Chapter

Mucosal Immunology

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Abstract

Approximately 80% of the pathogens that lead to deadly infections in humans choose mucosal tissue as the first site of infection. The mucosal surfaces of the body include the gastrointestinal tract, airways, oral cavity, and urogenital mucosa, which provide a large area conducive to the invasion and accumulation of many microorganisms and are of great importance in this regard. The large extent of mucus, as well as the accumulation of bacteria and countless foreign antigens in these areas, are the most important reasons for the importance of mucosal tissues. In addition to the myriad of symbiotic bacteria, large amounts of oral antigens (both pathogenic and non-pathogenic) enter a person's body daily and human mucosal tissues are exposed to these antigens. The function of the mucosal immune system is to distinguish pathogenic antigens from non-pathogenic ones. In this way, against a large number of oral antigens or co-tolerant microorganisms, and pathogenic antigens, a favorable (and even non-inflammatory, possible) immune response is produced. Mucosal tissue, as the largest lymphatic organ in the body, is home to 75% of the lymphocyte population and produces the highest amount of immunoglobulin. The amount of secreted IgA (sIgA) produced daily by mucosal surfaces is much higher than the IgG produced in the bloodstream. A 70 kg person produces more than 3 grams of IgA per day, which is about 70–60% of the total antibodies produced in the body. The first embryonic organ in which immune system cells are located in the intestine. Some researchers consider this organ (and specifically mucosal lymph nodes) to be the source of the human immune system.

Keywords: mucosal immunology, mucosa associated lymphoid tissues, organized mucosal associated lymphoid tissue, diffuse mucosal associated lymphoid tissue, innate lymphoid cells, M cell, poly immunoglobulin receptor, mucosal vaccination

1. Introduction

Mucosal surfaces interact directly with the outside of the body and interact with countless antigens. The need to establish an immune system in this tissue to fight pathogens is obvious, but the development of an immune response against native antigens or bacterial bacteria is an undesirable response. Therefore, the immune system in the mucosal tissues must be tolerant of many antigens, while maintaining the ability to respond to a small number of pathogenic antigens. Any tissue that can secrete mucus on the surface of the epithelial layer and can participate in the immune response is considered part of the mucosal lymphatic tissue (MALT). MALT is present in the gastrointestinal tract, airways, urogenital tract, conjunctiva, and endocrine glands (salivary and sweat glands), but has been studied mainly in the gastrointestinal tract, respiratory tract, and urogenital tract. Both innate and adaptive immune systems (humoral and cellular) are seen in these tissues. One of

the defense mechanisms in the mucosa is the physical and mechanical defense that acts as a non-specific barrier against infections, including the mucosal epithelial layer, intestinal peristaltic activity, and the mechanism of mucosal-mucosal clearance in the airways. The first line of defense in the mucosa is physical defense and innate immunity. Innate immune cells, such as tissue-resident macrophages and migrating neutrophils, are the first cells to act upon the onset of pathogen exposure. After innate immunity, adaptive immunity and its cells are activated by dendritic cells in the marginal lymph nodes (or in organized mucosal-associated lymphoid tissue) and called to the sites of infection. B cells in mucosal tissues produce and secrete antibodies, especially IgA. T lymphocytes also play a role in secreting pro-inflammatory cytokines or inducing cytotoxic activity. Moreover, mucosal tissues contain populations of Tαβ and Tγδ [1, 2].

1.1 Lymphatic tissues in the gastrointestinal tract

The human gastrointestinal tract consists of a tubular structure covered by a mucosal epithelial layer. Beneath the epithelial cells is the lamina propria, or lining of the mucosa, which contains the mucosal connective tissue (MALT), blood vessels, and lymph vessels. MALT located in the gastrointestinal tract is also called GALT[1]. MALT in this area also contains a large number of immune cells, which alone are larger than any other set of bone marrow, thymus, spleen, and lymph node cells. Mucosal lymph tissue is mainly composed of intraepithelial lymphocytes (IELs), lamina propria lymphocytes, IgA-producing plasma cells and macrophage antigen-presenting cells, dendritic cells, neutrophils, eosinophils, and mast cells. In certain areas of the mucosa, there are lymphoid follicles that contain T lymphocytes, B lymphocytes, etc. In general, it can be said that the intestine prevents the entry of bacteria and infectious agents in three ways, the first is through the mucosal layer that prevents the penetration of bacteria from the epithelium. The second barrier is the production and secretion of antimicrobial peptides in the intestinal lumen and killing them within the lumen. The third method of inhibition is the production of IgA from the plasma of lamina propria, which neutralizes pathogens within the intestinal lumen [3, 4].

2. The role and structure of mucosal lymph tissues

Mucous lymphatic tissues can be classified according to their structure and function. Structurally, mucosal lymph nodes are divided into two categories: organized or O-MALT[2] and diffuse or D-MALT[3]. Functionally, O-MALT is known as the site of induction of the immune response and D-MALT is the site of the immune response. In other words, immune responses are formed in O-MALT and perform their executive function in D-MALT. O-MALT is a place for antigen processing and production of effector and memory cells, after which the produced cells migrate to other mucosal diffuse lymph tissues such as D-MALT, leading to the protection of body surfaces. However, it has recently been shown that both types of lymph tissue play an important role in the production and differentiation of mucosal lymphocytes and mucosal immunity. Epithelial cells also play a role in the differentiation and production of cytotoxic T cells. It seems that intestinal mucosa and other mucosal surfaces affect bone marrow progenitor cells (T and B cells) and are effective in

[1] Gut Associated Lymphoid Tissue.

[2] Organized Mucosal Associated Lymphoid Tissue.

[3] Diffuse Mucosal Associated Lymphoid Tissue.

gene rearrangement of immunoglobulins and T cell receptors. The activation of the enzymatic machine required for the genetic synthesis of progenitor cells in the gut supports this theory. T cells also regulate the activity of epithelial cells. For example, intercellular permeability and ion secretion (by these cells) are affected by IFN-γ. Crypt cell proliferation in the small intestine and mucosal morphology are also regulated by T cell cytokines. O-MALT is called the afferent lymphoid region, which is the site of antigen entry and the formation of immune responses. While D MALT is an efferent lymphoid region and acts as a site of antigen interaction with differentiated cells (leading to antibody secretion and the activity of helper and cytotoxic lymphocytes) [1, 4, 5].

2.1 Organized mucosal associated lymphoid tissue (O-MALT)

O-MALT in the gastrointestinal tract includes Peyer's patches and isolated lymph follicles (ILF). The number and location of mucosal follicles vary greatly between species and in an individual also changes over time and exposure to antigens. Most of these centers are isolated and scattered throughout the airways and gastrointestinal tract, but their extent increases to the colon and rectum. Some of these lymphatic tissues together form large complexes such as the palatine, lingual, and pharyngeal tonsils called the Waldeyer's ring, mucosal follicles in the appendix, and Peyer's patches in the small intestine. Peyer's patches are more in the ileum (the last third of the small intestine) and less in the jejunum (not seen in the colon). Mucosal lymphoid follicles in both single form (ILF) and complex (Peyer's patches) are covered by a specific epithelium. The general structure of the lymph plaques of Peyer's patches is shown in **Figure 1**. Each Peyer's patches contain more than 100 lymphoid follicles, each with a dark border and a relatively lighter circular center. O-MALT in gastrointestinal lymphatic tissues includes Peyer's patches (in the ileum) and ILFs (in the colon).

