Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

2

Anatomical Uniqueness of the Mucosal Immune System (GALT, NALT, iBALT) for the Induction and Regulation of Mucosal Immunity and Tolerance

Aaron Silva-Sanchez and Troy D. Randall

Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL, United States

I. INTRODUCTION

The digestive and respiratory tracts are continuously exposed to antigens, commensals, and pathogens present in the lumen that can sometimes cross the mucosal epithelium, a barrier that is necessary for nutrient acquisition in the gut and for gas exchange in the respiratory tract. However, the exposure of the mucosal surface to exogenous materials also makes these tissues ports of entry for potentially pathogenic organisms. As a result, the immune system devotes enormous resources to the defense of mucosal surfaces. Although this defense needs to effectively prevent microbes from invading the underlying tissue, it must critically discriminate between innocuous antigens, commensal organisms, and pathogens in order to prevent inappropriate or inflammatory immune responses that might impair the physiological function of the host organ. Therefore mucosal lymphoid organs must have robust mechanisms to fine-tune immune responses to the types of antigen they encounter.

Immune responses to mucosal antigens are initiated in a variety of secondary lymphoid organs (SLOs) that collect antigens from mucosal surfaces, recruit naïve B and T cells from the blood, and utilize networks of fibroblastic stromal cells to organize encounters among various cell types and generate immune responses that are appropriate for the type of antigen and the tissue where the antigens are encountered. In the gut, the inductive tissues, gut-associated lymphoid tissues (GALT), include the Peyer's patches, cecal patches, colonic patches, isolated lymphoid follicles (ILFs), and cryptopatches, which, along with the gut-draining mesenteric

Mucosal Vaccines
DOI: https://doi.org/10.1016/B978-0-12-811924-2.00002-X

lymph nodes (MLNs), are inductive sites for adaptive immune responses to gut-derived antigens. In the respiratory tract, immune-inductive tissues include the nasopharyngeal-associated lymphoid tissue (NALT) and inducible bronchus-associated lymphoid tissue (iBALT), which, along with the cervical and mediastinal lymph nodes (LNs), generate adaptive immune responses to antigens encountered in the upper and lower respiratory tracts, respectively [1].

Mucosal lymphoid tissues and systemic lymphoid organs share basic architectural features that are required for the immune-inductive functions of these organs. For example, each lymphoid organ has mechanisms for recruiting leukocytes from the blood, mechanisms for spatially arranging lymphocytes and myeloid cells to maximize the efficiency of cellular interactions, mechanisms for sampling antigens from the surrounding tissue or from the luminal surface of the mucosal epithelium, and, finally, mechanisms for sending activated effector lymphocytes back into the periphery or to distal portions of the mucosal surface, where they eliminate pathogens or reside as memory cells (Chapter 3: Mucosal Antigen Sampling Across the Villus Epithelium by Epithelial and Myeloid Cells) and Chapter 5: Mucosal Immunity for Inflammation: Regulation of Gut-Specific Lymphocyte Migration by Integrins). Although these activities are generally similar in each type of mucosal lymphoid tissue, the mechanistic details that control each of these functions are often different, depending on the host organ (lung or gut), the location of the mucosal tissue in that organ, and the types of antigens that are encountered.

Although most mucosal lymphoid tissues are true SLOs and form at predictable sites during embryogenesis, independently of antigen or microbial signals, other mucosal lymphoid tissues form after birth at sites of infection or inflammation and require microbial or inflammatory signals for their development. These tissues are often referred to as ectopic or tertiary lymphoid organs (TLOs) [2]. Despite their different developmental origins, SLOs and TLOs share many overlapping developmental, architectural, and functional features. Nevertheless, the mechanistic details governing the immune functions of these organs are often different, in part owing to the stimuli that triggered their development and to the host organ in which they reside. Given that mucosal lymphoid organs can be involved in either pathogenesis, symbiosis or immune protection in a variety of clinical conditions and experimental disease models, it is essential to understand the mechanistic details controlling the development and function of each type of mucosal lymphoid organ in order to intervene in local mucosal immune responses. In this chapter, we will discuss the specific mechanisms that control the structure, development, and function of various mucosal lymphoid tissues in the respiratory and digestive tracts.

II. EVOLUTIONARY REQUIREMENT FOR MUCOSAL LYMPHOID ORGANS

The oldest known receptor-based, adaptive immune systems are found in jawless fish (lamprey and hagfish) and cartilaginous fish (shark) [3,4]. The immune systems of these organisms feature two distinct cell types that use genetic recombination to generate diverse repertoires of antigen receptors. For example, lampreys have one cell type that expresses variable lymphocyte receptors (VLR)-A/C and another cell type that expresses VLR-B [5,6]. These cell types are functionally analogous to the T and B cells that are found in higher vertebrates, and they express diverse repertoires of recombinant T cell antigen receptors (TCRs) and B cell antigen receptors (BCRs) [3,7].

Interestingly, all vertebrates with adaptive immune systems also have lymphoid aggregates along the gut tube (Fig. 2.1). For example, the gut lamina propria of jawless fish contains lymphoid aggregates that fulfill both

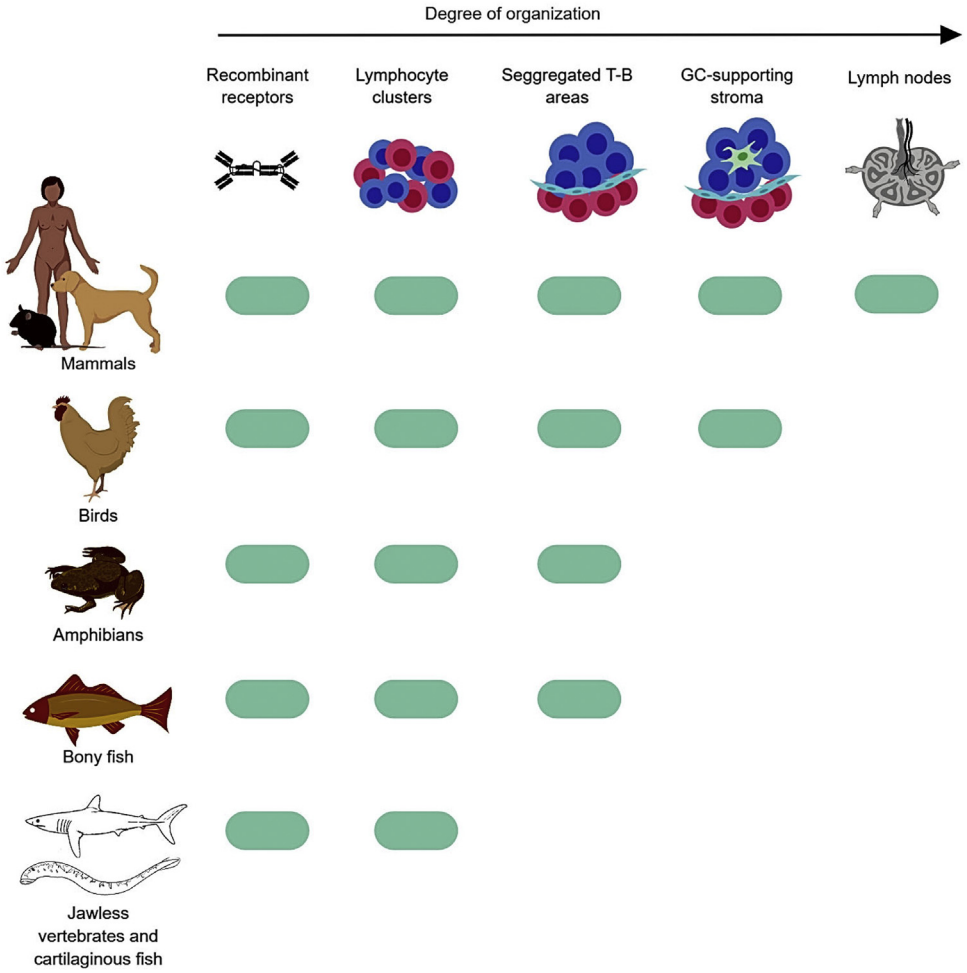

FIGURE 2.1 **Evolution of organized lymphoid tissues.** All classes of vertebrates possess some form of adaptive immune system using recombinant antigen receptors. These animals also form aggregates of immune cells along mucosal tissues. A higher degree of lymphocyte organization appears in the mucosa of bony fish and amphibians, in which T cell and B cell areas are segregated by fibroblastic stromal cells. However, specialized follicular dendritic cells within the B cell follicles are found only in birds and mammals. The presence of encapsulated lymph nodes is restricted to mammals.

hematopoietic and immune response functions, including the development of VLR-expressing cells as well as the proliferation and differentiation of those cells after antigenic encounter [8–10]. Similarly, the GALT of rabbits facilitates the diversification of the primary BCR repertoire and promotes antigen-dependent immune responses [11,12]. However, in other vertebrates, the generation of the primary lymphocyte repertoire is physically separated from the immune response to environmental antigens. Nevertheless, the GALT in all vertebrates is involved in immune responses to the luminal contents of the digestive tract.

Why should adaptive immune systems and complex lymphoid tissues coevolve? The

likeliest explanation is that, although a widely diverse repertoire of antigen receptors allows the recognition of a broad array of antigens, the frequency of any particular receptor that recognizes any particular antigen is very low, thereby reducing the probability that those few antigen-specific lymphocytes will encounter their cognate antigens. Given that mucosal tissues are continuously exposed to the external environment and that the digestive tract in particular is filled with a myriad of microorganisms, it makes sense that vertebrates evolved clusters of antigen-receptor-bearing lymphocytes directly beneath the mucosal epithelium, where they would be in close contact with potential antigens.

Although all vertebrates have mucosa-associated lymphoid aggregates, the highly organized lymphoid architecture observed in mammalian lymphoid tissues is a more recent evolutionary development [13]. For example, bony fish lack an obvious segregation of B and T cells in the lymphocyte clusters associated with the gut, gills, and nasopharynx [14–16], whereas amphibians and reptiles separate B and T cell areas in the spleen but not in the GALT [17]. In contrast, mucosal lymphoid tissues in birds and mammals are highly organized [13,14], with separated B and T cell areas, specialized fibroblastic stromal cells, strategically placed phagocytic and antigen-presenting cells, and well-demarcated germinal centers.

Why is lymphoid organization important? The evolution of specialized antigen-presenting cells, B cells and T cells, all of which need to interact for successful immune responses, requires mechanisms for these cells to find each other and additional mechanisms to support their proliferation and survival during immune responses [14]. In this regard, the development of specialized stromal cells, including fibroblastic reticular cells (FRCs) in the T cell area and follicular dendritic cells (FDCs) in the B cell area, is likely a key innovation. These cell types provide the scaffolding of lymphoid organs and help to spatially organize lymphocytes and antigen-presenting cells. For example, FRCs express the chemokine CCL19, which attracts T cells and activated dendritic cells (DCs) into the T cell zone [18,19], whereas FDCs express the chemokines CXCL12 and CXCL13, which attract B cells and T follicular helper (Tfh) cells and direct their spatial positioning in the B cell follicle and germinal center [20–23]. Although B and T cell separation occurs in amphibians and reptiles, identifiable stromal cells such as FDCs are observed only in birds and mammals [14] (Fig. 2.1), which probably explains why these species (and not others) are able to support germinal center responses and generate high-affinity antibodies [24,25]. Thus the mechanisms that control the differentiation of stromal cell elements are critical for the proper organization and function of lymphoid organs.

Not surprisingly, the genetic mechanisms that control lymphoid organ development and organization have coevolved with changes in immune architecture and function. For example, the development and organization of lymphoid tissues are regulated, in part, by the activities of tumor necrosis factor (TNF) superfamily members, TNFα, lymphotoxin-α (LT-α), and LT-β, and their receptors [26–28]. These genes are functionally expressed in lymphoid organs of higher vertebrates and sarcopterygian fish (lungfish and coelacanth) but not in teleost fish [16], an observation that correlates with lymphoid organization in each of these species. Interestingly, despite being evolutionarily advanced vertebrates, avian species appear to have lost the gene cluster encoding TNF, LT-α, and LT-β, as well as other TNF family members [29–31], which perhaps explains their inability to develop LNs [14]. However, avian species still develop highly organized mucosal lymphoid tissues, including cecal tonsil, esophageal tonsil, and Peyer's patches [32–35], suggesting that additional or alternative TNF family members contribute to the development and organization of these tissues.

In fact, a variety of TNF superfamily members and their ligands contribute in various ways to the development, organization, and maintenance of various systemic and mucosal lymphoid organs. These cytokines are important for the differentiation of FDCs [22,36] and FRCs [37–39], for the expression of lymphocyte-attracting chemokines [27,40,41], for the development and maintenance of high endothelial venules (HEVs) [42–44], for the survival of some DCs [45–47], for the activation of lymphocytes [48], and for the differentiation of antigen-transporting microfold (M) cells [49,50]. Importantly, the various TNF superfamily members and their receptors often have overlapping functions. As a result, different TNF superfamily ligand–receptor pairs are often used for similar purposes in different mucosal lymphoid tissues, depending on the cell types involved and the timing of the interactions. In the following sections, we will highlight which TNF superfamily members are important for the various features and functions of mucosal lymphoid tissues.

III. GUT-ASSOCIATED LYMPHOID TISSUE

The mucosal epithelium along the digestive tract is exposed to a vast array of foreign antigens derived from the diet as well as the community of microorganisms (the microbiota) that colonizes the gut. The gut microbiota is essential for the digestion of food and helps to catabolize nutrients that otherwise would be impossible for the host [51]. However, if not properly contained, the microbiota can breach the mucosal barrier and cause local or systemic infections that may lead to chronic or acute inflammation or even death [52]. Thus although commensal colonization is clearly beneficial to the host, noninflammatory immune control is required to prevent commensal encroachment into host tissues and simultaneously maintain microbial homeostasis at mucosal surfaces. To accomplish this goal, the digestive tract is lined with mucosa-associated lymphoid tissues (MALTs) that support adaptive immune responses to antigens derived from food and from the microbiota itself. These tissues include the cryptopatches, ILFs, Peyer's patches, cecal patches, colonic patches, MLNs, and even ectopic lymphoid tissues that can develop anywhere between the stomach and the colon.

A. Cryptopatches

Cryptopatches are the smallest and most numerous lymphoid aggregates in the mammalian gut, numbering around 30,000 in humans and around 1500 in mice [53]. Their distribution is not homogenous, with a higher frequency in the ileum and colon compared to the duodenum and jejunum. As their name suggests, cryptopatches are found beneath the crypts at the base of the intestinal villi; they consist of a small cluster (fewer than 1000 cells) of c-kit$^+$, IL-7R$^+$ lymphocytes, and CD11c$^+$CX3CR1$^+$ myeloid cells, but almost no B cells, T cells, or identifiable stromal cell networks [54] (Fig. 2.2). Initially, the c-kit$^+$IL-7R$^+$ cells were thought to be precursors of intestinal epithelial lymphocytes, and cryptopatches were thought to serve as sites of extrathymic T cell development [55]. However, more recent data show that the c-kit$^+$IL-7R$^+$ cells in cryptopatches are actually innate lymphoid cells (ILC3 cells) [56,57], which depend on the transcription factor RORγt and produce cytokines such as IL-17, IL-22, and GM-CSF [58,59] (Chapter 14: Innate Lymphoid Cells for the Control of Mucosal Immunity).

The expression of the chemokine receptors CCR6 and CXCR3 divide intestinal ILC3 cells into two populations. Those that express CXCR3 coexpress the transcription factors RORγt and T-bet and are located throughout the intestinal lamina propria [60–62], whereas those that express CCR6 also express NKp46 and c-kit and are the adult counterparts of fetal lymphoid

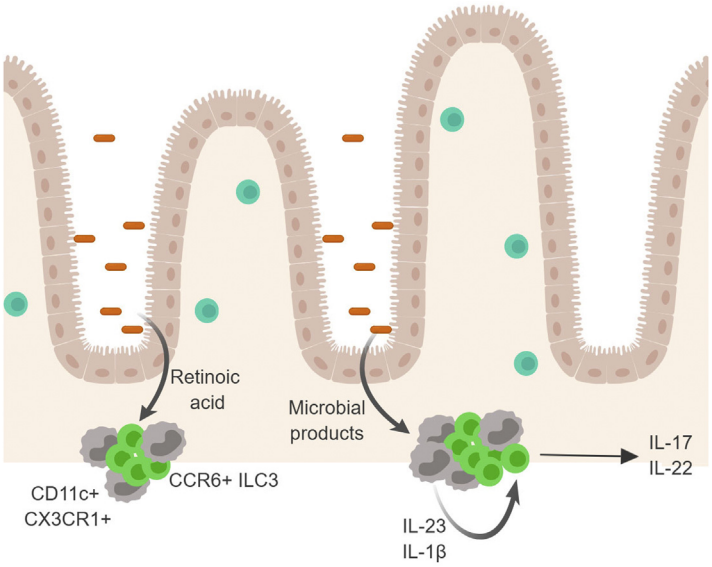

FIGURE 2.2 Organization and formation of cryptopatches. Cryptopatches (CPs) are located beneath the crypts at the base of the villi along the small and large intestines. The cryptopatches contain $CD11c^+CX3CR1^+$ myeloid cells and RORγt-dependent ILC3 cells that express either CCR6 or CXCR3. ILC3 cells in CPs are dependent on metabolites such as aromatic hydrocarbons and retinoic acid. Under homeostatic conditions, the $CCR6^+$ ILC3s function as lymphoid tissue inducer cells. Upon sensing of microbial products, $CX3CR1^+$ myeloid cells in the CPs produce IL-23, which induces ILC3 cells to secrete IL-17 and IL-22, thereby promoting granulocyte recruitment as well as epithelial cell proliferation and repair.

tissue inducer (LTi) cells [63–66]. Importantly, the $CCR6^+$ ILC3 cells reside in cryptopatches [54] and ILFs [67], where they control intestinal epithelial homeostasis and, following infection, promote intestinal inflammation.

