al.*, Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 1981–1986 (2004).

151. F. Barone *et al.*, IgA-Producing Plasma Cells Originate From Germinal Centers That Are Induced by B-Cell Receptor Engagement in Humans. *Gastroenterology*. **140**, 947–956 (2011).

152. J. Benckert *et al.*, The majority of intestinal IgA^+ and IgG^+ plasmablasts in the human gut are antigen-specific. *J. Clin. Invest.* **121**, 1946–1955 (2011).

153. C. Lindner *et al.*, Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. *J. Exp. Med.* **209**, 365–377 (2012).

154. M. Wei *et al.*, Mice carrying a knock-in mutation of Aicda resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense. *Nat. Immunol.* **12**, 264–270 (2011).

155. C. Perrier, N. Sprenger, B. Corthesy, B. Corthe, Glycans on Secretory Component Participate in Innate Protection against Mucosal Pathogens. *J. Biol. Chem.* **281**, 14280–14287 (2006).

156. A. Mathias, B. Corthésy, Recognition of gram-positive intestinal bacteria by hybridoma- and colostrum-derived secretory immunoglobulin A is mediated by carbohydrates. *J. Biol. Chem.* **286**, 17239–17247 (2011).

157. C. P. Quan, A. Berneman, R. Pires, S. Avrameas, J. P. Bouvet, Natural polyreactive secretory immunoglobulin A autoantibodies as a possible barrier to infection in humans. *Infect. Immun.* **65**, 3997–4004 (1997).

158. M. Shimoda, Y. Inoue, N. Azuma, C. Kanno, Natural polyreactive immunoglobulin A antibodies produced in mouse Peyer's patches. *Immunology*. **97**, 9–17 (1999).

159. S. Wang *et al.*, Manipulating the Selection Forces during Affinity Maturation to Generate Cross-Reactive HIV Antibodies Theory Manipulating the Selection Forces during Affinity Maturation to Generate Cross-Reactive HIV Antibodies. *Cell.* **160**, 785–797 (2015).

160. P. Bergqvist *et al.*, Re-utilization of germinal centers in multiple Peyer's patches results in highly synchronized, oligoclonal, and affinity-matured gut IgA responses. *Mucosal Immunol.* **6**, 122–135 (2012).

161. S. Casola *et al.*, B cell receptor signal strength determines B cell fate. *Nat. Immunol.* **5**, 317–327 (2004).

162. R. B. Sartor, Microbial Influences in Inflammatory Bowel Diseases. *Gastroenterology*. **134**, 577–594 (2008).

163. M. E. V Johansson *et al.*, Composition and functional role of the mucus layers in the intestine. *Cell. Mol. Life Sci.* **68**, 3635–3641 (2011).

164. M. E. V Johansson *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).

165. K. Masahata *et al.*, Generation of colonic IgA-secreting cells in the caecal patch. *Nat. Commun.* **5**, 3704 (2014).

166. W. R. Heath, F. R. Carbone, Dendritic cell subsets in primary and secondary T cell responses at body surfaces. *Nat. Immunol.* **10**, 1237–1244 (2009).

167. R. M. Steinman, Z. A. Cohn, Identification of a novel cell type in peripheral lymphoid organs of mice: I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* **137,** 1142–1162 (1973).

168. R. M. Steinman, Z. A. Cohn, Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J Exp Med*. **139**, 380–397 (1974).

169. R. M. Steinman, B. Gutchinov, M. D. Witmer, M. C. Nussenzweig, Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* **157**, 613–627 (1983).

170. L. Delamarre, M. Pack, H. Chang, I. Mellman, E. S. Trombetta, Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. *Science*. **307**, 1630–1634 (2005).

171. O. P. Joffre, E. Segura, A. Savina, S. Amigorena, Cross-presentation by dendritic cells. *Nat. Rev. Immunol.* **12**, 557–569 (2012).

172. D. Hashimoto, J. Miller, M. Merad, Dendritic Cell and Macrophage Heterogeneity In Vivo. *Immunity*. **35**, 323–335 (2011).

173. A. Mildner, S. Jung, Development and function of dendritic cell subsets. *Immunity*. **40**, 642–656 (2014).

174. M. Guilliams *et al.*, Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat. Rev. Immunol.* **14**, 571–578 (2014).

175. J. L. Coombes, F. Powrie, Dendritic cells in intestinal immune regulation. *Nat. Rev. Immunol.* **8**, 435–446 (2008).

176. C. L. Scott, A. M. Aumeunier, A. M. Mowat, Intestinal CD103^{+} dendritic cells: Master regulators of tolerance? *Trends Immunol.* **32**, 412–419 (2011).

177. C. Varol *et al.*, Intestinal Lamina Propria Dendritic Cell Subsets Have Different Origin and Functions. *Immunity*. **31**, 502–512 (2009).

