OPINION

Inducible lymphoid tissues in the adult gut: recapitulation of a fetal developmental pathway?

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Abstract | The intestinal immune system faces an extraordinary challenge from the large numbers of commensal bacteria and potential pathogens that are restrained by only a single layer of epithelial cells. Here, I discuss evidence that the intestinal immune system develops an extensive network of inducible, reversible lymphoid tissues that contributes to the vital equilibrium between the gut and the bacterial flora. I propose that this network is induced by cryptopatches, which are small clusters of dendritic cells and lymphoid cells that are identical to fetal inducers of lymph-node and Peyer's-patch development.

In the human intestine, there are 10^{14} bacteria, comprising more than 400 species, that feed on the food that we ingest and multiply¹, yet this 'infection' is contained. In fact, we allow these bacteria to thrive and use their fermentation products as nutrients, as stimulators of intestinal absorption and as protective agents against pathogens and cancer^{1–3}. Accordingly, the intestinal immune system is both vast and complex⁴. It has evolved not only to fight pathogens but also to confine the bacterial flora to the gut. To keep the digestive and absorptive functions of the gut at an optimum, a delicate equilibrium must be reached between the bacterial flora and the intestinal immune system^{2,3,5}. At this equilibrium, luminal bacteria do not cause considerable damage to, or invade, the single layer of gut epithelium, and the intestinal immune system

does not destroy the bacterial population. However, this subtle harmony can be perturbed by changes in dietary habits, by ingestion of toxic compounds or by infection with pathogens, all of which can result in alterations to the composition of the gut flora, damage to the epithelium, infection of the intestinal tissues and/or induction of inflammation.

The intestinal immune system has developed a unique arsenal to maintain this equilibrium^{4,6} (FIG. 1). Many different cell types — including members of the innate and the adaptive immune systems, and specialized epithelial and mesenchymal cells — constantly interact to target bacteria in the gut lumen, epithelium or lamina propria. In particular, dendritic cells (DCs) sample antigen or live bacteria from these compartments and migrate to the T-cell zones of inductive sites, such as the mesenteric lymph nodes and the PEYER'S PATCHES^{7,8} (see Glossary). There, DCs can induce adaptive immune responses that lead to the generation of effector T cells and of plasma cells that produce IgA, the main immunoglobulin that is manufactured by the immune system and secreted in large amounts into the gut lumen.

Recently, a new type of inductive site has been described, the isolated lymphoid follicle (ILF), which contains a single B-CELL FOLLICLE, as well as DCs and small numbers of T cells. ILFs are numerous in the lamina propria of the ANTI-MESENTERIC BORDER in mice, as well as in the most distal portion (the ileum) of the

mouse small intestine^{9–11} and the colon¹², and similar structures have been described for the human intestine^{13–15}. In contrast to lymph nodes, Peyer's patches¹⁶ and COLONIC PATCHES¹⁷, ILFs do not develop during fetal life but form after birth, when the intestine is colonized by bacteria^{9,10}.

It has been suggested that small clusters of lymphoid cells that reside between the CRYPTS of the mouse small intestine, known as cryptopatches, develop into ILFs when they are exposed to bacteria or pro-inflammatory stimuli¹². Most cryptopatch cells have a phenotype and developmental requirements that are identical to those of fetal lymphoid-tissue inducer (LTi) cells^{12,18} (also known as LTCs¹⁹ or LOICs), which are essential for the initiation of lymph-node and Peyer's-patch development in the fetus^{16,20,21}. Here, I propose that the young and adult intestinal immune system can recapitulate fetal lymphoid-tissue development and induce cryptopatches to form ILFs through the activation of LTi-like cells. The ability to form additional (and numerous) lymphoid follicles, and thereby markedly increase the number of lymphocytes that are present in the gut, might allow the intestine to adapt to a dynamic and potentially pathogenic gut flora and to maintain the crucial intestinal equilibrium.

Cryptopatches

Cryptopatches were first described following a study in mice by Ishikawa and colleagues, in 1996 (REF. 18). They consist of small clusters of lymphoid cells (10^2 – 10^3 cells) that are located between the crypts in the lamina propria of the small intestine and, to a lesser extent, the colon, and they contain two main types of cell. Whereas 20–30% of cryptopatch cells are CD11c⁺ DCs, most cryptopatch cells resemble early T-cell precursors²², as they express cKIT, the interleukin-7 receptor α -chain (IL-7R α), THY1 and CD44, but no T-cell receptor (TCR) or markers specific for the B-cell, macrophage, DC or granulocyte lineages^{12,18,23}. Throughout this review, I will refer to these