Given the lack of B and T cells in cryptopatches, these structures are probably not primary inductive sites for adaptive immune responses. Nevertheless, interactions between ILC3 cells and $CD11c^+CX3CR1^+$ myeloid cells in cryptopatches are an important component of intestinal immunity and homeostasis. For example, in response to microbial signals, the $CX3CR1^+$ myeloid cells in cryptopatches produce IL-23, which triggers the activation of ILC3 cells and their expression of cytokines such as IL-17 and IL-22 [68] (Fig. 2.2). These cytokines contribute to antimicrobial defense in the intestine by promoting the expression of antimicrobial molecules [69], triggering the recruitment of granulocytes [70], and enhancing epithelial proliferation and repair [71,72]. Upon the appropriate stimulation, $CX3CR1^+$ myeloid cells produce additional cytokines, such as IL-1β and TL1A, which also trigger ILC3 cell activation and the expression of IL-17 and IL-22 [73].

ILC3 cells are also potent producers of GM-CSF under both steady-state and inflammatory conditions [74–76]. Under homeostatic conditions, ILC3-derived GM-CSF helps to maintain immune tolerance by promoting the development of DCs that promote regulatory T cell (Treg) differentiation [75]. Conversely, the inflammatory activation of $CX3CR1^+$ myeloid cells leads to IL-23-driven activation and GM-CSF production by ILC3 cells, which in turn promotes colitis [76,77]. Moreover, GM-CSF feeds back on ILC3 cells, leading to their mobilization from cryptopatches and inflammation throughout the gut [76]. In fact, a variety of microbial signals trigger $CX3CR1^+$ myeloid cells to express inflammatory cytokines, including IL-1β, IL-23, and TL1A, that activate ILC3 cells to produce GM-CSF [74,75]. Thus the interactions between the microbiota, $CX3CR1^+$ myeloid cells, and ILC3 cells are important for intestinal homeostasis and inflammation.

In addition to inflammatory cytokines, microbe-derived metabolites are also essential for the formation and activation of ILC3 cells. For example, the production and maintenance of ILC3 cells depend on vitamin-A-derived

retinoic acid [78], which promotes expression of the transcription factor RORγt in developing ILC3 cells [79]. Similarly, the aryl hydrocarbon receptor, AHR, is expressed by ILC3 cells and senses soluble aromatic hydrocarbons that are produced by commensal bacteria or derived from the diet [80]. The AHR is essential for ILC3, for the development of ILC3 cells, and for their production of cytokines such as IL-22 [57]. ILC3 cells also sense oxysterols through the G-protein-coupled receptor, GPR183 [81], which is expressed by CCR6-expressing ILC3 cells in the cryptopatches and ILFs. Oxysterol compounds are constitutively produced by fibroblastic stromal cells [81], which recruit ILC3 cells to the cryptopatches and ILFs under steady-state conditions. Moreover, oxysterols are produced at higher levels during inflammatory responses, leading to local ILC3 activation and promoting inflammatory responses. Thus a variety of environmental triggers activate ILC3 cells in cryptopatches and help to maintain barrier function during homeostasis and trigger inflammatory responses following infection.

B. Isolated Lymphoid Follicles

Unlike the primitive structure of cryptopatches, the lymphoid architecture of ILFs is much more complex and, in many ways, resembles that of Peyer's patches, the classic MALT in the gut. ILFs, which are found throughout the small and large intestines of mice [53], consist of a single B cell follicle that often contains a germinal center and a network of CD21/35-expressing fibroblastic stromal cells, the FDCs. The B cell follicle of ILFs is positioned beneath a specialized dome epithelium containing M cells that transport antigen from the intestinal lumen to the leukocytes beneath [67] (Fig. 2.3). A small number of T cells, primarily $CD4^+$ cells, are present in the

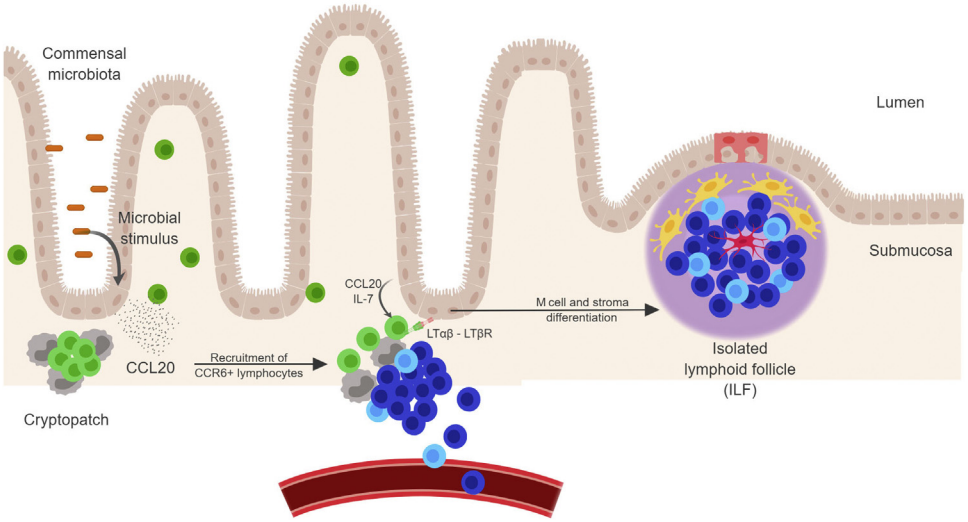

FIGURE 2.3 **Organization and formation of isolated lymphoid follicles (ILFs).** The transformation from cryptopatch to ILF entails the recruitment of lymphocytes, the development of specialized epithelial M cells, and the formation of FDC networks. Microbial products activate the intestinal epithelial cells to produce CCL20 and recruit $CCR6^+$ lymphocytes, including ILC3 cells, which in turn express membrane-bound LTαβ that reinforces M cell and FDC differentiation. Once formed, ILFs are maintained by constitutive expression of LTαβ on B cells, which interact with M cells and FDCs. Unlike the more complex Peyer's patches, ILFs consist mostly of B cells, forming a single B cell follicle positioned beneath a subepithelial dome formed by dendritic cells and a few $CD4^+$ T cells.

B cell follicle but do not form a discrete T cell zone. Dendritic cells are found beneath the dome and surrounding the B cell follicle [82]. Like cryptopatches, ILFs contain a significant fraction of CCR6-expressing ILC3 cells [83].

Although the structures of cryptopatches and ILFs are distinct, they are actually part of a spectrum of intestinal lymphoid tissues that ranges from nascent cryptopatches to fully developed ILFs [84,85], with cryptopatches vastly outnumbering ILFs. Interestingly, although cryptopatches appear only after birth, their development does not require signals from the microbiota [54] (Fig. 2.3). In contrast, the transition of cryptopatches to ILFs requires microbial exposure, which activates intestinal epithelial cells via innate sensing mechanisms, including peptidoglycan recognition by NOD1 in epithelial cells [86]. As a result, starting around 2 weeks after birth, some cryptopatches begin to recruit B cells and develop into ILFs [84,85].

The ability of lymphoid tissues to recruit lymphocytes is dependent on specialized vascular structures, known as HEVs, which display a variety of adhesion molecules and chemokines that allow circulating lymphocytes to adhere to the vascular wall and migrate into the tissue [44]. Although cryptopatches lack fully developed HEVs, MAdCAM-1-expressing HEVs are formed as cryptopatches become ILFs [87], thereby allowing the recruitment of $\alpha4\beta7$-expressing B and T cells. Although the blockade of $\alpha4\beta7$ has no effect on the numbers or cellularity of cryptopatches, it significantly impairs their ability to transition into ILFs [87].

Interactions between the chemokine receptor CCR6 and its ligand, CCL20, are also important for the formation and architecture of cryptopatches and ILFs [83] (Fig. 2.3). For example, mature ILFs have a dome epithelium with M cells that express CCL20 [86], which recruits specialized DCs, B and T cells [83,86], which cluster beneath the M cell-containing dome epithelium, where they await antigens that are transported across the epithelium. CCL20 expression by epithelial cells is also important for the development of ILFs, because it attracts $CCR6^+$ ILC3 cells, which express lymphotoxin and other activating ligands that promote the differentiation of M cells and the maturation of fibroblastic stromal cells that support the lymphoid structure of the ILF [83]. Epithelial cells also produce IL-7, which expands ILC3 cells and promotes their expression of lymphotoxin. In fact, cryptopatches and ILFs fail to develop in the absence of IL-7, primarily owing to the absence of ILC3 cells.

Whereas the cryptopatches primarily support innate immune responses due to a paucity of B and T cells, ILFs are important sites for the induction of adaptive immune responses, particularly the differentiation of IgA-producing B cells responding to the gut microbiota or food antigens [88,89]. Interestingly, ILFs are also an important source of T-cell-independent antimicrobial IgA [89], with no direct relation to the immune responses occurring in Peyer's patches [90]. Recent work has broadened our understanding of the role of ILFs, and a general consensus is that they act in a tolerogenic manner to control intestinal immune responses by generating both IgA-secreting plasma cells and regulatory T cells [91,92], both of which are involved in the noninflammatory containment of commensal organisms. Conversely, the absence of ILFs leads to poor IgA production and a dramatic expansion of the intestinal microbiota [88]. Thus ILFs are powerhouses of IgA production in response to microbial antigens [90].

C. Peyer's Patches, Cecal Patches, and Colonic Patches

The Peyer's patches, colonic patches, and cecal patches are among the largest MALT in the gut, and they are found in the submucosa on the antimesenteric side of the small

intestine, colon, and cecum, respectively (Fig. 2.4). Structurally, the various intestinal patches are very similar to one another, differing mostly in their location and number, although the fine details of the cecal and colonic patches have not been thoroughly studied. It is important to remember, however, that the density and composition of the gut microbiota vary dramatically from the stomach to the colon [93] and will certainly affect the development, activity, and functions of the various lymphoid organs in different locations.

Like ILFs, Peyer's, cecal, and colonic patches have a prominent dome epithelium or follicle-associated epithelium that consists of enterocytes as well as antigen-transporting M cells [1,94,95]. The M cells are large epithelial cells that lack microvilli on the luminal side and

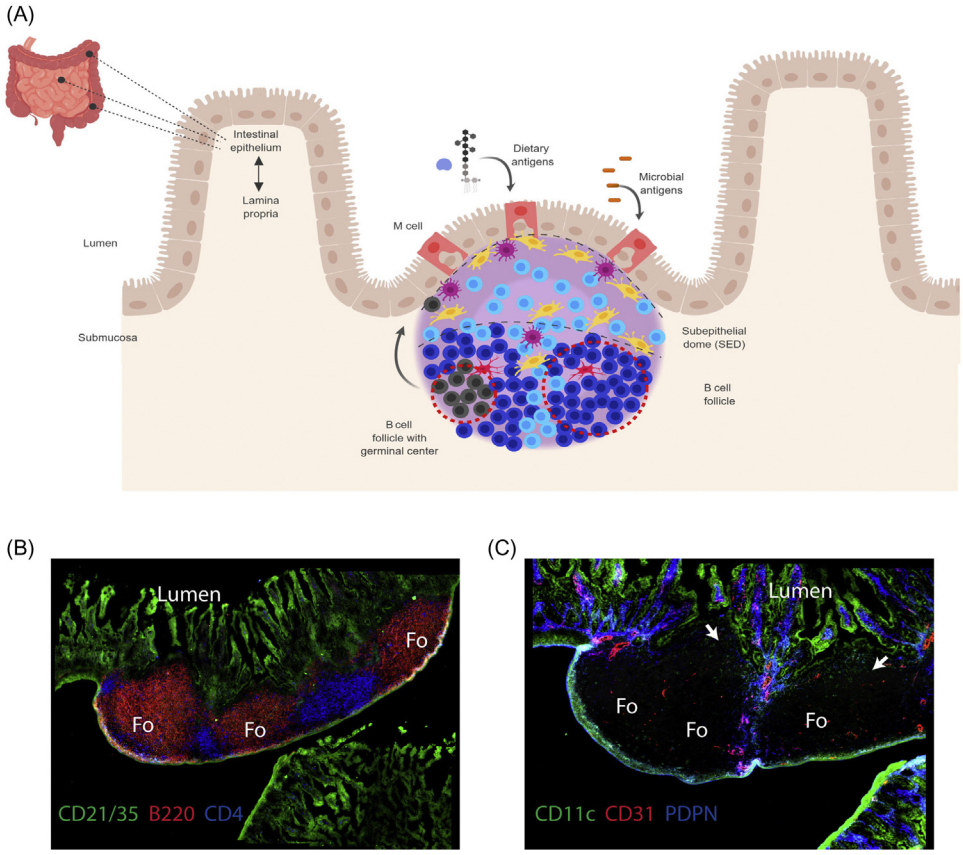

FIGURE 2.4 **Peyer's patches (PP) and large intestine patches.** Large, multifollicular lymphoid organs found in the submucosa of the small and large intestines are termed Peyer's patches (small intestine), cecal patches (cecum), or colonic patches (colon). (A) Peyer's patches are found beneath a specialized follicle-associated epithelial layer that contains M cells. The region immediately below the epithelium is called the subepithelial dome and contains $SIRPa^{+}XCR1^{-}$ dendritic cells that present antigen and help B cells switch to IgA. Monocyte-derived $CX3CR1^{+}$ macrophages in the dome region also contribute to the clearance and processing of lumen-derived antigens. B cell follicles separated by T-cell-rich interfollicular areas are found below the dome region. (B) Frozen sections of Peyer's patches showing B cell follicles (*red*), interfollicular T cell areas (*blue*), and the follicular dendritic cell network in B cell follicles (*green*). (C) White arrows indicate the dome region that contains $CD11c^{+}$ cells (*green*). Between B cell follicles (Fo), fibroblastic reticular cells (*blue*) surround HEVs (*red*).

have a deep invagination of the basolateral membrane (also known as the pocket) [49,96], which harbors a variety of DCs, macrophages, and lymphocytes that sample and respond to the transcytosed antigens. M cells are highly pinocytic and mediate the transcytosis of antigens from the luminal side to the basal side of the epithelium [49,96,97]. The area immediately below the epithelium, called the subepithelial dome, is where immune cells, mainly DCs and macrophages, can first encounter antigens transported from the lumen of the intestine (Chapter 3: Mucosal Antigen Sampling Across the Villus Epithelium by Epithelial and Myeloid Cells) and Chapter 28: M Cell-Targeted Vaccines).

Like ILFs, Peyer's patches have large B cell follicles, often with germinal centers, extending beneath the subepithelial dome toward the muscularis layer. Unlike ILFs however, which have a single B cell follicle and essentially no T cell zone, Peyer's patches have numerous follicles underneath their domes. Moreover, the B cell follicles of Peyer's patches are separated by interfollicular regions, which are functionally analogous to the T cell zones of LNs and contain both DCs and T cells [98]. Thus the Peyer's patches and other intestinal patches are structurally more complex than ILFs.

A variety of DCs reside in the Peyer's patches under homeostatic and inflammatory conditions [99–101]. Dendritic cells in regional LNs are broadly divided into those that migrated from peripheral tissues though afferent lymphatics (migratory DCs) and those that developed in the LN itself (resident DCs) [102]. In addition, both the migratory and resident DCs can be divided into those that express XCR1 (cDC1 cells) and those that express SIRPα (cDC2 cells) [103]. Since Peyer's patches lack afferent lymphatics, they lack migratory DCs (by definition) but have other DC subsets that perform similar functions. For example, the $CD11b^+$ DCs in the subepithelial dome region ($SIRP\alpha^+$ cDC2 cells) lack CCR7, but upon activation, they turn on CCR7 and migrate to the interfollicular region [100,104], where they present antigen to T cells.

