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Mucosal immune system: A brief review

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EL SISTEMA INMUNITARIO DE LAS MUCOSAS: UNA BREVE REVISIÓN

RESUMEN

La mayoría de los antígenos que entran en contacto con el sistema inmune durante la vida de un ser vivo lo hacen a través de la superficie de la mucosa de los tractos respiratorio, gastrointestinal y urogenital. Ocupan una superficie de 400 m2 y forman el área de mayor tamaño en contacto directo con el ambiente externo.

Las mucosas separan el ambiente externo del ambiente interno, estéril, y representan una primera línea de defensa. Esta barrera está en contacto tanto con patógenos que han desarrollado mecanismos eficaces para la colonización de epitelios e invasión de mucosas, como con antígenos inocuos, tales como comida, o la flora bacteriana comensal. En el primer caso se necesita una respuesta inmune eficaz y robusta, mientras que en el segundo se requiere una respuesta caracterizada por ignorancia o supresión activa.

En estas condiciones, las mucosas han desarrollado un complejo sistema inmune, con características anatómicas y funcionales particulares, capaz de generar rigurosas respuestas frente a antígenos patogénicos, mientras mantiene una situación de ignorancia o supresión activa frente a antígenos no patogénicos.

PALABRAS CLAVE: Inmunología de las mucosas / Inmunoglobulina A / Enterocitos / Placas de Peyer / Tejido linfoide asociado a mucosas.

ABSTRACT

Most of antigens that encounter the Immune System along life enter the body through mucosal surfaces of the respiratory, gastrointestinal and urogenital tract. These are the largest areas within the body in contact with the external environment and in adult humans protect some 400 m2 of surface.

Mucosal surfaces separate the external environment from the internal sterile environment and so represent a first line of defence system. This barrier faces environments rich in pathogens, which have developed effective mechanisms for colonisation of epithelial surfaces and invasion of mucosal tissues, but also harmless antigens such as food, airborne antigens or commensal bacterial flora. The latter represent the vast majority of the encountered antigens and require an appropriate response characterised by either ignorance or active suppression. However, for the former, a robust immune response is needed.

Under these influences, mucosae have developed a complex immune system, anatomical and functionally different from the systemic immune system, which is capable of mounting an immune response against pathogenic antigens while maintaining the required ignorance or active suppression against non-pathogenic antigens.

KEY WORDS: Mucosal Immunity / Immunoglobulin A / Enterocytes / Peyer´s patches / Mucosa-associated lymphoid tissue / MALT.

The mucosal immune system is the part of the immune system juxtaposed to the mucosal surfaces and in direct contact with the external antigenic environment. It is composed of the lymphoid tissues that are associated with mucosal surfaces (MALT or mucosa-associated lymphoid tissue) and can be separated into several components: gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT), nasopharinx-associated lymphoid tissue (NALT), the mammary and salivary glands and the genitounitary organs[1].

Mucosal tissues are heavily populated with cells of the immune system. It is estimated that the intestinal lining contains more lymphoid cells and produces more antibodies than any other organ in the body[2]. However, the immune cells are not alone in tasks of preventing infection. It is important to take into account also the various mechanical mechanisms, that some authors consider part of the innate immune system, present at these surfaces. They include the epithelial cell barrier and the extra-epithelial defences that the pathogens have to elude to gain access to the epithelial surface or to penetrate the underlying tissues.

EPITHELIAL AND EXTRAEPITHELIAL DEFENCES

The epithelial barrier constitutes a key element in preventing penetration of microorganisms. It possesses chemical and cellular defences, which differ slightly from one tract to another: only a single cell layer covers the intestinal mucosa, the airway tract varies from pseudoestratified to simple epithelium, and there is a multilayered squamous epithelial lining the oral cavity, pharynx, oesophagus, urethra and vagina[3]. The cells that compose the intestinal mucosal barrier are a self-renewing system undergoing continuous renewal from pluripotent stem cells located near the base of the crypts of Lieberkühn. The progeny of these stem cells undergo terminal differentiation into one of four cell lineages as they migrate toward the crypt surface (enterocyte, goblet cells or enteroendocrine cells) or to the crypt base (Paneth cells). Enterocytes are responsible for absorption of nutrients and secretion of electrolytes and also represent central components of the mucosal immune system[4].

Furthermore, the mechanical washing forces and cilial action create a current that rids the mucosal surface of organisms that enter the body and fail to bond early or well to the epithelium. If the microbes can adhere, alternative barriers (such as the mucus layer, composed of mucins secreted by globet cells) avoid the access. Additional mechanisms may inhibit the growth of microbes. These are the acidic pH of the stomach or some enzymes (lysozyme, lactoferrin, lactoperoxidase) secreted at mucosal surfaces that can kill bacteria, or anti- microbial peptides secreted by the epithelial cells such as defensins, cathelicidins and histatins. The commensal microflora provides colonisation resistance, either occupying potential binding sites, competing for nutrients or secreting inhibitory compounds (bacteriocins or metabolic products). Finally, and also included in the extra-epithelial defences are: 1) secretory IgA and IgM of limited antigen specificity, or natural antibodies, that trap the invading pathogen, 2) soluble pattern recognition receptors (PRRs) such as the mannose binding lectin (MBL), 3) the complement cascade components, 4) C-reactive protein, 5) lypopolysaccharide (LPS) binding protein (LBP) and CD14 (which can be also cell-associated), and 6) the cytokines and chemokines that orchestrate the immune system[1,5].