Figure 1 | The intestinal immune system. A single layer of epithelial cells separates the intestinal lamina propria from the gut flora. It is protected by a thick layer of mucus, bactericidal defensins, neutrophils and large amounts of antigen-specific secretory IgA⁸³. The intestinal immune system can be functionally divided into inductive sites, which include the mesenteric lymph nodes, Peyer's patches (in the small intestine), colonic patches^{17,89,90} and isolated lymphoid follicles (ILFs), and into effector sites, which include the epithelium and the lamina propria. In the epithelium, intraepithelial lymphocytes (IELs) monitor epithelial damage and might recognize microbial antigens^{26,27,91–93}. The lamina propria contains large numbers of T cells, IgA-producing plasma cells and macrophages. It also contains many dendritic cells (DCs), which migrate to mesenteric lymph nodes through the lymph and present antigens to T cells^{4,6}. The FOLLICLE-ASSOCIATED EPITHELIUM (FAE), which covers Peyer's patches, contains MICROFOLD (M) CELLS, which transport luminal antigens to the sub-epithelial dome of Peyer's patches for sampling by DCs that can also sample antigens from apoptotic epithelial cells^{94,95}. ILFs, similar to Peyer's patches, contain B cells, DCs and M cells (which are located in the adjacent FAE). Recent evidence indicates that cryptopatches, which contain DCs and Lin[−]cKIT⁺interleukin-7 receptor α -chain (IL-7R α)⁺ cells, differentiate into ILFs¹². Lin[−]cKIT⁺IL-7R α ⁺ cells are also found scattered in ILFs and in the sub-epithelial dome of Peyer's patches. HEV, high endothelial venule.

cells as lineage (Lin)[−]cKIT⁺IL-7R α ⁺ cryptopatch cells. In mice, cryptopatches appear 1–2 weeks after birth^{12,18}, and ~1,500 cryptopatches are found in the small intestine of adults^{18,23}. They develop independently of gut flora, and they also develop independently of B and T cells, as they are present in mice that are deficient in recombination-activating gene 2 (RAG2), or the β - or δ -chain of the TCR, and in severe combined immunodeficient (SCID) mice¹⁸. Their development also does not require signalling through the nuclear factor- κ B (NF- κ B) pathway¹⁸, which is in contrast to the development of lymph nodes and Peyer's patches¹⁶. However, cryptopatches are absent in mice that are deficient in IL-7R α ¹⁸ or the common cytokine-receptor γ -chain, a component of the IL-7R^{23,24}, indicating that IL-7 (which is produced by the

intestinal epithelium²⁵) has an essential role in the formation of cryptopatches.

The discovery of cryptopatches was akin to finding the holy grail for immunologists studying the intestinal immune system. For nearly two decades, the unique phenotype of CD8 $\alpha\alpha$ ⁺ INTRAEPITHELIAL LYMPHOCYTES (IELs) (FIG. 1) and their presence in athymic mice, as well as the results of numerous graft experiments, indicated an extrathymic yet unidentified origin for these cells^{26–28}. Several initial experiments indicated that cryptopatch cells were T-cell precursors and could differentiate into both $\alpha\beta$ and $\gamma\delta$ T cells^{23,29}. Nevertheless, these conclusions have recently been challenged²⁷, and genetic cell-fate mapping experiments show that cryptopatch cells are not T-cell precursors but, instead, might be adult counterparts of fetal LTi cells¹².

Do cryptopatches generate T cells? An extrathymic origin for IELs was first inferred from their apparent lack of dependence on negative selection in the thymus^{30–35} and from their presence in athymic mice³⁶. Furthermore, IELs could be derived from grafts of fetal liver or adult bone marrow given to athymic mice^{37–39}. Bone-marrow grafts generated cryptopatches that first contained CD11c⁺ DCs, then Lin[−]cKIT⁺IL-7R α ⁺ cells, and this was followed by repopulation of the host with $\alpha\beta$ -TCR⁺ and $\gamma\delta$ -TCR⁺ IELs²³. In addition, the adoptive transfer of Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells to SCID mice was shown to generate both $\alpha\beta$ -TCR⁺ and $\gamma\delta$ -TCR⁺ IELs, as well as T cells (but not B cells) in the lymph nodes, spleen and thymus²⁹. As further evidence that cryptopatch cells were T-cell precursors, a small proportion (0.1%) of cryptopatch cells were shown to express transcripts encoding CD3 ϵ , as well as the pre-TCR α -chain, RAG1 and RAG2 (REFS 24,40).

Several observations, however, favoured a thymic origin for IELs. The number of IELs, in particular the number of $\alpha\beta$ -TCR⁺ IELs, is severely decreased in athymic mice, and athymic mice that receive thymic grafts generate IELs^{39,41–44}. Moreover, recent experiments show that IELs can be positively selected in the thymus by agonist ligands, which negatively select for mature T cells in the thymus, spleen and lymph nodes⁴⁵. Similarly, re-aggregated thymic cultures generate IEL-like CD8 $\alpha\alpha$ ⁺ cells in the presence of agonist ligands⁴⁶. Further compelling evidence for a thymic origin of $\alpha\beta$ -TCR⁺ IELs in normal mice was obtained when the progeny of both CD4⁺CD8⁺ double positive (DP) immature thymocytes and Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells were determined by genetic cell-fate mapping¹². It was found that both DP thymocytes and Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells are unique in expressing the nuclear hormone receptor ROR- γ t (retinoic-acid-receptor-related orphan receptor- γ t), whereas DP thymocytes, but not Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells, were found to be able to express a transgene under the control of a recombinant *Cd4* promoter. Mice were then generated that expressed the Cre recombinase under the control of the *Rorc* locus (which encodes ROR- γ t) or the recombinant *Cd4* promoter, and these were crossed with a reporter mouse line that expressed green fluorescent protein only in cells harbouring Cre and their progeny⁴⁷. It was concluded from these experiments that all $\alpha\beta$ -TCR⁺ IELs, but not $\gamma\delta$ -TCR⁺ IELs, are derived from DP thymocytes and that cryptopatch cells generate neither $\alpha\beta$ T cells nor $\gamma\delta$ T cells.

