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REVIEW

# Human gut-associated lymphoid tissues (GALT); diversity, structure, and function

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Gut-associated lymphoid tissues (GALT) are the key antigen sampling and adaptive immune inductive sites within the intestinal wall. Human GALT includes the multi-follicular Peyer's patches of the ileum, the vermiform appendix, and the numerous isolated lymphoid follicles (ILF) which are distributed along the length of the intestine. Our current understanding of GALT diversity and function derives primarily from studies in mice, and the relevance of many of these findings to human GALT remains unclear. Here we review our current understanding of human GALT diversity, structure, and composition as well as their potential for regulating intestinal immune responses during homeostasis and inflammatory bowel disease (IBD). Finally, we outline some key remaining questions regarding human GALT, the answers to which will advance our understanding of intestinal immune responses and provide potential opportunities to improve the treatment of intestinal diseases.

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## INTRODUCTION

The human gut contains a diverse microbiota comprising approximately 40 trillion microorganisms[1] which provide essential benefits for human health, including prevention of colonization by pathogens[2], detoxification of bile acids[3], metabolism of non-digestible carbohydrates[4,5] and generation of key metabolites important for human health[6]. The intestinal immune system must respond appropriately to these beneficial microbes while providing active immunity against invading pathogens. Breakdown of this balance can lead to intestinal pathologies, including inflammatory bowel diseases (IBD; Crohn's disease (CD), and ulcerative colitis (UC)). Given these challenges, it is perhaps not surprising that the intestine contains the greatest number and diversity of immune compartments and immune cells in the body. Intestinal immune compartments can be broadly classified into inductive sites: the intestine-draining mesenteric lymph nodes (MLN) and the gut-associated lymphoid tissues (GALT)[7,8], in which adaptive immune cells undergo initial priming and differentiation, and effector sites: the intestinal lamina propria (LP) and epithelium in which primed adaptive immune cells localize and are maintained to promote barrier integrity and protective immunity.

The GALT of humans and mice includes both multi-follicular lymphoid tissues, such as Peyer's patches (PP) of the small intestine, and the far more numerous isolated lymphoid follicles (ILF) which are distributed along the length of the small and large intestines[7]. Additional types of GALT are specific to certain mammalian species, including the appendix in rabbits[9] and humans[10], the caecal and colonic patches in mice[11,12], and rectal lymphoid tissues in humans[13,14] and mice[15]. Most of our current understanding of the development, structure, and function of GALT comes from studies in mice (reviewed in[16]), and to what extent these observations are relevant to human GALT remains unclear. Limited understanding of human GALT is in part due to the inherent difficulty in obtaining human intestinal tissue, but also due to a lack of protocols allowing the isolation and analysis of human GALT. The current review focuses on human GALT, highlighting recent advances and discussing potential GALT functions in intestinal immune homeostasis and IBD. We compare findings to mouse GALT and highlight open questions regarding the importance of these enigmatic structures in human health and disease.

## MULTI-FOLLICULAR GALT OF THE HUMAN INTESTINE

Some large, multi-follicular GALT are visible to the naked eye without specific staining methods or magnification. The most prominent of these structures are the PP of the small intestine, first described by Konrad Peyer in 1677 as "distinct aggregated nodules"[17], together with the lymphoid follicles of the vermiform appendix, which were described during the 19th and early 20th century[18,19]. The following section will highlight our current understanding of the anatomy, location, and immune functions of these multi-follicular structures.

### Peyer's patches

Human PP contain tens to hundreds of individual follicles and are found on the anti-mesenteric wall (Fig. 1a), along the entire length of the small intestine. Their density increases towards the terminal ileum, where they form a lymphoid ring at the ileocecal junction[7,20–22]. Similar to murine PP development, which is initiated around day 16.5 of embryogenesis by local clustering of group 3 innate lymphoid cells (ILC3)[23], human PP development begins

