The bowel: A key component of the immune system

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INTRODUCTION

The gut immune system constitutes the most extensive and complex part of the immune system. It receives every day a huge antigenic load, and is able to distinguish between invasive pathogens and innocuous antigens from food and commensal bacteria. The gut has defence mechanisms that limit access for harmful substances to the body. This intestinal barrier is formed by several elements including digestive pancreatic enzymes, the intestinal epithelium, and bacteria making up the intestinal flora. However, the most effective barrier is the gut-associated lymphoid tissue (GALT). To understand how intestinal immune response develops and is regulated in the gut, and how this one can be extended to the rest of the organism.

GALT ANATOMY AND CELL COMPOSITION

Structurally, GALT is divided into two compartments (Fig. 1): a) Organized GALT, inductive site for intestinal immune response –formed by isolated lymphoid follicles, associated lymphoid follicles or Peyer’s patches, and mesenteric lymph nodes; and b) Diffuse GALT, effector of the immune response –formed by lymphocyte popula-
tions interspersed among epithelial cells (intraepithelial lymphocytes, IELs) or in the intestinal lamina propria (lamina propria lymphocytes, LPLs) (1).

Peyer's patches are formed by macroscopic lymphoid aggregates placed in the antimesenterial aspect of the intestinal mucosa. Lymphoid tissue is separated from the intestinal lumen by a monolayer of cells (follicle-associated epithelium, FAE) formed by columnar epithelial cells, M cells, IELs, and some mucus-secreting cells (goblet cells). M cells are enterocytes that lack glycocalix and have a folded luminal surface instead of the typical enterocytic microvilli, and are specialized in lumen antigen uptake. Under FAE there is a diffuse region, called the subepithelial dome, formed by dendritic cells and some macrophages. Interfollicular areas comprise T lymphocytes, mainly T helper (Th) in type, mature dendritic cells, and macrophages. Peyer’s patches contain numerous follicles composed of IgM+ B lymphocytes, which are plasma-cell precursors that produce IgA; it is in the germinal centers of these follicles that memory IgA+ B lymphocytes are generated. Unlike other lymphoid organs, Peyer’s patches only have efferent lymphatic vessels (1-3) (Fig. 1).

Mesenteric lymph nodes are placed in the intestinal mesentery, and are divided into three regions with different cell compositions: cortex, paracortex, and medulla (Fig. 2). The cortex has primary and secondary follicles, which are rich in B lymphocytes and dendritic cells. On the other hand, the paracortex is characterized by a high proportion of T lymphocytes and dendritic cells. The medulla, the deepest region in the node, is composed of T and B lymphocytes and plasma cells (4).

Circulating naïve T cells reach the lymph node through specialized postcapillary venules called high endothelial venules. The entrance of T lymphocytes into the paracortex is through these high endothelial venules and is guided by chemokines. These molecules are produced by endothelial, stromal, and dendritic cells, and bind a receptor on naïve T cells (Fig. 2) (4).

In the cortex, resident dendritic cells take up and process the antigens arriving in the lymph. Mature dendritic cells migrate then to the paracortex, and it is there that they present the antigen to naïve Th cells or T cytotoxic (Tc) cells. Thus are effector T cells generated, and then the adaptive immune response starts (4). While effector lymphocytes leave the lymph nodes and migrate to non-lymphoid tissues, some Th cells remain in the lymph node as memory cells or move to follicle germinal centers to promote the final process of B-cell differentiation (4).

IELs lie in the intestinal intraepithelial space, under the tight junctions and over the basal membrane (Fig. 1). Considering the large surface of intestinal mucosa (~400 m²) and its proportion with respect to epithelial cells (1:4-9), IELs represent an abundant population of immune cells (5). Although they are very heterogeneous, most of them have an atypical suppressor or cytotoxic phenotype that is specific of the mucosal compartment (CD8αα+). It differs from other lymphoid tissues where more conventional phenotypes (CD4+ and CD8αβ+) predominate (6). Although their origin and development are still much debated (7), it is known that IELs have an activated phenotype—typical of effector/memory cells—with immunoregulator ability, and provide an immediate and highly effective response to infected epithelial cells. All
in all, IELs play a key role in the prevention of luminal antigen priming, so they mediate the oral tolerance process (8).

On the other hand, the lamina propria, placed between the epithelium and *muscularis mucosa*, contains mature plasma cells that produce IgA, T lymphocytes (mainly Th), and other cell types including macrophages, dendritic cells, and mast cells (Fig. 1) (9). These cells are in continuous migration, differentiation, and renewal processes (10).

The two effector mucosal populations, IELs and LPLs, are under the influence of intestinal commensal bacteria, which help in the development of the immune function. In this sense, intestinal bacteria enable the expansion of intestinal epithelial lymphocytes, and the acquisition of their cytotoxic ability. Moreover, the flora plays an important role in the induction and maintenance of oral tolerance against antigens from the diet, by promoting IgA production by LPLs. Commensal bacteria also interact with antigen-presenting cells (APCs) in the epithelium and lamina propria, thus promoting a different interaction with Th cells, inducing the activation of regulatory cells, and stimulating tolerance against these bacteria (11).