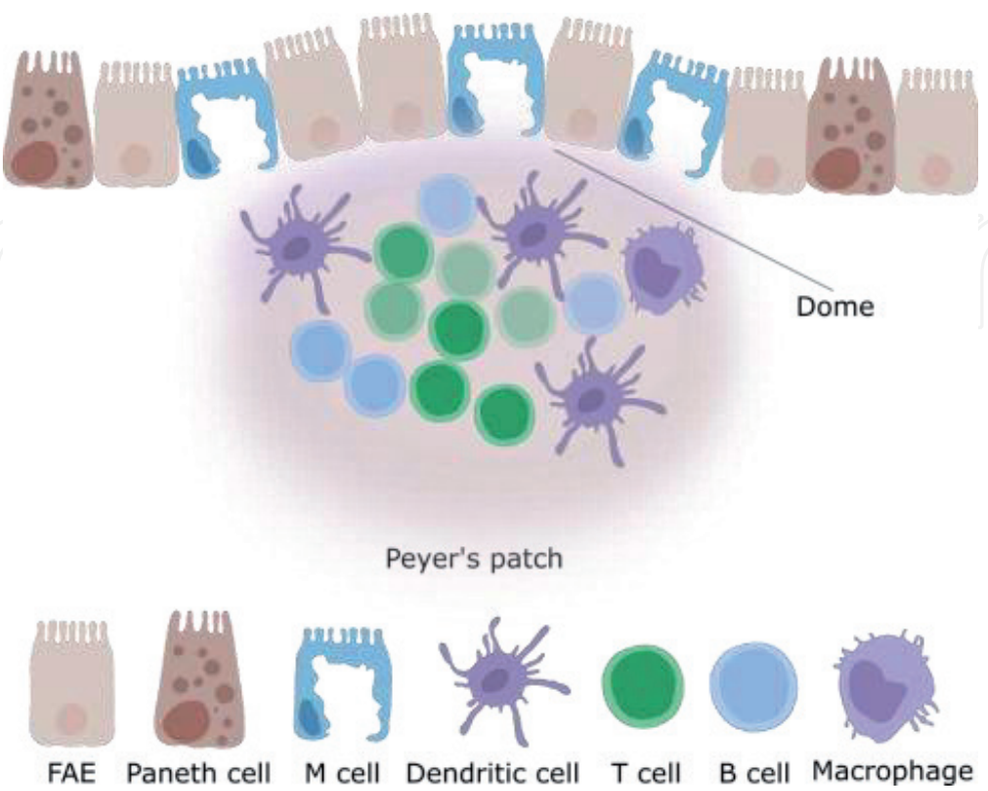

Figure 1.
The general structure of Peyer's patche lymph follicles. FAE, Follicule associated epithelium.

The follicles are separated from the mucosal epithelium by intercellular spaces and a dome-shaped area filled with lymphocytes called the corona. The mucosal surface above the corona of the follicle is free of villi on the surface of the epithelium of other areas and contains antigen-carrying cells or M cells (found only in this area). High endothelial venules (HEV) where lymphocytes leave the artery are located in the interfollicular section [1, 4, 5].

2.1.1 Lymphocytes in O-MALT

Lymphocytes in O-MALT and follicle associated Epithelium (FAE) has been studied in several species [6–8]. The follicles are the site of accumulation of B lymphocytes, dendritic cells, and macrophages. However, T lymphocytes are mainly predominant in the internal and parafollicular parts [6, 9]. Most of the parafollicular B lymphocytes and located in the corona are IgM^+ and the B cells in the germinal centers are IgA^+. CD4 + T lymphocytes are mostly found in the corona, below the epithelium of the dome area, and the parafollicular regions, and CD8 + T cells are often found in the interfollicular regions.

2.1.2 Antigen-presenting cells

Antigen-presenting cells in O-MALT (such as Peyer's patches) include follicular dendritic cells within the germinal center, interdigitating cells near lymphocytes of parafollicular regions, macrophages, and B cells. Macrophages are mostly concentrated in the coronal and B lymphocytes are often found in the follicular regions [7, 10, 11]. Antigen-presenting cells trap antigens of extracellular origin in endosomes. In these phagosomes, antigens are digested and processed by specific proteolytic enzymes, and finally the peptides are presented to T lymphocytes by MHC II. Cells isolated from Peyer Patch mice can be stimulated with antigen in vitro, resulting in a primary and secondary immune response, leading to the production of IgM class antibodies and IgG and IgA class antibodies, respectively [12].

2.2 Diffuse mucosal associated lymphoid tissue (D-MALT)

Diffuse lymphoid tissue is scattered throughout the mucosal surface and includes lymphocytes, diffuse plasma cells in the lamina propria, mucosal connective tissue, and intraepithelial lymphocytes (IELs). Some of these cells are derived from O-MALT and contain effector and memory lymphocytes. These cells are caused by antigen stimulation in areas such as Peyer's patches. In a regular process, antigen-stimulated cells begin to migrate from the site of stimulation and settle in other mucosal tissues [13, 14].

2.2.1 Intraepithelial lymphocytes (IELs)

Intraepithelial lymphocytes are often T cells located in the epithelial layer. About 15 to 20% of the population make up epithelial cells. These cells are considered guarding cells in the immune system and react with antigens earlier than others, and therefore show memory phenotype ($CD45RO^+$).

IELs are found in two types, Tαβ and Tγδ. The main function of these cells is to establish tolerance against symbiotic bacteria and to protect against pathogenesis. In humans, about 90% of IELs are Tαβ and only 10% are Tγδ. In mice, the percentage of Tγδ cells reaches 50%.

Most IEL cells are $CD8^+$ and are divided into two categories in terms of origin. Some of these are conventional Tαβ cells that have evolved in the thymus that

can express both the $CD4^+$ marker and the $CD8^+$ marker. The other group is the unconventional or natural Tαβ cells and Tγδ, which have evolved in environments other than the thymus, such as the intestine. These lymphocytes have the power of self-renewal and are restricted to non-classical MHC molecules. These unconventional IELs usually show a specific CD8 consisting of α chain homodimer. Most intraepithelial Tγδ cells, as well as many Tαβ lymphocytes in the gut, express the CD8αα homodimer. For this reason, the expression of CD8αα has been considered an indicator of intraepithelial T cells in the intestine compared to peripheral blood T cells.

Few IELs are found with the $CD4^+CD8^+$ or $CD4^-CD8^-$ phenotype. Unlike conventional TCRs, which have a wide variety, TCRs in IELs have limited variability.

Most IELs are dormant under normal conditions but react as soon as they are exposed to the antigen due to a memory phenotype. $T\alpha\beta CD8^+$ and Tγδ cells show cytotoxic activity against infection. Production and storage of perforins and granzymes can be done in IEL.

Conventional T cells, unlike unconventional T cells, must be activated to play their executive role. Both abnormal Tαβ and Tγδ cells in the intestinal epithelium detect antigens at the level of non-classical MHC molecules such as CD1, which allows factors expressed on the surface of damaged epithelial cells to respond to stress. Thus, Tγδ cells can also be activated in response to foreign antigen peptides and host cell-derived danger signals.

Tγδ cells have a more limited gene repertoire of TCR and in the gut often express the Vδ1 chain, which is different from blood Tγδ. Vδ1-expressing Tγδ cells can detect non-classical MHCs induced by MICA and MICB stress. MICA and MICB are known as the damage-associated molecular pattern (DAMP) and increase in response to cellular stress. Tγδ can respond to tissue damage in the shortest possible time. By secreting IFN-γ, these cells increase the cytotoxic response against virus-infected cells and enhance the neutrophilic response against bacteria.