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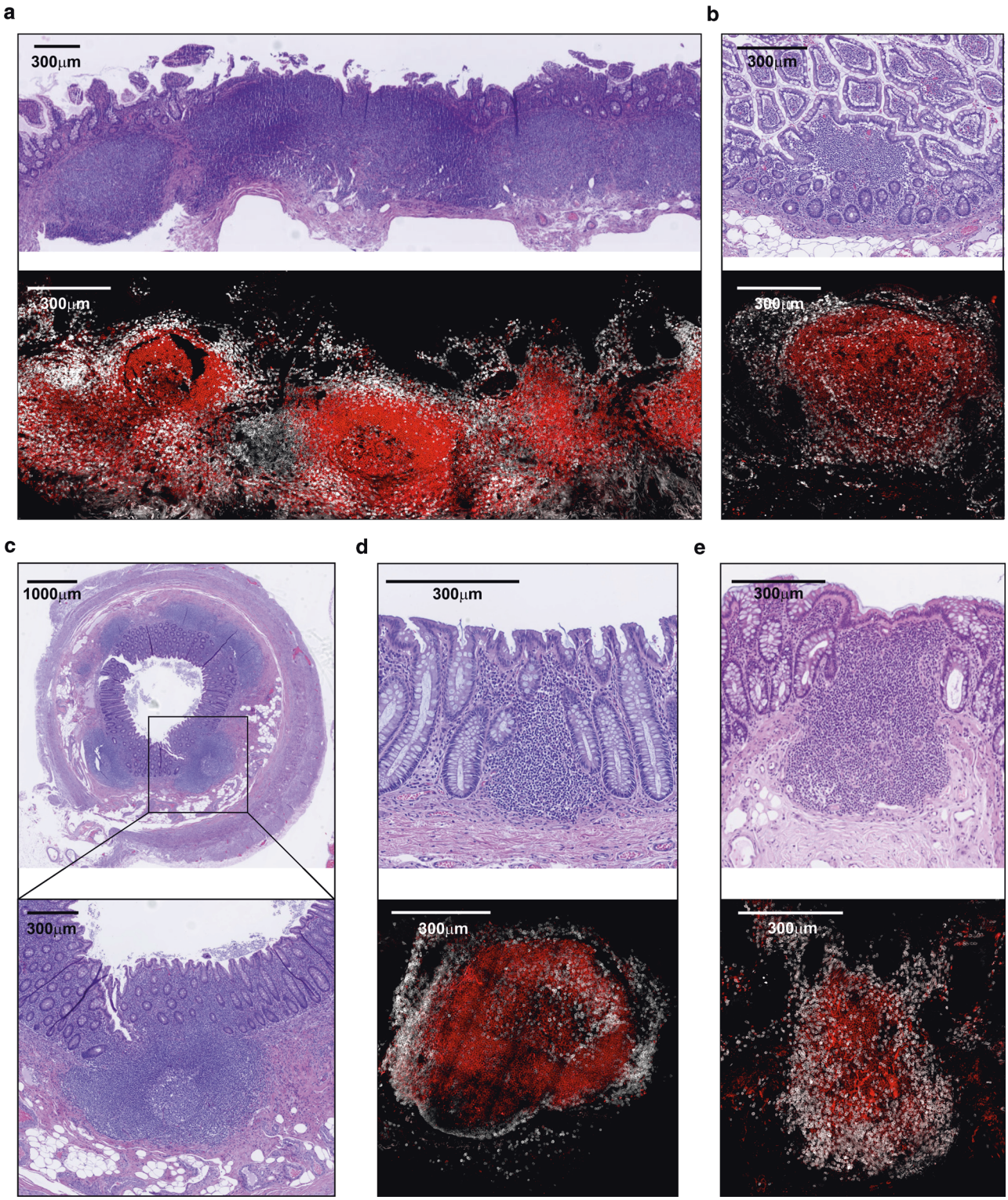


**Fig. 1 The structure, size and organization of human GALT.** H&E and immunohistochemical stainings of a human (**a**) Peyer's patch, (**b**) ileal mucosal ILF (M-ILF), (**c**) vermiform appendix, (**d**) sigmoid colon M-ILF and (**e**) sigmoid colon submucosal ILF (SM-ILF). The immunohistochemistry images depict $CD3^+$ T cells (white) and $CD19^+$ B cells (red), showing the general organization of GALT with a central B cell cluster and a surrounding marginal T cell zone. PP and appendiceal GALT typically exist as a chain of lymphoid follicles located in the mucosa and submucosa of the intestine. M-ILF and SM-ILF both consist of a solitary follicle, which for M-ILF is entirely located in the mucosa and for SM-ILF is mostly located in the submucosa possessing only a small interface with the intestinal lumen.

prenatally from around 14–16 weeks of gestation (reviewed in[24]). PP number and size continue to increase during childhood, peaking during early adolescence at around 240 PP, and then declining with age[20]. While the immune cell composition and function[25–27], as well as the size[20,28] of PP may change during aging, the full extent of these changes remains to be investigated (Box 1).

PP are dedicated adaptive immune priming sites and contain several specialized microanatomical niches for the efficient initiation and propagation of immune responses (Fig. 2, reviewed in ref. [29]). Overlying the luminal surface of PP is a specialized follicle-associated epithelium (FAE), which is sparsely covered by mucus but rich in specialized epithelial cells known as microfold (M) cells[30]. In mice, M cells have been shown to play a key role in shuttling luminal particulate antigens, including those from bacteria, viruses and SIgA-bound antigen into the PP parenchyma via transcytosis[31–35], and this process is required for optimal IgA responses to luminal antigen[36]. Whether M cells in the FAE of human PP perform similar functions remains to be demonstrated directly, but experiments with in vitro differentiated human M cells support this idea[37]. Human FAE also contains intraepithelial lymphocytes, although their function remains unclear[38,39]. On their basolateral side, M cells form pockets which allow direct interaction with the immune cells found in the underlying subepithelial dome (SED)[40]. In humans, these pockets are primarily inhabited by B and T cells, the former mostly expressing IgM (a third of which also express IgD), and the latter being primarily memory $CD4^+$ T cells[40]. The SED is dominated by professional antigen-presenting dendritic cells (DC)[41–43] and other myeloid cells[39,44], some of which have been observed to penetrate the basement membrane of the FAE[43] and which, in mice, extend dendrites through M-cell specific transcellular pores to sample luminal antigen[45]. The SED of human PP also contains diverse populations of memory $CD4^+$ T cells, and B cells of IgA, IgM, and IgG isotypes[38,44,46]. The latter include activated $FcRL4^+$ B cells[47], which secrete antibody in response to cytokines[48] and TLR-stimulation[49], rather than in response to BCR ligation[48,49]. Studies in mice have suggested that the SED may serve as a region of $CD4^+$ T cell priming[50,51] and DC-dependent IgA class-switching[52]. Activated B cells in the SED of mice have been shown to acquire antigen from M cells via their BCR and shuttle it to the germinal center (GC)[53], potentially promoting the induction of IgA responses. Whether such processes occur in the SED of human PP remains unclear.