Intestinal epithelial cells form a highly selective barrier. Transcellular and paracellular fluxes are tightly controlled by membrane pumps, ion channels and tight junctions, which adapts permeability to physiological needs. Besides this mechanical protective function, the epithelium provides the immune system with a continuous flux of information about the external environment. A correct function of the mucosal immune system requires a transport of molecules and antigens through the epithelial barrier and the establishment of collaboration among the epithelial cell, the professional antigen presenting cell and lymphoid cells[6]. Tolerance against commensals and immunity against pathogens require intact antigen uptake recognition, processing and response mechanisms[7].

ORGANISATION OF THE MUCOSAL IMMUNE SYSTEM

The lymphoid elements of different mucosal tissues (MALT) can be morphologically and functionally subdivided into two major parts: the organised mucosa associated lymphoid tissue, consisting of mucosal follicles, which are responsible for the induction phase of the immune response, and the diffuse mucosa associated lymphoid tissue, which consists of widespread leukocytes scattered throughout the epithelium and the *lamina propria* of the mucosa, and constitute the effector sites[3,8].

Organised Mucosa Associated Lymphoid Tissue

The organized MALT, characterised by mucosal lymphoid follicles, occurs in the tonsils, bronchi and intestines. Single follicles occur along the entire length of the gastrointestinal tract, with increasing frequency in the colon and rectum[9,10]. Aggregated follicles occur in the lingual and palatine tonsils and adenoids of the oral and nasopharynx tract, in the Peyer's patches of the small intestine and in the appendix[10].

Some mucosal tissues, such as the vagina, have no local organized MALT but rely on antigen uptake and transport into lymph nodes that drain the mucosa[3].

The best studied mucosal inductive sites are the GALT structures, including the ileal Peyer´s patches, the mesenteric lymph nodes and the appendix, and can be used as a model for the other mucosa-associated components.

Peyer's patches

The Peyer´s patches (PP) are macroscopic lymphoid aggregates that are found in the submucosa along the length of the small intestine. Mature Peyer´s patches consist of collections of large B-cell follicles and intervening T-cell areas. B naïve cells form the germinal centre of the follicle, supported or connected by follicular dendritic cells. These follicular dendritic cells are not bone marrow-derived and are different from the dendritic cells that present antigens to the naïve T cells[11]. Each follicle is surrounded by a parafollicular area rich in T cells, where a large number of high endothelium venules exist, allowing cellular migration and lymphoid recirculation[6]. Peyer´s patches differ from lymph nodes elsewhere in the body because they lack afferent lymphatics. This characteristic is in keeping with the notion that antigen is sampled from the lumen via the overlying epithelium[12].

The lymphoid areas are separated from the intestinal lumen by a single layer of columnar epithelial cells, known as the follicle associated epithelium (FAE), and a more diffuse area immediately below the epithelium, known as the subepithelial dome (SED). The FAE differs from the epithelium that covers the mucosa as it has lower levels of digestive enzymes, a less pronounced brush border and a total absence of receptor for polymeric IgA. It is infiltrated by large numbers of B cells, T cells, macrophages and dendritic cells. The most notable feature of the FAE is the presence of M (microfold) cells. M cells are specialised enterocytes with poorly developed brush borders and a thin overlying glycocalyx, and thus are adapted to antigen uptake function. M cells differentiate from enterocytes under the influence of membrane-bound lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) that is present on local lymphoid cells, mainly B cells[8].

For some authors a Peyer's patch, by definition, comprises five or more activated B-cell follicles[13], but some other authors consider that it is reasonable to categorize even a single nodule as a Peyer's patch, provided follicle associated epithelium (FAE) is present[14].

Mesenteric lymph nodes

Mesenteric lymph nodes (MLN) are the largest lymph nodes in the body. Their development is distinct from that of both Peyer's patches and peripheral lymph nodes, as it is relatively unaffected by the absence of most of the factors that are involved in the ontogeny of these other organs, such as tumor necrosis factor (TNF), TNF receptor (TNFR), $LT\alpha_1\beta_2$ and lymphotoxin-β receptor (LTBR). These factors might have complementary roles in MLN development. Accumulation of lymphocytes in MLN also requires L-selectin and $\alpha_4\beta_7$ integrin adhesion molecules, which normally direct lymphocytes to enter peripheral and mucosal tissues, respectively. As a result of these unique anatomical features, the MLN might be a crossroads between the peripheral and mucosal recirculation pathways[8].