Tγδ lymphocytes in the gut play an important role in protecting mucosal surfaces from damage caused by immune responses. Tγδ lymphocytes also regulate immune responses by increasing TGFβ and limiting the migration of inflammatory leukocytes to the intestinal tract. In addition, these cells produce Insulin-like growth factor-1 (IGF-1) and keratinocyte growth factor (KGF).

The proportion of Tγδ cells is higher among IEL cells in infancy. As you age, the proportion of Tαβ cells increases, so Tγδ cells in infancy are likely to play an effective role in defending against pathogens [2, 5].

2.2.2 Lamina properia lymphocytes

Lamina Properia Lymphocytes include B cells (often transformed into plasma cells) and T lymphocytes. In mice, 40% of lamina properia lymphocytes are B cells that produce mainly IgA. 25% of the cell population are T lymphocytes (mainly with the CD4 + TH2 phenotype) [15–19]. Human lamina propria CD4 + T cells provide memory cell markers and do not proliferate in response to antigenic stimuli. Rather, they produce cytokines such as IFN-γ [20]. The predominant population of T lymphocytes in the lamina propria is CD4 + T (60–70%), the majority of which exhibit the TCRα/β phenotype. Most of these cells have the CD45RO (specific for memory cells) and are different from peripheral blood T lymphocytes in this respect. Lamina propria is an important center for IgA production. In these areas, O-MALT derived B lymphoblasts (such as Peyer's patches) are affected by cytokines such as IL-6 and undergo differentiation [21]. Lamina properia TH1 cells proliferate TH2 cells by secreting IL-2 and IFN-γ. On the other hand, TH2 cells, by producing IL-5 and IL-6, prepare for the differentiation of B cells into IgA-producing plasma cells [22].

The lymphocytes in the lamina propria are mainly in the late stages of differentiation and often turn into plasma cells. Furthermore, In the intestinal lamina propria cells such as macrophages, neutrophils, eosinophils. There are dendritic cells and mast cells. Lamina propria CD4 + T cells can react with these cells, enhancing their phagocytic and antimicrobial capacity. Macrophages may also be involved in the processing and delivery of antigens to T cells.

3. Innate lymphoid cells in intestinal mucosa

Innate Lymphoid Cells (ILCs) in the intestinal mucosa are involved in defense against pathogens, enhancing the function of the physical barrier, and tolerance to the microbial flora. There are two types of ILC2 and ILC3 in the mucosa, and ILC2 is involved in the defense against worms in the gut. Besides, in response to cytokines IL-33 and IL-25, they can secrete cytokines IL-5 and IL-13, the former of which is effective in activating eosinophils and the latter in increased mucus production and thus repelling worm parasites. ILC3 is also present in the gut and can produce the cytokines IL-17 and IL-22 in response to stimulation with IL-18 and IL-23 cytokines. The cytokines produced by these cells are involved in enhancing the physical function of the mucosa by stimulating the production of defensins and strengthening strong epithelial connections.

Other cells in the mucosa are Mucosal associated invariant T (MAIT), which are a subset of CD8 + T cells with invariant TCR Va7.2-Ja33. The main role of these cells is to defend against bacteria and fungi that cross the intestinal epithelial barrier and enter the bloodstream. Intestinal bacteria (normal flora or other bacteria) enter the liver through the portal vein and encounter the MAIT cells if they pass through the intestinal epithelium and enter the bloodstream. These cells detect fungal and bacterial metabolites through an MHC-like protein class 1 called MRI and, once activated, produce a cytotoxic role by producing inflammation-promoting cytokines. 50% of the population of T cells located in the liver belongs to this group [1, 4, 5].

3.1 Enterocytes and antigen-presenting

Mucosal epithelial cells (especially small intestinal enterocytes) act as antigen-presenting cells and present MHC II molecules [23–26]. Besides, CD1d (MHC I-like) molecules are present on the surface of these cells. Mature enterocytes from intestinal villi express class II molecules whereas crypt cell production may be affected by cytokines such as IFN-γ [27]. Enterocytes are able to present antigens to T cells in vitro. However, the T cell response is suppressive [28, 29] and this mechanism seems to be involved in mucosal tolerance.

4. Antigen penetration into O-MALT

4.1 Follicule associated epithelium (FAE)

The intestinal epithelium can be thought of as a complex of crypt-centered cells. In the small intestine, each crypt contains a large number of undifferentiated germ cells from which other cells are formed. Differentiated crypt cells then cover adjacent villi [30].

Goblet cells, enterochromaffin, and pIgR-containing enterocytes are located in the lateral wall of the villi. Cells that move from the crypt to the dome of the lymphoid follicles become pIgR-containing enterocytes and M cells [31].

The location of Peyer's patches and other parts of O-MALT in members of an animal species is known. Immature M cells remain even after lymphocyte depletion with radiotherapy [32, 33]. The formation of mucosal tissues is organized before birth [34]. However, the antigen transfer process causes the mucosal follicles to expand. In general, it can be said that the superficial components of epithelial cells together with local secretory products are involved in the formation of O-MALT.

4.2 M cells

The cytoplasm of M cells forms a thin membrane-like structure in the upper part of the cytoplasm that separates the inner space of the intestine from the space below the epithelium, hence it is also called the membrane epithelial cell. In other words, these cells have a large envelope in which many immune cells, such as antigen-supplying cells, are located in this envelope, closest to the intestinal tract (**Figure 2**).

An important role of M cells is the transfer of antigen to the O-MALT. These cells are not presenting of antigen, but only its transporter. These cells endocytose the antigen not specifically, but selectively, meaning that not every antigen can pass through M cells. M cells select and pass antigens based on molecular load, hydrophobicity, and viability.

Since the transfer of antigen by M cells can play an important role in the first stage of the immune response, the factors that affect this transfer are very important in choosing a mucosal immunization strategy. M cells make up between 10% in humans and animals and up to 50% in rabbits around the follicular epithelial cells (FAE) [35]. Areas specific to endocytosis are present between irregular or shallow short microvilli on the upper surface of the M cell [36]. These cells lack some of the digestive enzymes present on the anterior membrane of enterocytes. However, M cell membranes contain many glycoconjugates compounds that can be suitable binding sites for lectin-like microbial surface molecules [36–38]. These cells endocytose and transmit microorganisms, particles, and lectins that selectively attach to their apical membrane with high efficiency [36], in other words, substances that bind to mucosal surfaces elicit a strong secretory response. For example, oral administration of lectin leads to the production of anti-lectin-specific IgA. While