Nevertheless, it remains possible that a subset of $\text{Lin}^{-}\text{cKIT}^{+}\text{IL-7R}\alpha^{+}$ cryptopatch cells generate $\alpha\beta$ and $\gamma\delta$ T cells on transfer to SCID mice, owing to the lymphopaenic state of these recipients. Accordingly, although extrathymic generation of IELs seems to be minimal in euthymic mice, in the absence of a thymus, this pathway is considerably more productive, in particular in the mesenteric lymph nodes⁴⁸. Finally, subsets of $\gamma\delta$ -TCR⁺ IELs are likely to derive from CD3⁺CD4⁺CD8⁺ triple-negative thymocytes^{27,49}, but an intestinal origin for $\gamma\delta$ -TCR⁺ IELs has not been ruled out by these studies. Interestingly, it has recently been shown that ($\alpha\beta$ -TCR⁺) DP thymocytes regulate the differentiation of $\gamma\delta$ T cells in the thymus by a lymphotoxin- β receptor (LT- β R)-dependent mechanism, and it was suggested that cryptopatch cells might similarly regulate the differentiation of intestinal $\gamma\delta$ T cells⁵⁰.

Do cryptopatches contain LTi-like cells? If cryptopatches do not contain T-cell precursors, then what are $\text{Lin}^{-}\text{cKIT}^{+}\text{IL-7R}\alpha^{+}$ cryptopatch cells? These cells express the nuclear hormone receptor ROR- γ ¹², as do DP thymocytes in the adult and LTi cells in the fetus²⁰. LTi cells are among the first haematopoietic cells to colonize developing lymph nodes and Peyer's patches (in regions known as the lymph-node and Peyer's-patch anlagen)^{20,51,52}; this occurs 1 week before birth and days before lymphocytes are recruited and segregate into B- and T-cell zones. LTi cells also express the membrane-bound heterotrimer lymphotoxin- $\alpha_1\beta_2$ (LT- $\alpha_1\beta_2$)^{51,52}, which is essential for the activation of local mesenchymal cells through the LT- β R^{53–56}. Activated mesenchymal cells are then involved in the formation of mature lymphoid tissues by recruiting (presumably through chemokines and adhesion molecules) new waves of LTi cells and, at a later stage of ontogeny, B cells, T cells and DCs¹⁶ (FIG. 2). Remarkably, fetal LTi cells, clustered in the lymph-node and Peyer's-patch anlagen, and adult intestinal $\text{Lin}^{-}\text{cKIT}^{+}\text{IL-7R}\alpha^{+}$ cells, clustered in cryptopatches, express an identical set of markers¹². In addition, the generation of both cell types depends on expression of ROR- γ and the inhibitor of basic helix-loop-helix transcription factors ID2 (inhibitor of DNA binding 2), but it is independent of RAG proteins and LT- α . There is also data that indicates a direct lineage relationship between fetal LTi cells and intestinal $\text{Lin}^{-}\text{cKIT}^{+}\text{IL-7R}\alpha^{+}$ cryptopatch cells. Although LTi cells are abundant in lymph-node and Peyer's-patch anlagen before birth, there are few of these cells present within days of birth, and they cannot be detected in the

Figure 2 | The function of lymphoid-tissue inducer cells. a | Lymphoid-tissue inducer (LTi) cells are required for initiation of lymph-node and Peyer's-patch development in the fetus. They were originally described as CD3⁺CD4⁺CD45⁺ cells⁹⁶, and they are derived from fetal liver-cell precursors^{97,98}. These cells are recruited to the lymph-node and Peyer's-patch anlagen, probably by chemokines — such as CXC-chemokine ligand 13 (CXCL13), which interacts with CXC-chemokine receptor 5 (CXCR5) — and they interact with specialized mesenchymal cells, through cell-surface-expressed α_4 -integrins¹⁶. LTi cells express lymphotoxin- $\alpha_1\beta_2$ (LT- $\alpha_1\beta_2$), the expression of which is upregulated by interleukin-7 (IL-7) or other ligands of the IL-7 receptor α -chain (IL-7R α)⁶⁴, and they activate mesenchymal cells through the LT- β receptor (LT- β R). As a result, mesenchymal cells produce more chemokines (including CXCL13, CC-chemokine ligand 19 (CCL19) and CCL21), express intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1), and establish the first positive-feedback loop for the recruitment of LTi cells. A few days before birth, lymphocytes and dendritic cells (DCs) are recruited to the lymph-node and Peyer's-patch anlagen. Because T cells and B cells themselves express LT- $\alpha_1\beta_2$ (REF. 80), recruitment of lymphocytes is amplified by a second positive-feedback loop and culminates in the formation of a mature lymph node or Peyer's patch, with segregated T-cell zones and B-cell follicles, high endothelial venules (HEVs) and additional specialized mesenchymal cells. **b** | Evidence indicates that the formation of isolated lymphoid follicles in the young and adult intestine recapitulates the fetal development of lymph nodes and Peyer's patches¹².