In either case, the first step in the induction of a mucosal immune response is the transport of antigens across the epithelial barrier. Following antigen processing and presentation in inductive sites, IgA-committed antigen specific B lymphoblasts proliferate locally and migrate via the bloodstream to local and distant mucosal and secretory tissues. There, they differentiate primarily into polymeric IgA-producing plasma cells. Dimeric or polymeric IgA antibodies are transported across epithelial cells into glandular and mucosal secretions via receptor-mediated transcytosis[15].

Diffuse mucosa-associated lymphoid tissue

The less organised lymphoid elements associated with the intestinal epithelium and the *lamina propria* contains both *lamina propria* mononuclear cells (LPMC) and intraepithelial lymphocytes (IELs).

Lamina propria mononuclear cells

The lamina propria is the layer of connective tissue between the epithelium and the muscularis mucosa. This layer is made up of smooth muscle cells, fibroblasts, lymphatics and blood vessels[8].

Adult human large and small intestinal *lamina propria* is infiltrated of lymphoid and myeloid cells. T cells, B cells, macrophages, dendritic cells, neutrophils, other granulocytes and mast cells are found[1]. The large numbers of macrophages, dendritic cells and T cells in the *lamina propria* make it likely that antigens crossing the epithelium may be processed and presented to lamina propria $CD4^+$ T cells.

IgA plasma cells make up 30-40% of the mononuclear cells in human intestinal lamina propria and small B cells make up 15-45% of the cells. In the normal intestine, around 80% of the total plasma cells secrete IgA, and low numbers of IgM and IgG secreting plasma cells are present as well. In contrast to plasma cells, most of the non-plasma B cells in mouse *lamina propria* are $sIgM^+$ $sIgD^+$ B cells.

T cells in the *lamina propria* are mainly $CD4^+$ (60-70%) and the vast majority express TcRαβ (95%). About 10% of

them are $CD25^+$, and most express CD45RO, indicating a memory phenotype[14]. $CD4^+$ T cells in the *lamina propria* are of particular importance to local immune regulation. They are generally unresponsive to TcR-mediated proliferative signals, but in humans they can be induced to proliferate when CD2 is used as an accessory molecule. They produce large amounts of cytokines, particularly IFN-γ, but also IL-4 and IL-10. *Lamina propria* $CD8^+$ T cells can also have potent cytolitic T-lymphocyte activity. Some of the antigen-experienced *lamina propria* T cells might be true effector cells, and might help local B cells to produce IgA. Alternatively they can be effector memory cells. Finally, *lamina propria* T cells might be regulatory T cells and therefore responsible for maintaining local tolerance to environmental antigens[8].

Intraepithelial lymphocytes

The average number of IELs in adult human jejunum is 20 per 100 absorptive cells and decreases distally in the gut. These cells are located above the basal lamina in the epithelial layer and separated from adjacent enterocytes by a 10 to 20 nm space. These lymphocyte-epithelial cell contacts have no junctional structure[16].

Intraepithelial cells are a functionally heterogeneous population that contains cells with antitumour activity, natural killer activity, allospecific cytotoxic T lymphocytes (CTL), precursors of CTL and mast cells[17]. Almost all IELs are $CD3^+$. Among these cells, only 5-15% express CD4 (helper/inducer phenotype) and the remaining cells express CD8 (cytotoxic/supressor phenotype). A large fraction of these express a CD8αα homodimer, which is essentially absent from the circulation. Of the few $CD4^+$ T cells present in the small intestine, many also express CD8αα. These «double positive» cells are also unprecedented in the systemic circulation. $CD4^-$ $CD8^-$ «double negative» cells account for more than 10% of murine small intestinal IELs and the majority of IELs in other compartments[18]. Although the T cell receptors that mediate antigen recognition are composed predominantly of αβ chains, the proportion of $\gamma\delta^+$ lymphocytes in the intestinal epithelium is much larger than in the peripheral blood and *lamina propria*. Thus, TcR$\gamma\delta^+$ lymphocytes are thought to have a special role in the intestinal epithelium. The microenvironment within the intestinal epithelium may influence the differentiation of IELs and although their functions are unclear, some possibilities are cytotoxicity, cytokine secretions, regulation of renewal of mucosal epithelium and tolerance[16].

Several groups have demonstrated the presence of $CD3^-CD7^+$ IELs in the intestinal epithelium. This subset represents a substantial proportion (42%±20) of the total IEL population in small intestinal biopsies from children. Within this population only 10% expressed CD8αα. Phenotypically they resemble natural killer cells and have been termed natural killer-like intraepithelial lymphocytes. Most of them express CD161, 45% are $CD56^+$ and only 12% express CD16, contrasting with the $CD56^+16^+$ expression found in classical circulating peripheral blood NK cells, but resembling the phenotype of NK cells isolated from early pregnant decidua[19]. This population contains abundant perforin intracytoplasmic granules and possesses lytic potential, showing the existence of functional natural killer cells within the gut epithelium[19,20].