Histological studies of human PP have shown that B cell follicles containing active germinal centers (GC) sit in close proximity to the SED[44,54–57]. Surrounding the GC is a mantle zone of naive $IgD^+$ B cells, which is in turn surrounded by a larger marginal zone containing memory B cells, predominantly of the IgA and IgM isotypes[44,58,59]. The marginal zone also contains $IgM^{hi}$ $IgD^+$ B cells[44], which are phenotypically similar to the splenic marginal zone B cells that generate innate-like responses to T-independent antigens[60,61].

Along with B cell-rich follicles, human PP contain perifollicular T cell zones which serve as reservoirs for naïve and memory T cells (Table 1)[25,62,63]. Memory $CD4^+$ T cells within the human PP are enriched in central memory $CD4^+$ T cells (Tcm), $FOXP3^+$ T regulator cells ($T_{reg}$) and T follicular helper cells ($T_{fh}$), and contain fewer poly-functional cytokine-producing cells compared with the surrounding mucosa[25,62]. In mice, lymphocytes enter PP from the circulation across high endothelial venules (HEV), in a process which requires expression of MAdCAM-1 by HEV and its ligand α4β7 on circulating lymphocytes[64–66]. HEV in human PP also express MAdCAM-1[63], which binds α4β7 on human lymphocytes[67], suggesting that similar processes may govern the entry of circulating lymphocytes into human PP. Consistent with this notion, vedolizumab (anti-α4β7) treatment of patients with mild IBD and concomitant HIV-1 infection was recently shown to reduce the size of GALT[68]. Finally, while human PP lack afferent lymphatics, diffuse efferent lymphatics are present on the submucosal side of PP and may act as exit points for immune cells[63,69].

In mice, PP GCs are considered key sites for the generation of intestinal antibody responses by providing specialized niches for B cells to undergo class switch recombination (CSR) and somatic hypermutation (SHM)[70,71]. The findings that intestinal CSR is restricted to PP[70], requires functional T cells[72] and CD40 signaling[70,71], and that intestinal $IgA^+$ plasma cells possess heavily mutated IgA heavy chains, indicative of SHM[73], suggest that plasma cells in murine PP are predominantly generated via GC- and T cell-dependent mechanisms. Nevertheless, $CD40^{-/-}$ mice, that lack PP GC and have reduced levels of CSR[70], are still able to generate normal levels of intestinal IgA[71]. Thus, under certain conditions PP may support T cell-independent IgA plasma cell generation. Similar to their murine counterparts, human PP are also considered key sites for the induction of intestinal antibody responses[59,74], a notion mainly derived from comparative analyses showing that human and murine PP have a similar organization and cellular composition[44,75,76]. Further indication that human PP contribute to the intestinal plasma cell pool comes from findings that IgA clones present within human ileal PP are overrepresented in the LP of the ileum, compared with the LP of the colon[62]. This also suggests that, as in mice[11,77–79], human PP may preferentially drive immune responses restricted to the small intestine. Consistent with this, we found that $IgA^+$ plasmablasts of the small intestinal LP, but not of the colonic LP, express the small intestinal homing receptor CCR9[62], which in mice is induced selectively on adaptive immune cells during their priming in PP and in small intestinal MLN[80–82]. These findings underscore the important role of PP as immune priming sites of the small intestine, and as a key source of small intestinal LP $IgA^+$ plasma cells.

PP are also associated with human B cell maturation, for example by supporting the clonal expansion and somatic diversification of the systemic marginal zone B cell subset[59]. Additionally, it has been suggested that traffic through PP contributes to the maturation of immature transitional B cells and may play a role in the deletion of autoreactive B cell clones[64].

## Vermiform appendix

The human vermiform appendix has long been considered a vestigial organ and appendectomy is not associated with any long-term negative effects[83]. Nevertheless, the appendix has been proposed to act as a "sanctuary" for intestinal bacteria by offering protection from diarrheal clearing and intestinal peristalsis[10,84]. It may also function as an immune cell priming site similar to other GALT[10,59,85,86]. Interestingly, as discussed further below, appendectomy is protective against ulcerative colitis[87–90], suggesting that this organ may retain some physiological functions.

Similar to PP development, appendiceal GALT development begins around gestational week 15–16[91,92], although the appendix itself already starts developing around gestational week 8[93]. While the processes driving the development and maturation of lymphoid structures in the appendix are not well understood, these structures mature in the years after birth into a chain of T cell- and B cell-rich lymphoid follicles embedded in the submucosa and LP (Fig. 1c)[10,25,59,91,94]. Like their counterparts in PP, appendiceal GALT follicles possess a dome structure facing towards the intestinal lumen, overlaid with an FAE containing M cells[95]. Distinct perifollicular T cell zones and central B cell follicles can be found[59], as well as monocytes and activated $CD86^+$ DC[25,59,96] (Table 1). The appendiceal GALT is enriched in naïve and $T_{cm}$ cells[25] and possesses GC[10,59], together with B cells at different stages of development and activation, implying that the vermiform appendix plays a role as an adaptive immune inductive site. As in PP, appendiceal GC contain a central cluster of GC B cells surrounded by a thin mantle zone containing naïve B cells, as well as a marginal zone containing marginal zone B cells and naïve B cells[59,97]. Memory B cells are