178. M. Bogunovic *et al.*, Origin of the Lamina Propria Dendritic Cell Network. *Immunity*. **31**, 513–525 (2009).

179. Loo YM, Gale M, Immune Signaling by RIG-I-like Receptors. *Immunity*. **34**, 680–692 (2011).

180. E. Elinav, T. Strowig, J. Henao-Mejia, R. A. Flavell, Regulation of the Antimicrobial Response by NLR Proteins. *Immunity*. **34**, 665–679 (2011).

181. L. A. J. O'Neill, D. Golenbock, A. G. Bowie, The history of Toll-like receptors - redefining innate immunity. *Nat. Rev. Immunol.* **13**, 453–60 (2013).

182. T. Kondo, T. Kawai, S. Akira, Dissecting negative regulation of Toll-like receptor signaling. *Trends Immunol.* **33**, 449–458 (2012).

183. I. Botos, D. M. Segal, D. R. Davies, The structural biology of Toll-like receptors. *Structure*. **19**, 447–459 (2011).

184. M. S. Jin *et al.*, Crystal Structure of the TLR1-TLR2 Heterodimer Induced by Binding of a Tri-Acylated Lipopeptide. *Cell*. **130**, 1071–1082 (2007).

185. J. Y. Kang *et al.*, Recognition of Lipopeptide Patterns by Toll-like Receptor 2-Toll-like Receptor 6 Heterodimer. *Immunity*. **31**, 873–884 (2009).

186. L. Liu *et al.*, Structural basis of toll-like receptor 3 signaling with double-stranded RNA. *Science*. **320**, 379–381 (2008).

187. B. S. Park *et al.*, The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature*. **458**, 1191–1195 (2009).

188. S. -I. Yoon *et al.*, Structural Basis of TLR5-Flagellin Recognition and Signaling. *Science.* **335**, 859–864 (2012).

189. G. M. Barton, J. C. Kagan, R. Medzhitov, Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. *Nat. Immunol.* **7**, 49–56 (2006).

190. T. Haas *et al.*, The DNA Sugar Backbone 2′ Deoxyribose Determines Toll-like Receptor 9 Activation. *Immunity*. **28**,

315–323 (2008).

191. O. Takeuchi *et al.*, Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a toll-like receptor 2- and MyD88-dependent signaling pathway. *J. Immunol.* **164**, 554–557 (2000).

192. H. Oshiumi, M. Matsumoto, K. Funami, T. Akazawa, T. Seya, TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. *Nat. Immunol.* **4**, 161–167 (2003).

193. M. Yamamoto *et al.*, Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science*. **301**, 640–643 (2003).

194. T. Kawai, O. Adachi, T. Ogawa, K. Takeda, S. Akira, Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity*. **11**, 115–122 (1999).

195. T. Horng, G. M. Barton, R. A. Flavell, R. Medzhitov, The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature*. **420**, 329–333 (2002).

196. F. Hayashi *et al.*, The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*. **410**, 1099–1103 (2001).

197. H. Hemmi *et al.*, Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat. Immunol.* **3**, 196–200 (2002).

198. H. Häcker *et al.*, Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF)6. *J. Exp. Med.* **192**, 595–600 (2000).

199. S. Goriely, M. F. Neurath, M. Goldman, How microorganisms tip the balance between interleukin-12 family members. *Nat. Rev. Immunol.* **8**, 81–86 (2008).

200. K. L. Wong, F. C. Lew, P. A. MacAry, D. M. Kemeny, CD40L-expressing CD8 T cells prime CD8α^+ DC for IL-12p70 production. *Eur. J. Immunol.* **38**, 2251–2262 (2008).

201. O. Schulz *et al.*, CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity*. **13**, 453–462 (2000).

202. A. D. Edwards *et al.*, Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J. Immunol.* **169**, 3652–3660 (2002).

203. I. Monteleone, A. M. Platt, E. Jaensson, W. W. Agace, A. M. Mowat, IL-10-dependent partial refractoriness to Toll-like receptor stimulation modulates gut mucosal dendritic cell function. *Eur. J. Immunol.* **38**, 1533–1547 (2008).

204. B. C. Taylor *et al.*, TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J. Exp. Med.* **206**, 655–667 (2009).

205. T. Chinen *et al.*, Prostaglandin E2 and SOCS1 have a role in intestinal immune tolerance. *Nat. Commun.* **2**, 190 (2011).

206. S. Wang *et al.*, MyD88-dependent TLR1/2 signals educate dendritic cells with gut-specific imprinting properties. *J. Immunol.* **187**, 141–150 (2011).

207. I. Albrecht *et al.*, Toll-like receptors differentially induce nucleosome remodelling at the IL-12p40 promoter. *EMBO Rep.* **5**, 172–177 (2004).