Figure 3 | The formation of cryptopatches and isolated lymphoid follicles in the intestine of young mice. After birth, lymphoid-tissue inducer (LTi)-like Lin⁻cKIT⁺interleukin-7 receptor α -chain (IL-7R α)⁺ cells are present in the lamina propria of the small intestine and the colon, and they express the nuclear receptor ROR- γ t (retinoic-acid-receptor-related orphan receptor- γ t)¹². Frozen sections were prepared from the intestines of mice expressing the enhanced green fluorescent protein under control of the ROR- γ t promoter; therefore, Lin⁻cKIT⁺IL-7R α ⁺ cells expressing ROR- γ t were visible under fluorescent light (green). These sections were then stained to visualize CD11c, present at the cell surface of dendritic cells (DCs) (red), and B220, present at the cell surface of B cells (blue). One week after birth, ROR- γ t⁺ cells are scattered throughout the lamina propria, but during the second week, they cluster into cryptopatches, together with DCs. At the beginning of the third week, cryptopatches in the colon, but not in the small intestine, seem to differentiate into small isolated lymphoid follicles (ILFs) as they recruit B cells, and they develop further into large ILFs at the end of the third week. The formation of ILFs depends on the presence of gut flora, tumour-necrosis-factor receptor 1, IL-7R α and lymphotoxin- α , β ₂. The generation of Lin⁻cKIT⁺IL-7R α ⁺ cells, as well as the formation of cryptopatches and ILFs, depends on ROR- γ t.

lymph nodes and Peyer's patches of adults^{20,51}. However, isolated LTi cells are found in the intestinal lamina propria after birth, and they cluster into cryptopatches at 1–2 weeks of age¹² (FIG. 3). Therefore, it has been proposed that Lin⁻cKIT⁺IL-7R α ⁺ cryptopatch cells are the adult counterpart of fetal LTi cells¹².

Organizers of lymphoid tissue

I propose a novel hypothesis for the function of cryptopatches. Because cryptopatches do not generate T cells but, instead, might contain LTi-like cells, I suggest that cryptopatches are adult organizers of intestinal lymphoid tissue. Lin⁻cKIT⁺IL-7R α ⁺ cryptopatch cells might induce the formation of ILFs and might recapitulate the fetal induction of lymph-node and Peyer's-patch development by LTi cells^{16,21} (FIG. 2).

Several lines of evidence support this hypothesis. First, as mentioned earlier, intestinal Lin⁻cKIT⁺IL-7R α ⁺ cells cluster into cryptopatches during the second week after birth¹². At the beginning of the third week, the number of cryptopatches in the colon decreases (FIG. 3), and small ILFs appear, which develop into large ILFs at the end of the third week, shortly before weaning^{9,10} (FIG. 3). Second, in ROR- γ t-deficient mice, the lack of lymph nodes and Peyer's patches is a consequence of the absence of fetal LTi cells²⁰. ROR- γ t-deficient mice also fail to generate adult intestinal Lin⁻cKIT⁺IL-7R α ⁺ cells, as well as

cryptopatches and ILFs¹². Third, lymph-node and Peyer's-patch development requires the expression of LT- α β ₂ by LTi cells^{20,52,57}. By contrast, the formation of ILFs is independent of LT- α β ₂ expressed by fetal LTi cells but requires LT- α β ₂ after birth^{9,10,58}. Together, these data indicate that Lin⁻cKIT⁺IL-7R α ⁺ cells in cryptopatches are required for the formation of ILFs by an LT- α β ₂-dependent mechanism.

Additional factors that are involved in the induction of lymph-node and Peyer's-patch development by LTi cells also seem to be involved in ILF formation. For example, IL-7R α is required for the development of lymph nodes and Peyer's patches^{59,60}, as well as for the formation of ILFs⁹. Both LTi cells and Lin⁻cKIT⁺IL-7R α ⁺ cryptopatch cells express IL-7R α ^{18,51,61}, and signalling through this receptor has been shown to induce upregulation of LT- α β ₂ expression^{57,60,62}. In the fetus, IL-7 is expressed in the thymus, in the liver, in the intestine⁶³ and by the specialized mesenchymal cells of the Peyer's-patch anlagen⁶⁴. In the adult gut, IL-7 is produced by the epithelium^{25,65}. Nevertheless, because the development of Peyer's patches is initiated in IL-7-deficient mice but not in IL-7R α -deficient mice, it has been proposed that additional ligands of IL-7R α are involved in lymphoid-tissue development⁶⁴. Furthermore, fetal LTi cells activate the mesenchymal cells of lymph-node and Peyer's-patch anlagen to

express intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1)^{16,20}. Whereas cryptopatches do not express (or express only low levels of) ICAM1 and VCAM1, small immature and large mature ILFs express high levels of these adhesion molecules^{12,58}, implying that the mesenchymal cells in these ILFs are activated. Finally, tumour-necrosis factor (TNF) receptor 1 (TNFR1), which is involved in the development of Peyer's patches¹⁶, is also required for the formation of ILFs¹⁰. As well as TNF, other TNF-superfamily members — including LT- α ₃, TRANCE (TNF-related activation-induced cytokine) and LIGHT — and CXC-chemokine ligand 13 (CXCL13; also known as BLC) have also been shown to be involved in lymph-node and Peyer's-patch development¹⁶. It will be important to assess whether these factors are also involved in the formation of ILFs and whether the proposed differentiation of cryptopatches into ILFs recapitulates all of the aspects of lymph-node and Peyer's-patch development (FIG. 4).

Cryptopatches as inducible organizers

Despite these similarities, there is a fundamental difference between the formation of ILFs after birth and the fetal development of lymph nodes and Peyer's patches. The development of lymph nodes and Peyer's patches is initiated at precise time points and locations during fetal ontogeny¹⁶, independently of microbial stimulation. By contrast, the formation of ILFs is induced by intestinal bacteria^{9,10} or inflammation^{14,66,67} (FIG. 4). That is, lymph nodes and Peyer's patches are programmed secondary lymphoid tissues, whereas ILFs are inducible tertiary lymphoid tissues. Primary lymphoid tissues are sites of lymphocyte generation, such as the liver in the fetus and the bone marrow and thymus in the adult. Therefore, although Lin⁻cKIT⁺IL-7R α ⁺ cryptopatch cells might be functional equivalents of LTi cells, unlike fetal LTi cells, they seem to require activation to induce the formation of ILFs. For example, because the development of an intestinal flora is required for the formation of mature ILFs in young mice^{9,10}, it is possible that bacteria induce the expression of cytokines by the epithelium, by macrophages or by DCs present in the epithelium, lamina propria or cryptopatches. These cytokines could then activate the LTi-like function of Lin⁻cKIT⁺IL-7R α ⁺ cryptopatch cells.

The formation of ILFs in the colon after birth favours the hypothesis that cryptopatches are inducible organizers of ILFs. Cryptopatches are abundant in the small intestine of adult mice but are rare in the colon¹⁸.

Conversely, ILFs are abundant in the colon (REF. 12, and G.E., unpublished observations) but are found only in particular regions of the small intestine, such as the anti-mesenteric wall and the ileum^{9–11}. As discussed earlier, histological analysis indicates that, during the third week of life, colonic cryptopatches differentiate into ILFs (FIG. 3). Because the number of gut bacteria increases during the first weeks of life and is highest in the ileum and the colon, these observations indicate that ILFs develop from cryptopatches in response to an increased load of luminal bacteria. Furthermore, an abnormally high load of luminal bacteria can lead to ILF hyperplasia. Mice that are deficient in activation-induced cytidine deaminase (AID), which is required for somatic hypermutation and class-switch recombination of immunoglobulin genes, fail to produce IgA and affinity-matured IgM⁶⁸. As a consequence, these mice have 100-fold more bacteria present in the upper parts of the small intestine (the duodenum and jejunum), as well as increased numbers of ILFs that are also markedly hyperplastic. A similar phenotype is found in patients with a deficiency in AID⁶⁹. Conversely, germ-free mice have only immature ILFs that mainly contain Lin[−]cKIT⁺IL-7R α ⁺ cells⁹ and are structurally similar to cryptopatches.

The formation of ILFs not only is inducible but also is reversible. Adult mice treated with an LT- β R-immunoglobulin fusion protein (a decoy receptor for LT- $\alpha_1\beta_2$) have no ILFs in the small intestine, and mice treated with a TNFR1-immunoglobulin fusion protein (a decoy receptor for TNF and LT- α_3) have a 2–3-fold decrease in the number of ILFs that are present⁷⁰. Furthermore, treatment of AID-deficient mice with antibiotics to reduce the intestinal bacterial load abolishes ILF hyperplasia and reduces the number of ILFs⁶⁸. These observations show that signalling through TNFR1, as well as signals that are mediated by bacteria and LT- $\alpha_1\beta_2$, not only are required for the formation of ILFs but also are essential for their maintenance.

Mechanisms for the formation of ILFs

The gut flora induces ILF formation, and TNFR1 ligands (such as TNF and LT- α_3) are also required for this process^{9,10}. These observations could indicate that the formation of ILFs involves Toll-like receptors (TLRs), which bind microbial components and induce expression of pro-inflammatory factors, such as TNF, type I interferons, T-helper-1-cell cytokines and prostaglandins⁷¹. TLRs are expressed by many cell types, including intestinal epithelial cells, DCs and macrophages.