Almost all IELs, whatever their surface phenotype, bear the $\alpha_E\beta_7$ integrin (CD103), whose ligand is epithelial E-cadherin. Interactions between $\alpha_E\beta_7$ and E-cadherin may help anchor IELs in the epithelium and may also play a functional role[21,22].

Origin of IELs

Because of the heterogeneous surface phenotype of intraepithelial lymphocytes, Hayday and colleagues propose to simplify the understanding of IEL compartments by classifying them into two cell types: a and b. Type a includes TcRαβ$^+$ cells that primarily recognize antigens presented by conventional MHC class I and II, and are primed within the systemic circulation. Type b cells includes TcRαβ$^+$ CD8αα$^+$ IEls and TcRγδ$^+$ IELs, that respond to antigens not restricted by conventional MHC. Although TcRαβ$^+$ CD8αα$^+$ and TcRγδ$^+$ cells are different one from another, they share many features (expression of certain genes such as FcεRIγ or Ly49E), that distinguish them from the type a IELs[18].

Several groups have shown that foetal liver or bone marrow progenitors can reconstitute type b IELs in thymectomised recipients, and, as a result, these cells were classified as thymic-independent (TI) cells, while type a were considered thymic-dependent (TD) IELs. Nonetheless not all type b IELs in all species are TI, as some avian and murine $\gamma\delta^+$ IELs develop in the thymus.

Type a or TD IELs of the small intestine are mostly CD8αβ$^+$ TcRαβ$^+$ T cells, and a few are $CD4^+$. Numerous TcR gene rearrangements in these IELs are shared with gut *lamina propria* T cells and with thoracic duct $CD8^+$ T cell blasts. This supports the hypothesis that they are primed to antigen in Peyer's patches or in the extrafollicular areas of the gut, after which they drain, via mesenteric lymph nodes and the lymphatics, to the thoracic duct. After entering the blood, they home back to the *lamina propria* throughout the small and large intestine. From the *lamina propria* they enter the epithelium with variable efficiency and may continue to exchange across the two compartments. Within the epithelium particular clones will accumulate in response

to repeated priming to an antigen[18]. For these TD cells, the use of the preformed thymic repertoire would ensure the possibility of a rapid response to the appearance in the gut of occasional antigens, such as infections[23].

Type b cells do not recognise antigens presented by MHC on professional antigen presenting cells, and it is then conceivable that they are primed directly by epithelial cells *in situ*. These IELs may develop from double negative thymic progenitors that escape to the periphery before MHC-based selection of double positive thymocytes. Possibly these double negative cells acquire CD8αα$^+$ in the gut under the influence of factors such as TGF-β. It has been suggested that IELs develop in the foetal or newborn thymus before the establishment of efficient central deletion. After emigration from the thymus, those cells that populate the spleen or peripheral lymph nodes are tolerised by autoantigens but those that invade the intestine are positively selected[23].

Extrathymic differentiation of these IELS is not incompatible with the passage through the thymus of some TcR$^-$ progenitor cells, which might undergo T lineage commitment or expansion in this location. The release of cytokines such as IL-7 by the thymus or by antigenically stimulated thymus-derived cells may also influence the development of thymus-independent IELs[23]. IL-7, necessary for the earliest thymic lymphocyte precursors, has been detected abundantly in foetal intestinal epithelial cells, and CD117 (stem cell factor receptor) on occasional cells in the *lamina propria*. These findings, with the evidence that maturation and TcR recombination occur *in situ* in foetal intestine, support the idea of extrathymic T cell differentiation in the gut[24].

INDUCTION OF IMMUNE RESPONSE

There is abundant evidence that organised MALT plays a major role in antigen sampling and generation of lymphocytes, including specific IgA effector B cells, memory B cells and T cells. This involves active lymphocyte proliferation, local production of certain cytokines and continuous cellular trafficking[3]. Antigen uptake and initiation of an immune response is tightly regulated because an adequate immune response requires first access to antigen. These MALT structures lack a lymphatic supply of antigens but instead can sample foreign material from epithelial surfaces[13].

Initial mucosal antigen encounter / Antigen uptake

Stratified, nonkeratinised or parakeratinised epithelia lack tight junctions. In stratified and pseudoestratified epithelia, antigen-processing dendritic cells move into the epithelium, obtain samples of luminal antigens and migrate back to local or distant organised tissues. In simple intestinal and airway epithelia, whose intercellular spaces are sealed by tight junctions, antigens are preferentially taken up through the specialised areas of the follicle-associated epithelium. Specialised epithelial M cells deliver samples of foreign material by transepithelial transport from the lumen to organised lymphoid tissues within the mucosa[3]. M cells through its limited microvillus border, sparse glycocalyx and active transcytotic pathway, are clearly important in delivering certain types of antigens to Peyer's patches for both initiating immune responses characterized by active IgA secretion and contributing to the development of oral tolerance[25].