208. J. L. Coombes *et al.*, A functionally specialized population of mucosal CD103$^+$ DCs induces Foxp3$^+$ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J. Exp. Med.* **204**, 1757–1764 (2007).

209. J. J. Worthington, B. I. Czajkowska, A. C. Melton, M. A. Travis, Intestinal dendritic cells specialize to activate transforming growth factor-β and induce Foxp3$^+$ regulatory T cells via integrin $\alpha_v\beta_8$. *Gastroenterology*. **141**, 1802–1812 (2011).

210. H. Païdassi *et al.*, Preferential expression of integrin $\alpha_v\beta_8$ promotes generation of regulatory T cells by mouse CD103$^+$ dendritic cells. *Gastroenterology*. **141**, 1813–1820 (2011).

211. S. Uematsu *et al.*, Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat. Immunol.* **9**, 769–776 (2008).

212. C.-M. Sun *et al.*, Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T_{reg} cells via retinoic acid. *J. Exp. Med.* **204**, 1775–1785 (2007).

213. N. E. Welty *et al.*, Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *J. Exp. Med.* **210**, 2011–2024 (2013).

214. A. C. Melton *et al.*, Expression of $\alpha_v\beta_8$ integrin on dendritic cells regulates Th17 cell development and experimental autoimmune encephalomyelitis in mice. *J. Clin. Invest.* **120**, 4436–4444 (2010).

215. M. Acharya *et al.*, alphav Integrin expression by DCs is required for Th17 cell differentiation and development of experimental autoimmune encephalomyelitis in mice. *J. Clin. Invest.* **120**, 4445–4452 (2010).

216. A. Yokota *et al.*, GM-CSF and IL-4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity. *Int. Immunol.* **21**, 361–377 (2009).

217. O. Schulz *et al.*, Intestinal CD103$^+$, but not CX3CR1$^+$, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J. Exp. Med.* **206**, 3101–3114 (2009).

218. E. Jaensson *et al.*, Small intestinal CD103$^+$ dendritic cells display unique functional properties that are conserved between mice and humans. *J. Exp. Med.* **205**, 2139–2149 (2008).

219. M. Shan *et al.*, Mucus Enhances Gut Homeostasis and Oral Tolerance by Delivering Immunoregulatory Signals. *Science.* **342**, 447–453 (2013).

220. M. R. Neutra, N. J. Mantis, J. P. Kraehenbuhl, Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat. Immunol.* **2**, 1004–1009 (2001).

221. H. Lelouard, M. Fallet, B. De Bovis, S. Méresse, J. Gorvel, Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. *Gastroenterology*. **142**, 592–601 (2012).

222. J. R. McDole *et al.*, Goblet cells deliver luminal antigen to CD103^{+} dendritic cells in the small intestine. *Nature*. **483**, 345–349 (2012).

223. J. Farache *et al.*, Luminal Bacteria Recruit CD103^{+} Dendritic Cells into the Intestinal Epithelium to Sample Bacterial Antigens for Presentation. *Immunity*. **38**, 581–595 (2013).

224. J. H. Niess *et al.*, CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. **307**, 254–258 (2005).

225. E. Mazzini, L. Massimiliano, G. Penna, M. Rescigno, Oral Tolerance Can Be Established via Gap Junction Transfer of Fed Antigens from CX$_3$CR1^{+} Macrophages to CD103^{+} Dendritic Cells. *Immunity*. **40**, 248–261 (2014).

226. S. A. Houston *et al.*, The lymph nodes draining the small intestine and colon are anatomically separate and immunologically distinct. *Mucosal Immunol.* doi: 10.1038/mi.2015.77

227. V. Cerovic *et al.*, Intestinal CD103 − dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunol.* **6**, 104–113 (2012).

228. G. E. Diehl *et al.*, Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX$_3$CR1^{hi} cells. *Nature*. **494**, 116–120 (2013).

229. A. Iwasaki, B. L. Kelsall, Localization of Distinct Peyer's Patvh Dendritic Cell Subsets and Their Recruitment by Chemokines Macrophage Inflammatory Protein (MIP)-3α, MIP-3β, and Secondary Lymphoid Organ Chemokine. *J. Exp. Med.* **191**, 1381–1394 (2000).

230. A. J. Macpherson, T. Uhr, Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*. **303**, 1662–1665 (2004).

231. M. H. Jang *et al.*, CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J. Immunol.* **176**, 803–810 (2006).

232. D. Malhotra, A. L. Fletcher, S. J. Turley, Stromal and hematopoietic cells in secondary lymphoid organs: Partners in immunity. *Immunol. Rev.* **251**, 160–176 (2013).