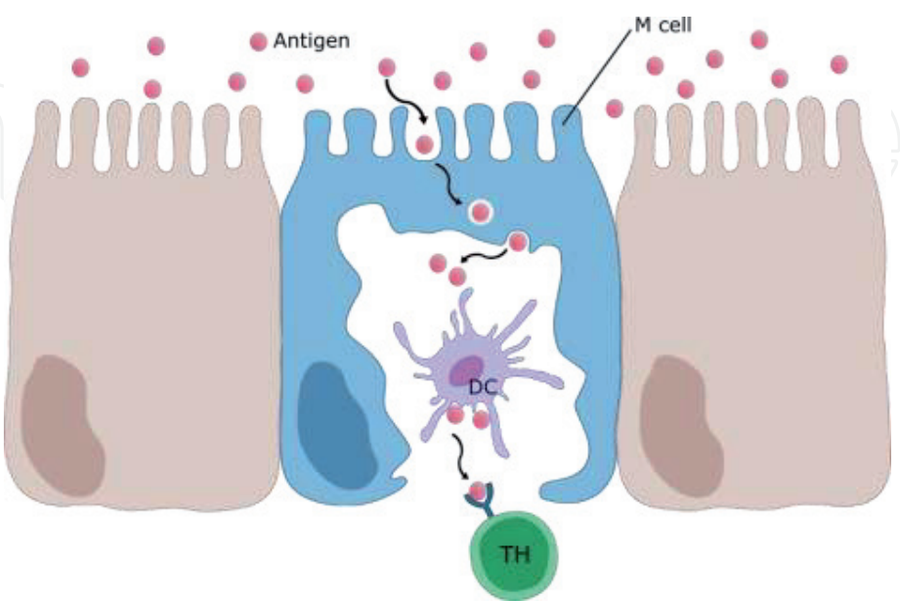

Figure 2.
M cell. The basement membrane of the M cell begins to form an intracellular envelope. M cells first transfer antigens from the airways and gastrointestinal tract to the envelope and then to the defense cells located beneath the epithelium.

the administration of the same amount of another immunogen that does not have adhesion and binding properties is ineffective [39].

The reason for the lack of adverse responses to food antigens and the normal intestinal flora should be sought in the inability of M cells to transmit soluble luminal antigens and nonadherent particles [40]. It seems that the introduction of small but frequent oral or inhaled amounts of soluble immunogens leads to tolerance [41].

Some viruses, bacteria, and protozoa, such as Cryptosporidium, selectively attach to M cells and transmit well. Among these viruses, only reovirus type I, poliovirus, and HIV 1 bind specifically to the upper membrane of M cells. These viruses do not attach to cell surfaces in the FAE or the epithelium of the villi.

In reovirus type I, one of the outer capsid proteins (δ1 or μ1), after being activated by the proteolytic process in the gastrointestinal tract, causes the virus to contact the M cell.

In animals, large numbers of gram-negative pathogens and *Streptococcus pyogenes* bind selectively or preferably to M cells. Some viruses (such as rotaviruses and transmissible gastroenteritis viruses), as well as bacteria such as *Escherichia coli* [42], Yersinia pseudo-tuberculosis, *Vibrio cholerae* [43–45], Shigella [46], *Yersinia enterocolitica* [47], and *Campylobacter jejuni* [48], have proliferated in M cells after infiltration and they cause local infection and inflammation. M cells use a carbohydrate-lectin detection system with multiple receptors to identify a variety of pathogenic microorganisms in the gut.

The cell surface of M is increased due to the presence of accessible membrane regions and specific binding regions of large ligands and is therefore different from other epithelial cells.

In the gut, immunoglobulins also bind specifically to M cells [49], so that for the first time in suckling rabbits, accumulation of milk sIgA was observed on M cells of Peyer's patches.

Both Fc and Fab IgG fragments attach to the M cell. Lectins present on the surface of M cells identify abundant oligosaccharides present on immunoglobulins. Specific binding and transport of immunoglobulins by M cells may be involved in the regulation of immune responses. sIgA usually prevents antigens and microorganisms from coming into contact with mucosal surfaces. The Fc Domain IgA molecule is hydrophilic (hydrophilic and hair-phosphatic) and binds IgA (attached to microorganisms) to epithelial cells. The Fc properties of the IgA molecule prevent the colonization of pathogens (without causing inflammation).

Antigens of these complexes are reabsorbed and evaluated by macrophages and lymphocytes inside or below the epithelium (containing Fcα receptors) [50–52]. This event intensifies the secretory immune response against pathogens that have not been effectively eliminated from the gut. However, convincing evidence of the ultimate fate of IgA or IgA-Antigen complexes is not available after uptake by M cells but it is speculated that Fcα receptors on the surface of mucosal cells may play a role in other stages of the mucosal immune response. IgA reacts with lactoferrin and lactoperoxidase through the FC region, thereby enhancing the function of these nonspecific defense elements.

4.3 Antigen transfer

M cells absorb adhesive molecules such as lectins and ferritin through membrane clathrin vesicles and discharge them into vesicular or tubular structures similar to the cytoplasmic apex endosomes (above the epithelial pocket) [36]. In this part of the cell structure, vesicular endosomes are rarely found and no structures are containing acid phosphatase [53]. During transfers, endocytic materials do

not decompose extensively. However, the presence of endosomal hydrolase in M cell transport vesicles has not been ruled out. The apical vesicles of M cells are acidic [54].

Proteins and microbes that have entered the M cell vesicles are discharged out of the epithelial cell by the exocytosis membrane up to 10 minutes after vertebral endocytosis [36, 55].

Exocytic vesicles originate from endosomal intermediate components and structures. Lysosomes are present in the pericardial Golgi of M cells, but endocytic materials of the apical membrane have not been observed in these areas.

M cells shorten their transport path by lifting the lateral membrane toward the apex and shortening the lateral endosomes directly to specific regions of the lateral base (**Figure 2**). The intraepithelial membrane of M cells is different from the lateral membrane (which attaches to the adjacent cell) and the basement membrane (which attaches to the basal lamina).

For example, it has been shown that Na/K ATPase pumps are concentrated in the lateral part (not in the envelope membrane of M cells. It is said that the presence of a specific population of lymphocytes in the M cell envelope indicates the presence of specific lymphocyte receptors in the envelope membrane (**Figure 2**). The mechanism of distribution of specific lymphoid cells in this area is still unknown The pattern of glycosylation determines the specificity of M cells. The structure of LPS in *salmonella typhi* morium fimbriae plays a role in binding to M cells.

M cells, make up a small population of epithelial cells. However, their ability to transmit intestinal adhesive particles is remarkable.

4.4 M cells, areas of infiltration of pathogenic microorganisms

M cells have developed their non-specific mechanisms for binding and absorption of intestinal material so that the mucosal immune system can access a variety of microorganisms and particles. The ability of M cells to bind to bacteria such as *Vibrio cholerae* allows the immune system to sample these non-invasive pathogens well and to organize the appropriate secretory immune response. The secretion of sIgA anti-cholera toxin (CT) plays an important role in limiting the course of the disease and preventing the recurrence of infection [56–58].

Many pathogenic bacteria and viruses that attach to M cells use this intraepithelial transport pathway as an invasion pathway. For example, reoviruses and polioviruses reach the Peyer's patches by selectively binding to the apex of the M cells [59, 60]. *Salmonella typhimurium* in mice and *Salmonella typhi* in humans are gram-negative pathogens that transmit to M cells attached to Peyer's patches and cause disease [45]. An effective mucosal immune response against Salmonella cannot prevent the organism from spreading to the liver and spleen. Therefore, with intestinal infiltration into the host, the systemic spread of the disease will occur. In addition, early transport of *Shigella flexneri* [46] and *Yersinia enterocolitica* [47] causes these organisms to enter the lamina propria by invading the lateral basal surfaces of epithelial cells and infecting mucosal macrophages.