The other migratory DCs found in the intestine are the cDC1 $CD103^+$ DCs [105,106], which migrate through afferent lymphatics to MLNs, where they cross-present antigens to $CD8^+$ T cells [107,108]. The $CD103^+$ DCs also efficiently promote the expression of the gut-homing receptors, CCR9 and α4β7 integrin, on responding T cells [109,110]. Importantly, under steady-state conditions, $CD103^+$ DCs can trigger naive T cells to differentiate into Foxp3-expressing regulatory T cells through a mechanism that requires TGF-β and retinoic acid signaling [111–113]. Although $CD103^+$ DCs are found in Peyer's patches [114], these cells are most likely resident $CD8\alpha^+$ DCs found in the interfollicular region. The interfollicular region also has $CD8\alpha^-CD11b^-$ (double negative) resident DCs near the base of the dome area near the follicle [115]. These double negative DCs are most likely cDC2 cells, since they express SIRPα [115] but are distinct from the $CD11b^+$ DCs in the dome region.

Additional antigen-presenting cells found in the Peyer's patches, but not in conventional LNs, express lysozyme and are known as either Lyso-DCs or Lyso-macrophages, the latter of which can be further divided on their expression of the apoptotic cell receptor TIM4. All three of these cell types express the chemokine receptor CX3CR1 [115], a feature identifying them as monocyte-derived cells. Lyso-DCs that express CD11b and Lyso-Macs that express CD4 but not TIM4 are located in the dome region, whereas $CD4^+$ $Tim4^+$ lyso-Macs are located in the interfollicular region [115].

As it does in other SLOs, the fibroblastic stromal architecture of Peyer's patches acts as a scaffolding that supports the structure of the tissue and produces a variety of factors that promote the survival of lymphocytes and regulate their position. For example, the FDC networks of Peyer's patches express, or at least display, the chemokine CXCL13, which attracts and organizes B cells and Tfh cells in germinal

centers [21,116]. In fact, nearly every follicle in Peyer's patches has a germinal center filled with rapidly proliferating, antigen-responsive B cells, most of which have switched to IgA [104]. The FDCs in Peyer's patches express typical markers such as CD21/35 and FDCM1, as well as the Fcα/μR, which is important for restraining germinal centers and the production of serum IgA [117].

Peyer's patches also have dense networks of FRCs, which primarily define the T cell zone (the interfollicular area in Peyer's patches) [19,116], where they express CCL19, the high-affinity ligand for CCR7, which attracts naïve T cells and activated DCs. The FRCs also facilitate T cell trafficking within the interfollicular region [19,21] and help to coordinate interactions between antigen-presenting DCs and naïve T cells. Importantly, FRCs in the interfollicular areas express IL-7 [118,119], a cytokine necessary for the homeostatic maintenance of naïve T cells. Although FRCs are primarily associated with the T cell zones of lymphoid organs, they also extend into the B cell follicles, where they express IL-7 and BAFF [120], which maintains the viability of B cells. Finally, FRCs in Peyer's patches modulate the activity of ILC1 cells through the transpresentation of IL-15 [121]. Thus FRCs are essential for the placement, movement, and survival of a variety of hematopoietic cell types.

In addition to the chemokines made by fibroblastic stromal cells, chemokines made by epithelial cells in the dome are also important for cellular positioning in Peyer's patches [100]. For example, dome epithelial cells express CCL20 and CCL9 [100], which attract subsets of immature CCR6- and CCR1-expressing DCs to the subepithelial dome of Peyer's patches [100]. CCL20 is also important for attracting B cells to the dome, where they interact with $CD11b^+$ DCs and isotype-switch to IgA [88,122]. Another chemokine, CXCL16, is also expressed by the dome epithelium of Peyer's patches, ILFs, and cecal patches [123], whereas its receptor, CXCR6, is observed on subpopulations of activated and memory $CD4^+$ and $CD8^+$ T cells in the dome region. Thus the chemokines expressed by the dome epithelial cells are critical for the placement and juxtaposition of antigen-presenting cells and subsets of activated and memory lymphocytes beneath the antigen-transporting dome epithelium.

Although M cells nonspecifically sample luminal antigens by pinocytosis, they also acquire antigens by receptor mediated endocytosis [124]. In fact, M cells express a variety of receptor-like molecules that help them sample a wide array of microbial pathogens and antigens. Some of these receptors have been identified by comparing gene expression profiles of M cells with those of enterocytes [125]. For example, the GPI-anchored glycoprotein 2 (GP2) is expressed on the luminal surface of M cells and acts as a receptor for type I piliated bacteria, including *Escherichia coli* and *Salmonella typhimurium* [126]. Similarly, a homolog of GP2, the urinary protein uromodulin, is also expressed on M cells [127,128], where it also binds type I piliated *E. coli*. Another GPI-anchored protein, the cellular prion protein (PrPc), is also abundantly expressed on the luminal surface of M cells [125,129]. PrPc interacts with Hsp60 of *Brucella abortus* and helps its internalization into M cells [130]. Other M-cell-expressed proteins with potential antigen-sampling activity include ANXA5, which binds the lipid A domain of LPS and facilitates the uptake of Gram-negative bacteria [131], and the peptidoglycan recognition protein (PGLRP)-1, which binds to bacterial peptidoglycans [132] (Chapter 28: M Cell-Targeted Vaccines).

Although M cells use their receptors to sample antigens and microorganisms in the gut, the reverse is also true, and some pathogens have evolved mechanisms to use M cells as a point of entry. For example, *Yersinia enterocolitica*, *Listeria monocytogenes*, *S. typhimurium*, human immunodeficiency virus, influenza virus, polio virus, and reovirus all use M cells and the

receptors they express as ports of entry [133–135]. Moreover, the enterotoxin of *Clostridium perfringens* (CPE) binds to members of the Claudin tight-junction protein family, one of which, Claudin 4, is expressed in the cytoplasm of M cells, where it facilitates endocytosis [136]. In fact, peptides from the c-terminal domain of CPE binds to Claudin 4 can be used to target vaccine antigens to M cells [137]. Similarly, a peptide from the outer membrane protein H (OmpH β1α1) of *Y. enterocolitica* binds to C5aR on the luminal surface of M cells, and it can be used to enhance antigen uptake and mucosal vaccine responsiveness [138]. Thus the natural antigen-sampling activity of M cells can be exploited to enhance mucosal vaccination and tolerance.

Of course, one of the main functions of MALT in the gut, including ILFs, Peyer's patches, cecal patches, and colonic patches, is the production of IgA in response to antigens and commensal organisms in the gut lumen [74,95,104,139]. The process by which naïve B cells become activated and switch to IgA is very dependent on the structural architecture and cell types in GALT. In particular, activated B cells use the chemokine receptor CCR6 to migrate to the dome region of Peyer's patches, where they interact with DCs that prompt B cells to undergo isotype-switching to IgA [88]. Switching to IgA requires B cells to encounter active TGF-β, which is converted from the latent form by integrin αvβ8-expressing DCs in the dome epithelium [99]. Isotype-switching to IgA also requires ILC3 cells [56,80], which provide lymphotoxin-β receptor (LTβR)-dependent signals to DCs and stromal cells. A similar process likely occurs in ILFs, although much of the IgA production in ILFs occurs independently of T cells [89].

IV. DEVELOPMENT OF GUT-ASSOCIATED LYMPHOID TISSUE

The development of the GALT in many ways is similar to the development of conventional LNs. Both types of lymphoid organs develop according to a developmentally programmed series of cellular interactions that occurs independently of exogenous antigen or inflammation [1]. This process begins during fetal development and requires interactions between lymphoid tissue inducer (LTi) cells of hematopoietic origin [63] and lymphoid tissue organizer (LTo) cells of mesenchymal origin [140,141]. LTi cells are a subset of ILC3 cells that express the transcription factor RORγt [66] and produce cytokines such as TNF, LT-α, LT-β, IL-17, and IL-22 [59], all of which are involved in some aspect of lymphoid organ development or maturation. The development of lymphoid organs and GALT in particular has been previous reviewed [1,53], and we will summarize some of the most pertinent aspects here.

In Peyer's patches, the first step in development occurs around day 12.5 of embryogenesis and involves the activation of lymphoid tissue initiator (LTin) cells [142]. LTin cells are defined as $CD45^+CD11c^+c\text{-}kit^+$ cells that express the LTβR and the tyrosine kinase receptor, RET [140]. LTin cells are activated by the recognition of the RET ligands, leading to LTin accumulation around $VCAM^+$ LTo cells, which become activated and express CXCL13, thereby prompting the recruitment and clustering of CXCR5-expressing LTi cells [143]. The reciprocal interactions between LTαβ-expressing LTi cells and LTβR-expressing LTo cells reinforce the expression of CXCL13 and leads to the development of the Peyer's patch anlagen. Once the Peyer's patch anlagen is established, the developing stromal compartment recruits additional leukocytes via the induced expression of cytokines (IL-7, VEGF-C, and RANK ligand) [27], chemokines (CXCL12, CXCL13, CCL19, and CCL21), and adhesion molecules (VCAM-1 and ICAM-1) [144]. Taken together, these molecules help to recruit leukocytes and lymphocytes, maintain their survival, and promote their spatial positioning.

The organogenesis of Peyer's patches strictly requires the function of LTi cells [66]. As a result, mice lacking the transcription factors RORγt and Id2 lack LTi cells; consequently, they do not develop LNs or Peyer's patches [142]. Similarly, mice lacking either CXCL13 or its receptor, CXCR5, fail to develop LNs or Peyer's patches, owing to the inability of LTi cells to cluster in the lymphoid anlagen [23]. Moreover, mice lacking LT-α, LT-β, or LTβR also fail to form LNs or Peyer's patches, owing to an inability of LTi cells to activate LTo cells and promote their eventual differentiation into mature lymphoid stroma [145,146]. Importantly, TNFR1 and LTβR are coupled to both the canonical and noncanonical NFκB signaling pathways [147], both of which are important in Peyer's patch development [148,149].

The final steps of Peyer's patch development occur after birth when T and B cells begin to populate their corresponding niches. At this time, LTi cells downregulate the expression of CXCR5 and upregulate the expression of CXCR4 [116], thereby changing their migration away from the B cell follicle and toward the interfollicular region. This process is coordinated by TGF-β and is necessary for the differentiation of FRCs [150]. Once Peyer's patches are fully developed and populated, leukocytes help to maintain stromal cell differentiation; B cells are particularly important for the maintenance of FDCs [151], whereas DCs are important for the maintenance of FRCs [152]. Finally, the interactions between the microbiota and epithelial cells are arguably as important as the interactions between leukocytes and stromal cells for the maintenance of Peyer's patches [153], particularly for the differentiation of M cells [154], the expression of epithelial chemokines, and the organization of the dome region [129].

Although the mechanisms controlling the development of Peyer's patches are well defined, those controlling the development of colonic patches and cecal patches have not been studied in depth; the assumption is that the development of these organs will be similar to that of Peyer's patches. However, the muscularis layer is different in the small intestine and colon, and the density of commensal organisms in the colon greatly exceeds the density of those in the small intestine [53]. Thus the mechanisms that control the development, maturation, or function of these organs may be different as well.

In this regard, there are notable differences in the organogenesis and regulation of ILFs in the small intestine and colon [94]. For example, although the development and maturation of ILFs in the small intestine require microbial colonization, the ILFs in the colon develop independently of microbial colonization and signaling through CCR6, RANK, and CXCL13 [94]. In contrast, colonic ILFs have specific requirements that the small intestine ILFs do not. For example, colonic ILFs require MyD88-dependent signals for maturation [94], whereas small intestine ILFs do not. Similarly, colonic ILFs require IL-23 signaling [155] but are suppressed by IL-25 signaling. These observations suggest that the molecular mediators of ILF maturation differ between the small and large intestines, with colonic ILFs more dependent on microbial detection and small intestine ILFs more dependent on danger signals.

The mechanisms controlling the development of cryptopatches are different from those controlling the development of Peyer's patches or LNs [53], in part because cryptopatches lack B and T cell domains. In fact, the cryptopatches in adults are in some ways analogous to the Peyer's patch anlagen in developing embryos. However, instead of primary interactions between CXCR5-expressing LTi cells and CXCL13-expressing LTo cells, the formation of cryptopatches is dependent on interactions between CCR6-expressing ILC3 cells and CCL20-expressing epithelial cells [83], which also produce IL-7 and thereby help to maintain ILC3 cells [54]. Moreover, like the development

of most lymphoid tissues, the formation of cryptopatches is entirely dependent on RORγt [87], which is essential for ILC3 differentiation. Interestingly, although the development of cryptopatches requires lymphotoxin signaling, it uses only the canonical pathway and does not require RelB [156].

The maturation of cryptopatches into ILFs often begins with microbial signals to the epithelium, which increases expression of CCL20 and IL-7 [86], leading to the activation of ILC3 cells [83]. Alternatively, ILC3 cells can be activated directly by metabolic products that engage the AHR [57] or by IL-23 generated from activated myeloid cells [155]. Once ILC3 cells are activated, they express LTαβ and trigger the differentiation of LTβR-expressing stromal cell precursors, which upregulate CXCL13, CCL19, and CCL21 as well as VCAM-1 and ICAM-1 for the recruitment and retention of lymphocytes [157]. Further accumulation of B cells, recruitment of T cells, and differentiation of follicle-associated epithelium (FAE) mark the maturation of an ILF. The cytokine RANKL (receptor activator of NF-KB ligand) and its receptor (RANK) are crucial for the maturation of ILFs [158] but not for the formation of CPs, primarily because of the role of RANK signaling in the differentiation of M cells, which will be discussed in the next section.

V. M CELL DIFFERENTIATION IN GUT-ASSOCIATED LYMPHOID TISSUE

The development of M cells in the dome epithelium is a specific feature of mucosal lymphoid tissues and, not surprisingly, involves reciprocal interactions between various cell types and signaling through receptors in the TNFR family. For example, M cell differentiation requires signaling through the TNFR family member RANK, which is expressed on epithelial stem cells [159]. RANK signaling activates TRAF6 and NF-KB in epithelial cells [160] and triggers the expression of the transcription factor Spi-B, which is required for the final maturation and accumulation of M cells in the dome epithelium [160]. Interestingly, the systemic administration of RANKL induces the ectopic differentiation of enterocytes into M cells throughout the villous epithelium [161], suggesting that any enterocyte is capable of differentiating into M cells but that the normal availability of RANK ligand is spatially restricted to the epithelium overlaying lymphoid clusters or FAE. In fact, RANK ligand is primarily expressed by subepithelial stromal cells in areas of lymphoid tissue (e.g., Peyer's patches) [162].

Interestingly, stromal cells in developing LNs express RANK ligand following interactions with lymphotoxin-expressing LTi cells [27]. In turn, RANK ligand maintains the survival of LTi cells and increases their expression of lymphotoxin, generating a positive feedback loop. Given that lymphotoxin is important for the differentiation of the M cell-containing FAE [49] and that RORγt-expressing ILC3 cells are necessary for the differentiation of M cell-containing ILFs [89], it makes sense that the ILC3 cells in GALTs participate in M cell differentiation.

The expression of CCL20 in the intestinal epithelium is rapidly induced by RANK ligand stimulation and enhanced by TNFR and LTβR stimulation [163]. Other signals triggered by enteroinvasive bacteria, including *S. typhimurium*, *Salmonella enteritidis*, and *L. monocytogenes*, also induce CCL20 expression by intestinal epithelial cells [164]. The pathogen-mediated expression of CCL20 and β-defensin-2 by epithelial cells may defend against bacterial infections by attracting CCR6-expressing Th17 cells [165,166]. However, pathogens may also exploit this mechanism by stimulating the differentiation of enterocytes into M cells and thereby facilitating their translocation from the gut lumen.

B cells and DCs are also involved in M cell maturation; however, neither of these cell types expresses RANK ligand. Moreover, B-cell-derived TNF and lymphotoxin are each dispensable for the development of the FAE and M cells [163]. Nevertheless, the expression of CCR6 on B cells and DCs is required for their attraction to the dome epithelium [99] and for the final maturation of GP2-expressing M cells [159]. In fact, the basolateral pocket of M cells in Peyer's patches usually contains at least one B lymphocyte [167]. Moreover, adding B cells to cultures of Caco-2 intestinal epithelial cells promotes their differentiation into M cells [168,169], and mice that lack B lymphocytes appear to lack M cell transcytosis function [170]. The factors provided by B cells (or other hematopoietic cells) that stimulate M cell differentiation include signaling between B cells and M cells via CD137L/CD137 [50] or macrophage migration inhibitory factor [171].

VI. LYMPHOID TISSUES OF THE RESPIRATORY TRACT

The mucosal lining the upper and lower respiratory tracts is constantly exposed to inhaled antigens and has endogenous microbial communities [172], although the density of the respiratory microbiota is dramatically less than that in the gut, and the species that compose these communities are also different [173]. As a result, MALTs that trigger local immune responses are present in the upper respiratory tract and nasal passages as well as in the lower respiratory tract and lung. Like the MALT of the gut (or GALTs), the mucosal tissues of the respiratory tract can generate local immune responses to pathogens and inflammatory antigens and help to maintain homeostatic symbiosis with the local microbial communities.