233. M. Bajénoff *et al.*, Stromal Cell Networks Regulate Lymphocyte Entry, Migration, and Territoriality in Lymph Nodes. *Immunity*. **25**, 989–1001 (2006).

234. S. E. Acton *et al.*, Podoplanin-Rich Stromal Networks Induce Dendritic Cell Motility via Activation of the C-type Lectin Receptor CLEC-2. *Immunity*. **37**, 276–289 (2012).

235. R. L. Lindquist *et al.*, Visualizing dendritic cell networks in vivo. *Nat. Immunol.* **5**, 1243–1250 (2004).

236. R. S. Allan *et al.*, Migratory Dendritic Cells Transfer Antigen to a Lymph Node-Resident Dendritic Cell Population for Efficient CTL Priming. *Immunity*. **25**, 153–162 (2006).

237. T. R. Mempel, S. E. Henrickson, U. H. Von Andrian, T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature*. **427**, 154–159 (2004).

238. H. Spits *et al.*, Innate lymphoid cells--a proposal for uniform nomenclature. *Nat. Rev. Immunol.* **13**, 145–9 (2013).

239. D. Artis, H. Spits, The biology of innate lymphoid cells. *Nature*. **517**, 293–301 (2015).

240. N. Serafini, C. A. J. Vosshenrich, J. P. Di Santo, Transcriptional regulation of innate lymphoid cell fate. *Nat. Rev. Immunol.* **15**, 415–428 (2015).

241. K. Moro *et al.*, Innate production of T_H2 cytokines by adipose tissue-associated c-Kit$^+$Sca-1$^+$ lymphoid cells. *Nature*. **463**, 540–544 (2010).

242. D. R. Neill *et al.*, Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. **464**, 1367–1370 (2010).

243. A. E. Price *et al.*, Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 11489–11494 (2010).

244. C. J. Oliphant *et al.*, MHCII-Mediated Dialog between Group 2 Innate Lymphoid Cells and CD4$^+$ T Cells Potentiates Type 2 Immunity and Promotes Parasitic Helminth Expulsion. *Immunity*. **41,** 283-295 (2014).

245. M. R. Hepworth *et al.*, Innate lymphoid cells regulate CD4$^+$ T-cell responses to intestinal commensal bacteria. *Nature*. **498**, 113–7 (2013).

246. M. R. Hepworth *et al.*, Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4$^+$ T cells. *Science*. **348**, 1031–1035 (2015).

247. S. N. Waggoner *et al.*, Roles of natural killer cells in antiviral immunity. *Curr. Opin. Virol.* **16**, 15–23 (2016).

248. A. Fuchs *et al.*, Intraepithelial Type 1 Innate Lymphoid Cells Are a Unique Subset of IL-12- and IL-15-Responsive IFN-γ-Producing Cells. *Immunity*. **38**, 769–781 (2013).

249. C. S. N. Klose *et al.*, Differentiation of Type 1 ILCs from a Common Progenitor to All Helper-like Innate Lymphoid Cell Lineages. *Cell*. **157**, 340–356 (2014).

250. N. K. Crellin *et al.*, Regulation of cytokine secretion in human CD127$^+$ LTi-like innate lymphoid cells by toll-like receptor 2. *Immunity*. **33**, 752–764 (2010).

251. M. Cella, K. Otero, M. Colonna, Expansion of human NK-22 cells with IL-7, IL-2, and IL-1beta reveals intrinsic functional plasticity. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 10961–10966 (2010).

252. M. Coccia *et al.*, IL-1 mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4$^+$ Th17 cells. *J. Exp. Med.* **209**, 1595–1609 (2012).

253. A. Mortha *et al.*, Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science*. **343**, 1249288 (2014).

254. N. Satoh-Takayama *et al.*, Microbial flora drives interleukin 22 production in intestinal NKp46$^+$ cells that provide innate mucosal immune defense. *Immunity*. **29**, 958–970 (2008).

255. L. Van Maele *et al.*, TLR5 signaling stimulates the innate production of IL-17 and IL-22 by CD3$^-$CD127$^+$ immune

cells in spleen and mucosa. *J. Immunol.* **185**, 1177–1185 (2010).

256. M. A. Kinnebrew *et al.*, Interleukin 23 Production by Intestinal CD103^{+}CD11b^{+} Dendritic Cells in Response to Bacterial Flagellin Enhances Mucosal Innate Immune Defense. *Immunity*. **36**, 276–287 (2012).

257. Y. Goto, S. E. Domino, P. Kulig, B. Becher, Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science.* **345**, 1254009 (2014).

258. C. Vonarbourg *et al.*, Regulated expression of nuclear receptor RORγt confers distinct functional fates to NK cell receptor-expressing RORγt^{+} innate lymphocytes. *Immunity*. **33**, 736–751 (2010).