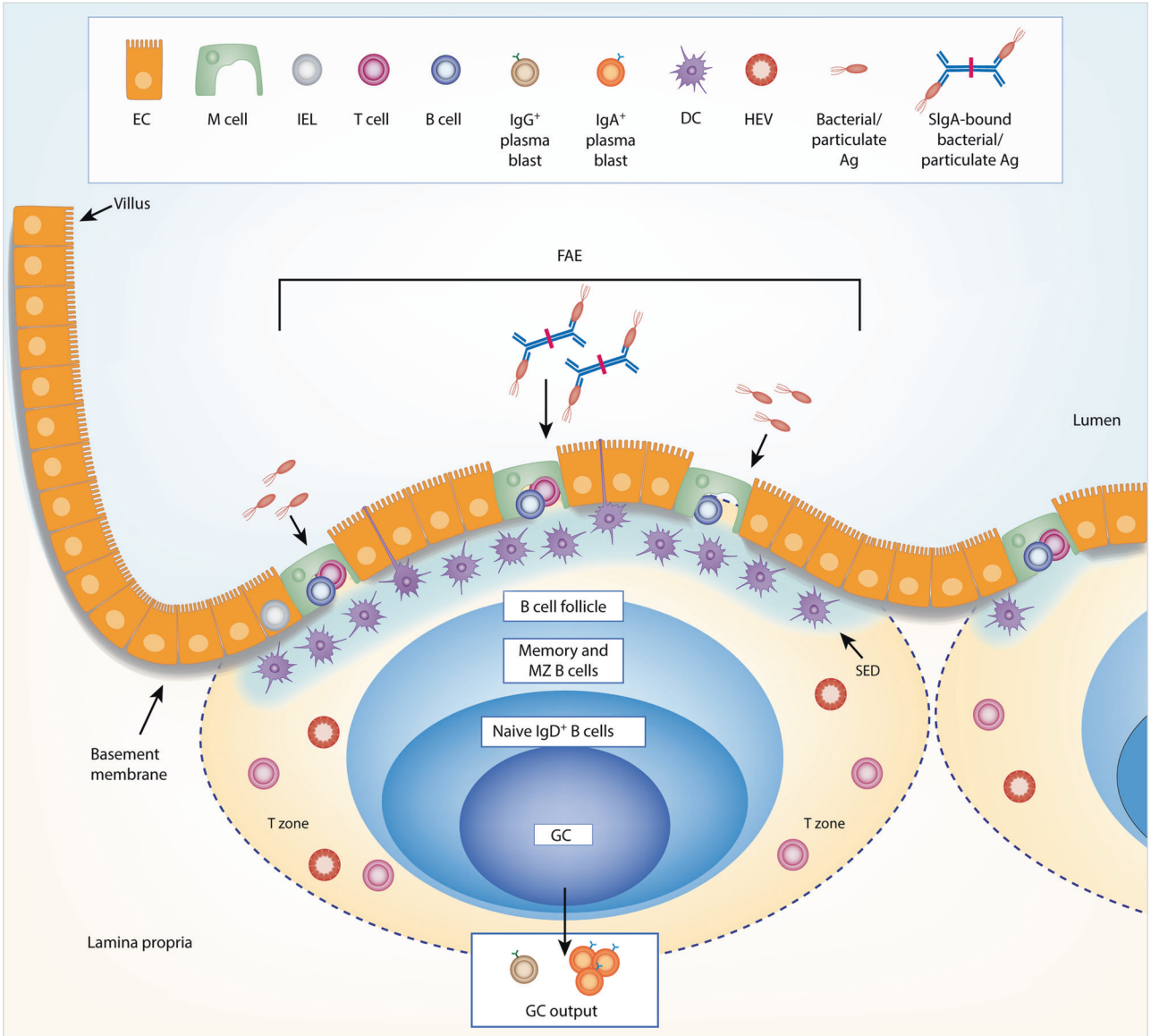


**Fig. 2 Cellular composition of a human PP follicle as example of human GALT.** PP follicles are in intimate association with the intestinal lumen and possess a specialized FAE containing M cells that shuttle free and IgA-bound antigen into the PP. Below the FAE, PP possess a SED region rich in antigen-presenting cells. Tightly associated with the SED is the underlying B cell follicle consisting of an outer marginal zone harboring memory and marginal zone B cells, a thin mantle zone accommodating naïve B cells, and a central GC, contributing to the generation of $IgA^+$ plasmablasts that seed the surrounding small intestine. B cell follicles of PP are separated by perifollicular T cell zones (dotted lines). FAE follicle-associated epithelium, SED subepithelial dome, GC germinal center, Ag antigen.

located in the SED and periphery of the follicle in proximity to the FAE[46,59]. Class-switched $IgA^+$ cells can be detected in appendiceal GALT, but they also contain memory $IgM^+$ B cells[86] and more $IgG^+$ B cells than the surrounding LP[46,98]. Thus, the appendix may serve as a local environment for class-switching and as a reservoir of class-switched memory B cells.

Together, these findings suggest that the human vermiform appendix represents a *bona fide* adaptive immune inductive site with a similar structure and immune cell composition to PP. It remains unclear whether immune responses initiated within this site differ from those in the PP, and whether the human appendix supports site-specific regional immunity within the intestine, as recently suggested for human PP[62].

### Other multi-follicular GALT

GALT consisting of more than one lymphoid follicle are relatively rare in the human large intestine during homeostasis, although a few poorly characterized multi-follicular structures have been described in the cecum and proximal colon[62], together with rare multi-follicular structures around the anal canal, sometimes referred to as the rectal tonsil[13,14]. The exact structure, function, development, cellular composition, and prevalence of these multi-follicular GALT remains unclear.