A. Nasopharyngeal-Associated Lymphoid Tissue

The upper respiratory tract has a variety of mucosal lymphoid organs whose location and structure differ greatly among the various mammalian species. For example, humans lack constitutively organized lymphoid tissues in the nasal passages but often have diffuse lymphoid tissues (likely ectopic lymphoid aggregates) [174]. Instead, humans have well-developed mucosal lymphoid tissues in the nasopharynx (adenoids) and the oropharynx (tonsils), which together are termed Waldeyer's ring [175]. Although sheep, goats, pigs, dogs, and cats lack detectable lymphoid tissues in the nose [175], horses have nasal structures similar to ILFs [175,176], and rabbits have nasal-associated mucosal tissues as well as a variety of ILF-like structures in the nasal passages [177]. By contrast, rodents develop constitutively organized lymphoid tissues in the nasal cavity that are termed nasal-associated lymphoid tissue (NALT) [178].

In mice, NALT is found on the dorsal side of the soft palate at the bottom of the nasal passages [179] (Fig. 2.5). In cross section, NALT appears as two bell-shaped structures located on the ventral region of the nasal passages above the hard palate [175,179]. NALT extends along the length of the nasal passages and consists of multiple B cell follicles arranged like a string of beads separated by interfollicular areas [175,179]. Like Peyer's patches, the interfollicular areas contain numerous T cells and DCs that are arranged on a stromal network of CCL19-expressing FRCs, whereas the B cell follicles and germinal centers are organized around stromal networks of CXCL13-expressing FDCs [175,180].

Similar to MALT in the gut, NALT has a dome epithelium that expresses the chemokines CCL20 and CCL9 [181,182]. However, unlike Peyer's patches, it does not express CXCL16 [123]. M cells with short microvilli and

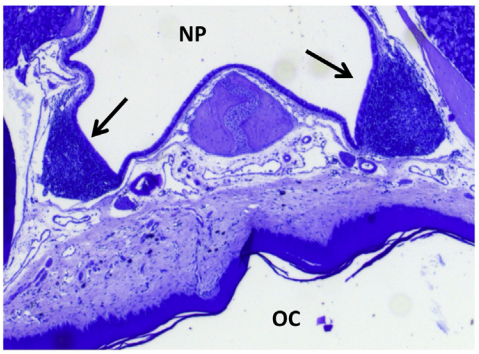

FIGURE 2.5 **Nasopharyngeal-associated lymphoid tissue (NALT).** A cross section of a decalcified mouse nose shows NALT areas (*arrows*) on either side of the nasal passage (NP), above the oral cavity (OC).

microfold morphology are easily identified in the dome epithelium of NALT as well as turbinate epithelium of nasal cavity [96,183] and efficiently acquire luminal antigens and particulates [184], which they transcytose across the epithelium. Not surprisingly, NALT M cells express markers, including GP2, Tnfaip2, and Spi-B [184], which are also expressed in M cells of the intestinal tract. CCR6-expressing DCs and B cells fill the dome region under the epithelium and are closely associated with M cells [185]. As in Peyer's patches, interactions between CCR6-expressing leukocytes and M cells are important for the maintenance of NALT structure and function [182].

The HEVs of NALT are found in the interfollicular regions or at the T cell–B cell boundary [186]. Interestingly, NALT HEVs express peripheral node addressin (PNAd) [179,187] alone or mostly associated with mucosal addressin cell adhesion molecule (MAdCAM) [179], a difference that seems to be dependent on the molecular details of lymphotoxin signaling [188]. Hence MAdCAM expression on the HEVs is expressed only in conjunction with PNAd [179]. HEVs in NALT also express the chemokine CCL21 [181], which facilitates lymphocyte trafficking from the blood.

Like other mucosal lymphoid organs, NALT lacks afferent lymphatic vessels. However, efferent lymphatic vessels are found at the outside edge of the interfollicular T cell areas of NALT, where they collect effector cells that were primed and expanded in NALT [180]. Interestingly, the lymphatic vasculature of NALT connects with the lymphatic vasculature draining the ocular region that contains another mucosal lymphoid tissue, the lacrimal-duct-associated lymphoid tissue (LDALT) [180,189]. Thus ocular and nasal lymphatics seem to converge on their way to downstream cervical LNs.

The ontogeny of NALT is unique among mucosal lymphoid organs, perhaps because of its position in the nasal passages. Although the development of NALT resembles that of ILFs in the sense that it occurs after birth in response to antigenic or microbial encounter [190], there are important differences in terms of the cell types and molecular pathways required for its development. For example, LTi cells seem dispensable for NALT formation [191] as well as for LDALT formation [189], since mice that are deficient in the transcription factor RORγt (and therefore deficient in ILC3 cells, including LTi cells [191,192]) still have apparently normal NALT structures. Similarly, mice lacking IL-7 (which fail to support LTi cells as well as most lymphocytes) still have rudimentary NALT structures. In contrast, mice lacking the

transcription factor Id2 (which lack all ILC cells [142]) completely fail to develop NALT [191]. Thus it seems that an ILC population other than ILC3 cells or LTi cells is required for NALT development. In this regard, ILC2 cells are more prominently represented in the respiratory tract than are ILC3 cells [1,193].

The requirement of lymphotoxin signaling is also different in NALT than it is in most lymphoid tissues. For example, unlike the development of Peyer's patches and essentially all other lymphoid organs, the development of NALT still occurs in the absence of LT-α [192,194,195]. Although NALT remains in LT-α-deficient mice, its structure is abnormal, with poorly defined B and T cell domains, a lack of FDCs, and reduced expression of chemokines (CXCL13, CCL19, CCL21, and CCL20) [194], which normally maintain the lymphoid architecture of NALT. This result is likely due to the fact that LTi cells are not involved in NALT development and that LT-α is the primary mediator of LTi cell activity in lymphoid organ development.

NALT development also occurs normally mice lacking CXCL13 [181], which is required for the development of most lymphoid organs because LTi cells use CXCR5 to find and interact with LTo cells. Again, however, the organization of NALT is impaired in the absence of CXCL13 or CXCR5, as FDCs fail to develop and stromal cells are unable to organize a B cell follicle [192,194]. However, increasing antigenic encounter leads to the further organization of NALT, even in the absence of CXCR5 or LT-α [181,194,195], suggesting that the structural maturation of NALT can use alternative pathways, such as CD40-dependent signaling [194], which may substitute for LT-α signaling by engaging the noncanonical NFκB pathway [196].

The differentiation of M cells and the formation of a dome region are also important for the proper structure and function of NALT. As in Peyer's patches, the differentiation of NALT M cells requires interactions between RANK, expressed on epithelial cells, and its ligand, expressed by stromal cells in the subepithelial dome region [162,197]. As in the intestine, the administration of RANK ligand to the nasal passages activates RelB and Spi-B in epithelial cell progenitors [184], ultimately leading to the differentiation of additional $GP2^{+}Tnfaip2^{+}$ epithelial M cells in the nasal passages [127,160,184,197]. Interestingly, unlike the development of M cells in the intestine, which are derived from proliferating precursors in the crypts, the M cells in NALT appear to differentiate in the absence of proliferation [184].

Interactions with lymphocytes are also an important part of M cell maturation in NALT, as the morphology of M cells and the basolateral pocket are lost when lymphocytes are depleted [198]. The interactions between leukocytes and M cells utilizes CCR6 [182,185], as the number of M cells is reduced in the NALT of CCR6-deficient mice. Moreover, the antigen-transporting ability of the M cells remaining in CCR6-deficient mice is also impaired [182], leading to poor IgA responses to nasally administered antigens. Although interactions with B cells are not required for the initial development of M cells, B cells trigger the TNFR family member CD137 (commonly known as 4-1BB) on M cells by its ligand on B cells [50], which maintains M cell morphology, promotes the formation of the basolateral pocket, and facilitates their ability to transport antigens and particulates [50].

The antigen-transporting activity of M cells in NALT also allows pathogen entry. For example, pathogens such as mouse mammary tumor virus (MMTV) [199], group A streptococcus [200,201], and *Burkholderia pseudomallei* [202] all target NALT as a portal of entry. Similarly, molecules expressed on the surface of NALT M cells can be targeted to enhance the uptake and response to vaccine antigens. For example, the Claudin-4-targeting peptide of CPE promotes the uptake of nanoparticles through NALT M

cells and enhances local IgA responses [137]. Moreover, M-cell-binding lectins, including *Griffonia simplicifolia* I isolectin B4 (GSI-B4) [203], *Dolichos biflorus* agglutinin (DBA) [203], and *Ulex europaeus* agglutinin (UEA) [203], and M-cell-binding proteins such as reovirus protein σ1 [204] promote antigen uptake by M cells in NALT and, when used in combination with adjuvants such as cholera toxin [205] or heat-labile toxin IIa-B5 [206], elicit potent IgA responses in the nasal passages.

As a mucosal lymphoid organ, it might seem that NALT would preferentially generate IgA responses following vaccination. However, mucosal lymphoid tissues in the respiratory and digestive tracts are not functionally identical [207]. However, immunization or infection of the nasal passages typically elicits as much IgG as IgA [208]. These responses are clearly biased by the type of adjuvant involved, as immunization with cholera toxin typically leads to IgA responses [205,209], whereas protein-containing microparticles primarily elicit IgG responses [210]. Infection with common respiratory viruses, such as influenza [211] and reovirus [212], elicits both IgG- and IgA-secreting cells in NALT. Nevertheless, B cells responding to influenza more often switch to IgA in NALT than in mediastinal LNs, lung, or spleen [213]. Intriguingly, IgA memory B cells generated in NALT accumulate more somatic mutations than do IgG memory cells [214], suggesting a selective entry into the memory compartment, perhaps by interactions with the CCR6-expressing DCs in the subepithelial dome region (Chapter 7: Induction and Regulation of Mucosal Memory B Cell Responses).

B. Bronchus-Associated Lymphoid Tissue

The lower respiratory tract includes the trachea, bronchi, bronchioles, and alveoli, which are continuously exposed to inhaled antigens and pathogens, including common respiratory viruses such as influenza virus, respiratory syncytial virus, and rhinovirus. Therefore the lower respiratory tract has to be protected by robust immunological mechanisms. However, the lung is also the primary site of gas exchange, which is most efficient when the distance between the respiratory epithelium of the alveoli and the vascular endothelium of the capillaries is minimized. As a result, the delicate structures of the lower lung are susceptible to damage by inflammatory responses, implying that immune responses in the lung must be appropriately tempered to provide protection against pathogens without causing undue inflammation and damage.

Given the exposure of the lung to inhaled antigens and pathogens, one might expect that the lower respiratory tract would be filled with mucosal lymphoid tissues along the bronchi and bronchioles. In fact, some species have mucosal lymphoid structures, termed BALT, along the respiratory tract [215–217]. However, the lungs of healthy humans have relatively few BALT-like areas [218,219], and the lungs of mice from clean animal facilities completely lack such structures [220]. Nevertheless, humans with chronic lung diseases such as chronic obstructive pulmonary disease [221–223], hypersensitivity pneumonitis [224], and even pulmonary complications of rheumatoid arthritis [225–227] have many well-developed BALT-like areas that seem to react with local antigens. Moreover, mice also develop BALT-like structures in their lungs following pulmonary infection or inflammation [228] (Fig. 2.6). These findings suggest that the BALT-like areas in humans and mice are not true SLOs that develop during embryogenesis but instead are ectopic or tertiary lymphoid tissues that develop in response to local inflammation [228,229]. Interestingly, some mammals, such as pigs and goats, develop BALT during gestation [215,230–233], apparently independent of exposure to antigen or microbes, suggesting that in these species, BALT is a true SLO. Therefore to distinguish between BALT formed according to a developmental program

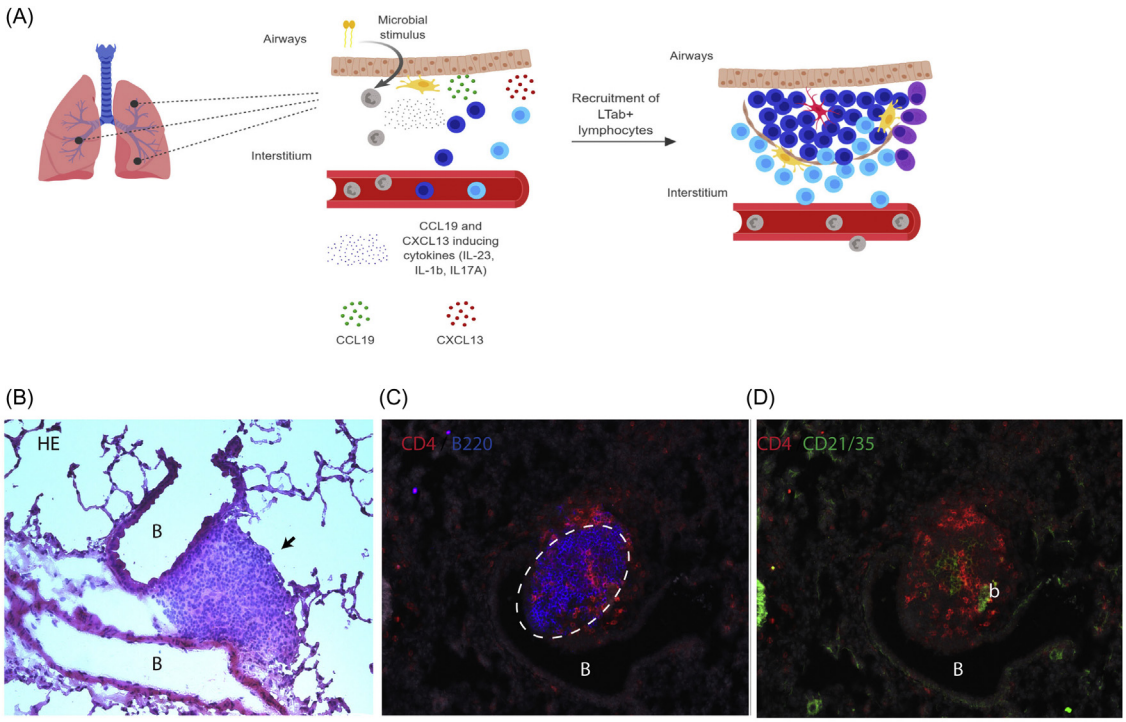

FIGURE 2.6 **Inducible bronchus-associated lymphoid tissue (iBALT).** (A) The formation of iBALT requires pulmonary inflammation and the recruitment and activation of neutrophils, macrophages, and dendritic cells. Inflammatory cytokines such as IL-17A, IL-1α, and IL-23 promote the production of CCL19 and CXCL13 by fibroblastic cells, which recruit $CCR7^+$ T cells and $CXCR5^+$ B cells. Activated lymphocytes provide LTαβ, which promotes the differentiation of fibroblast and epithelial cells into lymphocyte-organizing stromal cells. The structure of iBALT resembles that of other lymphoid organs in which segregated B cell and T cell areas are located near epithelia and are supported by specialized stromal populations. (B) H&E staining of lung sections showing iBALT. (C) Immunofluorescence staining of $B220^+$ B cells (*blue*) and $CD4^+$ T cells (*red*). The follicle is highlighted (*dashed line*). (D) $CD4^+$ T cells (*red*) and $CD21/35^+$ FDC network (*green*). *B*, Bronchi; *b*, blood vessel.

and the lung-associated lymphoid tissues that are inducible and are formed after inflammation, we coined the term "inducible BALT" (iBALT) [225,234,235].

As the name suggests, iBALT forms along the bronchi and bronchioles in the lung, often at branch points of the bronchial tree and usually next to or even surrounding a pulmonary artery [228,234,236–238]. In fact, the structure of iBALT often fills the perivascular space and, depending on how the lung is sectioned, may appear to be exclusively perivascular [237,239]. BALT-like lymphoid aggregates in the lung have a spectrum of phenotypes, beginning with a small, loosely organized cluster of B cells that lacks FDCs and ending with a dense lymphoid accumulation that includes multiple germinal-center-containing B cell follicles arranged on networks of CD21-expressing FDCs and separated by interfollicular T cell areas with networks of podoplanin-expressing FRCs [229,240,241]. In some cases, iBALT areas can be found with extensive FDC networks and germinal-center-containing B cell follicles but no discernible T cell areas [229,241]. In other cases, however, iBALT areas can be observed

with dense clusters of T cells and podoplanin-expressing FRCs but no B cell follicles [229,241]. These different structures are undoubtedly distinct at a functional level; however, the underlying reasons why different types of structures are formed remain unclear.

Although BALT areas in some species seem to have a specialized dome epithelium with M cells [96,242], the iBALT observed in mice and humans typically lacks this feature [1], consistent with the idea that iBALT is an ectopic lymphoid tissue rather than a truly MALT of the airway. However, the absence of M cells in the iBALT of mice and humans raises the question of how these tissues acquire antigen. In this regard, it is clear that areas of iBALT have associated lymphatic vessels around the B cell follicle at the edge of the T cell area [243,244]. In fact, the inflammatory processes that trigger iBALT formation also trigger the growth of new lymphatic vessels [245]. It seems likely that some of these vessels are efferent lymphatic vessels that carry effector cells away from iBALT and toward the downstream draining mediastinal LN. However, some of these vessels may also be afferent lymphatics that carry antigen and DCs from distal portions of the lung into iBALT. In fact, nasally administered DCs rapidly migrate into the T cell areas of iBALT, most probably via afferent lymphatic vessels [43,246,247].

