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Review

Generation of Immunoglobulin diversity in human gut-associated lymphoid tissue

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ABSTRACT

The organised gut associated lymphoid tissue (GALT) exists adjacent to an extensive and diverse luminal flora. The follicle associated epithelium and associated dendritic cells and lymphocytes form a tightly fortified gateway between the flora and the host that permits connectivity between them and chronic activation of the lymphoid compartment. As a consequence, plasma cell precursors are generated continuously, and in abundance, in GALT by clonal proliferation. Clonal proliferation alone on this scale would reduce the spectrum of B cell specificity. To compensate, GALT also houses molecular machinery that diversifies the receptor repertoire by somatic hypermutation, class switch recombination and receptor revision. These three processes of enhancing the diversity of mature B cells ensure that although clonally related plasma cells may secrete immunoglobulin side by side in the mucosa they rarely have identical antigen binding sites.

1. The conflicting requirements of an IgA response: quantity and diversity

Most immunological effector cells in the body are located on the mucosal front line; there are more plasma cells in the subepithelial connective tissue of the human intestinal lamina propria than in the rest of the immune system combined, and most of these secrete IgA. More than 3 g of IgA is transported into the human intestinal lumen daily [1–3]. The target of this IgA is luminal microbial flora that consists of more than 10^{12} bacteria per gram of lumen contents in the lower bowel, comprising over 1000 species, each of which is antigenically diverse [4,5]. Complexes of IgA bind to the flora forming a protective matrix of intestinal mucus, bacteria and antibody [6]. In order to achieve this equilibrium IgA, like the flora, must be not only abundant but also diverse. Clonal proliferation of plasma cell precursors occurs in order to achieve the observed number of IgA secreting cells, but proliferation alone would logically compromise diversity by generating more similar clonotypes. A potential consequence of chronic cellular proliferation is the generation of a 'bland' repertoire with limited diversity that would not match the diversity of the flora. In this review we will consider sites and mechanisms generating and disseminating the IgA response and generating diversity and potential specificity within it.

It is important to bear in mind that the mucosal immune system is highly active and dynamic. Gowans and Knight described a population of dividing cells in the thoracic duct lymph of rats, most of which is derived from the gut, that were destined to migrate and lodge in the intestinal lamina propria [7]. This paper, that became the seminal text for cellular mucosal immunology, provided the first evidence for chronic homing of intestinal plasma cell precursors. This concept was supported by subsequent studies that drained the thoracic duct lymph of rats and saw an acute fall in the intestinal plasma cell population [8,9]. The constant flow of intestinal plasma cell precursors via the thoracic duct lymph promotes the idea of dynamism in the mucosal immune system – of death and renewal of lamina propria effector cells – the renewal being facilitated by the major B and T cell proliferative compartment; the gut-associated lymphoid tissue (GALT) [10–12].

2. Gut associated lymphoid tissues and their relationship to the bacterial flora

Germ free animals and newborn humans have no IgA plasma cells in the lamina propria [13–15]. Colonisation, even with a single species, induces IgA plasma cell generation [16,17]. In humans,

Abbreviations: AID, activation-induced cytidine deaminase; APRIL, A-proliferation and inducing ligand; BAFF, B cell-activating factor belonging to the TNF family; BCMA, B-cell maturation antigen; CDR, complementarity determining region; D, diversity region; DC, dendritic cell; FAE, follicle associated epithelium; GALT, gut associated lymphoid tissue; HSPG, heparan sulfate proteoglycans; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; ILF, isolated lymphoid follicles; iNOS, inducible Nitric Oxide synthase; J, joining region; Kde, kappa deleting element; LGC, lymphoglandular complexes; LTi, lymphoid tissue inducer cells; M cells, microfold cells; MALT, mucosa-associated lymphoid tissue; NOD-1, nucleotide-binding oligomerisation domain 1; PP, Peyer's patches; RALDH1, retinaldehyde dehydrogenase 1; RAR, retinoic acid receptor; RSS, recombination signal sequence; TACI, transmembrane activator and calmodulin interactor; TLR, toll-like receptor; V, variable region; VIP, vasointestinal peptide.

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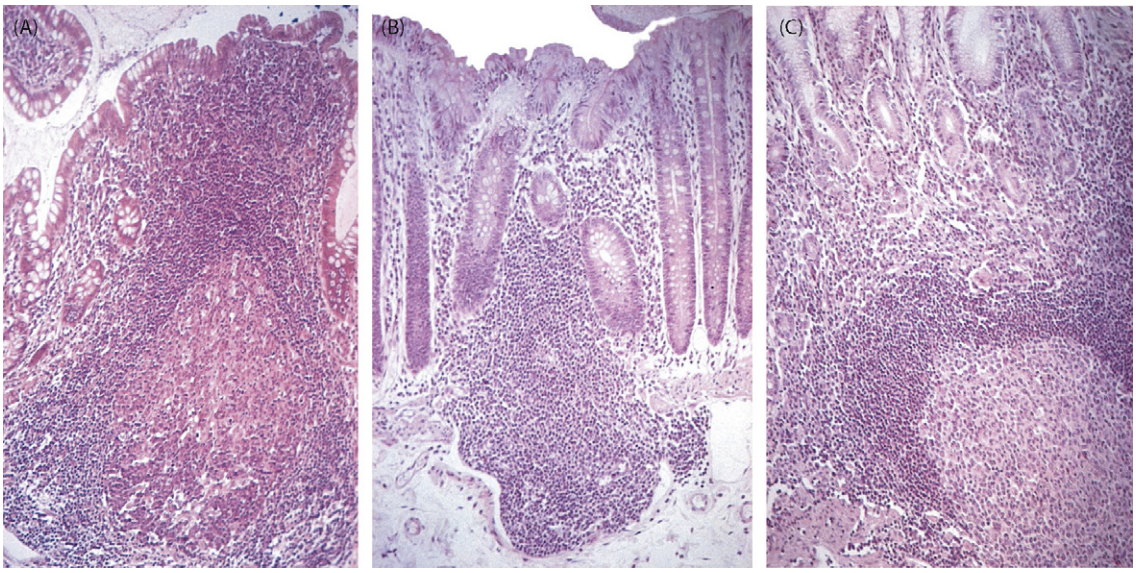