## ISOLATED LYMPHOID FOLLICLES

Consisting of a single lymphoid follicle with a diameter between 0.1 and 1.3 mm[62,99], ILF are substantially smaller than PP or the follicle chain of the vermiform appendix. However, as there are an estimated 30,000 ILF in the human intestine[100–103], they collectively constitute a major GALT compartment in humans. ILF are found along the length of the intestine, although their cellular composition, density and location within the gut wall varies between intestinal segments. Using novel techniques to

**Table 1.** Immune cell composition of human GALT.

| Cell | Subset | Small intestine | | Large intestine | | |
|---|---|---|---|---|---|---|
| | | Peyer's patch | M-ILF | Appendix | SM-ILF | M-ILF |
| **T cells** | CD3$^+$ | **36 (± 9)** [c] | **43 (20–69)** [f], **47 (± 9)** [c] | **ND** | **54 (41–62)** [f], **68 (± 10)** [c] | **59 (± 12)** [c] |
| | CD4$^+$ | 82 (± 6) [c] | 59 (37–77) [f], 79 [e], 80 (± 6) [c] | ND | 69 (55–83) [f], 88 (± 6) [c] | 81 (± 2) [c] |
| | *Naïve* | 7 (± 5) [d], 12 (± 14) [c] | 6 (± 6) [c] | 23 (± 15) [d] | 36 (± 18) [c] | 8 (± 8) [c] |
| | *CM* | 26 (± 11) [d], 49 (± 11) [c] | 49 (± 13) [c] | 39 (± 15) [d] | 52 (± 18) [c] | 44 (± 14) [c] |
| | *EM* | 38 (± 12) [c], 66 (± 16) [d] | 45 (± 16) [c] | 38 (± 18) [d] | 11 (± 7) [c] | 46 (± 29) [c] |
| | *Tfh* | 5 (± 4) [c] | ND | ND | 1 (± 1) [c] | ND |
| | *Treg* | 8 (± 4) [c] | ND | ND | 12 (± 5) [c] | ND |
| | CD8$^+$ | 18 (± 6) [c] | 13 (9–38) [f], 19 [e], 20 (± 6) [c] | ND | 12 (± 6) [c], 12 (3–19) [f] | 19 (± 2) [c] |
| | *Naive* | 8 (± 10) [c], 12 (± 4) [d] | 4 (± 4) [c] | 27 (± 17) [d] | 28 (± 13) [c] | 16 (± 19) [c] |
| | *CM* | 6 (± 4) [d], 15 (± 10) [c] | 18 (± 13) [c] | 7 (± 5) [d] | 28 (± 12) [c] | 23 (± 20) [c] |
| | *EM* | 65 (± 23) [c], 75 (± 11) [d] | 64 (± 21) [c] | 62 (± 21) [d] | 33 (± 9) [c] | 52 (± 30) [c] |
| | *EMRA* | 7 (± 6) [d], 11 (± 6) [c] | 14 (± 7) [c] | 4 (± 4) [d] | 10 (± 5) [c] | 11 (± 4) [c] |
| | TCRγδ$^+$ | ND | 1 (0–3) [f] | ND | 3 (0–6) [f] | ND |
| **B cells** | **CD19$^+$** | **57 (± 4)** [c] | **45 (24–57)** [f], **47 (± 14)** [c], **58** [e] | **ND** | **32 (± 6)** [c], **34 (18-64)** [f] | **39 (± 8)** [c] |
| | Naïve | 18 (8-48) [g], 23 (± 13) [c] | 28 (± 19) [c] | 25 (12-45) [g] | 23 (3-59) [g], 32 (± 19) [c] | 11 (± 9) [c] |
| | Memory | 50 (23-77) [g], 61 (± 14) [c] | 58 (± 14) [c] | 53 (24-73) [g] | 52 (13-78) [g], 65 (± 23) [c] | 64 (± 32) [c] |
| | GC | 12 (7-20) [g], 13 (± 11) [c] | 9 (± 7) [c] | 8 (1-24) [g] | 3 (± 3) [c], 10 (1-23) [g] | 7 (± 15) [c] |
| **Innate** | **Mast cells** | **1.9 (± 1.2)** [b] | **1.5 (± 1)** [b] | **ND** | **0.5 (± 0.25)** [b] | **1 (± 1.25)** [b] |
| | **Gran** | **2.7 (± 2)** [b] | **2 (± 2)** [b] | **ND** | **0.25 (± 0.25)** [b] | **1.25 (± 1)** [b] |
| | **DCs** | **0.024** [a], **0.6 (± 0.4)** [b] | **0.75 (± 0.5)** [b] | **0.055** [a] | **0.5 (± 0.25)** [b] | **1.5 (± 0.5)** [b] |
| | cDC1 | 25 (± 8) [a] | ND | 36 (± 15) [a] | ND | ND |
| | cDC2 | 62.5 (± 17) [a] | ND | 55 (± 9) [a] | ND | ND |
| | pDC | 12.5 (± 8) [a] | ND | 9 ± 4 [a] | ND | ND |
| | Mo/mac | **0.3 (± 0.25)** [a], **0.4 (± 0.3)** [b] | **0.5 (± 0.5)** [b] | **0.4 (± 0.2)** [a] | **0.5 (± 0.5)** [b] | **1.5 (± 0.5)** [b] |
| | ILC3 | **0.5 (± 0.25)** [b] | **1.75 (± 1.25)** [b] | **ND** | **1.25 (± 0.5)** [b] | **6.5 (± 3.5)** [b] |