Well-developed areas of BALT also have HEVs in the T cell area next to the T cell−B cell boundary [248,249]. Consistent with the idea that iBALT is an ectopic rather than a classical MALT, the HEVs in iBALT express CCL21 and PNAd but not MAdCAM-1 [248,249], suggesting that $\alpha4\beta7$-expressing mucosal-homing lymphocytes are not preferentially recruited to iBALT. Together with the lack of a dome epithelium, these observations reinforce the conclusion that iBALT is an ectopic rather than a mucosal lymphoid organ.

The lymphoid chemokines CXCL13, CCL19, and CCL21 are expressed by fibroblastic stromal cells in iBALT and are important for its structure and function. For example, although iBALT develops in CXCL13-deficient mice and even has separated B and T cell areas [241], it lacks FDCs and fails to form true B cell follicles or germinal centers. In contrast, the iBALT that forms in plt/plt mice (lacking both CCL19 and CCL21 [240,250]), has B cell follicles with FDCs but fails to develop T cell areas and cannot form germinal centers [250], probably owing to the failure of Tfh differentiation. Mice lacking all three chemokines fail to form any iBALT at all [250]. Unlike Peyer's patches and ILFs, CCR6 is not important for the formation or function of iBALT [1], consistent with the lack of an M cell-containing dome epithelium (or FAE) in this tissue.

Numerous inflammatory or infectious stimuli can trigger the formation of iBALT via a variety of pathways [228]. However, many of the core mechanisms involved in SLO development are also involved in the formation of iBALT. In general, iBALT is formed most easily during the neonatal period in both mice and humans [219,240], perhaps owing to increased frequencies of ILCs or decreased frequencies of Tregs [240,251,252]. In fact, CCR7-deficient mice spontaneously develop iBALT, owing to the inability of Tregs to suppress pulmonary inflammation [251,253,254]. Conversely, the reconstitution of CCR7-deficient mice with WT Tregs prevents the formation of iBALT [251]. These observations are likely pertinent for the development of iBALT and other ectopic lymphoid tissues in autoimmune patients, who often have defects in Treg function.

Although MALT in the intestine (or GALT), including cryptopatches, ILFs, Peyer's patches, cecal patches, and colonic patches, requires ILC3 cells, these cells are not required for iBALT development, as iBALT forms easily in mice lacking the transcription factor RORγt [240]. This observation implies that other cell types such as ILC2 cells, which are common in neonatal lungs [255], may play important roles

in iBALT formation. Despite the nonessential role of ILC3 cells, both IL-17 and IL-22 are important for the formation of iBALT in response to the inert inflammatory molecules, LPS [240]. These cytokines are provided by γδ T cells and follicular-homing Th17 cells during iBALT development [240]. The role of these cytokines is somewhat surprising, given that iBALT forms independently of RORγt, which is normally required for the differentiation of ILC3 cells, γδ T cells, and Th17 cells. Thus it seems that there are redundant mechanisms for iBALT formation when IL-17 is limiting.

The formation of iBALT in response to IL-17 involves neutrophil accumulation via the expression of the inflammatory chemokines, CXCL9, CXCL10, and CXCL11 [252,256]. Neutrophils and other granulocytes cause damage, in part because of the release of proteases [257–259], which trigger the expression of homeostatic and inflammatory chemokines and lead to iBALT formation [259]. Similarly, IL-17 directly promotes the expression of the homeostatic chemokines CXCL13 and CCL19 [240,260,261], which are important for the recruitment of B and T cells and for the formation of iBALT. Although IL-17 promotes the expression of these chemokines under inflammatory conditions, lymphotoxin signaling maintains the expression of these chemokines under homeostatic conditions [240,241,261]. Thus once iBALT is formed, it no longer requires IL-17 but instead requires lymphotoxin-expressing lymphocytes and DCs to maintain stromal architecture, chemokine expression, and HEV differentiation.

Importantly, IL-17 is required for iBALT formation only under some conditions, most notably bacterial infections or exposure to bacterial products [228]. However, under other conditions, such as intranasal infection with modified vaccinia virus Ankara (MVA), iBALT forms independently of IL-17 [241]. As might be expected, iBALT formation in response to bacterial infection is dependent on TLR signaling through MyD88 and Trif [241], whereas iBALT formation in response to viral infection is independent of these pathways. Nevertheless, all of the inflammatory pathways eventually converge on the differentiation and maintenance of stromal cells via lymphotoxin and the expression of homeostatic chemokines.

Other types of inflammatory stimuli trigger iBALT formation via other mechanisms. For example, pulmonary exposure to alum or silica particles trigger dying macrophages to release IL-1α, which acts as an alarmin that promotes the recruitment of eosinophils, macrophages, neutrophils, DCs, and T cells to the lungs and ultimately leads to iBALT formation [262]. In another example, pulmonary infection with *Pneumocystis murina* elicits the expression of IL-25 and IL-17, which indirectly trigger the expression of CXCL13 by lung fibroblasts via IL-13 and IL-6 [261]. Interestingly, the IL-25 and IL-17 receptor complexes share a common subunit, the IL-17Rα [263,264].

Once past the inflammatory stage, the mechanisms that maintain iBALT parallel those of most lymphoid tissues. For example, the loss of DCs leads to the shrinkage of iBALT areas [247,265], perhaps because DCs are required for the maintenance of HEVs [266–268] and, without them, lymphocytes fail to be recruited and the structures fall apart. Fibroblastic stromal cells and lymphatic endothelial cells also contribute to the maintenance of iBALT, in part by expression of homeostatic chemokines that maintain the B and T cell areas [43] but also by expression of cytokines such as IL-7 and BAFF [120] that promote the survival of naïve and memory T and B cells. Many of these homeostatic mechanisms require lymphotoxin or TNF signaling between leukocytes and stromal cells [269], similar to what occurs in other lymphoid organs.

The areas of iBALT clearly function like other lymphoid organs and can independently support primary and secondary B and T cell responses to pulmonary antigens [1,2,270,271]. This functional capacity is apparent in mice

lacking conventional LNs and spleen [2,234]. However, the initial activation of T cells can also be observed in iBALT following the intranasal administration of antigen-loaded DCs [247]. Moreover, the formation of germinal centers in iBALT is a clear sign that antigen-specific B cells are being primed locally [265]. Furthermore, antigen-specific plasmablasts accumulate at the border between the B cell follicle and the T cell area [265], suggesting that they were generated locally. Interestingly, immune responses triggered in BALT do not seem to bias the antibody class switch toward a particular isotype, such as IgA [265]. This observation might be explained by the lack of a dome epithelium (or FAE) and specialized DCs that facilitate the activation of TGF-β and promote IgA switching. Again, this observation is consistent with the idea that iBALT is an ectopic lymphoid tissue rather than a MALT.

Even though iBALT is not a constitutive SLO in the lung, its presence has a strong impact on the success of pulmonary immune responses. In the context of pulmonary infection, the presence of iBALT is uniformly beneficial and usually leads to faster clearance of the pathogen with less pulmonary and systemic pathology [228]. For example, immune responses against pulmonary infection with influenza virus, SARS corona virus, *Coxiella burnetii*, *Francisella tularensis*, and *Mycobacterium tuberculosis* are all more effective when iBALT is present in the lung [235,265,272–274]. In part, this success can be attributed to an accelerated B cell and antibody response [234,273,275]. Given the location of antigen-specific germinal center B cells and plasmablasts, this faster B cell response may be simply due to proximity to antigen. Alternatively, the local differentiation of T cells may be biased toward Tfh cells rather than effector cells. Given the unique functional attributes of iBALT, it may be desirable to develop vaccines that transiently induce iBALT for the initiation of antigen-specific humoral and/or cellular immunity against respiratory pathogens.

In contrast to its beneficial role in responding to pulmonary infection, the presence of iBALT in inflammatory lung diseases may be detrimental to the host, because enhanced local immune responses to autoantigens or environmental antigens would likely exacerbate inflammation and tissue damage. For example, areas of iBALT are found in patients with hypersensitivity pneumonitis [224], rheumatic lung disease [225,227], chronic obstructive pulmonary disease [223,276,277], and even idiopathic pulmonary fibrosis [254,278]. These diseases are associated with chronic inflammation and continual or repeated exposure to antigens. Moreover antigen-specific B and T cells are found in the iBALT areas [235], suggesting that they are contributing to disease pathogenesis. Despite the link between chronic lung disease and the formation of iBALT, it is not clear whether iBALT is exacerbating disease or is actually attenuating inflammation by sequestering antigens and effector cells.

Finally, despite the fact that iBALT is an ectopic lymphoid tissue, it is clearly a critical component of the pulmonary immune system and has the ability to fine-tune local immune responses and either ameliorate or exacerbate pulmonary pathology. Given that iBALT is formed in response to environmental exposures, particularly during early life, it seems likely that the type and frequency of these exposures may set the tone of pulmonary immunity for many years and perhaps the life span of the individual [218,279]. Therefore it is essential that we understand the mechanisms controlling the development and function of iBALT in order to harness its infection-preventing properties during vaccination and to restrain its pathological activities in inflammatory diseases.

VII. CONCLUDING REMARKS

In summary, the mucosal lymphoid tissues of the intestinal and respiratory tracts share

many developmental, architectural, and functional features that allow them to sample mucosal antigens and generate appropriate immune responses. However, the mechanistic details that regulate how each of these tissues functions are often slightly different, in part owing to the location in which they reside, the number and type of microbial communities to which they are exposed, and the cell types involved in their function. As a result, vaccines that target particular mucosal sites or immune-modulating therapies that are directed toward particular organs must account for these differences in order to be effective.

References

[1] Randall TD, Mebius RE. The development and function of mucosal lymphoid tissues: a balancing act with micro-organisms. Mucosal Immunol 2014;7:455–66.

[2] Carragher DM, Rangel-Moreno J, Randall TD. Ectopic lymphoid tissues and local immunity. Semin Immunol 2008;20:26–42.

[3] Kasahara M, Sutoh Y. Two forms of adaptive immunity in vertebrates: similarities and differences. Adv Immunol 2014;122:59–90.

[4] Danilova N. The evolution of adaptive immunity. Adv Exp Med Biol 2012;738:218–35.

[5] Das S, Li J, Hirano M, Sutoh Y, Herrin BR, Cooper MD. Evolution of two prototypic T cell lineages. Cell Immunol 2015;296:87–94.

[6] Alder MN, Herrin BR, Sadlonova A, Stockard CR, Grizzle WE, Gartland LA, et al. Antibody responses of variable lymphocyte receptors in the lamprey. Nat Immunol 2008;9:319–27.

[7] Kishishita N, Nagawa F. Evolution of adaptive immunity: implications of a third lymphocyte lineage in lampreys. Bioessays 2014;36:244–50.

[8] Matsunaga T, Rahman A. In search of the origin of the thymus: the thymus and GALT may be evolutionarily related. Scand J Immunol 2001;53:1–6.

[9] Shields JW. The functional evolution of GALT: a review. Lymphology 2000;33:47–57.

[10] Finke D, Meier D. Molecular networks orchestrating GALT development. Curr Top Microbiol Immunol 2006;308:19–57.

[11] Pospisil R, Mage RG. Rabbit appendix: a site of development and selection of the B cell repertoire. Curr Top Microbiol Immunol 1998;229:59–70.

[12] Vajdy M, Sethupathi P, Knight KL. Dependence of antibody somatic diversification on gut-associated lymphoid tissue in rabbits. J Immunol 1998;160:2725–9.

[13] Boehm T, Hess I, Swann JB. Evolution of lymphoid tissues. Trends Immunol 2012;33:315–21.

[14] Neely HR, Flajnik MF. Emergence and evolution of secondary lymphoid organs. Annu Rev Cell Dev Biol 2016;32:693–711.

[15] Salinas I. The mucosal immune system of teleost fish. Biology (Basel) 2015;4:525–39.

[16] Tacchi L, Larragoite ET, Munoz P, Amemiya CT, Salinas I. African lungfish reveal the evolutionary origins of organized mucosal lymphoid tissue in vertebrates. Curr Biol 2015;25:2417–24.

[17] Colombo BM, Scalvenzi T, Benlamara S, Pollet N. Microbiota and mucosal immunity in amphibians. Front Immunol 2015;6:111.

[18] Link A, Vogt TK, Favre S, Britschgi MR, Acha-Orbea H, Hinz B, et al. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. Nat Immunol 2007;8:1255–65.

[19] Comerford I, Harata-Lee Y, Bunting MD, Gregor C, Kara EE, McColl SR. A myriad of functions and complex regulation of the CCR7/CCL19/CCL21 chemokine axis in the adaptive immune system. Cytokine Growth Factor Rev 2013;24:269–83.

[20] Li L, Choi YS. Follicular dendritic cell-signaling molecules required for proliferation and differentiation of GC-B cells. Semin Immunol 2002;14:259–66.

[21] Rodda LB, Bannard O, Ludewig B, Nagasawa T, Cyster JG. Phenotypic and morphological properties of germinal center dark zone Cxcl12-expressing reticular cells. J Immunol 2015;195:4781–91.

[22] Aguzzi A, Kranich J, Krautler NJ. Follicular dendritic cells: origin, phenotype, and function in health and disease. Trends Immunol 2014;35:105–13.

[23] Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature 2000;406:309–14.

[24] Mebius RE, Kraal G. Structure and function of the spleen. Nat Rev Immunol 2005;5:606–16.

[25] Yasuda M, Taura Y, Yokomizo Y, Ekino S. A comparative study of germinal center: fowls and mammals. Comp Immunol Microbiol Infect Dis 1998;21:179–89.

[26] Ruddle NH. Lymphoid neo-organogenesis: lymphotoxin's role in inflammation and development. Immunol Res 1999;19:119–25.

[27] Vondenhoff MF, Greuter M, Goverse G, Elewaut D, Dewint P, Ware CF, et al. LTbetaR signaling induces cytokine expression and up-regulates lymphangiogenic factors in lymph node anlagen. J Immunol 2009;182:5439–45.

[28] Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol 2006;7:344–53.
[29] Kaiser P, Poh TY, Rothwell L, Avery S, Balu S, Pathania US, et al. A genomic analysis of chicken cytokines and chemokines. J Interferon Cytokine Res 2005;25:467–84.
[30] Magor KE, Miranzo Navarro D, Barber MR, Petkau K, Fleming-Canepa X, Blyth GA, et al. Defense genes missing from the flight division. Dev Comp Immunol 2013;41:377–88.
[31] Dalloul RA, Long JA, Zimin AV, Aslam L, Beal K, Blomberg Le A, et al. Multi-platform next-generation sequencing of the domestic turkey (*Meleagris gallopavo*): genome assembly and analysis. PLoS Biol 2010;8. Available from: http://dx.doi.org/10.1371/journal.pbio.1000475.
[32] Bienenstock J, Befus D. Gut- and bronchus-associated lymphoid tissue. Am J Anat 1984;170:437–45.
[33] Olah I, Nagy N, Magyar A, Palya V. Esophageal tonsil: a novel gut-associated lymphoid organ. Poult Sci 2003;82:767–70.
[34] Casteleyn C, Doom M, Lambrechts E, Van den Broeck W, Simoens P, Cornillie P. Locations of gut-associated lymphoid tissue in the 3-month-old chicken: a review. Avian Pathol 2010;39:143–50.
[35] Gomez Del Moral M, Fonfria J, Varas A, Jimenez E, Moreno J, Zapata AG. Appearance and development of lymphoid cells in the chicken (*Gallus gallus*) caecal tonsil. Anat Rec 1998;250:182–9.
[36] Jarjour M, Jorquera A, Mondor I, Wienert S, Narang P, Coles MC, et al. Fate mapping reveals origin and dynamics of lymph node follicular dendritic cells. J Exp Med 2014;211:1109–22.
[37] Bajenoff M, Germain RN. B-cell follicle development remodels the conduit system and allows soluble antigen delivery to follicular dendritic cells. Blood 2009;114:4989–97.
[38] Lu TT, Browning JL. Role of the lymphotoxin/LIGHT system in the development and maintenance of reticular networks and vasculature in lymphoid tissues. Front Immunol 2014;5:47.
[39] Zhao L, Chen J, Liu L, Gao J, Guo B, Zhu B. Essential role of TNF-alpha in development of spleen fibroblastic reticular cells. Cell Immunol 2015;293:130–6.
[40] Ngo VN, Korner H, Gunn MD, Schmidt KN, Riminton DS, Cooper MD, et al. Lymphotoxin alpha/beta and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. J Exp Med 1999;189:403–12.
[41] Yu P, Wang Y, Chin RK, Martinez-Pomares L, Gordon S, Kosco-Vibois MH, et al. B cells control the migration of a subset of dendritic cells into B cell follicles via CXC chemokine ligand 13 in a lymphotoxin-dependent fashion. J Immunol 2002;168:5117–23.
[42] Onder L, Danuser R, Scandella E, Firner S, Chai Q, Hehlgans T, et al. Endothelial cell-specific lymphotoxin-beta receptor signaling is critical for lymph node and high endothelial venule formation. J Exp Med 2013;210:465–73.
[43] Stranford S, Ruddle NH. Follicular dendritic cells, conduits, lymphatic vessels, and high endothelial venules in tertiary lymphoid organs: parallels with lymph node stroma. Front Immunol 2012;3:350.
[44] Ager A. High endothelial venules and other blood vessels: critical regulators of lymphoid organ development and function. Front Immunol 2017;8:45.
[45] Josien R, Wong BR, Li HL, Steinman RM, Choi Y. TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. J Immunol 1999;162:2562–8.
[46] Baldwin HM, Ito-Ihara T, Isaacs JD, Hilkens CM. Tumour necrosis factor alpha blockade impairs dendritic cell survival and function in rheumatoid arthritis. Ann Rheum Dis 2010;69:1200–7.
[47] Thorne A, Tomic S, Pavlovic B, Mihajlovic D, Dzopalic T, Colic M. Tumor necrosis factor-alpha promotes survival and phenotypic maturation of poly(I:C)-treated dendritic cells but impairs their Th1 and Th17 polarizing capability. Cytotherapy 2015;17:633–46.
[48] Fu YX, Huang G, Wang Y, Chaplin DD. Lymphotoxin-alpha-dependent spleen microenvironment supports the generation of memory B cells and is required for their subsequent antigen-induced activation. J Immunol 2000;164:2508–14.
[49] Debard N, Sierro F, Browning J, Kraehenbuhl JP. Effect of mature lymphocytes and lymphotoxin on the development of the follicle-associated epithelium and M cells in mouse Peyer's patches. Gastroenterology 2001;120:1173–82.
[50] Hsieh EH, Fernandez X, Wang J, Hamer M, Calvillo S, Croft M, et al. CD137 is required for M cell functional maturation but not lineage commitment. Am J Pathol 2010;177:666–76.
[51] Brestoff JR, Artis D. Commensal bacteria at the interface of host metabolism and the immune system. Nat Immunol 2013;14:676–84.
[52] Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature 2011;474:298–306.
[53] Agace WW, McCoy KD. Regionalized development and maintenance of the intestinal adaptive immune landscape. Immunity 2017;46:532–48.
[54] Kanamori Y, Ishimaru K, Nanno M, Maki K, Ikuta K, Nariuchi H, et al. Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of