Intestinal epithelial cells express varied levels of TLR2 (a receptor for lipoproteins and glycolipids), TLR3 (a receptor for double-stranded RNA), TLR4 (a receptor for lipopolysaccharide and heat-shock proteins) and TLR5 (a receptor for flagellin)^{71–74}. DCs express different types of TLR depending on the subset and their state of maturity, whereas macrophages express most TLRs⁷¹. Given this, DCs that are present in cryptopatches (constituting 20–30% of cryptopatch cells) might bind bacterial products or components through TLRs, after the penetration of these molecules or live bacteria into the lamina propria. Additional mechanisms could be involved in the detection of intestinal bacteria and the production of pro-inflammatory signals, such as the uptake of live bacteria⁷⁸ or bacteria opsonized with IgA⁷⁵ by neutrophils, myeloid cells and DCs.

So, in the model proposed here, how would cryptopatches integrate pro-inflammatory signals and induce the formation of ILFs? Cryptopatches are organized as a central cluster of Lin[−]cKIT⁺IL-7R α ⁺ cells (which constitutes 70–80% of cryptopatch cells) and a ring of DCs¹⁸. TNF that is produced by various cells that have been activated through TLRs, or by DCs in cryptopatches, might directly activate Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells. In an indirect manner, TNF might also induce the

maturation of DCs⁷⁶ in cryptopatches, which would then activate Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells. As a consequence, activated Lin[−]cKIT⁺IL-7R α ⁺ cryptopatch cells might upregulate expression of LT- $\alpha_1\beta_2$, and similar to the function of LTi cells in the development of lymph nodes and Peyer's patches¹⁶ (FIG. 2), this could then induce LT- β R-expressing cryptopatch mesenchymal cells to secrete lymphocyte-recruiting chemokines — such as CXCL13, CC-chemokine ligand 19 (CCL19; also known as ELC) and CCL21 (also known as SLC) — and to express high levels of ICAM1 and VCAM1. Similar to lymph-node and Peyer's-patch development, this would culminate in the formation of an organized lymphoid tissue (FIG. 4).

Not only can both intestinal flora and pathogens induce the production of TNF, but high levels of TNF are also produced during inflammatory bowel disease (IBD). This cytokine is an important factor in IBD, because administration of TNF-specific antibody is now a standard treatment for IBD⁷⁷. In chemically⁶⁶ or genetically⁷⁸ induced colitis in mice, ILFs become enlarged and are increased in number. Moreover, in ulcerative colitis and Crohn's disease in humans, hyperplastic lymphoid aggregates have been observed in the colonic lamina propria, and these mainly

Figure 4 | The formation of isolated lymphoid follicles. Fetal intestinal lymphoid-tissue inducer (LTI) cells might persist in the lamina propria of newborns, where they could cluster into cryptopatches, together with dendritic cells (DCs), during the second week of life. The formation of cryptopatches depends on the interleukin-7 receptor α -chain (IL-7R α) and on lymphotoxin- $\alpha_1\beta_2$ (LT- $\alpha_1\beta_2$), which is possibly expressed by LTI-like cells, but it is independent of the gut flora^{12,18,58}. In response to a growing local gut flora, colonic cryptopatches differentiate into isolated lymphoid follicles (ILFs) during the second to third week of life^{9,10} (FIG. 3). The formation of ILFs requires LTI-like cells, LT- $\alpha_1\beta_2$ and IL-7R α , as well as the gut flora and tumour-necrosis factor (TNF)^{9,10,12}. It remains to be determined whether chemokines and adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1), expressed by mesenchymal cells are required for ILF formation, similar to lymph-node and Peyer's-patch development (FIG. 2). Increased numbers of ILFs, which are also hyperplastic, form in the small intestine in response to excessive numbers of gut flora⁶⁸ or during inflammation^{14,66,67,79}, both of which are perturbations that could result in the production of TNF. Pathogens, such as *Salmonella* spp. and *Yersinia* spp., are taken up by DCs present in ILFs and can elicit antigen-specific IgA responses¹⁵. ILFs might also produce IgA to maintain homeostasis of the gut flora, as well as in response to inflammation. M cell, microfold cell.

contain B cells, T cells and DCs^{14,67,79}. These structures are similar to ILFs, and they indicate that the large amount of TNF produced during IBD might induce the hyperplasia of ILFs. It should be noted that TNF and other pro-inflammatory factors (such as LT- α , β_2 and LT- α_3) can be expressed by various cells, including activated T and B cells that are recruited to the site of chronic inflammation^{80,81}. So, it is possible that these activated cells induce ILF formation independently of Lin⁻cKIT⁺IL-7R α ⁺ cryptopatch cells.