Values represent mean percentages (± SD or range) based on flow cytometry analysis of indicated organs from non-inflamed intestinal tissues. Bold numbers represent percentage of CD45$^+$ cells and remaining numbers are a percentage of parent populations (underlined). Differences in cellular proportions between studies likely reflect variations between patient cohorts, gating strategy, and contamination with the surrounding tissue
*cDC* conventional dendritic cell, *CM* central memory, *DC* dendritic cell, *EM* effector memory, *GC* germinal center, *Gran* granulocytes, *ILC3* group 3 innate lymphoid cell, *M-ILF* mucosal isolated lymphoid follicle, *Mo/Mac* monocytes and macrophages, *ND* not determined, *pDC* plasmacytoid dendritic cell, *SM-ILF* submucosal isolated lymphoid follicle, *TCR* T cell receptor, *Tfh* T follicular helper, *Treg* T regulatory, *EMRA* effector memory re-expressing CD45RA. a = ref. [96], ileum and colon. b = ref. [99], terminal ileum and proximal colon. c = ref. [62], terminal ileum and proximal colon. d= ref. [25], ileum, distal PP, and colon. e = ref. [137], terminal ileum M-ILF, using numbers from non-allografted tissues. f = ref. [134], terminal ileum and colon; colonic ILF assumed to be SM-ILF. g= ref. [59], terminal ileum and colon; colonic follicles assumed to be SM-ILF. a–e, SD given in brackets. f and g, range given in brackets

identify and quantify ILF in the human intestine, we recently described the presence of distinct kinds of ILF, one of which resides solely within the mucosal LP (hereafter termed mucosal ILF, M-ILF), while the other protrudes into the overlying LP but resides mainly in the submucosa (hereafter termed SM-ILF)[62,99] (Fig. 1b, d, and e). The distribution of M-ILF and SM-ILF differs along the length of the intestine, with M-ILF largely restricted to the ileum and distal colon, while SM-ILF are distributed along the colon and are only rarely found in the ileum[62].

## ILF Development

While ILF have been identified in a wide range of mammalian species[104–107], our current understanding of their development derives primarily from studies in mice. Murine ILF development begins 1–3 weeks after birth with the formation of cryptopatches (CP)[108], which are pericryptal lymphoid aggregates whose development requires RORγt$^+$c-kit$^+$ ILC3, and which also contain CD11c$^+$ DC and VCAM1$^+$PDPN$^+$ mesenchymal stromal cells[12,72,109,110]. CP maturation into ILF depends on the sensing of intestinal AhR[111] and microbiota-derived ligands[112,113]. The transition to mature ILF is associated with the formation of a central B cell cluster making up to 50–70% of total cells[108,114,115], surrounded by a ring of ILC3 that constitute approximately 25% of total cells[114].

Whether CP-like structures exist in the human intestine remains unclear. This likely reflects difficulties in obtaining pediatric intestinal tissues for analysis[116], although it has also been suggested that humans may completely lack CP[104,117]. Structures described as "lymphocyte-filled villi" have been proposed as possible developmental precursors of mature human ILF[117] and, more recently, clusters of cKit$^+$CCR6$^+$ putative ILC3 were identified in the human colon and were suggested to be CP, although their cellular content and organization were not assessed in detail[118]. Of note, ILC3 are enriched in human M-ILF and SM-ILF compared with LP[99], suggesting that ILC3 may be involved in the development, maintenance and/or function of human ILF, as they are in mice[111,119,120]. The time window of ILF development differs substantially between species however: in the human intestine, lymphocyte accumulations reminiscent of ILF can be observed before and around birth[121–123], in line with a recent finding that stromal cells with a phenotype reminiscent of GALT stromal cells are also already detectable in the human fetal colon[124]. This suggests that a host microbiome is not required for the development of at least some human ILF.

The signals driving ILF development in the small intestine and colon in mice are partially distinct, with microbiota driving ILF maturation in the small intestine, but inhibiting this process in the colon[12,113]. Murine small intestinal ILF also uniquely depend on RANKL[125] and CXCL13[12], while colonic ILF development is uniquely promoted by IL-23 and suppressed by IL-25[113]. Although the role of these factors in human ILF development is unknown, these findings suggest that ILF development is distinct in different parts of the intestine, perhaps reflecting variation in the local microenvironment such as abundance of food or microbiota-derived metabolites including short chain fatty acids[126] and retinoic acid[127], as well as microbial load and diversity[7,128,129]. These factors together may contribute also to the distinct development and distribution of human M-ILF and SM-ILF along the length of the intestine.

After birth, ILF can be found at all ages in humans[25,101], with their total numbers peaking in the first few years of life[25,102], indicating a process of postnatal maturation. Importantly, none of the above-mentioned human studies distinguished M-ILF from SM-ILF, and whether these different structures develop during similar or distinct developmental stages remains to be determined. Future studies applying the techniques for isolating anatomically distinct GALT[99], in individuals of different ages, may help resolve these issues.