The M cell basolateral surface is deeply invaginated to form a large intraepithelial pocket into which transcytosed particles and macromolecules are delivered. Antigens are efficiently endocytosed or phagocytosed by multiple mechanisms in the apical membrane of the M cells. Each of these mechanisms results in antigen transport into endosomal tubules and vesicles and large multivesicular bodies, and to their subsequent release by exocytosis into the pocket. It is not known whether M cells participate in the processing and presentation of antigens nor if they express MHC class II molecules[3,8]. Instead they are believed to pass on intact antigens to professional APCs either in the epithelium or in the underlying dome region.

Specific subpopulations of lymphocytes migrate into the pocket and associate closely with the pocket membrane, forming apparent adhesion sites. In rodents, rabbits and humans, B and T lymphocytes, with a small number of macrophages, have been identified. Most of the T cells are CD4$^+$, none were TcRγδ$^+$ and, in humans, most of them express the early activation marker CD69, typical of memory cells, although in some species naïve T cells are observed. B cells in the pockets express the naïve cell marker CD45RA, along with HLA-DR, suggesting that the M cell pocket is a site of interaction of T cells with antigen-presenting B cells. Nevertheless, memory B cells, which may originate from the underlying B cell follicles, have been found in human M cells pockets. This interaction might lead to IL-2 secretion and promote T-cell survival and proliferation, or, anergy and hence tolerance, should this naïve T cell lack the necessary costimulatory molecules[13,26].

B cells in the M pockets are of the same type as the subepithelial B cells associated with the underlying follicle. It has been suggested that B lymphoblast traffic into the M cell pocket may allow continued antigen exposure and extension and diversification of the immune response. The cells in the pocket may interact early with the incoming

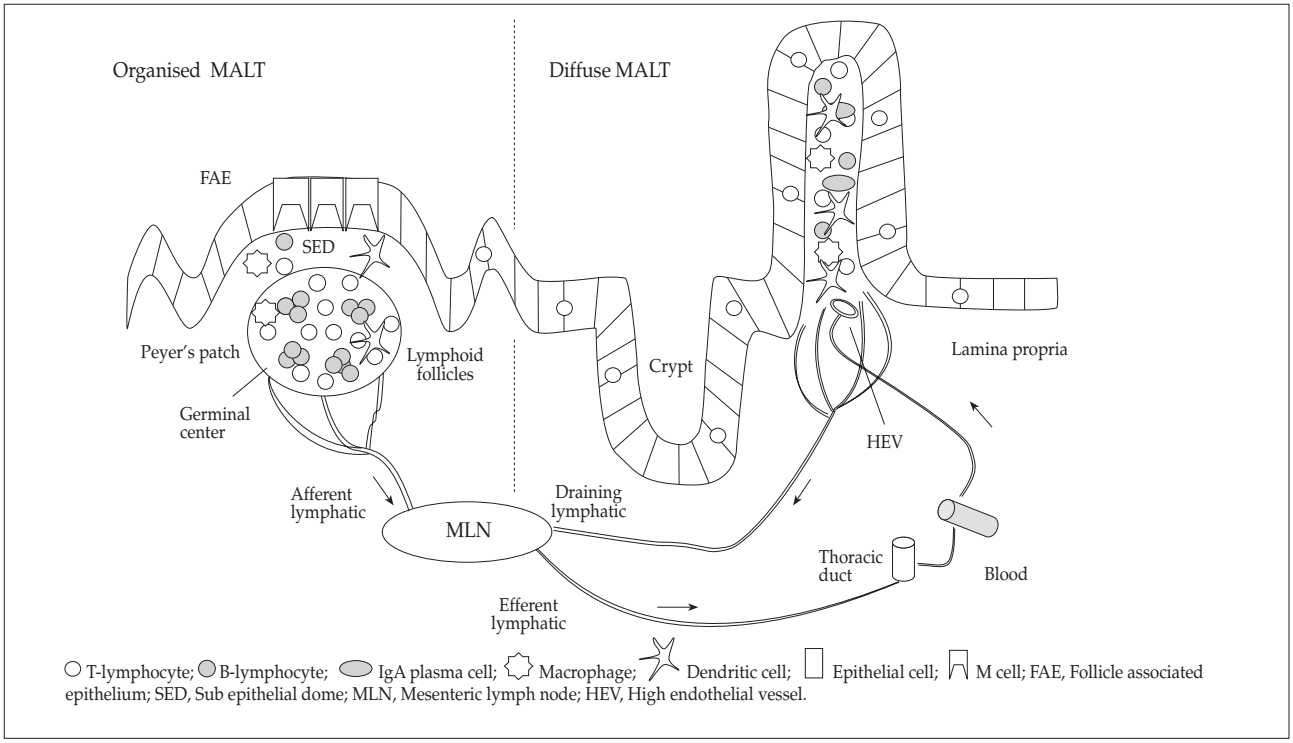

Figure 1. *Schematic representation of the organised and diffuse mucosa-associated lymphoid tissue (MALT) in the intestinal epithelium. Organised tissue represents inductive sites of the immune response whereas diffuse tissue represents effector sites, where primed cells arrive after passage through lymphatic drainage to blood circulation and, finally to lamina propria via blood vessels possessing high endothelial vessels (HEV).*

antigens, in an environment sequestered from the modulating influence of systemic humoral immunity.