**INTESTINAL IMMUNE RESPONSE**

**Luminal antigen uptake**

Luminal antigens can penetrate the intestinal mucosa and reach GALT through different pathways. Antigen uptake by M cells, which are present in Peyer’s patches, constitutes the best known gateway. The apical membrane of M cells is designed to favor the adhesion and uptake of luminal antigens such as macromolecules, adhesive particles, viruses, and bacteria (12). M cells can also sample certain food proteins and IgA (13,14). Once the antigen is taken up, the process of transcytosis begins: M cells internalize luminal antigens by endocytosis or phagocytosis, and transport them in their vesicles towards the basolateral membrane, where they are released to the extracellular matrix. The basolateral membrane of M cells has a profound invagination (intraepithelial pocket) that contains lymphocytes and macrophages devoted to antigen processing for presentation (12).

Enterocytes constitute a second possible route for antigen uptake. They are less accessible than M cells due to their glycocalyx layer, rich in hydrolytic enzymes that block the entry of macromolecular aggregates and microorganisms. At present, it is accepted that enterocytes are not only capable of taking up soluble antigens coming to their surface, but also of processing and presenting them to T lymphocytes (15). Luminal antigen uptake can also occur by means of a paracellular mechanism through spaces between enterocytes, where dendritic cells project their dendrites due to the expression of tight junction-associated proteins (16).

**Immune response induction**

M cells take up and transport luminal antigens towards APCs located inside the dome in Peyer’s patches. APCs internalize and process luminal antigens, converting them into antigenic peptides that will be expressed in the plasmatic membrane associated to major histocompatibility molecules (MHC), in order to be recognized by the T-cell receptor (TCR). Activated APCs can interact with interfollicular T lymphocytes of the Peyer’s patch or migrate towards mesenteric lymph nodes via lymphatic vessels.

Upon activation, Th lymphocytes can mainly differentiate into two effector subpopulations called Th1 and Th2 with different functions based on the cytokine profile they secrete (Fig. 3).

**Fig. 3. Classification of activated T helper lymphocytes. The type of stimulus determines cytokines to be secreted at the time of antigenic recognition, thus favoring T-lymphocyte differentiation into a certain effector or regulatory subpopulation.**

Th1 lymphocytes are characterized by the secretion of γ interferon (IFNγ), interleukin 2 (IL-2), and lymphotoxin (LT or TNFβ), and their main function is phagocyte-mediated defence against infections, especially those produced by intracellular microorganisms (viruses, bacteria, and certain protozoa). On the other hand, Th2 lymphocytes, IL-4, IL-5, and IL-13 producers, act as allergic response mediators and defenders against infections produced by helminths and arthropods (Fig. 3) (17). Cytokines produced by these subpopulations not only determine their effector functions (18), but also participate in their own development and clonal expansion. Thus,
each subpopulation amplifies itself and also exerts a regulatory role on the other one (19).

The existence of a third effector subpopulation called Th17 has been recently described, characterized by the secretion of IL-17 and IL-6 (20). Although its biological functions are not totally clear, this effector population seems to be involved in the defence against bacterial and fungal infections not totally covered by Th1 and Th2 responses (21).

Apart from these three effector subpopulations, the presence of regulatory T lymphocytes is now established: Tr1 lymphocytes, mainly IL-10 producers, and Th3 lymphocytes characterized by the secretion of transforming growth factor β (TGFβ). These lymphocytes are especially important in the intestine due to their regulatory effects on the immune response during inflammatory and infectious processes. Moreover, they exert a key role in the development of oral tolerance against innocuous antigens coming from the diet and the microbiota—oral tolerance is the absence of immune response against an antigen to which an individual has been previously exposed through the gastrointestinal tract (22).

As mentioned before, naïve T-lymphocyte differentiation into effector subpopulations is conditioned by the type of stimulation and especially by cytokines secreted during antigenic recognition (Fig. 3). IL-12 is the main factor responsible for Th1 differentiation, whereas IL-4 promotes Th2 subpopulations (23). Some extracellular bacteria drive Th17 differentiation by inducing IL-23 secretion by APCs (20). In addition, regulatory T lymphocytes are expanded in response to IL-10 and/or TGFβ (24).

Certain cytokines such as IL-4, IL-5 and TGFβ induce IgA synthesis by follicular B lymphocytes in Peyer’s patches. These B lymphocytes, plasma-cell precursors, migrate towards mesenteric lymph nodes where maturation and clonal expansion take place. Then, these lymphocytes reach the bloodstream through the thoracic duct (1). After several recirculations these lymphocytes migrate to effector tissues, such as the intestinal lamina propria, where they will exert their function (Fig. 1). A great variety of factors influence this migration: General factors such as tissular irritation, inflammation, innervation, and hormonal signals, and also specific factors such as adhesion molecule expression, stromal signals, cytokines, antigens, and chemokine production by the endothelium (10).