Role of cryptopatches and ILFs

From the structural similarity between ILFs and B-cell follicles in lymph nodes and Peyer's patches, and from the location of ILFs beneath the intestinal epithelium^{9,10,12}, it can be inferred that ILFs have a role in the intestinal immune system that is similar to that of Peyer's patches: that is, they are immune inductive sites. Indeed, it has recently been reported that ILFs can induce the production of antigen-specific IgA^{15,70}. However, in contrast to Peyer's patches, ILFs are inducible structures^{9,10,68}. Their size varies from clusters that are the size of cryptopatches (10²–10³ cells), in the lower part of the normal small intestine, to clusters that are approximately one-quarter the size of normal Peyer's-patch follicles, in Peyer's-patch-deficient mice¹⁰ and in the colon¹². Most markedly, in AID-deficient mice, ILFs in the upper small intestine reach

the size of normal Peyer's-patch follicles (10⁵–10⁶ cells)⁶⁸. On average, a mouse small intestine has 8–12 Peyer's patches, each of which contains 5–7 B-cell follicles; therefore, there are a total of 40–84 follicles, which contain a total of 0.5 $\times$ 10⁷–10⁸ cells, in the small intestine. By contrast, an estimated 150–300 ILFs are present in the small intestine^{9,10}, which contain a total of $\sim$ 10⁶ cells in normal mice and almost 10⁸ cells in AID-deficient mice⁶⁸, thereby possibly outnumbering the cells present in the Peyer's patches. Therefore, in response to a new threat, the inducible nature of ILFs allows the mobilization of a large number of lymphocytes (mainly B cells⁹) in addition to those that are already present in the Peyer's patches and mesenteric lymph nodes.

ILFs are inducible^{9,10,68} and reversible lymphoid tissues^{68,70}, which can generate IgA-mediated antigen-specific responses^{15,70}. Because these responses quench their own source of induction, ILFs might self-regulate their number and size. For example, an increase in the number of bacteria present in the lumen induces an increase in the number and size of ILFs, which in turn generates an IgA-mediated response that targets the bacteria. However, as bacteria are neutralized, the induction of ILFs decreases, leading to an equilibrium between bacteria and ILFs. In AID-deficient mice, the number of luminal bacteria present in the small intestine is

100-fold greater than in normal mice; in response, the number and size of ILFs are also markedly increased⁶⁸. Again, both parameters stabilize and reach an equilibrium; however, this is different from the equilibrium in normal mice. Nevertheless, AID-deficient mice have seemingly normal intestinal functions^{68,82} and have no intestinal inflammation, showing the exceptional flexibility of intestinal structure and function. Cryptopatches and ILFs might therefore have an essential role in the equilibrium that is reached between the gut flora and the immune system.

The specific role of cryptopatches and ILFs in immunity to intestinal pathogens and in establishing tolerance is starting to be addressed. Lorenz and Newberry¹⁵ have recently shown that ILFs can induce the production of *Salmonella typhimurium*-specific IgA after oral infection with these bacteria. They also showed that *Yersinia enterocolitica* is found in mature ILFs 24 hours after oral infection. It will be important to assess further the role of cryptopatches and ILFs during infection with intestinal pathogens (such as *Shigella* spp., *Toxoplasma gondii* and reoviruses^{1,83}) and to determine the involvement of cryptopatches and ILFs in the establishment of tolerance to orally administered antigens^{84,85}.

By contrast, cryptopatches and ILFs might also be involved in intestinal immunopathology. As ILF hyperplasia is induced during IBD^{14,66,67,79}, it is possible that cryptopatches and ILFs exacerbate IBD pathology by establishing a positive-feedback loop for inflammation. In this case, if the differentiation of cryptopatches into ILFs could be inhibited, this could lead to a novel class of treatments for IBD. For example, because ROR- γ t is specifically expressed by intestinal Lin⁻cKIT⁺IL-7R α ⁺ cells (and by immature thymocytes)¹² and because it is required for the formation of cryptopatches and ILFs, it has been proposed that ROR- γ t could be a pharmacological target for the treatment of IBD¹².

Conclusions

In this Opinion article, I have developed the idea that cryptopatches are intestinal 'sensors' of the gut flora, as well as of intestinal infection and injury. Pro-inflammatory signals might activate LT α i-like cryptopatch cells to induce the formation of ILFs, recapitulating the fetal pathway of lymph-node and Peyer's-patch development. The intestinal cryptopatch–ILF pathway could thereby ensure the formation of the number of inductive lymphoid tissues that is appropriate to the number of luminal bacteria or to the challenge posed by

Glossary

ANTI-MESENTERIC BORDER

The mesentery is the membrane that attaches the small intestine to the back of the abdominal cavity. It also contains vessels that carry lymph and blood towards and away from the intestine. The anti-mesenteric border of the intestine is opposite to where the mesentery is attached.

B-CELL FOLLICLE

An aggregate of B cells in lymphoid tissues. It contains naive B cells, as well as activated, proliferating and maturing B cells in germinal centres. B-cell follicles are contiguous with T-cell zones.

COLONIC PATCHES

Lymphoid tissues that are similar to Peyer's patches and are present in the mucosa of the colon. Owing to the thick colonic muscle layer, colonic patches are not readily visible at the surface of the colon.

CRYPTS

Crypts are tubular invaginations of the intestinal epithelium. At the base of the crypts, there are Paneth cells, which produce bactericidal defensins, and stem cells, which continuously divide and are the source of all intestinal epithelial cells. Contiguous with the crypts are the villi. These are projections into the lumen that have an outer layer that mainly consists of mature, absorptive enterocytes but also contains mucus-secreting goblet cells.