c-kit + IL-7R + Thy1 + lympho-hemopoietic progenitors develop. J Exp Med 1996;184:1449–59.
[55] Lundqvist C, Baranov V, Hammarstrom S, Athlin L, Hammarstrom ML. Intra-epithelial lymphocytes. Evidence for regional specialization and extrathymic T cell maturation in the human gut epithelium. Int Immunol 1995;7:1473–87.
[56] Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science 2011;334:1561–5.
[57] Lee JS, Cella M, McDonald KG, Garlanda C, Kennedy GD, Nukaya M, et al. AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. Nat Immunol 2011;13:144–51.
[58] Nagasawa M, Spits H, Ros XR. Innate lymphoid cells (ILCs): cytokine hubs regulating immunity and tissue homeostasis. Cold Spring Harb Perspect Biol 2018;10.
[59] Zhong C, Zheng M, Zhu J. Lymphoid tissue inducer-A divergent member of the ILC family. Cytokine Growth Factor Rev 2018;. Available from: http://dx.doi.org/10.1101/cshperspect.a028563.
[60] Luci C, Reynders A, Ivanov II, Cognet C, Chiche L, Chasson L, et al. Influence of the transcription factor RORgammat on the development of NKp46 + cell populations in gut and skin. Nat Immunol 2009;10:75–82.
[61] Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C, et al. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46 + cells. Nat Immunol 2009;10:83–91.
[62] Satoh-Takayama N, Vosshenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, et al. Microbial flora drives interleukin 22 production in intestinal NKp46 + cells that provide innate mucosal immune defense. Immunity 2008;29:958–70.
[63] Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4 + CD3- LTbeta + cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. Immunity 1997;7:493–504.
[64] Sawa S, Cherrier M, Lochner M, Satoh-Takayama N, Fehling HJ, Langa F, et al. Lineage relationship analysis of RORgammat + innate lymphoid cells. Science 2010;330:665–9.
[65] Eberl G, Littman DR. Thymic origin of intestinal alphabeta T cells revealed by fate mapping of RORgammat + cells. Science 2004;305:248–51.
[66] Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nat Immunol 2004;5:64–73.
[67] Hamada H, Hiroi T, Nishiyama Y, Takahashi H, Masunaga Y, Hachimura S, et al. Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. J Immunol 2002;168:57–64.
[68] Savage AK, Liang HE, Locksley RM. The development of steady-state activation hubs between adult LTi ILC3s and primed macrophages in small intestine. J Immunol 2017;199:1912–22.
[69] Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 2006;203:2271–9.
[70] Aden K, Rehman A, Falk-Paulsen M, Secher T, Kuiper J, Tran F, et al. Epithelial IL-23R signaling licenses protective IL-22 responses in intestinal inflammation. Cell Rep 2016;16:2208–18.
[71] Li Z, Hodgkinson T, Gothard EJ, Boroumand S, Lamb R, Cummins I, et al. Epidermal Notch1 recruits RORgamma(+) group 3 innate lymphoid cells to orchestrate normal skin repair. Nat Commun 2016;7:11394.
[72] Eyerich S, Wagener J, Wenzel V, Scarponi C, Pennino D, Albanesi C, et al. IL-22 and TNF-alpha represent a key cytokine combination for epidermal integrity during infection with *Candida albicans*. Eur J Immunol 2011;41:1894–901.
[73] Longman RS, Diehl GE, Victorio DA, Huh JR, Galan C, Miraldi ER, et al. CX(3)CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. J Exp Med 2014;211:1571–83.
[74] Magri G, Miyajima M, Bascones S, Mortha A, Puga I, Cassis L, et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. Nat Immunol 2014;15:354–64.
[75] Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. Science 2014;343:1249288.
[76] Pearson C, Thornton EE, McKenzie B, Schaupp AL, Huskens N, Griseri T, et al. ILC3 GM-CSF production and mobilisation orchestrate acute intestinal inflammation. eLife 2016;5:e10066.
[77] Eken A, Singh AK, Treuting PM, Oukka M. IL-23R + innate lymphoid cells induce colitis via interleukin-22-dependent mechanism. Mucosal Immunol 2014;7:143–54.
[78] Goverse G, Labao-Almeida C, Ferreira M, Molenaar R, Wahlen S, Konijn T, et al. Vitamin A controls the presence of RORgamma + innate lymphoid cells and lymphoid tissue in the small intestine. J Immunol 2016;196:5148–55.

[79] van de Pavert SA, Ferreira M, Domingues RG, Ribeiro H, Molenaar R, Moreira-Santos L, et al. Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. Nature 2014;508:123–7.
[80] Kiss EA, Diefenbach A. Role of the aryl hydrocarbon receptor in controlling maintenance and functional programs of RORgammat(+) innate lymphoid cells and intraepithelial lymphocytes. Front Immunol 2012;3:124.
[81] Emgard J, Kammoun H, Garcia-Cassani B, Chesne J, Parigi SM, Jacob JM, et al. Oxysterol sensing through the receptor GPR183 promotes the lymphoid-tissue-inducing function of innate lymphoid cells and colonic inflammation. Immunity 2018;48 120–32 e128.
[82] McDonald KG, McDonough JS, Dieckgraefe BK, Newberry RD. Dendritic cells produce CXCL13 and participate in the development of murine small intestine lymphoid tissues. Am J Pathol 2010;176:2367–77.
[83] Lugering A, Ross M, Sieker M, Heidemann J, Williams IR, Domschke W, et al. CCR6 identifies lymphoid tissue inducer cells within cryptopatches. Clin Exp Immunol 2010;160:440–9.
[84] Lorenz RG, Chaplin DD, McDonald KG, McDonough JS, Newberry RD. Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin beta receptor, and TNF receptor I function. J Immunol 2003;170:5475–82.
[85] Pabst O, Herbrand H, Friedrichsen M, Velaga S, Dorsch M, Berhardt G, et al. Adaptation of solitary intestinal lymphoid tissue in response to microbiota and chemokine receptor CCR7 signaling. J Immunol 2006;177:6824–32.
[86] Bouskra D, Brezillon C, Berard M, Werts C, Varona R, Boneca IG, et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 2008;456:507–10.
[87] Wang C, McDonough JS, McDonald KG, Huang C, Newberry RD. Alpha4beta7/MAdCAM-1 interactions play an essential role in transitioning cryptopatches into isolated lymphoid follicles and a nonessential role in cryptopatch formation. J Immunol 2008;181:4052–61.
[88] Lin YL, Ip PP, Liao F. CCR6 deficiency impairs IgA production and dysregulates antimicrobial peptide production, altering the intestinal flora. Front Immunol 2017;8:805.
[89] Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivanov II, et al. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. Immunity 2008;29:261–71.
[90] Knoop KA, Newberry RD. Isolated lymphoid follicles are dynamic reservoirs for the induction of intestinal IgA. Front Immunol 2012;3:84.
[91] Russler-Germain EV, Rengarajan S, Hsieh CS. Antigen-specific regulatory T-cell responses to intestinal microbiota. Mucosal Immunol 2017;10:1375–86.
[92] Wichner K, Fischer A, Winter S, Tetzlaff S, Heimesaat MM, Bereswill S, et al. Transition from an autoimmune-prone state to fatal autoimmune disease in CCR7 and RORgammat double-deficient mice is dependent on gut microbiota. J Autoimmun 2013;47:58–72.
[93] Pere-Vedrenne C, Flahou B, Loke MF, Menard A, Vadivelu J. Other Helicobacters, gastric and gut microbiota. Helicobacter 2017;22(Suppl. 1). Available from: http://dx.doi.org/10.1111/hel.12407.
[94] Baptista AP, Olivier BJ, Goverse G, Greuter M, Knippenberg M, Kusser K, et al. Colonic patch and colonic SILT development are independent and differentially regulated events. Mucosal Immunol 2013;6:511–21.
[95] Masahata K, Umemoto E, Kayama H, Kotani M, Nakamura S, Kurakawa T, et al. Generation of colonic IgA-secreting cells in the caecal patch. Nat Commun 2014;5:3704.
[96] Wang M, Gao Z, Zhang Z, Pan L, Zhang Y. Roles of M cells in infection and mucosal vaccines. Hum Vaccin Immunother 2014;10:3544–51.
[97] Brayden DJ, Baird AW. A distinctive electrophysiological signature from the Peyer's patches of rabbit intestine. Br J Pharmacol 1994;113:593–9.
[98] Pabst O, Herbrand H, Worbs T, Friedrichsen M, Yan S, Hoffmann MW, et al. Cryptopatches and isolated lymphoid follicles: dynamic lymphoid tissues dispensable for the generation of intraepithelial lymphocytes. Eur J Immunol 2005;35:98–107.
[99] Reboldi A, Arnon TI, Rodda LB, Atakilit A, Sheppard D, Cyster JG. IgA production requires B cell interaction with subepithelial dendritic cells in Peyer's patches. Science 2016;352:aaf4822.
[100] Zhao X, Sato A, Dela Cruz CS, Linehan M, Luegering A, Kucharzik T, et al. CCL9 is secreted by the follicle-associated epithelium and recruits dome region Peyer's patch CD11b + dendritic cells. J Immunol 2003;171:2797–803.
[101] Da Silva C, Wagner C, Bonnardel J, Gorvel JP, Lelouard H. The Peyer's patch mononuclear phagocyte system at steady state and during infection. Front Immunol 2017;8:1254.
[102] Austyn JM. Dendritic cells in the immune system-history, lineages, tissues, tolerance, and immunity. Microbiol Spectr 2016;4.
[103] Guilliams M, Dutertre CA, Scott CL, McGovern N, Sichien D, Chakarov S, et al. Unsupervised high-

dimensional analysis aligns dendritic cells across tissues and species. Immunity 2016;45:669–84.
[104] Reboldi A, Cyster JG. Peyer's patches: organizing B-cell responses at the intestinal frontier. Immunol Rev 2016;271:230–45.
[105] Luda KM, Joeris T, Persson EK, Rivollier A, Demiri M, Sitnik KM, et al. IRF8 transcription-factor-dependent classical dendritic cells are essential for intestinal T cell homeostasis. Immunity 2016;44:860–74.
[106] Ohta T, Sugiyama M, Hemmi H, Yamazaki C, Okura S, Sasaki I, et al. Crucial roles of XCR1-expressing dendritic cells and the XCR1-XCL1 chemokine axis in intestinal immune homeostasis. Sci Rep 2016;6:23505.
[107] Bekiaris V, Persson EK, Agace WW. Intestinal dendritic cells in the regulation of mucosal immunity. Immunol Rev 2014;260:86–101.
[108] Persson EK, Scott CL, Mowat AM, Agace WW. Dendritic cell subsets in the intestinal lamina propria: ontogeny and function. Eur J Immunol 2013;43:3098–107.
[109] Mora JR, Bono MR, Manjunath N, Weninger W, Cavanagh LL, Rosemblatt M, et al. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. Nature 2003;424:88–93.
[110] Johansson-Lindbom B, Agace WW. Generation of gut-homing T cells and their localization to the small intestinal mucosa. Immunol Rev 2007;215:226–42.
[111] Paidassi H, Acharya M, Zhang A, Mukhopadhyay S, Kwon M, Chow C, et al. Preferential expression of integrin alphavbeta8 promotes generation of regulatory T cells by mouse CD103+ dendritic cells. Gastroenterology 2011;141:1813–20.
[112] Boucard-Jourdin M, Kugler D, Endale Ahanda ML, This S, De Calisto J, Zhang A, et al. Beta8 integrin expression and activation of TGF-beta by intestinal dendritic cells are determined by both tissue microenvironment and cell lineage. J Immunol 2016;197:1968–78.
[113] Worthington JJ, Czajkowska BI, Melton AC, Travis MA. Intestinal dendritic cells specialize to activate transforming growth factor-beta and induce Foxp3+ regulatory T cells via integrin alphavbeta8. Gastroenterology 2011;141:1802–12.
[114] Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Forster R, et al. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. J Exp Med 2005;202:1063–73.
[115] Bonnardel J, Da Silva C, Wagner C, Bonifay R, Chasson L, Masse M, et al. Distribution, location, and transcriptional profile of Peyer's patch conventional DC subsets at steady state and under TLR7 ligand stimulation. Mucosal Immunol 2017;10:1412–30.
[116] Okada T, Ngo VN, Ekland EH, Forster R, Lipp M, Littman DR, et al. Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. J Exp Med 2002;196:65–75.
[117] Kurita N, Honda S, Shibuya A. Increased serum IgA in Fcalpha/muR-deficient mice on the (129 x C57BL/6) F1 genetic background. Mol Immunol 2015;63:367–72.
[118] Onder L, Narang P, Scandella E, Chai Q, Iolyeva M, Hoorweg K, et al. IL-7-producing stromal cells are critical for lymph node remodeling. Blood 2012;120:4675–83.
[119] Hara T, Shitara S, Imai K, Miyachi H, Kitano S, Yao H, et al. Identification of IL-7-producing cells in primary and secondary lymphoid organs using IL-7-GFP knock-in mice. J Immunol 2012;189:1577–84.
[120] Cremasco V, Woodruff MC, Onder L, Cupovic J, Nieves-Bonilla JM, Schildberg FA, et al. B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. Nat Immunol 2014;15:973–81.
[121] Gil-Cruz C, Perez-Shibayama C, Onder L, Chai Q, Cupovic J, Cheng HW, et al. Fibroblastic reticular cells regulate intestinal inflammation via IL-15-mediated control of group 1 ILCs. Nat Immunol 2016;17:1388–96.
[122] McDonald KG, Wheeler LW, McDole JR, Joerger S, Gustafsson JK, Kulkarni DH, et al. CCR6 promotes steady-state mononuclear phagocyte association with the intestinal epithelium, imprinting and immune surveillance. Immunology 2017;152:613–27.
[123] Hase K, Murakami T, Takatsu H, Shimaoka T, Iimura M, Hamura K, et al. The membrane-bound chemokine CXCL16 expressed on follicle-associated epithelium and M cells mediates lympho-epithelial interaction in GALT. J Immunol 2006;176:43–51.
[124] Mantis NJ, Cheung MC, Chintalacharuvu KR, Rey J, Corthesy B, Neutra MR. Selective adherence of IgA to murine Peyer's patch M cells: evidence for a novel IgA receptor. J Immunol 2002;169:1844–51.
[125] Nakato G, Fukuda S, Hase K, Goitsuka R, Cooper MD, Ohno H. New approach for m-cell-specific molecules screening by comprehensive transcriptome analysis. DNA Res 2009;16:227–35.
[126] Hase K, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. Nature 2009;462:226–30.
[127] Sato S, Kaneto S, Shibata N, Takahashi Y, Okura H, Yuki Y, et al. Transcription factor Spi-B-dependent and -independent pathways for the development of Peyer's patch M cells. Mucosal Immunol 2013;6:838–46.
[128] Yanagihara S, Kanaya T, Fukuda S, Nakato G, Hanazato M, Wu XR, et al. Uromodulin-SlpA binding