Activated T lymphocytes in GALT exhibit a pattern of adhesion molecules and chemokine receptors different from that of activated lymphocytes in peripheral lymphatic organs, which promotes their mobilization towards mucosal tissues and, especially, to those where response was initiated.

Lymphocytes reaching the lamina propria of the intestine are distributed in different compartments. Plasma cells remain in the lamina propria, where they finalize their maturation into IgA-secreting cells. Th lymphocytes also remain along the villous border and crypts, whereas Te lymphocytes preferably migrate to the epithelium, thus becoming IELs. Both activated lymphocyte subsets are maintained in a latent state as memory cells, and upon antigen reencountering they exert their programmed effector functions (8).

Mucosal immunoglobulins

IgA is the most abundant immunoglobulin in the intestinal mucosa (80-90%) and exerts an important role as first defence line against toxins, and colonization and invasion by pathogens. It is mainly synthesized in the lamina propria of the intestine in response to T-lymphocyte activation in Peyer’s patches. Structurally, two IgA isoforms are distinguished: monomeric and polymeric (25).

Polymeric IgA, secreted (S-IgA) in the intestinal mucosa, is composed of two IgA molecules covalently linked through their constant region and associated with one molecule of the joining J chain. In addition, it contains a secretory component consisting of a segment of polymeric Ig receptor (pIgR). Polymeric IgA (p-IgA) is the main Ig in mucosal secretions, whereas monomeric IgA (mIgA) is predominant in the serum (25). pIgA is transported towards the mucosal surface by means of epithelial transcytosis. In this process, mIgA that contains J chain binds to the polymeric Ig receptor present in the basolateral membrane of epithelial cells. This IgA-pIg complex is internalized and transported by vesicles to the apical membrane of epithelial cells in order to be released to the intestinal lumen. During the releasing process, pIgR is cleaved and the extracellular domain, the secretory component, remains bound to pIgA, providing resistance against proteases in the intestinal lumen (Fig. 4) (5). Mucosal IgA production is regulated by the cytokine milieu. Thus, IL-5, IL-6, and IL-10 favor the final differentiation phase of B lymphocytes into IgA-secreting plasma cells (26).

Given that IgA synthesis takes place mainly at the intestinal level, and its transport to the intestinal lumen is very efficient, this isotype constitutes a minor component of non-mucosal immunity in comparison to IgG and IgM. S-IgA, besides being resistant to intraluminal proteolysis, does not trigger inflammatory responses and is therefore an ideal mechanism for intestinal mucosa protection (27). In the lumen, S-IgA can form immune complexes with antigens, thus preventing the entry of microorganisms and dietary antigens (28). IgA can also act at the intraepithelial and subepithelial levels blocking antigens across the intestinal barrier (29).

IgM is also present in the intestinal surface (6-18%), but in a lower proportion than IgA due to the existence of a lower number of mucosal plasma cells and a less efficient IgM transport to the intestinal lumen (30). S-IgM is composed of 5 IgM molecules linked to a J chain...
sincerely to IgA. IgM transport is also performed through p-IgR, but contrary to IgA, IgM is non-cova-
ently linked to the secretory component, thus being more susceptible to proteolytic enzymes (5) (Fig. 4). S-
IgM is more abundant in early life, and can become the main isotype in IgA-deficient patients, since IgM pro-
duction and transport is usually increased as a compensatory mechanism. However, S-IgM cannot totally re-
place S-IgA function (5).

IgG, coming from local synthesis and the bloodstream, constitutes a minor isotype in the intestinal mucosa. It is exclud-
ively found as a monomeric form, and despite the fact that it is not subjected to external active transport, it can reach the intestinal lumen through a paracellular pathway. This isotype can also be increased in IgA-defi-
cient patients (27) (Fig. 4).

CONCLUSION

GALT plays a very important defensive role in the in-
testine, which is constantly exposed to a high antigenic burden. Its unique structure, which is composed of orga-
nized tissue (Peyer’s patches and mesenteric lymph nodes) and diffuse tissue (IELs and LPLs), allows the de-
velopment of efficient and appropriate responses accord-
ing to each type of stimulus. It slows down invasive pathogenes and induces oral tolerance in response to in-
nocuous antigens coming from the diet and the intestinal epithelium. Secretory antibodies, mainly the IgA isotype, also constitute a defence mechanism that is characteristic and common in all bodily mucosas.

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Fig. 4. Transport of IgA, IgM and IgG to the intestinal lumen. IgA and IgM are synthesised in the intestine in their polymeric isoforms and they are transported to the lumen due to their interaction with the polymeric Ig receptor (pIgR). However, IgG comes mainly from the blood stream and reaches the lumen via a paracellular way. SC: S ecretory component. Adapted from (5).