259. J. H. Bernink *et al.*, Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat. Immunol.* **14**, 221–229 (2013).

260. C. S. N. Klose *et al.*, A T-bet gradient controls the fate and function of CCR6^{-}RORγt^{+} innate lymphoid cells. *Nature*. **494**, 261–265 (2013).

261. S. Buonocore *et al.*, Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature*. **464**, 1371–1375 (2010).

262. N. Powell *et al.*, The Transcription Factor T-bet Regulates Intestinal Inflammation Mediated by Interleukin-7 Receptor^{+} Innate Lymphoid Cells. *Immunity*. **37**, 674–684 (2012).

263. T. Hoyler *et al.*, The Transcription Factor GATA-3 Controls Cell Fate and Maintenance of Type 2 Innate Lymphoid Cells. *Immunity*. **37**, 634–648 (2012).

264. J. Mjösberg *et al.*, The Transcription Factor GATA3 Is Essential for the Function of Human Type 2 Innate Lymphoid Cells. *Immunity*. **37**, 649–659 (2012).

265. R. Yagi *et al.*, The transcription factor GATA3 is critical for the development of all IL-7Rα-expressing innate lymphoid cells. *Immunity*. **40**, 378–388 (2014).

266. Y. Motomura *et al.*, Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. *Immunity*. **40**, 758–771 (2014).

267. J.-E. Turner *et al.*, IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J. Exp. Med.* **210**, 2951–2965 (2013).

268. S. A. Saenz *et al.*, IL-25 simultaneously elicits distinct populations of innate lymphoid cells and multipotent progenitor type 2 (MPPtype2) cells. *J. Exp. Med.* **210**, 1823–1837 (2013).

269. M. Salimi *et al.*, A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J. Exp. Med.* **210**, 2939–2950 (2013).

270. B. S. Kim *et al.*, TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci. Transl. Med.* **5**, 170ra16 (2013).

271. X. Yu *et al.*, TNF superfamily member TL1A elicits type 2 innate lymphoid cells at mucosal barriers. *Mucosal*

Immunol. **7**, 730–740 (2014).

272. F. Meylan *et al.*, The TNF-family cytokine TL1A promotes allergic immunopathology through group 2 innate lymphoid cells. *Mucosal Immunol.* **7**, 958–968 (2014).

273. T. Y. F. Halim, R. H. Krauß, A. C. Sun, F. Takei, Lung Natural Helper Cells Are a Critical Source of Th2 Cell-Type Cytokines in Protease Allergen-Induced Airway Inflammation. *Immunity*. **36**, 451–463 (2012).

274. T. Y. F. Halim *et al.*, Retinoic-Acid-Receptor-Related Orphan Nuclear Receptor Alpha Is Required for Natural Helper Cell Development and Allergic Inflammation. *Immunity*. **37**, 463–474 (2012).

275. B. S. Kim *et al.*, Basophils Promote Innate Lymphoid Cell Responses in Inflamed Skin. *J. Immunol.* **193**, 3717–3725 (2014).

276. G. Eberl *et al.*, An essential function for the nuclear receptor RORγt in the generation of fetal lymphoid tissue inducer cells. *Nat. Immunol.* **5**, 64–73 (2004).

277. M. Tsuji *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).

278. E. Scandella *et al.*, Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone. *Nat. Immunol.* **9**, 667–675 (2008).

279. J. A. Dudakov *et al.*, Interleukin-22 Drives Endogenous Thymic Regeneration in Mice. *Science.* **336**, 91–95 (2012).

280. L. A. Monticelli *et al.*, Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* **12**, 1045–1054 (2011).

281. A. B. Molofsky *et al.*, Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J. Exp. Med.* **210**, 535–549 (2013).

282. D. Wu *et al.*, Eosinophils Sustain Adipose Alternatively Activated Macrophages Associated with Glucose Homeostasis. *Science.* **332**, 243–247 (2011).

283. E. Hams, R. M. Locksley, A. N. J. McKenzie, P. G. Fallon, Cutting edge: IL-25 elicits innate lymphoid type 2 and type II NKT cells that regulate obesity in mice. *J. Immunol.* **191**, 5349–5353 (2013).

284. J. R. Brestoff *et al.*, Group 2 innate lymphoid cells promote beiging of white Adipose Tissue and Limit Obesity. *Nature* **519,** 242–246 (2014).

285. S. P. Spencer *et al.*, Adaptation of Innate Lymphoid Cells to a Micronutrient Deficiency Promotes Type 2 Barrier Immunity. *Science.* **343**, 432–437 (2014).

286. J. C. Nussbaum *et al.*, Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature*. **502**, 245–248 (2013).

287. E. A. Kiss *et al.*, Natural Aryl Hydrocarbon Receptor Ligands Control Organogenesis of Intestinal Lymphoid Follicles. *Science.* **334**, 1561–1565 (2011).