### Structure and composition of human ILF

Human M-ILF and SM-ILF both contain an FAE with putative antigen-sampling M cells[62,130,131], although M cell-dependent uptake of particulate luminal antigens has yet to be formally demonstrated in both mouse and human ILF. Underlying the FAE of both M-ILF and SM-ILF is an SED which is rich in $CD11c^+$ antigen-presenting cells[62], a central B cell follicle which may contain a GC and is encircled by T cells[62,130], a vascular network that includes both $MAdCAM\text{-}1^+$ and $MAdCAM\text{-}1^-$ vessels[40,44,62], and a surrounding lymphatic network[62,117,130]. Due to the lack of a clear boundary between ILF and the surrounding LP, there is a "mixed cell zone" at their interface, with decreasing cellular density away from the follicle[101,130,132,133]. More than 90% of cells within M-ILF and SM-ILF are lymphocytes, with a slightly higher proportion of T cells compared with B cells[62] (Table 1). This is in marked contrast to murine ILF, which consist mostly of B cells and only a few scattered T cells[104,108,109], although it should be noted that T cell numbers increase in murine ILF with ageing[115]. Most T cells in human ILF are $CD4^+$ and, similar to PP, both $CD4^+$ and $CD8^+$ T cell populations in ILF contain more $T_{cm}$ and naïve T cells than in the LP[25,62]. As in PP, memory $CD4^+$ T cells in ILF contain fewer poly-functional cytokine-producing cells and larger proportions of $FOXP3^+$ Tregs compared to surrounding LP[25,62]. Finally, both M-ILF and SM-ILF contain $PD\text{-}1^+$ putative $T_{fh}$ cells[25,62], consistent with the presence of GC. The B cell compartment of ILF is also distinct from that of the LP, as it lacks plasma cells and contains high proportions of naïve and memory B cells, as well as small numbers of GC and marginal zone-like B cells[59,62]. Clonal analysis suggests that memory B cells recirculate between different GALT[59] and consistent with this, the proportions of $IgM^+$, $IgG^+$, and $IgA^+$ cells within the memory B cell compartment of ILF are similar between ILF from the same patient[62].

Despite these similarities, the structure and cellular composition of M-ILF and SM-ILF differ in several aspects[62,117,130]. Anatomically, SM-ILF are located in mucosal invaginations with most of their mass in the intestinal submucosa, with only a small area in the mucosa accessible to the intestinal lumen[56,62,99,130]. On the other hand, M-ILF are fully located in the mucosa and present a large domed FAE surface to the lumen[56,62,99]. M-ILF are also on average smaller[132], but with a larger SED[130], contain more FAE intraepithelial lymphocytes[132], have a higher B:T cell ratio[132,134], higher frequencies of GC B cells[62], and are enriched in ILC3 compared with SM-ILF[99]. M cells present in the FAE of M-ILF but not SM-ILF express the mature M cell marker GP-2[62], which in mice has been shown to promote the uptake of type-1 fimbriated bacteria[135], indicating potential differences in M cell function between these sites. Additionally, SM-ILF contain a greater proportion of naïve T cells compared with both M-ILF and PP, even when comparing SM-ILF and M-ILF from the same intestinal segment[62]. Whether such differences in cellular composition reflect differences in the type or amount of luminal antigens sampled by these structures remains to be determined (Box 1).

### Function of human ILF in immune homeostasis

Murine ILF are thought to be an important source of T-cell-independent IgA responses[72], and a similar role has been proposed for human ILF on the basis that they contain dendritic cells and macrophages expressing *A proliferation-inducing ligand* (APRIL), as well as B cells expressing *Transmembrane activator* and *CAML interactor* (TACI)[41,121]. However, human intestinal $IgA^+$ clones show evidence of extensive somatic hypermutation[73,136], indicating that T-cell-independent IgA differentiation in humans is probably limited. Indeed the presence of Tfh cells[62,137], GC[56,117,122,137,138], follicular dendritic cells[62], *CD40L expression*[121], and high levels of the enzyme required for somatic hypermutation in GC B cells, activation-induced deaminase (AID)[41,86,121,138], suggest that human ILF act as key priming sites for T-cell-dependent B cell responses. Consistent with this notion, recent analyses of GC B cell populations indicate that ILF may support the generation of IgA1, IgA2, IgG, and IgM B cell responses[59,62,86]. Whether the acquisition of a particular isotype is influenced by an ILF's location within or along the length of the intestine, and the nature of the signals driving these different responses, remain to be determined (Box 1). Notably, we recently found that IgA clones present in colonic SM-ILF were overrepresented in the colonic LP compared with the ileal LP[62], indicating that colonic SM-ILF contribute to local B cell responses in the colon, reflecting the role of PP in the small intestine. Furthermore, we found that $IgA^+$ plasma cells in the colon, but not in the small intestine, expressed GPR15[62], a receptor which in mice has been shown to mediate T cell recruitment to the colon LP[139,140]. Whether GPR15 is induced on newly generated plasmablasts in colonic SM-ILF remains unclear.

Despite the above findings, many important questions remain about human ILF function in intestinal homeostasis (Box 1), including their contribution to the generation of effector and regulatory T cell responses, whether M-ILF and SM-ILF have similar or distinct immune functions, and the functional implications of the distinct distribution of M-ILF and SM-ILF along the intestine.

## GALT IN PROTECTION FROM MUCOSAL PATHOGENS

While studies in mice have demonstrated a key role for GALT in the induction of local immune responses and protection against mucosal pathogens[141–143], evidence that human GALT are involved in similar processes remains circumstantial. For example, M cells in GALT appear to be the principal route of entry for several human pathogens such as *Salmonella*[144], *Yersinia*[145,146], and *Shigella spp*[147], poliovirus[148], mycobacteria[149] and prions[150], and oral vaccines against many of these pathogens are more effective than their parenteral equivalents[151–155]. Addressing the direct involvement of human GALT in such processes is likely to be complicated by the difficulty in obtaining appropriate GALT material from infected or vaccinated individuals (Box 1).