Below the epithelium of the dome lies an extensive network of macrophages and dendritic cells intermingled with $CD4^+$T cells and B cells from the underlying follicle. These cell populations are presumably active in uptake of incoming pathogens as well as in processing, presentation and perhaps storage of antigens. Several DC subsets have been described in mouse Peyer's patches[3].

From there, the antigen presenting cells move to the T-cell areas and/or B-cell follicles, where they can interact with naïve lymphocytes. In the follicle, B cells undergo immunoglobulin class switching from expression of IgM to IgA under the influence of several local factors, including transforming growth factor (TGF-β), IL-10 and cellular signals delivered by dendritic and T cells.

The lymphocytes primed in the Peyer's patches exit through the draining lymphatics to the mesenteric lymph nodes where they reside for an undefined period of further differentiation before they migrate into the bloodstream through the thoracic duct and finally accumulate in the mucosa (Fig. 1). Lymphocytes primed in the GALT lose expression of L-selectin and selectively upregulate expression of $\alpha_4\beta_7$ integrin, whose ligand, mucosal addressin cell adhesion molecule 1 (MAdCAM 1), is expressed at high levels by the vasculature of mucosal surfaces[8].

Dendritic cells in antigen uptake

Antigen presenting cells such as macrophages and dendritic cells are positioned immediately below M cells and thus are ideally located to sample transported antigens that are subsequently presented to lymphoid follicles. These sites are relatively rare within the epithelial surface, and they alone may not be sufficient to representatively sample the vast lumenal content[27].

Castagnoli and colleagues reported that the adaptive immune system appears to display an additional, M-cell independent, mechanism that allows dendritic cells to sample environmental microorganisms without compromising the epithelial barrier function, and to deliver them into lymphoid tissues where an efficient immune response can be mounted.

Their study has shown, in an *in vitro* model, that dendritic cells extend processes through epithelial tight junctions into the lumen and are able to sample lumenal content. The dendritic cell processes slide between intestinal epithelial

cells preserving the epithelial cell barrier. The authors propose that dendritic cells may be able to make effective tight junctions with intestinal epithelial cells, since dendritic cells are shown to express tight junction proteins themselves, and there is not detectable alteration in tight junction permeability in their model. Under resting conditions, infiltrating dendritic cells would establish loose contacts with pre-existing epithelial tight junctions. Upon bacterial infection, dendritic cells would be recruited from the blood and activated, probably via epithelial signals. They then would up-regulate the expression of tight junction proteins and distribute them to the cell surface and dendrites. This would allow dendritic cell to compete for epithelial proteins and open up the tight junction. Infiltrating dendritic cell then would face the gut lumen and could directly sample bacteria. Bacterial components, such as LPS, trigger reorganization of tight junction proteins, allowing dendritic cells to detach from the junctions with epithelial cells and to migrate into the draining lymph nodes, after a change in the chemokine receptor program also triggered by bacterial components.

Because dendritic cells are migratory cells, they can transport pathogens to the mesenteric lymph nodes and the spleen for the induction of systemic responses, which suggests that this alternative route of bacterial internalisation has an important physiological relevance. It suggests the implication of dendritic cells in direct IgA induction against commensal bacteria in the *lamina propria* and they could also be involved in the transport of apoptotic intestinal epithelial cells to T cell areas of MLN without perturbing the integrity of the epithelial barrier[27-29].

Epithelial cells in antigen uptake

The vast majority of the mucosal surface is comprised of the absorptive intestinal epithelium, which is predicted to be the major site of antigen contact. The availability of antigens via this cell barrier is regulated by soluble mediators including cytokines and toxins, which increase permeability (IL-4, IL-13, TNF, IFN-γ), and cytokines that enhance the barrier formation (TGF-β, IL-15). Paracellular permeability is an indiscriminate mechanism of antigen uptake, which delivers antigens directly to the basolateral surface of the intestinal epithelial cell, and to professional antigen-presenting cells such as macrophages and dendritic cells. More important are the transcellular mechanisms, nonreceptor-mediated (fluid phase) or receptor-mediated. The former is predominantly degradative with a minor fraction traversing the cell intact, although given the large surface area this pathway may be biologically relevant. Receptor mediated mechanisms serve to transport intact molecules. Both processes have the capability of delivering macromolecules into endolysosomal compartments associated with MHC class II antigen presentation in epithelial cells[25].