O-MALT contains IgA-producing plasma cell precursors and is the center of the mucosal IgA response. After being transfected by M cells, the antigens first encounter the antigen-presenting cells and the lymphocytes in the cell's inner envelope [7]. In the dome area below the FAE, IgM^{+}B cells, CD4 + T cells, dendritic cells, and macrophages form a cellular network by which antigens are absorbed, processed, and delivered to lymphocytes. After activation, the process of maturation and differentiation of B cells occurs in O-MALT.

5. Dendritic cells in the gastrointestinal tract

In mucosal tissues, dendritic cells are known to be the main controllers of immune responses These cells act as a protective system and, by identifying pathogens, can stimulate naive T and B cells. Both O-MALT and D-MALT tissues contain dendritic cells. There are several subgroups of DCs in the mucosa, each with unique properties. DCs in the Peyer's patches are often located in the M cell envelope and the subepithelial dome (SED) and are $CD11b^{+}$, $CD8\alpha^{-}$, $CCR1^{+}$, and $CCR6^{+}$. CCR1 and CCR6 receptors bind to CCL9 (MIP-1γ) and CCL20 (MIP-3α) chemokines, respectively.

CCL9 and CCL20 are continuously secreted from FAE cells and are located by the CCR1 and CCR6 receptors, causing these DCs to be located in the Peyer's patches epithelium.

DCs of Peyer's patches secrete 10-IL in the absence of infection in response to the uptake of dietary antigens or microbiome, which inhibits the inflammatory response to these antigens. When exposed to pathogens, these DCs are rapidly recalled below the FAE by increasing CCL20 secretion from the epithelium. Microbial products cause the expression of co-stimulatory molecules on the surface of DCs, and excited DCs lead to the activation and differentiation of naive T cells into effector cells. In Peyer's patches, in addition to the above-mentioned DCs, there is another DC subclass, which, unlike the first type, is $CD11b^{-}$, $CD8a^{+}$ and $CCR6^{-}$. These cells are found in T cell-rich areas in Peyer's patches and produce IL-12 inflammatory cytokines.

A major route of antigen transport to Peyer's patches (O-MALT) is M cells. Other ways to transport antigens to the O-MALT region include the entry of food and soluble antigens through the epithelium. Moreover, the presence of FcRn on the surface of enterocytes enables these cells to detect IgA-coated antigens. The binding of FcRn to the antigen and antibody complex can trigger the entry of immune complexes from the luminal surface to the basal surface of enterocytes by transcytosis. When apoptosis kills pathogen-infected enterocytes, antigens can penetrate the subepithelial layer. More specifically, DCs uptake apoptotic cell debris and associated antigens (**Figure 3**).

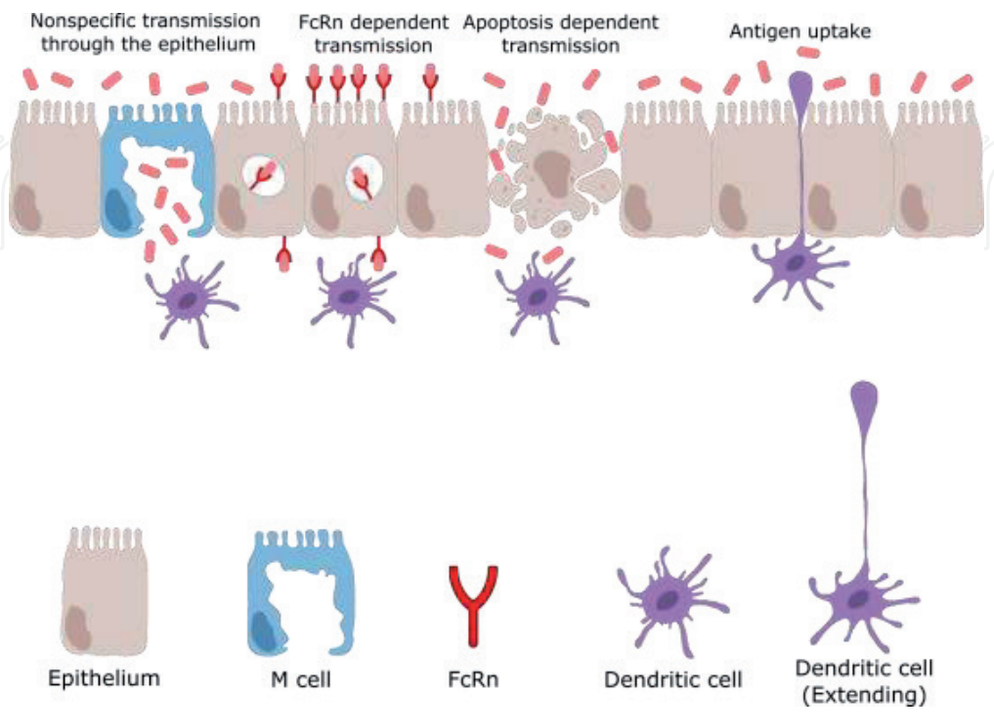

Figure 3.
Different ways antigen enters mucosal tissues.

Another way to pick up antigens in the gastrointestinal tract is through DCs and macrophages, can send their appendages into the intestinal lumen without disrupting the integrity of the epithelial cells and actively sampling the antigens in the lumen, thereby transporting the antigen to the Transmit lamina propria.

Lamina propria dendritic cells (LPDCs) that pick up antigens in ways other than M cells play an important role in maintaining tolerance to non-pathogenic intestinal antigens.

LPDCs express the CD103 index (integrin α_E: B7) at their surface and can migrate to T-cell-rich regions of the mesenteric lymph nodes through afferent lymphatics. In the mesenteric lymph nodes, LPDCs can react with naive T cells and activate them, inducing intestinal homing characteristics in these cells. As a result, active T cells can return to the gut and differentiate into effector cells. The migration of $CD103^+$DCs to the lymph nodes is dependent on CCR7 expression. CCR7 is constantly expressed on the surface of these DCs, but its expression increases during infection. When there is no infectious agent, about 5 to 10 percent of mucosal DCs migrate to the mesenteric lymph nodes.

$CD103^+$ dendritic cells produce the non-protein retinoic acid (RA) molecule that is involved in cell signaling. RA is the product of the effect of retinal dehydrogenase enzyme on vitamin A. RA production from these DCs induces CCR9 and integrin α4: β7 markers on the surface of B and T cells, which is effective in implanting these cells in the intestine. LPDCs respond poorly to inflammatory stimuli such as TLR ligands and produce more IL-10. For this reason, the migration of $CD103^+$ DCs into the mesenteric lymph nodes in the absence of an infectious agent causes differentiation into Treg $FoxP3^+$ (iTreg) cells. RA secreted from DCs and TGFβ plays an important role in differentiating these Treg. TGFβ is abundantly produced by intestinal cells. In addition, intestinal DCs produce a substance called Indoleamine 2, 3-Dioxygenase (IDO). This enzyme catalyzes tryptophan and leads to the differentiation and induction of Treg cells in the intestine.

$CD103^+$ DCs in the small intestinal mucosa are effective in combating inflammation. Factors such as RA, TGFβ, PGE2, and TSLP[4] are effective in perpetuating this anti-inflammatory response. TSLP, RA, and TGFβ are made by intestinal epithelial cells.

Macrophages located in mucosal tissue naturally produce IL-10. This cytokine deactivates DCs and preserves mucosal Tregs.