dictates *Lactobacillus acidophilus* uptake by intestinal epithelial M cells. Int Immunol 2017;29:357–63.
[129] Ohno H. Intestinal M cells. J Biochem 2016;159:151–60.
[130] Nakato G, Hase K, Suzuki M, Kimura M, Ato M, Hanazato M, et al. Cutting edge: *Brucella abortus* exploits a cellular prion protein on intestinal M cells as an invasive receptor. J Immunol 2012;189:1540–4.
[131] Wood MB, Rios D, Williams IR. TNF-alpha augments RANKL-dependent intestinal M cell differentiation in enteroid cultures. Am J Physiol Cell Physiol 2016;311: C498–507.
[132] Lo D, Tynan W, Dickerson J, Mendy J, Chang HW, Scharf M, et al. Peptidoglycan recognition protein expression in mouse Peyer's Patch follicle associated epithelium suggests functional specialization. Cell Immunol 2003;224:8–16.
[133] Sansonetti PJ, Phalipon A. M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. Semin Immunol 1999;11:193–203.
[134] Clark MA, Hirst BH, Jepson MA. M-cell surface beta1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. Infect Immun 1998;66:1237–43.
[135] Jensen VB, Harty JT, Jones BD. Interactions of the invasive pathogens *Salmonella typhimurium*, *Listeria monocytogenes*, and *Shigella flexneri* with M cells and murine Peyer's patches. Infect Immun 1998;66:3758–66.
[136] Matsumura T, Sugawara Y, Yutani M, Amatsu S, Yagita H, Kohda T, et al. Botulinum toxin A complex exploits intestinal M cells to enter the host and exert neurotoxicity. Nat Commun 2015;6:6255.
[137] Rajapaksa TE, Stover-Hamer M, Fernandez X, Eckelhoefer HA, Lo DD. Claudin 4-targeted protein incorporated into PLGA nanoparticles can mediate M cell targeted delivery. J Control Release 2010;142:196–205.
[138] Kim SH, Jung DI, Yang IY, Kim J, Lee KY, Nochi T, et al. M cells expressing the complement C5a receptor are efficient targets for mucosal vaccine delivery. Eur J Immunol 2011;41:3219–29.
[139] Tsuji M, Suzuki K, Kinoshita K, Fagarasan S. Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis. Semin Immunol 2008;20:59–66.
[140] Veiga-Fernandes H, Coles MC, Foster KE, Patel A, Williams A, Natarajan D, et al. Tyrosine kinase receptor RET is a key regulator of Peyer's patch organogenesis. Nature 2007;446:547–51.
[141] White A, Carragher D, Parnell S, Msaki A, Perkins N, Lane P, et al. Lymphotoxin a-dependent and -independent signals regulate stromal organizer cell homeostasis during lymph node organogenesis. Blood 2007;110:1950–9.
[142] Cherrier M, Sawa S, Eberl G. Notch, Id2, and RORgammat sequentially orchestrate the fetal development of lymphoid tissue inducer cells. J Exp Med 2012;209:729–40.
[143] Patel A, Harker N, Moreira-Santos L, Ferreira M, Alden K, Timmis J, et al. Differential RET signaling pathways drive development of the enteric lymphoid and nervous systems. Sci Signal 2012;5:ra55.
[144] Adachi S, Yoshida H, Kataoka H, Nishikawa S. Three distinctive steps in Peyer's patch formation of murine embryo. Int Immunol 1997;9:507–14.
[145] Rennert PD, Browning JL, Mebius R, Mackay F, Hochman PS. Surface lymphotoxin alpha/beta complex is required for the development of peripheral lymphoid organs. J Exp Med 1996;184:1999–2006.
[146] De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, et al. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science 1994;264:703–7.
[147] Matsushima A, Kaisho T, Rennert PD, Nakano H, Kurosawa K, Uchida D, et al. Essential role of nuclear factor (NF)-kappaB-inducing kinase and inhibitor of kappaB (IkappaB) kinase alpha in NF-kappaB activation through lymphotoxin beta receptor, but not through tumor necrosis factor receptor I. J Exp Med 2001;193:631–6.
[148] Piao JH, Yoshida H, Yeh WC, Doi T, Xue X, Yagita H, et al. TNF receptor-associated factor 2-dependent canonical pathway is crucial for the development of Peyer's patches. J Immunol 2007;178:2272–7.
[149] Futterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K. The lymphotoxin beta receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. Immunity 1998;9:59–70.
[150] Nakagawa R, Togawa A, Nagasawa T, Nishikawa S. Peyer's patch inducer cells play a leading role in the formation of B and T cell zone architecture. J Immunol 2013;190:3309–18.
[151] Endres R, Alimzhanov MB, Plitz T, Futterer A, Kosco-Vilbois MH, Nedospasov SA, et al. Mature follicular dendritic cell networks depend on expression of lymphotoxin beta receptor by radioresistant stromal cells and of lymphotoxin beta and tumor necrosis factor by B cells. J Exp Med 1999;189:159–68.
[152] Saito Y, Respatika D, Komori S, Washio K, Nishimura T, Kotani T, et al. SIRPalpha(+) dendritic cells regulate homeostasis of fibroblastic reticular cells via TNF receptor ligands in the adult spleen. Proc Natl Acad Sci USA 2017;114:E10151–60.

[153] Eberl G, Lochner M. The development of intestinal lymphoid tissues at the interface of self and microbiota. Mucosal Immunol 2009;2:478–85.
[154] Yamanaka T, Helgeland L, Farstad IN, Fukushima H, Midtvedt T, Brandtzaeg P. Microbial colonization drives lymphocyte accumulation and differentiation in the follicle-associated epithelium of Peyer's patches. J Immunol 2003;170:816–22.
[155] Donaldson DS, Bradford BM, Artis D, Mabbott NA. Reciprocal regulation of lymphoid tissue development in the large intestine by IL-25 and IL-23. Mucosal Immunol 2015;8:582–95.
[156] Taylor RT, Lugering A, Newell KA, Williams IR. Intestinal cryptopatch formation in mice requires lymphotoxin alpha and the lymphotoxin beta receptor. J Immunol 2004;173:7183–9.
[157] Honda K, Nakano H, Yoshida H, Nishikawa S, Rennert P, Ikuta K, et al. Molecular basis for hematopoietic/mesenchymal interaction during initiation of Peyer's patch organogenesis. J Exp Med 2001;193:621–30.
[158] Knoop KA, Butler BR, Kumar N, Newberry RD, Williams IR. Distinct developmental requirements for isolated lymphoid follicle formation in the small and large intestine: RANKL is essential only in the small intestine. Am J Pathol 2011;179:1861–71.
[159] Kimura S, Yamakami-Kimura M, Obata Y, Hase K, Kitamura H, Ohno H, et al. Visualization of the entire differentiation process of murine M cells: suppression of their maturation in cecal patches. Mucosal Immunol 2015;8:650–60.
[160] Kanaya T, Hase K, Takahashi D, Fukuda S, Hoshino K, Sasaki I, et al. The Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold cells. Nat Immunol 2012;13:729–36.
[161] Donaldson DS, Sehgal A, Rios D, Williams IR, Mabbott NA. Increased abundance of M cells in the gut epithelium dramatically enhances oral prion disease susceptibility. PLoS Pathog 2016;12:e1006075.
[162] Knoop KA, Kumar N, Butler BR, Sakthivel SK, Taylor RT, Nochi T, et al. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. J Immunol 2009;183:5738–47.
[163] Sehgal A, Kobayashi A, Donaldson DS, Mabbott NA. c-Rel is dispensable for the differentiation and functional maturation of M cells in the follicle-associated epithelium. Immunobiology 2017;222:316–26.
[164] Regan T, Nally K, Carmody R, Houston A, Shanahan F, Macsharry J, et al. Identification of TLR10 as a key mediator of the inflammatory response to *Listeria monocytogenes* in intestinal epithelial cells and macrophages. J Immunol 2013;191:6084–92.
[165] Ghannam S, Dejou C, Pedretti N, Giot JP, Dorgham K, Boukhaddaoui H, et al. CCL20 and beta-defensin-2 induce arrest of human Th17 cells on inflamed endothelium in vitro under flow conditions. J Immunol 2011;186:1411–20.
[166] Hirata T, Osuga Y, Takamura M, Kodama A, Hirota Y, Koga K, et al. Recruitment of CCR6-expressing Th17 cells by CCL 20 secreted from IL-1 beta-, TNF-alpha-, and IL-17A-stimulated endometriotic stromal cells. Endocrinology 2010;151:5468–76.
[167] Ermak TH, Bhagat HR, Pappo J. Lymphocyte compartments in antigen-sampling regions of rabbit mucosal lymphoid organs. Am J Trop Med Hyg 1994;50:14–28.
[168] Kernéis S, Bogdanova A, Kraehenbuhl JP, Pringault E. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science 1997;277:949–52.
[169] Araujo F, Sarmento B. Towards the characterization of an in vitro triple co-culture intestine cell model for permeability studies. Int J Pharm 2013;458:128–34.
[170] Kobayashi A, Donaldson DS, Erridge C, Kanaya T, Williams IR, Ohno H, et al. The functional maturation of M cells is dramatically reduced in the Peyer's patches of aged mice. Mucosal Immunol 2013;6:1027–37.
[171] Man AL, Lodi F, Bertelli E, Regoli M, Pin C, Mulholland F, et al. Macrophage migration inhibitory factor plays a role in the regulation of microfold (M) cell-mediated transport in the gut. J Immunol 2008;181:5673–80.
[172] Wang J, Li F, Tian Z. Role of microbiota on lung homeostasis and diseases. Sci China Life Sci 2017;60:1407–15.
[173] Tipton L, Ghedin E, Morris A. The lung mycobiome in the next-generation sequencing era. Virulence 2017;8:334–41.
[174] Sepahi A, Salinas I. The evolution of nasal immune systems in vertebrates. Mol Immunol 2016;69:131–8.
[175] Pabst R. Mucosal vaccination by the intranasal route. Nose-associated lymphoid tissue (NALT)-Structure, function and species differences. Vaccine 2015;33:4406–13.
[176] Mair TS, Batten EH, Stokes CR, Bourne FJ. The histological features of the immune system of the equine respiratory tract. J Comp Pathol 1987;97:575–86.
[177] Casteleyn C, Broos AM, Simoens P, Van den Broeck W. NALT (nasal cavity-associated lymphoid tissue) in the rabbit. Vet Immunol Immunopathol 2010;133:212–18.
[178] Bienenstock J, McDermott MR. Bronchus- and nasal-associated lymphoid tissues. Immunol Rev 2005;206:22–31.

[179] Csencsits KL, Jutila MA, Pascual DW. Nasal-associated lymphoid tissue: phenotypic and functional evidence for the primary role of peripheral node addressin in naive lymphocyte adhesion to high endothelial venules in a mucosal site. J Immunol 1999;163:1382–9.

[180] Lohrberg M, Pabst R, Wilting J. Co-localization of lymphoid aggregates and lymphatic networks in nose- (NALT) and lacrimal duct-associated lymphoid tissue (LDALT) of mice. BMC Immunol 2018;19:5.

[181] Rangel-Moreno J, Moyron-Quiroz J, Kusser K, Hartson L, Nakano H, Randall TD. Role of CXC chemokine ligand 13, CC chemokine ligand (CCL) 19, and CCL21 in the organization and function of nasal-associated lymphoid tissue. J Immunol 2005;175:4904–13.

[182] Date Y, Ebisawa M, Fukuda S, Shima H, Obata Y, Takahashi D, et al. NALT M cells are important for immune induction for the common mucosal immune system. Int Immunol 2017;29:471–8.

[183] Kim DY, Sato A, Fukuyama S, Sagara H, Nagatake T, Kong IG, et al. The airway antigen sampling system: respiratory M cells as an alternative gateway for inhaled antigens. J Immunol 2011;186:4253–62.

[184] Mutoh M, Kimura S, Takahashi-Iwanaga H, Hisamoto M, Iwanaga T, Iida J. RANKL regulates differentiation of microfold cells in mouse nasopharynx-associated lymphoid tissue (NALT). Cell Tissue Res 2016;364:175–84.

[185] Fukuyama Y, Tokuhara D, Sekine S, Aso K, Kataoka K, Davydova J, et al. Potential roles of CCR5(+) CCR6 (+) dendritic cells induced by nasal ovalbumin plus Flt3 ligand expressing adenovirus for mucosal IgA responses. PLoS One 2013;8:e60453.

[186] Ying X, Chan K, Shenoy P, Hill M, Ruddle NH. Lymphotoxin plays a crucial role in the development and function of nasal-associated lymphoid tissue through regulation of chemokines and peripheral node addressin. Am J Pathol 2005;166:135–46.

[187] Ohmichi Y, Hirakawa J, Imai Y, Fukuda M, Kawashima H. Essential role of peripheral node addressin in lymphocyte homing to nasal-associated lymphoid tissues and allergic immune responses. J Exp Med 2011;208:1015–25.

[188] Browning JL, Allaire N, Ngam-Ek A, Notidis E, Hunt J, Perrin S, et al. Lymphotoxin-beta receptor signaling is required for the homeostatic control of HEV differentiation and function. Immunity 2005;23:539–50.

[189] Nagatake T, Fukuyama S, Kim DY, Goda K, Igarashi O, Sato S, et al. Id2-, RORgammat-, and LTbetaR-independent initiation of lymphoid organogenesis in ocular immunity. J Exp Med 2009;206:2351–64.

[190] Kunisawa J, Fukuyama S, Kiyono H. Mucosa-associated lymphoid tissues in the aerodigestive tract: their shared and divergent traits and their importance to the orchestration of the mucosal immune system. Curr Mol Med 2005;5:557–72.

[191] Fukuyama S, Hiroi T, Yokota Y, Rennert PD, Yanagita M, Kinoshita N, et al. Initiation of NALT organogenesis is independent of the IL-7R, LTbetaR, and NIK signaling pathways but requires the Id2 gene and CD3(-) CD4(+)CD45(+) cells. Immunity 2002;17:31–40.

[192] Rangel-Moreno J, Carragher D, Randall TD. Role of lymphotoxin and homeostatic chemokines in the development and function of local lymphoid tissues in the respiratory tract. Immunologia 2007;26:13–28.

[193] Nagatake T, Fukuyama S, Sato S, Okura H, Tachibana M, Taniuchi I, et al. Central role of core binding factor beta2 in mucosa-associated lymphoid tissue organogenesis in mouse. PLoS One 2015;10:e0127460.

[194] Krege J, Seth S, Hardtke S, Davalos-Misslitz AC, Forster R. Antigen-dependent rescue of nose-associated lymphoid tissue (NALT) development independent of LTbetaR and CXCR5 signaling. Eur J Immunol 2009;39:2765–78.

[195] Harmsen A, Kusser K, Hartson L, Tighe M, Sunshine MJ, Sedgwick JD, et al. Cutting edge: organogenesis of nasal-associated lymphoid tissue (NALT) occurs independently of lymphotoxin-α (LT α) and retinoic acid receptor-related orphan receptor-γ, but the organization of NALT is LT α dependent. J Immunol 2002;168:986–90.

[196] Hostager BS, Bishop GA. CD40-mediated activation of the NF-kappaB2 pathway. Front Immunol 2013;4:376.

[197] de Lau W, Kujala P, Schneeberger K, Middendorp S, Li VS, Barker N, et al. Peyer's patch M cells derived from Lgr5(+) stem cells require SpiB and are induced by RankL in cultured "miniguts". Mol Cell Biol 2012;32:3639–47.

[198] Ermak TH, Steger HJ, Strober S, Owen RL. M cells and granular mononuclear cells in Peyer's patch domes of mice depleted of their lymphocytes by total lymphoid irradiation. Am J Pathol 1989;134:529–37.

[199] Velin D, Fotopoulos G, Luthi F, Kraehenbuhl JP. The nasal-associated lymphoid tissue of adult mice acts as an entry site for the mouse mammary tumor retrovirus. J Exp Med 1997;185:1871–6.

[200] Park HS, Cleary PP. Active and passive intranasal immunizations with streptococcal surface protein C5a peptidase prevent infection of murine nasal mucosa-associated lymphoid tissue, a functional homologue of human tonsils. Infect Immun 2005;73:7878–86.