Numerous reports have described a low expression of HLA class II antigens on the surface of normal epithelial cells, and have demonstrated increased expression of these molecules associated with pathological conditions such as IBD, graft versus host disease and celiac disease. These observations suggested that the intestinal epithelium might function in the immigration or regulation of $CD4^+$ T cell response in the mucosa.

Several groups have described the presentation of antigens by intestinal epithelial cells in human[30], rat[31] and mouse[32]. Heishberg and colleagues describe two distinct pathways for antigen processing by intestinal epithelial cells that distinguish between activated and nonactivated states. The first pathway is similar to that seen in conventional antigen presenting cells and uses similar proteases, invariant chain (Ii) and HLA-DMαβ. This pathway facilitates efficient antigen presentation even at low antigen concentrations and occurs in the presence of the pro-inflammatory cytokine IFN-γ. The second pathway functions independent of Ii and HLA-DMαβ, occurs in the absence of IFN-γ, and requires high concentration of antigen to elicit T-cell stimulation. This non-conventional pathway could be associated with oral tolerance[33].

Intestinal epithelial cell lines can also process apically absorbed antigen and present it on their basal surface to $CD4^+$T cells *in vitro*. As enterocytes are MHC class-II-positive in most species, but normally do not express the co-stimulatory molecules that are required for full T-cell activation, they are good candidates for tolerogenic APCs *in vivo*. Presentation of antigens by enterocytes to adjacent $CD4^+$T cells might help to explain local tolerance. However, naïve $CD4^-$T cells are rare in the *lamina propria*. In addition, lamina propria T cells do not migrate out of the gut and therefore, it seems unlikely that this pathway could contribute to systemic tolerance. It remains possible that presentation of antigens to *lamina propria* $CD4^+$T cells by MHC class-II-expressing enterocytes could be involved in maintaining the survival and activity of previously primed regulatory of effector T cells, thereby maintaining local tolerance to environmental antigens or sustaining chronic inflammatory conditions[8].

Human intestinal epithelial cells express classical MHC class I, as well as a number of non-classical MHC molecules (MICA, MICB, HLA-E, Hfe, CD1d, FcRn). The *in vivo* role of classical MHC class I is presumably related to immunosurveillance of intracellular infections, given the presence of MHC class I restricted, antigen-specific cytolitic T-cell effector cells within the epithelium[34]. A similar function

is likely attributable to MICA and MICB. The information to date suggests that this pathway of recognition does not involve intracellular processing of antigens.

CD1d expression on epithelium has been demonstrated in rodents and humans. It functions in the presentation of lipid antigens to NK-T cells ($CD4^+$ and double negative T cells bearing an invariant T cell receptor α chain (Vα24JαQ) in association with Vβ11, and expressing the natural killer cell marker NKRP1A) *in vitro*. If such response occurs *in vivo*, CD1d might function in the acquisition of lipid antigens apically and presentation basally to local T cells homologous to the hypothetical role of the MHC class II pathway for presentation of intraluminal protein antigens[25].

In addition to expressing antigen presenting molecules and the machinery necessary for antigen presentation functions, intestinal epithelial cells express a wide variety of cytokines, cytokine receptors and molecules involved in T-cell binding and potentially costimulation[35-37]. Regulated production of a repertoire of inducible/inflammatory and constitutive/homeostatic chemokines by the intestinal epithelium orchestrates and coordinates trafficking of mucosal inflammatory and immune effector cells. Enterocyte-derived chemokines likely play a major role in regulating leukocyte migration in chronic disregulated inflammation, characteristic of inflammatory bowel disease, and during infection with microbial pathogens[4].

Synthesis of Immunoglobulin A (IgA)

B cells primed in the Peyer´s patches home back to the *lamina propria* from the arterial blood after passing through the MLN, the lymphatic system and finally the bloodstream. Once the in *lamina propria*, the full maturation is achieved, and it transforms into an immunoglobulin-secreting active plasma cell.

IgA is the most important immunoglubulin in the intestine and other mucosal surfaces. Secretion of other isotypes (IgM, IgG or IgE) is augmented only if the tissues are inflamed or diseased, or if there is a deficiency of IgA. IgA2 subclass predominates in the normal intestinal mucosa, in contrast to the upper aerodigestive tract, tonsils and lymph nodes, where most of the IgA is IgA1[1].

In the intestine, the secretory form of IgA is synthesised by plasma cells in the *lamina propria*. It is a dimer of two IgA molecules covalently linked together through a joining (J) chain molecule between the alpha heavy chains. The complex is then translocated through intestinal epithelial cells and transferred to the intestinal lumen. The translocation mechanism depends on the binding of the J chain to the polymeric immunoglobulin receptor (pIgR), expressed by intestinal epithelial cells. The complex is endocytosed at the basolateral surface of enterocytes into a vesicle with the IgA attached, and then the vesicle is delivered to the apical surface by proteolysis of the receptor, part of which (secretory component) remains attached to the free immunoglobulin.