Studies have indicated that $DC103^+$DCs, located in the large intestine, play a role in maintaining tolerance and the immune response to symbiotic bacteria and are rarely seen in Peyer's patches. In addition to $CD103^+$ DCs, other myeloid cells are found in the lamina propria, which stimulate inflammatory responses. These cells produce cytokines such as IL 6, IL 23, TNF a, and nitric oxide (NO), which are involved in differentiation into executive TH17 cells and class switching to IgA in B lymphocytes. These $CD103^-$ DCs are stimulated by TLR5 and express the CX3CR1 index, which is the receptor, and chemokine fractalkine. The aforementioned cells cannot migrate to the lymph nodes and are not able to present antigen to the naive T cell and produce RA. Furthermore, in addition, they are not classified as classical DCs, are more like macrophages, and are involved in the production of inflammatory cytokines.

6. Adaptive immunity in the gastrointestinal tract

Humoral immunity and mucosal IgA production are the main forms of acquired immunity in the gastrointestinal tract. Secretory IgA dimer into the lumen, IgG and

[4] Thymic Stromal Lymphopoietin.

IgM participate in the defense against pathogens. The role of cellular immunity in the gastrointestinal tract is to control responses in the gut with the help of Treg and TH17 cells.

After capturing the antigen, dendritic cells migrate to the mesenteric lymph nodes and Peyer's patches, and acquired intestinal immune responses are formed[5]. Active T and B lymphocytes enter the bloodstream through the lymph flow at the site of the thoracic duct. They then settle in the mucosal tissues through the appearance of implanted surface molecules in the intestinal mucosa [1, 4].

6.1 Mucosal B lymphocytes and IgA production

In Peyer's patches, most B cells in the corona and dark zone of the follicular germinal centers are IgM^+/ IgD^+, while in the light zone the germinal centers cells are more than 90% IgA^+ cells. IgA cells in the germinal centers leave the O-MALT, enter the mesenteric lymphatic ducts, and then the blood flows from there to the mucosal and glandular areas of different parts of D-MALT and become IgA-producing plasma cells. IgA cells in the germinal centers are called immune cells. Unlike villi capillaries, which allow the release of serum proteins into lamina propria, capillaries in Peyer's patches have no pores and are impermeable to serum proteins. Therefore, it can be said that immune response interactions such as antibody response, cell accumulation, and secretion of cytokines against intestinal O-MALT antigens are not affected by systemic processes. Based on this, it can be acknowledged that circulating IgA is unable to prevent viral invasion of Peyer's patches and the proliferation of infectious agents in the mucosa. Class switching to IgA occurs in O-MALT. The predominant class of antibodies in the gastrointestinal mucosa is the IgA dimer. In humans, two IgA subclasses are encoded in the genome by two separate and distant sequences. Class switching is associated with the removal of genes upstream of the CH fragment.

In the intestinal mucosa, by two mechanisms dependent or independent of T cells, the class is selectively switched to lgA. Cytokines are extremely important in any phenomenon of class change. In the gut, TGFβ also plays an important role in switching classes to IgA. If class switching is T-dependent, IgA is produced with a higher affinity for the antigen. The DCs capture the antigen, move it to the interfollicular zone (in Peyer's patches) or the mesenteric lymph nodes, and deliver it to the naive CD4 + T. CD4 + T cells are then activated and differentiated into TFH (follicular helper T cells). Then, they react with B IgM^+/ IgD^+ cells and induce class switching to IgA. The prerequisite for this is TGFβ and CD40L binding of T cell surface to CD40 expressed in B cell. NO production from dendritic cells can increase the expression of TGFβ receptor on B cells. In T-cell-independent switching, active dendritic cells produce cytokines such as APRIL[6], BAFF[7], and TGFβ, leading to the induction of class switching in B IgM^+ / IgD^+ cells (especially B1 cells). In this case, IgA is produced with less binding affinity than in the T cell-dependent state.

In the process of differentiating $BIgA^+$ cells into IgA-producing plasma cells, the cell secretory system is fully developed, α-CH fusion occurs at the mRNA level, and a J chain is produced. IL-2 is involved in regulating J chain production in B lymphocytes and plasma cells. In vitro, B cells committed to producing IgA of O-MALT origin undergo 6-IL differentiation in the final stages of differentiation. But in vivo studies do not confirm this finding. Therefore, it can be concluded that there are no

[5] Inductive Sites.

[6] A proliferation-inducing ligand.

[7] B-cell activating factor of the TNF family.

factors required for IgA differentiation and secretion. By migrating these lymphocytes to D-MALT regions and effector sites, the conditions for differentiation into end-cell cells are provided [1, 4, 5].

6.2 The role of secretory IgA in the regulation of immune responses

IgA B cells do not differentiate in O-MALT and therefore IgA concentration is low in these areas. Serum immunoglobulin concentrations are also very low in these areas [61]. However, sIgA located in the lamina propria and glandular secretions enter the O-MALT by binding to the apical membrane of M cells in the FAE [62].

T cells containing the Fc receptor in Peyer's patches act as helper cells and increase BIgA + cells. Fcα receptor T and B cells are involved in the specific regulation of the isotype of the mucosal immune system [63].

Antigen-IgA complexes are also transported to O-MALT by M cells [62], so it can be said that the Fcα receptor of B cells or macrophages enhances the immune response by increasing antigen uptake and processing. In conclusion, IgA reabsorption by M cells and reaction with Fcα receptors are involved in modulating the immune response [64].

Also, in mucous secretions and glands, anti-idiotypes can enhance the immune response by such a mechanism. This clarifies the reason for the reaction of breastfed infants (sIgA absorption) to oral and injectable vaccines [65].

6.3 Lymphocyte migration and homing

Lymphocyte and monocyte migration and implantation play an important role in the mucosal immune response. This process causes a set of specific cells to migrate to areas such as the Peyer's patches where antigens are present, and the widespread effector and memory cells to different parts of the mucosal surface provide comprehensive protection for the body.

Numerous molecules and receptors are involved in the lymphocytes homing into the intestinal mucosa, including homing receptors, cell adhesion molecules (integrins) of chemokines, and chemokine receptors.

Naive lymphocytes enter the mesenteric lymph node and O-MALT (Peyer's patches) through HEV. Lymphatic tissues facilitate the entry of naive lymphocytes expressing CCR7 and L-selectin by secreting CCL19 and CCL21. If in O-MALT and lymph nodes, these lymphocytes are exposed to specific antigens presented at the APC, the incidence of CCR7 and L-selectin is reduced. Once the cells are activated, they leave the mesenteric lymph nodes through the lymph and Peyer's patches and enter the bloodstream through the thoracic duct. Dendritic cells in the mucosa can induce specific molecules to localize activated lymphocytes in the gastrointestinal tract. Activated lymphocytes increase the expression of α4: β7 integrins that bind to MadCAM1 on their surface. MadCAM1 is expressed on the endothelial surface lining the blood vessels of the intestine and its associated lymphatic tissues. Due to this interaction, it provides the conditions for the adhesion of active lymphocytes to the endothelial vessels of the gastrointestinal tract. Activated T and B cells express the CCR9 chemokine receptor on their surface after initial exposure to antigen in the small intestine. This receptor binds to TECK (CCL25) at the epithelial surface of the small intestine, leading to the re-implantation of these cells in this area. Primary activation of lymphocytes in the colon leads to the development of the chemokine receptor CCR10, which binds to the MEG (CCL28) surface of the colon epithelial cells. Furthermore, CCL28 can be secreted by the mammary and salivary glands [1, 2].