FOLLICLE-ASSOCIATED EPITHELIUM

(FAE). The epithelium that overlies mucosal lymphoid tissues, such as the Peyer's patches and the isolated lymphoid follicles in the intestine. Lymphoid tissues induce the differentiation of normal intestinal epithelium into FAE, which is specialized in antigen capture and transport.

INTRAEPITHELIAL LYMPHOCYTES

(IELs). Intraepithelial lymphocytes are T cells that reside on the basolateral side of the intestinal epithelium. They express either an $\alpha\beta$ -TCR (T-cell receptor) or a $\gamma\delta$ -TCR, as well as the CD8 $\alpha\alpha$ homodimer. This is in contrast to most conventional CD8⁺ T cells, which express the CD8 $\alpha\beta$ heterodimer. CD8 $\alpha\alpha$ is a ligand of the non-classical MHC class I molecule thymus leukaemia antigen (TL), which is expressed by the intestinal epithelium.

MICROFOLD CELLS

(M cells). Differentiated epithelial cells that are present in follicle-associated epithelium. They transport antigens from the intestinal lumen into lymphoid tissues. Mucosal lymphoid tissues induce the differentiation of epithelial cells into M cells.

PEYER'S PATCHES

Protruding lymphoid tissues that are present in the mucosa of the small intestine. In mice, they are composed of five to seven closely packed B-cell follicles. They are named after Johann Conrad Peyer (1653–1712), a Swiss anatomist.

infection or injury. In addition, cryptopatches might regulate the differentiation of intestinal T cells^{24,40,50}.

Tertiary lymphoid tissues also form in chronic autoimmune lesions^{86,87} and in the lungs after infection with influenza virus⁸⁸. In the latter case, however, the formation of inducible bronchus-associated lymphoid tissues is independent of ROR- γ t, so it presumably does not require LTi-like cells. It is possible that, during infection and chronic inflammation, the recruitment of high numbers of activated T and B cells expressing LT- α β , TNF⁸⁰ or other pro-inflammatory factors bypasses the requirement for LTi-like cells in the formation of tertiary lymphoid tissue.

So, the intestine might have maintained the unique fetal characteristics of lymphoid-tissue development. Nevertheless, it is an optimal and physiological model to study the formation and the function of tertiary, inducible lymphoid tissues. Evolution of the intestinal cryptopatch–ILF network can be readily monitored and studied in response to bacterial colonization of the gut and perturbations in the gut flora, as well as in response to well-characterized pathogens and tumours, and during chronic inflammatory disease. Eventually, the cryptopatch–ILF model could generate knowledge that leads to the design of new therapies that increase specific immunity through the local formation of tertiary lymphoid tissues or that prevent chronic inflammation in IBD and autoimmunity.

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Competing interests statement

The author declares no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to Entrez Gene:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
CCL19 | CCL21 | CXCL13 | ICAM1 | IL-7 | IL-7R α | LT- α , β_2 | LT- β R | ROR- γ t | TNF | TNFR1 | VCAM1

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OPINION

A worm's eye view of the immune system: consequences for evolution of human autoimmune disease

David W. Dunne and Anne Cooke

Abstract | Humans and the many parasites that we can host have co-evolved over millions of years. This has been compared to an arms race in which the immune armoury of the human has evolved to deal with potential pathogens and the pathogen has evolved strategies to evade, and in some cases use, the immune system of the human host. Recently, there have been marked changes in the exposure of individuals in the developed world to both microorganisms and metazoan parasites, so the immune stimuli such organisms provide no longer have a role in our lives. As we discuss here, this is a marked perturbation, and the absence of the associated immunomodulation might have led to the increased emergence of autoimmune diseases.

Autoimmune disease arises when tolerance to self-tissue breaks down and pathology develops. With the exception of rare autoimmune diseases that result from mutations in a single gene, such as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED)¹, most autoimmune diseases are influenced by variations at several genetic loci and by environmental factors. A role for the environment in the development

of most autoimmune diseases is indicated by the less than 100% concordance of disease development in monozygotic twins; for example, the concordance rate for **type 1 diabetes** in monozygotic twins is only ~40% (REF. 2). The incidence of some autoimmune diseases, such as type 1 diabetes and **systemic lupus erythematosus**, has been markedly increasing in the developed world on a time-scale that rules out genetic change as an explanation. In the case of type 1 diabetes, which is a T helper 1 (T_H1)-cell-mediated autoimmune disease with juvenile onset, this is particularly important, as it was lethal until Banting and Best³ discovered insulin, in the 1920s. This implies that humans have retained potentially lethal allelic variants of certain genes, either because they have historically conferred a strong selective advantage or because they are in linkage disequilibrium with advantageous alleles. Although the increased incidence of type 1 diabetes could be attributed to exposure to a novel environmental agent, such as a virus, given the lethality of this autoimmune disease, it is more probable that the increase is a consequence of the current lack of infection, which forestalled the onset of diabetes in the past. In recent years, the following proposition has been gaining ascendancy: that, over the past