288. G. F. Sonnenberg *et al.*, Innate Lymphoid Cells Promote Anatomical Containment of Lymphoid-Resident Commensal

Bacteria. *Science.* **336**, 1321–1325 (2012).

289. L. V Hooper, J. Xu, P. G. Falk, T. Midtvedt, J. I. Gordon, A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 9833–9838 (1999).

290. S. Sawa *et al.*, RORγt^+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat. Immunol.* **12**, 320–326 (2011).

291. L. L. Korn *et al.*, Conventional $CD4^+$ T cells regulate IL-22-producing intestinal innate lymphoid cells. *Mucosal Immunol.* **7**, 1045–1057 (2014).

292. J. Qiu *et al.*, The Aryl Hydrocarbon Receptor Regulates Gut Immunity through Modulation of Innate Lymphoid Cells. *Immunity*. **36**, 92–104 (2012).

293. N. von Burg *et al.*, Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 12835–12840 (2014).

294. A. A. Kruglov *et al.*, Nonredundant function of soluble LTα3 produced by innate lymphoid cells in intestinal homeostasis. *Science.* **342**, 1243–1246 (2013).

295. G. Magri *et al.*, Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. *Nat. Immunol.* **15**, 354–364 (2014).

296. A. M. C. Faria, H. L. Weiner, Oral Tolerance: Therapeutic Implications for Autoimmune Diseases. *Clin. Dev. Immunol.* **13**, 143–157 (2006).

297. D. Mucida *et al.*, Reciprocal Th17 and regulatory T cell differentiation mediated by retinoic acid. *Science.* **317**, 256–260 (2007).

298. M. J. Benson, K. Pino-Lagos, M. Rosemblatt, R. J. Noelle, All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J. Exp. Med.* **204**, 1765–1774 (2007).

299. M. Guilliams *et al.*, Skin-draining lymph nodes contain dermis-derived $CD103^-$ dendritic cells that constitutively produce retinoic acid and induce $Foxp3^+$ regulatory T cells. *Blood*. **115**, 1958–1968 (2010).

300. G. Matteoli *et al.*, Gut $CD103^+$ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut*. **59**, 595–604 (2010).

301. V. Cerovic *et al.*, Lymph-borne $CD8\alpha^+$ dendritic cells are uniquely able to cross-prime $CD8^+$ T cells with antigen acquired from intestinal epithelial cells. *Mucosal Immunol.* **8,** 38–48 (2014).

302. T. Fukaya *et al.*, Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific $CD4^+Foxp3^+$ regulatory T cells in the establishment of oral tolerance. *Blood*. **116**, 2266–2276 (2010).

303. K. M. Murphy, A. B. Heimberger, D. Y. Loh, Induction by antigen of intrathymic apoptosis of $CD4^+CD8^+TCR^{lo}$ thymocytes in vivo. *Science.* **250**, 1720–1723 (1990).

304. J. C. Miller *et al.*, Deciphering the transcriptional network of the dendritic cell lineage. *Nat. Immunol.* **13**, 888–899 (2012).

305. T. L. Denning *et al.*, Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. *J. Immunol.* **187**, 733–747 (2011).

306. Y. S. Choi, D. Eto, J. A. Yang, C. Lao, S. Crotty, Cutting Edge: STAT1 Is Required for IL-6-Mediated Bcl6 Induction for Early Follicular Helper Cell Differentiation. *J. Immunol.* **190**, 3049–3053 (2013).

307. Y. Hiramatsu *et al.*, c-Maf activates the promoter and enhancer of the IL-21 gene, and TGF-beta inhibits c-Maf-induced IL-21 production in $CD4^+$ T cells. *J. Leukoc. Biol.* **87**, 703–712 (2010).

308. A. T. Bauquet *et al.*, The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and T_H17 cells. *Nat. Immunol.* **10**, 167–175 (2009).

309. M. A. Kroenke *et al.*, Bcl6 and Maf Cooperate To Instruct Human Follicular Helper CD4 T Cell Differentiation. *J. Immunol.* **188**, 3734–3744 (2012).

310. N. Schmitt *et al.*, Human Dendritic Cells Induce the Differentiation of Interleukin-21-Producing T Follicular Helper-like Cells through Interleukin-12. *Immunity*. **31**, 158–169 (2009).

311. C. S. Ma *et al.*, Early commitment of naïve human $CD4^+$ T cells to the T follicular helper (T_{FH}) cell lineage is induced by IL-12. *Immunol. Cell Biol.* **87**, 590–600 (2009).

312. L. Wei, A. Laurence, K. M. Elias, J. J. O'Shea, IL-21 Is Produced by Th17 Cells and Drives IL-17 Production in a STAT3-dependent Manner. *J. Biol. Chem.* **282**, 34605–34610 (2007).