Lymphocytes that have first been exposed to the antigen and have detected it on the surface of intestinal mucosal DCs have identified implantation molecules and can implant in the gastrointestinal mucosa. For this reason, it seems that vaccination against intestinal infections requires the administration of the vaccine in the mucosa because DCs in the mucosa will have the power to induce specific implantation molecules [4].

With the passage of active lymphocytes through the vascular endothelium, the expression of α4: β7 integrins stops on their surface, and instead another integrin called α_E: β7 appears on their surface. α_E: β7 can attach to the cadmium E molecule on the surface of intestinal mucosal epithelial cells. In this way, the lymphocytes are kept in the vicinity of the epithelial cells after entering the lamina propria (**Figure 4**).

6.4 Secretory IgA

In an adult human, more than 3 grams of IgA is secreted daily in the mucosa and glands. Secretory IgA is made up of two interconnected molecules (each containing four immunoglobulin chains).

In mice, rats, and rabbits there is only one IgA isotype, but in humans, there are two isotopes IgA1 and IgA2 encoded by two separate genes [66].

IgA2 is often made by mucosal plasma cells, and the lack of 13 specific amino acids in the α2 chain makes IgA2 resistant to specific anti-IgA1 proteases produced by purulent bacteria.

dimeric IgA also contains the J chain and the secretory component (SC). The carboxylic part of Fc is the two IgA molecules next to each other and their Fab is outward. In humans, mice, and rabbits, the penultimate cysteine of the two α chains binds to the cysteine J chain through disulfide bonding.

The J chain has an Ig-like domain and the SC has five Ig-like domains. A complete sIgA molecule consists of two IgA monomeric molecules of a J chain and a secretory component. The secretory component covers areas sensitive to proteolytic digestion and the IgA hinge, and the secretory variants of this immunoglobulin are highly resistant to proteases [1, 4].

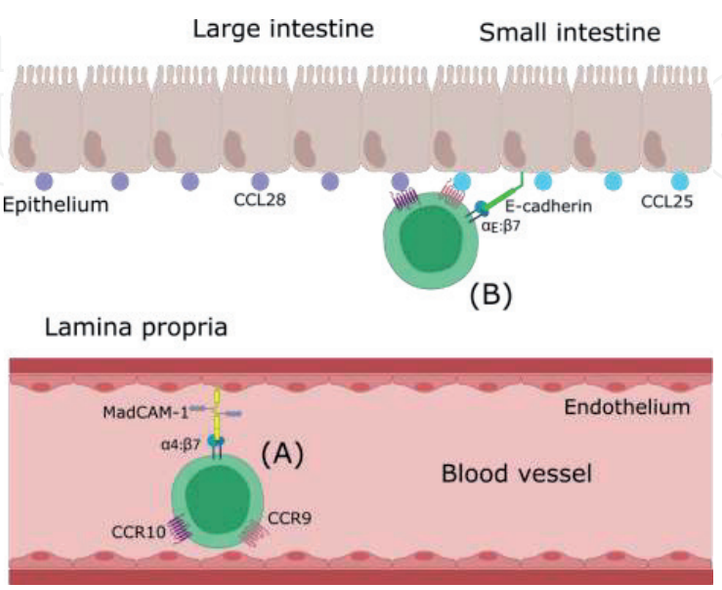

Figure 4.
Homing in gastrointestinal mucosa. Effector T lymphocytes attach to MadCAM-1 surface endothelial cells for homing in the gut (A). Intestinal epithelial cells express specific chemokines for T cells that intend to home in the gut (B).

7. Intraepithelial IgA transport

7.1 Poly immunoglobulin receptor (pIgR)

The transfer of IgA from the production site in the mucosal and glandular tissue areas to the secretions takes place in an active process with the involvement of membrane polymer receptors (**Figure 5**).

The pIgR receptor is a membrane glycoprotein consisting of five Ig-like domains (reinforced with disulfide bounds) at the cell surface, an intramembrane fragment, and a 100-amino acid sequence within the cytoplasm.

The human immunoglobulin polymer receptor gene is located on chromosome 1. The genes of these receptors in epithelial cells are affected by cytokines such as IFN-γ in vitro and are expressed on the surface of these cells. Therefore, it can be said that mucosal inflammation has an aggravating role in the transfer of sIgA to secretions [1, 66].

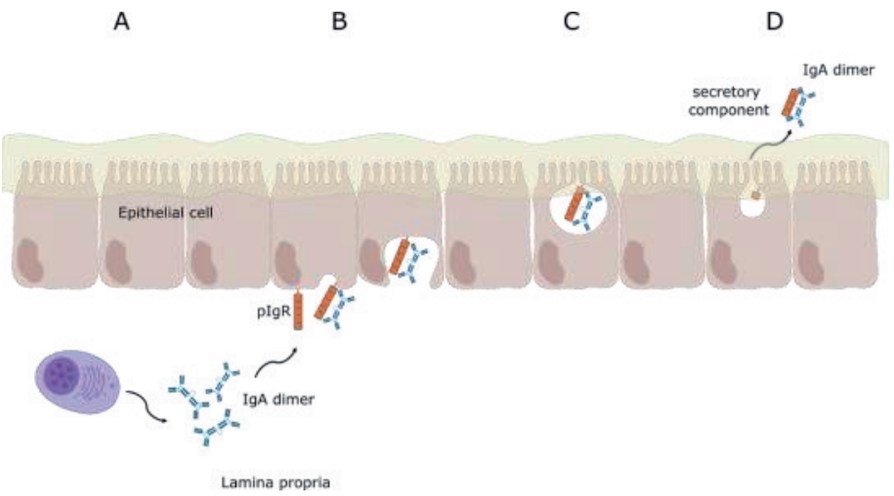

Figure 5.
Mechanism of IgA dimer production in lamina propria and its transmission by epithelial cells. Lamina propria plasma cells produce IgA dimers (A). These antibodies are transported into the epithelial cells via pIgR at the basal surface (B). Following the release of IgA from the luminal surface of these cells by the mechanism of transcytosis (C), due to proteolytic cleavage, part of the receptor remains attached to the IgA dimer, which is the secretory component or SC (D).

7.2 Binding of IgA to the immunoglobulin receptor

IgA binds to the first Immunoglobulin-like domain of the Poly-Ig receptor. Following the separation of pIgR from the epithelial cell, a disulfide bond is established between the cysteine of the fifth SC region and the Fc portion of one of the IgA monomer monomers. Domains 2, 3, and 4 of the secretory component do not participate in the binding but are necessary for the establishment of the two cysteine roots [66].

8. Mechanisms of secretory IgA protection

8.1 Immune exclusion

Secretory IgA dimer is responsible for binding to microorganisms in the intestine and mucosal surfaces of the gastrointestinal tract, respiratory tract, and genital tract [67, 68].

The slgA-antigen complex can be easily trapped in mucus, excreted by bowel movements, and the beat of cilia of the respiratory tract. Also, the sIgA can directly block the microbial binding sites to epithelial cells [69].

The basic way of protection by sIgA is the same as immune exclusion. Therefore, the presence of appropriate levels of specific sIgA can only cause protection (even in the absence of other immunological mechanisms) [2].

9. Respiratory mucosa

The airways are an important route for the entry of pathogen antigens, allergens, and airborne particles. The upper respiratory tract mucosa contains the nasal lymphatic tissue (NALT), the bronchial lymphatic tissue (BALT), and the airway lymph nodes, and the lower respiratory tract mucosa contains the smaller airway lymph nodes and alveoli.