Fig. 1. Paraffin sections of human GALT stained using Haematoxylin and Eosin. (A) Peyer's patch with prominent germinal center and intimate association with the FAE. (B) Colonic LGC with small or absent germinal center and a narrower FAE. Unlike the Peyer's patches that are exclusively in the mucosa, LGC cross the *muscularis mucosa*. (C) MALT, with a large germinal center, in stomach. Normal stomach does not contain lymphoid tissue, but MALT can be acquired in response to infection with *Helicobacter pylori*.

the IgA response is propagated in the organised lymphoid tissues; B lineage cell division occurs almost exclusively in the organised gut-associated lymphoid tissue (GALT) that is located beneath specialised follicle-associated epithelium (FAE) [12]. GALT is uncapsulated and spreads laterally between the adjacent crypts where it merges with the lamina propria. In contrast to the organised lymphoid tissue, lamina propria contains predominantly effector cells and few B cells that retain the mature B cell antigen CD20 and little evidence of cell division *in situ* [12,18].

GALT in humans is comprised predominantly of clusters of organised lymphoid tissue in the terminal ileum (Peyer's patches [PP]), appendix and isolated lymphoid follicles (ILF) that occur beneath the epithelium throughout the gut [19–21]. ILFs were described in human colon as lymphoglandular complexes (LGC) by O'Leary and Sweeney [22]. In addition to constitutive GALT, it can be acquired in humans in response to infection. For example, mucosa associated lymphoid tissue (MALT) is acquired in the stomach, that normally does not contain lymphoid tissue, in response to infection with the pathogenic gastric bacterium, *Helicobacter pylori* [23].

In humans, PP, LGCs and appendix have been described in fetal intestine as organised structures, identifiable from 22 weeks of gestation, though at this time there is no germinal center formation [22–25]. Germinal centers are acquired in the Peyer's patches, rectal and appendiceal follicles after birth, where they precede the appearance of plasma cells in the lamina propria [13,22,25], presumably in parallel with the colonisation of the gut by bacterial flora. In contrast, most LGCs in adults do not have germinal centers. In adult human colon, only 1% of human LGCs have germinal centers, although the frequency of LGCs that contain germinal centers increases towards the distal colon and within 10 cm of a colon carcinoma [22]. The microanatomical components of constitutive and acquired human GALT are illustrated in Figs. 1 and 2.

In mice, as in humans, PP are concentrated in the terminal ileum and ILF are dispersed throughout the intestine [26]. In mice, PP and ILF develop sequentially. Whereas the PP are constitutive and induced to develop before birth by lymphoid inducer cells (LTi) [27], ILF develop after birth in response to the flora through colonisation of clusters of LTi-like cells termed cryptopatches [28–30]. Nucleotide-binding oligomerisation domain 1 (NOD1) is crucially involved in maturation of murine ILF as the gut becomes colonised, through recognition of bacterial peptidoglycans [31].

The FAE forms the interface between GALT and the lumenal flora. The FAE contains Microfold cells, or M cells, that are specialised epithelial cells that sample the lumen and thus expose the associated lymphoid tissues to the luminal contents including particulate material and IgA associated antigens [32,33]. The frequency of M cells in the FAE depends on species and age. Humans have relatively few, but like other components of the FAE, M cells are present before birth [25,34,35]. The FAE is disrupted by a B and T cell infiltrate, the composition of which is distinct from the intraepithelial lymphocyte population in epithelia that are not associated with organised lymphoid tissues [19,20,36]. The lymphocytic component of the FAE is apparent without antigenic exposure (Fig. 2D), but the infiltrate increases in rats as the FAE matures in response to microbial colonisation [24,25,37]. M cells interface with lymphocytes creating a microenvironment that resembles a germinal center in that the B cells express high levels of costimulatory molecules CD80/CD86 [38]. The FAE function itself is regulated by toll-like receptors (TLRs) and associates intimately with a multifunctional dendritic cell (DC) component that is central to IgA responses and that will be discussed in detail below [39–41].

Some IgA plasma cells in mice originate from a peritoneal B1 B cell population that has no direct equivalent in humans [42,43]. The precise contribution of the B1 B cell subset to the plasma cell population is not clear, but estimates of up to 50% have been suggested. B1 B cells are a self-renewing and self-sustaining B cell subset associated with innate immunity through their production of polyspecific antibodies. It is thought that their function may be related to the ability of dendritic cells to extend dendritic processes through the intestinal epithelium and sample antigens [44].

3. Dendritic cells; pluripotent orchestrators of IgA responses

GALT contains abundant DCs that can be induced to extend processes through the FAE and contact the lumen contents [45]. Interaction between DC and luminal contents has three potential consequences that directly affect B cell function through T cell dependent and independent routes.

First, native lumenal antigens may be identified through innate receptors, internalised and transported to the lymphoid tissues [46]. The coccoid form of *H. pylori* for example has been observed