During the transport, IgA can be forming an immunocomplex with antigen, so that antigens that have passed the epithelial barrier can be cleared into the lumen. Monomeric IgA or IgG, which are not substrates for the pIgR, can be transported as part of the immunocomplexes. Specific IgA can also neutralise viral replication within the epithelial cells themselves during the transit process[38].

Most of the adaptive secretory IgA secreted in the intestine is generated in Peyer´s patches, and the progeny migrates to the *lamina propria*. However, it has been shown that a secretory response can occur in the absence of Peyer´s patches and it has been suggested that about 25% of the secretory IgA response is T- independent and largely of the B1 lineage. In humans, this pathway is less important. IgA plasma cells are highly mutated, and that implies selection and affinity maturation in the Peyer´s patches germinal centres[22].

MUCOSAL TOLERANCE

Mucosal antigen delivery can either up-regulate or down-regulate systemic immune responses. In the latter instance, a state of systemic unresponsiveness, also called mucosal tolerance, is achieved. Inhibition of antigen-specific immune responses in systemic compartments by mucosal antigen delivery is important for the prevention of overstimulation of responses to frequently encountered, and hypersensitivity responses to food proteins and allergens. Furthermore, this system could potentially be applied to the prevention and treatment of autoimmune diseases by feeding relevant antigens.

Oral administration of a single high dose or repeated oral delivery of low doses of proteins have been shown to induce systemic unresponsiveness, presumably in the presence of mucosal IgA antibody responses. Likewise, nasal administration of proteins may be equally effective.

T cells are the major cell type involved in the induction of mucosally induced tolerance, and they are involved in the generation of active suppression, clonal anergy or deletion. High doses of orally fed antigens induce clonal deletion or anergy, characterised by the absence of T-cell proliferation and diminished IL-2 production, and by IL-2 receptor expression. On the contrary, frequently administered low doses of antigen induce active suppression by $CD4^+$ or $CD8^+$ T cells that secreted cytokines such as TGF-α, IL-4, and IL-10, which are also known to up-regulate IgA production, and is thus compatible with the observation that mucosal

immune responses and systemic tolerance may concur simultaneously.

T cell priming occurs in the MLNs, either due to the antigen itself reaching the MLNs in the draing lymph or as a result of APCs that have acquired unprocessed antigen from M cells and then migrated to the MLNs. T cells that are primed in the MLNs, would then differentiate and migrate to the mucosa to induce local immune responses. Because the MLNs can act as a crossover point between the peripheral and systemic immune systems, this pathway might also explain the induction of systemic immunity or tolerance in response to intestinal antigens[38]. A subpopulation of DCs has been identified in the GALT that takes up apoptotic epithelial cell remnants and transports them to T-cell areas of MLNs. Such constitutive DC migration in the absence of overt inflammation might represent a mechanism by which antigen can be presented in a form that can induce tolerance to both self-proteins and innocuous luminal antigens derived from either dietary proteins or the normal gut flora[39].

Three possibilities of antigen uptake during oral tolerance induction can be proposed. First, antigen may be pinocytosed into the epithelial cells themselves, and interactions with IELs may influence oral tolerance. Second, antigen may selectively enter the GALT via M cells and lead to APC-T cell interactions that down-regulate T- and B-cell responses. Finally, oral antigen may not perturb the gastrointestinal tract immune system at all but simply enter and cross the epithelium in a paracellular manner and reach the bloodstream, in which tolerance would be induced.

The αβ T cells appear to be the crucial players in down-regulation of systemic immune responses to orally administered antigens. It is generally agreed that the status of oral tolerance can be explained by (a) clonal anergy, (b) clonal deletion of T cells, or (c) active suppression by T regulatory cells (either Th3, Tr1 or $CD4^+$ $CD25^+$) through the secretion of inhibitory cytokines (TGFβ, IL-4, and IL-10). Low doses of oral antigen tend to favour the third form of inhibition, whereas high doses of feeding induce clonal anergy of immunocompetent T cells. These two forms of oral tolerance are not mutually exclusive and may occur simultaneously after oral administration of antigens.

In addition, the role of γδ T cells in the achievement of systemic tolerance must be taken into account. Several studies have provided evidence that these cells are part of the essential T regulatory cell network for the induction and maintenance of antigen-specific IgA response in the presence of systemic unresponsiveness induced by prolonged oral administration of protein antigen[40,41].

Because oral tolerance is specific for the antigen initially ingested or inhaled and does not influence the development of systemic immune responses against other antigens, its manipulation has become an increasingly attractive strategy for preventing and possibly treating illnesses associated with or resulting from the development of immune reactions against specific antigens encountered or expressed (autoantigens) in non mucosal tissues.

Increasing attention is being paid to the role it could play in the prevention or treatment of autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, uveitis, type 1 diabetes, and contact hypersensitivity. Pilot clinical trials of oral tolerance have been conducted in patients with autoimmune diseases, and promising clinical benefits have been reported[42].

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