[201] Park HS, Costalonga M, Reinhardt RL, Dombek PE, Jenkins MK, Cleary PP. Primary induction of CD4T

cell responses in nasal associated lymphoid tissue during group A streptococcal infection. Eur J Immunol 2004;34:2843–53.
[202] Owen SJ, Batzloff M, Chehrehasa F, Meedeniya A, Casart Y, Logue CA, et al. Nasal-associated lymphoid tissue and olfactory epithelium as portals of entry for *Burkholderia pseudomallei* in murine melioidosis. J Infect Dis 2009;199:1761–70.
[203] Takata S, Ohtani O, Watanabe Y. Lectin binding patterns in rat nasal-associated lymphoid tissue (NALT) and the influence of various types of lectin on particle uptake in NALT. Arch Histol Cytol 2000;63:305–12.
[204] Wu Y, Wang X, Csencsits KL, Haddad A, Walters N, Pascual DW. M cell-targeted DNA vaccination. Proc Natl Acad Sci USA 2001;98:9318–23.
[205] Shukla A, Katare OP, Singh B, Vyas SP. M-cell targeted delivery of recombinant hepatitis B surface antigen using cholera toxin B subunit conjugated bilosomes. Int J Pharm 2010;385:47–52.
[206] Yuki Y, Kiyono H. New generation of mucosal adjuvants for the induction of protective immunity. Rev Med Virol 2003;13:293–310.
[207] Kiyono H, Fukuyama S. NALT- versus Peyer's-patch-mediated mucosal immunity. Nat Rev Immunol 2004;4:699–710.
[208] Kataoka K, Fujihashi K, Oma K, Fukuyama Y, Hollingshead SK, Sekine S, et al. The nasal dendritic cell-targeting Flt3 ligand as a safe adjuvant elicits effective protection against fatal pneumococcal pneumonia. Infect Immun 2011;79:2819–28.
[209] Matsuo K, Yoshikawa T, Asanuma H, Iwasaki T, Hagiwara Y, Chen Z, et al. Induction of innate immunity by nasal influenza vaccine administered in combination with an adjuvant (cholera toxin). Vaccine 2000;18:2713–22.
[210] Heritage PL, Underdown BJ, Brook MA, McDermott MR. Oral administration of polymer-grafted starch microparticles activates gut-associated lymphocytes and primes mice for a subsequent systemic antigen challenge. Vaccine 1998;16:2010–17.
[211] Tamura S, Iwasaki T, Thompson AH, Asanuma H, Chen Z, Suzuki Y, et al. Antibody-forming cells in the nasal-associated lymphoid tissue during primary influenza virus infection. J Gen Virol 1998;79(Pt 2):291–9.
[212] Zuercher AW, Coffin SE, Thurnheer MC, Fundova P, Cebra JJ. Nasal-associated lymphoid tissue is a mucosal inductive site for virus-specific humoral and cellular immune responses. J Immunol 2002;168:1796–803.
[213] Boyden AW, Legge KL, Waldschmidt TJ. Pulmonary infection with influenza A virus induces site-specific germinal center and T follicular helper cell responses. PLoS One 2012;7:e40733.
[214] Shimoda M, Nakamura T, Takahashi Y, Asanuma H, Tamura S, Kurata T, et al. Isotype-specific selection of high affinity memory B cells in nasal-associated lymphoid tissue. J Exp Med 2001;194:1597–607.
[215] Barman NN, Bhattacharyya R, Upadhyaya TN, Baishya G. Development of bronchus-associated lymphoid tissue in goats. Lung 1996;174:127–31.
[216] Watt NJ, MacIntyre N, Collie D, Sargan D, McConnell I. Phenotypic analysis of lymphocyte populations in the lungs and regional lymphoid tissue of sheep naturally infected with maedi visna virus. Clin Exp Immunol 1992;90:204–8.
[217] Delventhal S, Hensel A, Petzoldt K, Pabst R. Effects of microbial stimulation on the number, size and activity of bronchus-associated lymphoid tissue (BALT) structures in the pig. Int J Exp Pathol 1992;73:351–7.
[218] Delventhal S, Brandis A, Ostertag H, Pabst R. Low incidence of bronchus-associated lymphoid tissue (BALT) in chronically inflamed human lungs. Virchows Arch B Cell Pathol Incl Mol Pathol 1992;62:271–4.
[219] Gould SJ, Isaacson PG. Bronchus-associated lymphoid tissue (BALT) in human fetal and infant lung. J Pathol 1993;169:229–34.
[220] Kolopp-Sarda MN, Bene MC, Massin N, Moulin JJ, Faure GC. Immunohistological analysis of macrophages, B-cells, and T-cells in the mouse lung. Anat Rec 1994;239:150–7.
[221] Johnson JD, Houchens DP, Kluwe WM, Craig DK, Fisher GL. Effects of mainstream and environmental tobacco smoke on the immune system in animals and humans: a review. Crit Rev Toxicol 1990;20:369–95.
[222] Meuwissen HJ, Hussain M. Bronchus-associated lymphoid tissue in human lung: correlation of hyperplasia with chronic pulmonary disease. Clin Immunol Immunopathol 1982;23:548–61.
[223] Tschernig T, Pabst R. Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases. Pathobiology 2000;68:1–8.
[224] Suda T, Chida K, Hayakawa H, Imokawa S, Iwata M, Nakamura H, et al. Development of bronchus-associated lymphoid tissue in chronic hypersensitivity pneumonitis. Chest 1999;115:357–63.
[225] Rangel-Moreno J, Hartson L, Navarro C, Gaxiola M, Selman M, Randall TD. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. J Clin Invest 2006;116:3183–94.
[226] Sato A, Hayakawa H, Uchiyama H, Chida K. Cellular distribution of bronchus-associated lymphoid tissue in rheumatoid arthritis. Am J Respir Crit Care Med 1996;154:1903–7.

[227] Shilling RA, Williams JW, Perera J, Berry E, Wu Q, Cummings OW, et al. Autoreactive T and B cells induce the development of bronchus-associated lymphoid tissue in the lung. Am J Respir Cell Mol Biol 2013;48:406–14.
[228] Hwang JY, Randall TD, Silva-Sanchez A. Inducible bronchus-associated lymphoid tissue: taming inflammation in the lung. Front Immunol 2016;7:258.
[229] Fleige H, Forster R. Induction and analysis of bronchus-associated lymphoid tissue. Methods Mol Biol 2017;1559:185–98.
[230] Pabst R, Binns RM. The immune system of the respiratory tract in pigs. Vet Immunol Immunopathol 1994;43:151–6.
[231] Vranckx K, Maes D, Marchioro SB, Villarreal I, Chiers K, Pasmans F, et al. Vaccination reduces macrophage infiltration in bronchus-associated lymphoid tissue in pigs infected with a highly virulent *Mycoplasma hyopneumoniae* strain. BMC Vet Res 2012;8:24.
[232] Zamri-Saad M, Effendy AW. The effects of dexamethasone on the response of bronchus-associated lymphoid tissue to intranasal administration of formalin-killed *Pasteurella haemolytica* A2 in goats. Vet Res Commun 1999;23:467–73.
[233] Effendy AW, Zamri-Saad M, Maswati MA, Ismail MS, Jamil SM. Stimulation of the bronchus-associated lymphoid tissue of goats and its effect on in vitro colonization by *Pasteurella haemolytica*. Vet Res Commun 1998;22:147–53.
[234] Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, Goodrich S, et al. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. Nat Med 2004;10:927–34.
[235] Wiley JA, Richert LE, Swain SD, Harmsen A, Barnard DL, Randall TD, et al. Inducible bronchus-associated lymphoid tissue elicited by a protein cage nanoparticle enhances protection in mice against diverse respiratory viruses. PLoS One 2009;4:e7142.
[236] Plesch BE, Gamelkoorn GJ, van de Ende M. Development of bronchus associated lymphoid tissue (BALT) in the rat, with special reference to T- and B-cells. Dev Comp Immunol 1983;7:179–88.
[237] Gadaleanu V. Ovalbumin-induced immunomorphologic reaction of rat lung with emphasis on bronchus-associated lymphoid tissue (BALT). Morphol Embryol (Bucur) 1982;28:65–9.
[238] Gregson RL, Davey MJ, Prentice DE. The response of rat bronchus-associated lymphoid tissue to local antigenic challenge. Br J Exp Pathol 1979;60:471–82.
[239] van der Brugge-Gamelkoorn GJ, Plesch BE, Sminia T, Langevoort HL. Specific antibody-forming cells in bronchus-associated lymphoid tissue (BALT) and lung of the rat after intratracheal challenge with horseradish peroxidase. Virchows Arch B Cell Pathol Incl Mol Pathol 1985;49:269–76.
[240] Rangel-Moreno J, Carragher DM, de la Luz Garcia-Hernandez M, Hwang JY, Kusser K, Hartson L, et al. The development of inducible bronchus-associated lymphoid tissue depends on IL-17. Nat Immunol 2011;12:639–46.
[241] Fleige H, Ravens S, Moschovakis GL, Bolter J, Willenzon S, Sutter G, et al. IL-17-induced CXCL12 recruits B cells and induces follicle formation in BALT in the absence of differentiated FDCs. J Exp Med 2014;211:643–51.
[242] Gebert A, Hach G. Vimentin antibodies stain membranous epithelial cells in the rabbit bronchus-associated lymphoid tissue (BALT). Histochemistry 1992;98:271–3.
[243] Kawamata N, Xu B, Nishijima H, Aoyama K, Kusumoto M, Takeuchi T, et al. Expression of endothelia and lymphocyte adhesion molecules in bronchus-associated lymphoid tissue (BALT) in adult human lung. Respir Res 2009;10:97.
[244] Shinoda K, Hirahara K, Iinuma T, Ichikawa T, Suzuki AS, Sugaya K, et al. Thy1 + IL-7 + lymphatic endothelial cells in iBALT provide a survival niche for memory T-helper cells in allergic airway inflammation. Proc Natl Acad Sci USA 2016;113:E2842–2851.
[245] Baluk P, Adams A, Phillips K, Feng J, Hong YK, Brown MB, et al. Preferential lymphatic growth in bronchus-associated lymphoid tissue in sustained lung inflammation. Am J Pathol 2014;184:1577–92.
[246] Muniz LR, Pacer ME, Lira SA, Furtado GC. A critical role for dendritic cells in the formation of lymphatic vessels within tertiary lymphoid structures. J Immunol 2011;187:828–34.
[247] Halle S, Dujardin HC, Bakocevic N, Fleige H, Danzer H, Willenzon S, et al. Induced bronchus-associated lymphoid tissue serves as a general priming site for T cells and is maintained by dendritic cells. J Exp Med 2009;206:2593–601.
[248] Otsuki Y, Ito Y, Magari S. Lymphocyte subpopulations in high endothelial venules and lymphatic capillaries of bronchus-associated lymphoid tissue (BALT) in the rat. Am J Anat 1989;184:139–46.
[249] Xu B, Wagner N, Pham LN, Magno V, Shan Z, Butcher EC, et al. Lymphocyte homing to bronchus-associated lymphoid tissue (BALT) is mediated by L-selectin/PNAd, alpha4beta1 integrin/VCAM-1, and LFA-1 adhesion pathways. J Exp Med 2003;197:1255–67.
[250] Rangel-Moreno J, Moyron-Quiroz JE, Hartson L, Kusser K, Randall TD. Pulmonary expression of CXC chemokine ligand 13, CC chemokine ligand 19, and CC chemokine ligand 21 is essential for local

immunity to influenza. Proc Natl Acad Sci USA 2007;104:10577–82.
[251] Kocks JR, Davalos-Misslitz AC, Hintzen G, Ohl L, Forster R. Regulatory T cells interfere with the development of bronchus-associated lymphoid tissue. J Exp Med 2007;204:723–34.
[252] Foo SY, Zhang V, Lalwani A, Lynch JP, Zhuang A, Lam CE, et al. Regulatory T cells prevent inducible BALT formation by dampening neutrophilic inflammation. J Immunol 2015;194:4567–76.
[253] Kocks JR, Adler H, Danzer H, Hoffmann K, Jonigk D, Lehmann U, et al. Chemokine receptor CCR7 contributes to a rapid and efficient clearance of lytic murine gamma-herpes virus 68 from the lung, whereas bronchus-associated lymphoid tissue harbors virus during latency. J Immunol 2009;182:6861–9.
[254] Trujillo G, Hartigan AJ, Hogaboam CM. T regulatory cells and attenuated bleomycin-induced fibrosis in lungs of CCR7-/- mice. Fibrogenesis Tissue Repair 2010;3:18.
[255] de Kleer IM, Kool M, de Bruijn MJ, Willart M, van Moorleghem J, Schuijs MJ, et al. Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung. Immunity 2016;45:1285–98.
[256] Das A, Kole L, Wang L, Barrios R, Moorthy B, Jaiswal AK. BALT development and augmentation of hyperoxic lung injury in mice deficient in NQO1 and NQO2. Free Radic Biol Med 2006;40:1843–56.
[257] Bashir A, Shah NN, Hazari YM, Habib M, Bashir S, Hilal N, et al. Novel variants of SERPIN1A gene: interplay between alpha1-antitrypsin deficiency and chronic obstructive pulmonary disease. Respir Med 2016;117:139–49.
[258] Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The role of neutrophil elastase inhibitors in lung diseases. Chest 2017;152:249–62.
[259] Solleti SK, Srisuma S, Bhattacharya S, Rangel-Moreno J, Bijli KM, Randall TD, et al. Serpine2 deficiency results in lung lymphocyte accumulation and bronchus-associated lymphoid tissue formation. FASEB J 2016;30:2615–26.
[260] Jones GW, Jones SA. Ectopic lymphoid follicles: inducible centres for generating antigen-specific immune responses within tissues. Immunology 2016;147:141–51.
[261] Eddens T, Elsegeiny W, Garcia-Hernadez ML, Castillo P, Trevejo-Nunez G, Serody K, et al. Pneumocystis-driven inducible bronchus-associated lymphoid tissue formation requires Th2 and Th17 immunity. Cell Rep 2017;18:3078–90.
[262] Kuroda E, Ozasa K, Temizoz B, Ohata K, Koo CX, Kanuma T, et al. Inhaled fine particles induce alveolar macrophage death and interleukin-1alpha release to promote inducible bronchus-associated lymphoid tissue formation. Immunity 2016;45:1299–310.
[263] Gaffen SL. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 2009;9:556–67.
[264] Ho AW, Gaffen SL. IL-17RC: a partner in IL-17 signaling and beyond. Semin Immunopathol 2010;32:33–42.
[265] GeurtsvanKessel CH, Willart MA, Bergen IM, van Rijt LS, Muskens F, Elewaut D, et al. Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. J Exp Med 2009;206:2339–49.
[266] Martinet L, Filleron T, Le Guellec S, Rochaix P, Garrido I, Girard JP. High endothelial venule blood vessels for tumor-infiltrating lymphocytes are associated with lymphotoxin beta-producing dendritic cells in human breast cancer. J Immunol 2013;191:2001–8.
[267] Martinet L, Girard JP. Regulation of tumor-associated high-endothelial venules by dendritic cells: a new opportunity to promote lymphocyte infiltration into breast cancer? Oncoimmunology 2013;2:e26470.
[268] Moussion C, Girard JP. Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. Nature 2011;479:542–6.
[269] Matsumoto M. Role of TNF ligand and receptor family in the lymphoid organogenesis defined by gene targeting. J Med Invest 1999;46:141–50.
[270] Moyron-Quiroz JE, Rangel-Moreno J, Hartson L, Kusser K, Tighe MP, Klonowski KD, et al. Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. Immunity 2006;25:643–54.
[271] Chiavolini D, Rangel-Moreno J, Berg G, Christian K, Oliveira-Nascimento L, Weir S, et al. Bronchus-associated lymphoid tissue (BALT) and survival in a vaccine mouse model of tularemia. PLoS One 2010;5:e11156.
[272] Khader SA, Guglani L, Rangel-Moreno J, Gopal R, Junecko BA, Fountain JJ, et al. IL-23 is required for long-term control of *Mycobacterium tuberculosis* and B cell follicle formation in the infected lung. J Immunol 2011;187:5402–7.
[273] Richert LE, Harmsen AL, Rynda-Apple A, Wiley JA, Servid AE, Douglas T, et al. Inducible bronchus-associated lymphoid tissue (iBALT) synergizes with local lymph nodes during antiviral CD4+ T cell responses. Lymphat Res Biol 2013;11:196–202.
[274] Saif LJ. Mucosal immunity: an overview and studies of enteric and respiratory coronavirus infections in a swine model of enteric disease. Vet Immunol Immunopathol 1996;54:163–9.

[275] Foo SY, Phipps S. Regulation of inducible BALT formation and contribution to immunity and pathology. Mucosal Immunol 2010;3:537–44.
[276] John-Schuster G, Hager K, Conlon TM, Irmler M, Beckers J, Eickelberg O, et al. Cigarette smoke-induced iBALT mediates macrophage activation in a B cell-dependent manner in COPD. Am J Physiol Lung Cell Mol Physiol 2014;307:L692–706.
[277] McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. N Engl J Med 2011;365:1567–75.
[278] Jupelli M, Shimada K, Chiba N, Slepenkin A, Alsabeh R, Jones HD, et al. Chlamydia pneumoniae infection in mice induces chronic lung inflammation, iBALT formation, and fibrosis. PLoS One 2013;8:e77447.
[279] Richmond I, Pritchard GE, Ashcroft T, Avery A, Corris PA, Walters EH. Bronchus associated lymphoid tissue (BALT) in human lung: its distribution in smokers and non-smokers. Thorax 1993;48:1130–4.