## GALT IN IBD PATHOGENESIS

CD and UC are the two major subtypes of IBD, both involving chronic immune responses against the intestinal microbiota, and both associated with aberrant lymphocyte responses[156–159]. However, it is not known where and how these pathological

**Box 1:** Some remaining key questions in GALT research

While recent studies have substantially advanced our understanding of human GALT, many questions remain with potentially important implications for our understanding of intestinal immunity. These include:

- What signals drive the development and distribution of GALT structures along the intestine?
- What is the impact of ageing on GALT and the associated immune cell compartment?
- Do different types of GALT play distinct roles in intestinal immune responses?
- What antigens do distinct GALT sample and respond to, and does this change in IBD?
- What role do GALT play in the initiation and maintenance of IBD?
- What role do GALT play in immunity to intestinal pathogens?

responses are initiated. In the following paragraphs we discuss the potential role of GALT in the onset and perpetuation of IBD.

GALT in Crohn's disease

While CD can affect all layers of the gut wall at any site from the mouth to the anus, CD inflammation occurs most frequently in the ileum and proximal colon[160], and it is characterized by "skip lesions" in which areas of inflammation are interrupted by non-involved areas[160]. The earliest signs of CD which can be detected by colonoscopy are inflammatory lesions in the FAE of ILF and PP, known as aphthous or aphthoid ulcers[161–164]. These can progress into the large transmural lesions responsible for the characteristic symptoms and high morbidity of CD[161,164], suggesting that GALT may act as key sites of disease initiation in CD.

Recent single-cell RNA-sequencing studies have demonstrated that the affected intestine of CD patients is enriched in cell types associated with organized lymphoid structures, including HEV[158,165,166], naïve and memory B cells, $T_{reg}$, and naïve or central memory T cells[158]. Whether this is due to enlarged GALT or *de novo* generation of tertiary lymphoid organs (TLO) in the intestine of CD patients remains unclear, since there are currently no definite criteria to distinguish ILF from TLO (Box 2). Nevertheless, increased numbers of lymphocytes have been reported in the ILF of CD patients[133] and we have recently shown that presumptive SM-ILF are markedly enlarged in the inflamed large intestine of CD patients[99], indicating that ILF are sites of adaptive immune activation in CD. Whether these processes play a pathogenic role or help counteract inflammation in established CD remains an open question (Box 1). In support of the former, mutations in *NOD2*, which is a major genetic risk factor for CD, have recently been associated with increased retrograde transport of bacteria-IgA complexes from the lumen into PP, potentially through a direct effect on M cells[167], and perhaps promoting the inflammation of CD.

GALT in ulcerative colitis

UC usually starts in the rectum and spreads along the large intestine, but rarely progresses beyond the ileocecal junction into the small intestine[160,168]. In contrast to CD, skip lesions are not seen in UC, while the inflammation is normally restricted to the mucosal layer and does not involve GALT-associated aphthoid ulcers[160,163]. However, structures called "basal lymphoid aggregates" have been described in the inflamed mucosa of UC patients[169–173]. Consistent with this, the inflamed colon of UC patients is enriched in cells and molecules associated with organized lymphoid structures, including HEV[166], TNF-producing $T_{reg}$[156,159,165], $T_{CM}$[165], follicular B cells[159], the T cell-recruiting chemokine CCL19[174], and lymphoid tissue-associated stromal cells[159,174]. Additionally, the numbers and size of visible GALT have been shown to increase in UC-affected tissues[130,133,175]. However, it remains unclear whether these basal lymphoid aggregates represent enlarged ILF or inflammation-induced TLO (Box 2). UC is also often preceded by appendiceal inflammation, and surgical removal of the vermiform appendix correlates with a lower risk of the development of UC, as well as less severe symptoms after disease onset[87–90]. While further studies are required to assess potential causality, a possible explanation for these findings is that appendiceal GALT contributes to the generation of pathogenic adaptive immune responses in UC.

**Box 2:** The structure and functions of TLO

TLO are lymphocyte accumulations within peripheral tissues that develop *de novo* in response to local inflammation[177] and they can be found in all layers of the gut wall during IBD[177,178]. As TLO include structures with varying degrees of organization, sometimes including clearly-delineated T and B cell zones[177], it may be challenging to distinguish between ILF and intestinal TLO. Resolving the potential structural and functional differences between ILF and TLO, and whether they play similar or distinct immunological roles in IBD might reveal novel disease-driving mechanisms but will require careful analysis of their structure and cellular composition in the early diseased intestine.

Collectively, these studies suggest that human GALT could be involved in the initiation and perpetuation of inflammation in IBD, potentially through the generation of aberrant T cell[156,158,159,169] and B cell responses[157,158,176]. However, it remains possible that GALT activation in IBD is merely a consequence of sustained intestinal inflammation, rather than initiating and driving the inflammatory process (Box 1).

## CONCLUDING REMARKS AND REMAINING QUESTIONS

The existence of GALT within the human intestine has been known for centuries[17,101,102], and recent studies have substantially advanced our knowledge of their heterogeneity, abundance, composition, and function. Despite these findings, major knowledge gaps remain regarding their development and role in intestinal homeostasis, infection, and inflammatory disease (Box 1). Answering such questions will likely lead to an enhanced understanding of how immune responses are initiated and regulated within the human intestine, and with it the potential of identifying novel mucosal vaccination strategies and ways to treat chronic intestinal disease.

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## AUTHOR CONTRIBUTIONS

U.M., P.J., and T.F. and W.A. wrote the manuscript, which was revised and approved by all authors. N.B. designed the figures, J.S. discussed literature and gave input on the manuscript, L.R. acquired the histological images.

## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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