313. A. Suto *et al.*, Development and characterization of IL-21-producing $CD4^+$ T cells. *J. Exp. Med.* **205**, 1369–1379 (2008).

314. O. Dienz *et al.*, The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by $CD4^+$ T cells. *J. Exp. Med.* **206**, 69–78 (2009).

315. S. Nakayamada *et al.*, Early Th1 Cell Differentiation Is Marked by a Tfh Cell-like Transition. *Immunity*. **35**, 919–931 (2011).

316. R. I. Nurieva *et al.*, Generation of T Follicular Helper Cells Is Mediated by Interleukin-21 but Independent of T Helper 1, 2, or 17 Cell Lineages. *Immunity*. **29**, 138–149 (2008).

317. G. Trinchieri, A. Sher, Cooperation of Toll-like receptor signals in innate immune defence. *Nat. Rev. Immunol.* **7**, 179–190 (2007).

318. M. B. Lutz *et al.*, An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods*. **223**, 77–92 (1999).

319. M. Ebisawa *et al.*, $CCR6^{hi}CD11c^{int}$ B cells promote M-cell differentiation in Peyer's patch. *Int. Immunol.* **23**, 261–269 (2011).

320. N. Hanagata, Structure-dependent immunostimulatory effect of CpG oligodeoxynucleotides and their delivery system. *Int. J. Nanomedicine*. **7**, 2181–2195 (2012).

321. R. M. Salazar-Gonzalez *et al.*, CCR6-Mediated Dendritic Cell Activation of Pathogen-Specific T Cells in Peyer's Patches. *Immunity*. **24**, 623–632 (2006).

322. N. Fasnacht *et al.*, Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. *J. Exp. Med.* **211**, 2265–2279 (2014).

323. C. Parham *et al.*, A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* **168**, 5699–5708 (2002).

324. Z. Chen *et al.*, Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 8137–8142 (2006).

325. L. Wei *et al.*, Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation. *Immunity*. **32**, 840–851 (2010).

326. N. Hirata *et al.*, Selective synergy in anti-inflammatory cytokine production upon cooperated signaling via TLR4 and TLR2 in murine conventional dendritic cells. *Mol. Immunol.* **45**, 2734–2742 (2008).

327. M. Krummen *et al.*, Release of IL-12 by dendritic cells activated by TLR ligation is dependent on MyD88 signaling, whereas TRIF signaling is indispensable for TLR synergy. *J. Leukoc. Biol.* **88**, 189–199 (2010).

328. S. M. Mäkelä, M. Strengell, T. E. Pietilä, P. Osterlund, I. Julkunen, Multiple signaling pathways contribute to synergistic TLR ligand-dependent cytokine gene expression in human monocyte-derived macrophages and dendritic cells. *J. Leukoc. Biol.* **85**, 664–672 (2009).

329. D. Mitchell *et al.*, Dual stimulation of MyD88-dependent Toll-like receptors induces synergistically enhanced production of inflammatory cytokines in murine bone marrow-derived dendritic cells. *J. Infect. Dis.* **202**, 318–329 (2010).

330. G. Napolitani, A. Rinaldi, F. Bertoni, F. Sallusto, A. Lanzavecchia, Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* **6**, 769–776 (2005).

331. X. Ouyang *et al.*, Cooperation between MyD88 and TRIF pathways in TLR synergy via IRF5 activation. *Biochem. Biophys. Res. Commun.* **354**, 1045–1051 (2007).

332. D. P. Simmons *et al.*, Mycobacterium tuberculosis and TLR2 agonists inhibit induction of type I IFN and class I MHC antigen cross processing by TLR9. *J. Immunol.* **185**, 2405–2415 (2010).

333. Y. C. Liu *et al.*, TLR2 signaling depletes IRAK1 and inhibits induction of type I IFN by TLR7/9. *J. Immunol.* **188**, 1019–1026 (2012).

334. S. Uematsu *et al.*, Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal $CD11c^+$ lamina propria cells. *Nat. Immunol.* **7**, 868–874 (2006).

335. F. Re, J. L. Strominger, IL-10 released by concomitant TLR2 stimulation blocks the induction of a subset of Th1

cytokines that are specifically induced by TLR4 or TLR3 in human dendritic cells. *J. Immunol.* **173**, 7548–7555 (2004).

336. K. A. Bode, F. Schmitz, L. Vargas, K. Heeg, A. H. Dalpke, Kinetic of RelA activation controls magnitude of TLR-mediated IL-12p40 induction. *J. Immunol.* **182**, 2176–2184 (2009).

337. C.-Y. Zhang *et al.*, TLR2 signaling subpathways regulate TLR9 signaling for the effective induction of IL-12 upon stimulation by heat-killed Brucella abortus. *Cell. Mol. Immunol.* **9**, 324–333 (2012).

338. C. G. Vinuesa *et al.*, A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature.* **435**, 452–8 (2005).

339. M. A. Linterman *et al.*, Follicular helper T cells are required for systemic autoimmunity. *J. Exp. Med.* **206**, 561–576 (2009).

340. B. M. E. Neurath, I. Fuss, B. L. Kelsall, E. Sti, W. Strober, Antibodies to Interleukin 12 Abrogate Established Experimental Colitis in Mice. *J. Exp. Med.* **182**, 1281–1290 (1995).

341. N. J. Davidson *et al.*, IL-12, but not IFN-gamma, plays a major role in sustaining the chronic phase of colitis in IL-10-deficient mice. *J. Immunol.* **161**, 3143–3149 (1998).

342. S. Hue *et al.*, Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J. Exp. Med.* **203**, 2473–2483 (2006).

343. D. Yen *et al.*, IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J. Clin. Invest.* **116**, 1310–1316 (2006).

344. R. E. Ley, D. A. Peterson, J. I. Gordon, Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell.* **124**, 837–848 (2006).

345. I. Yusuf *et al.*, Germinal Center T Follicular Helper Cell IL-4 Production Is Dependent on Signaling Lymphocytic Activation Molecule Receptor (CD150). *J. Immunol.* **185**, 190–202 (2010).

346. M. Kuraoka, M. Hashiguchi, S. Hachimura, S. Kaminogawa, $CD4^{-}$c-$kit^{-}CD3\varepsilon^{-}$IL-$2R\alpha^{+}$ Peyer's patch cells are a novel cell subset which secrete IL-5 in response to IL-2: Implications for their role in IgA production. *Eur. J. Immunol.* **34**, 1920–1929 (2004).

347. M. L. Robinette *et al.*, Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nat. Immunol.* **16,** 306–317 (2015).

348. J. A. Walker, J. L. Barlow, A. N. J. McKenzie, Innate lymphoid cells--how did we miss them? *Nat. Rev. Immunol.* **13**, 75–87 (2013).

349. K. Moro, K. N. Ealey, H. Kabata, S. Koyasu, Isolation and analysis of group 2 innate lymphoid cells in mice. *Nat. Protoc.* **10**, 792–806 (2015).

350. S. Hapfelmeier *et al.*, Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science.* **328**, 1705–1709 (2010).

351. J. I. Kim, I.-C. Ho, M. J. Grusby, L. H. Glimcher, The Transcription Factor c-Maf Controls the Production of Interleukin-4 but Not Other Th2 Cytokines. *Immunity*. **10**, 745–751 (1999).

352. M. Y. Kim *et al.*, Heterogeneity of lymphoid tissue inducer cell populations present in embryonic and adult mouse lymphoid tissues. *Immunology*. **124**, 166–174 (2008).

353. T. Kurosaki, K. Kometani, W. Ise, Memory B cells. *Nat. Rev. Immunol.* **15**, 149–159 (2015).

354. M. A. Linterman *et al.*, IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. *J. Exp. Med.* **207**, 353–363 (2010).

355. D. Zotos *et al.*, IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J. Exp. Med.* **207**, 365–378 (2010).

356. R. L. Reinhardt, H.-E. Liang, R. M. Locksley, Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat. Immunol.* **10**, 385–393 (2009).

357. I. L. King, M. Mohrs, IL-4-producing $CD4^+$ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J. Exp. Med.* **206**, 1001–1007 (2009).

358. K. Ozaki *et al.*, A critical role for IL-21 in regulating immunoglobulin production. *Science*. **298**, 1630–1634 (2002).

359. B. León *et al.*, Regulation of T_H2 development by $CXCR5^+$ dendritic cells and lymphotoxin-expressing B cells. *Nat. Immunol.* **13**, 681–690 (2012).

360. T. Kuwahara *et al.*, The lifestyle of the segmented filamentous bacterium: A non-culturable gut-associated immunostimulating microbe inferred by whole-genome sequencing. *DNA Res.* **18**, 291–303 (2011).

361. K. W. Beagley *et al.*, Recombinant murine IL-5 induces high rate IgA synthesis in cycling IgA-positive Peyer's patch B cells. *J. Immunol.* **141**, 2035–2042 (1988).

362. R. Matsumoto *et al.*, Interleukin-5 induces maturation but not class switching of surface IgA-positive B cells into IgA-secreting cells. *Immunology*. **66**, 32–38 (1989).

363. T. Hiroi *et al.*, Deficiency of IL-5 receptor alpha-chain selectively influences the development of the common mucosal immune system independent IgA-producing B-1 cell in mucosa-associated tissues. *J. Immunol.* **162**, 821–828 (1999).

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