The immune system is present in the airways like other mucous membranes and plays an important role in regulating homeostasis and preventing harmful immune responses to harmless antigens. The respiratory system also contains specialized and organized mucosal tissues such as the palatine, lingual, pharyngeal, and adenoids, which form a ring-like structure called the "Waldeyer's ring" in the pathway of air and food antigens (**Figure 6**).

The extensive vascular network of the respiratory system provides a favorable environment for the migration of lymphocytes and the passage of blood vessels to the lung tissue. Leukocytes do not follow the conventional method of homing in lymphoid tissues and do not have processes such as rolling and attaching to the endothelium and passing through the HEV.

One of the defense mechanisms in the mucosa is physical and mechanical defense, which is seen in the respiratory system as a mechanism of clearance of the ciliary mucosa (mucociliary). The most abundant cells in the upper airways are ciliated epithelial cells that form the physical barrier [2, 5].

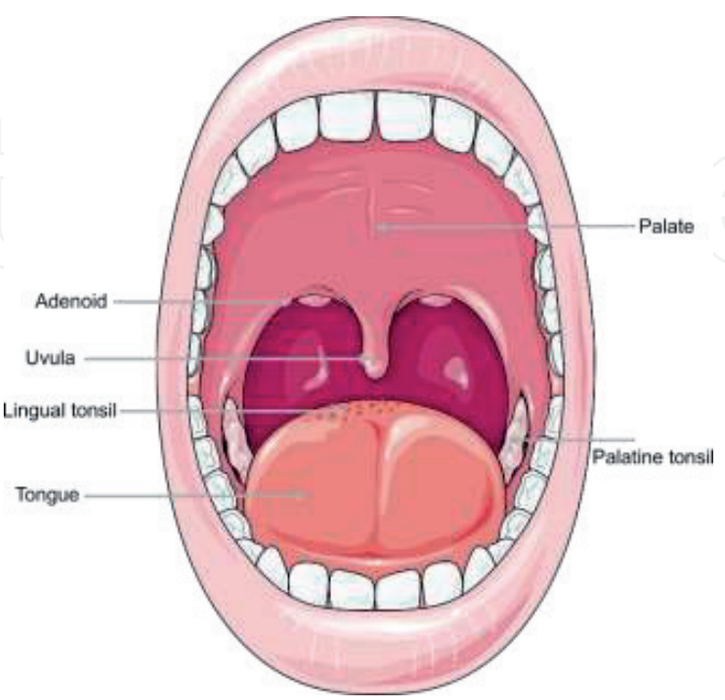

Figure 6.
Waldeyer's ring. The tonsils and adenoids form a ring of lymphatic tissue in the gastrointestinal tract and airways called the Waldeyer's ring.

Goblet cells are present in the margins of ciliated epithelial cells and are responsible for secreting mucus.

The mucus layer is directed to the upper respiratory tract by the movement of the cilium, so that suspended particles and pathogens are excreted or swallowed through sneezing and coughing which is called mucociliary clearance. Various cells in the respiratory tract, such as ciliated epithelial cells, alveoli, and immune cells located subepithelial, can produce and secrete antimicrobial peptides such as defensins, cathelicidins, collectins, and protease inhibitors [5].

9.1 Waldeyer's ring

The tonsils and adenoids are a great place to trap antigens from the mouth and nose. In humans, the Waldeyer's ring forms a network of lymphatic tissue in the nasopharyngeal mucosa, which is the structure of NALT. The epithelial surface of the tonsils and adenoids is the site of antigen entry due to its proximity to the external environment.

The palatine tonsils are two oval masses of secondary lymphatic tissue that are located in pairs behind the oral cavity and at the beginning of the oropharynx and are the entry point for respiratory and gastrointestinal antigens. The tonsils have several depressions called crypts. The presence of crypts increases the surface of the tonsils and the ability to remove antigens. The outer layer of each crypt is composed of epithelial cells, which have M-like cells present and perform the function of antigen uptake and transport through the epithelium. Below the epithelium of each crypt is one or more secondary lymph follicles.

Most cell populations of NALT lymphatic structures are composed of T and B lymphocytes and to a lesser extent dendritic cells and macrophages. NALT is structurally similar to MALT and has FAE-containing cells similar to M goblet and IELs. Lymphatic follicles are also seen in the subepithelial layer. Most tonsils located in the tonsils are B cells that turn into antibody-producing plasma cells (often IgA). The number of CD4 + T cells in this area is very low and IEL lymphocytes CD8 + T is found as CD8 + αβT or in the unusual phenotypes CD8αα + αβ T and CD8αα + γδ T.

Lymphatic tissues along the airways form the BALT structure. The upper airways have more organized lymphatic structures than the lower airways. In the lungs, active immune cells migrate mainly to the mediastinal and cervical lymph nodes, which enlarge in the face of infectious agents. In the BALT structure, the number of M and IEL cells in the overlying epithelium is very rare and there are no goblet cells in this area. In BALT, similar to MALT, lymph follicles are seen. B cells located inside the follicles usually have a memory phenotype and are mostly IgA^{+}. In the absence of infection, BALT is difficult to detect. Therefore, BALT is considered a secondary structure in cases of infection [4, 5].

9.2 Regulation of immune responses by airway epithelial cells

Airway epithelial cells specialize in regulating immune responses in the respiratory tract. While these cells can detect pathogenic microbes, they do not respond to harmless antigens and cause respiratory homeostasis These cells produce antimicrobial peptides, inflammatory cytokines, and chemokines, and express much lower levels of TLRs than the gastrointestinal epithelium, However, the expression of these TLRs is strongly influenced by TNF-α and IFN-γ [5].

9.3 Dendritic cells in the respiratory mucosa

BALT and NALT have a large number of DCs. These cells help maintain homeostasis by detecting and differentiating between pathogenic and harmless antigens

and by inducing tolerance to their antigens. Airway DCs are often of the myeloid class, but plasmacytoid DCs are rarely seen.

There is also a population of positive langerin DCs in the upper airways that are somewhat similar to cutaneous Langerhans cells and are involved in immune surveillance. In the lower airways and lung tissue, there are lung parenchymal dendritic cells (LPDCs) or interstitial DCs that are scattered in the alveolar epithelium and the alveolar space or the connective tissue between the epithelium and the arteries. LPDCs are often $CD11b^{+}$ and belong to the myeloid class.

DCs in the respiratory tract are considered strong cells in antigen uptake but have weak power in stimulating T lymphocytes. Airway DCs mainly direct the response to T2 and Treg, and by producing TGFβ lead to the switching of B cell class to IgA-producing plasma cells. In other words, airway dendritic cells regulate and modulate the immune response. Similar to MALT, dendritic cells meet and stimulate T cells by moving to the lymph nodes in the lungs. The lymph cells, then activated by lymph flow and then blood flow, return to the position of the lungs and participate in the immune response [4].

9.4 Lymphocyte homing in the respiratory mucosa

Integrins play an important role, especially α4 (α4: β7 and α4:β1) in the process of lymphocyte homing in the respiratory mucosa. E-cadherins are prominent in lung and intestinal cells and bind to αE:β7 integrins and are involved in the establishment of lymphocytes. Active T lymphocytes attach to CCL5 (RANTES) by expressing the CCR5 chemokine receptor at their surface and are located in the parenchyma of lung tissue. CCL5 is a chemotactic agent that is naturally secreted from lung tissue and increases during inflammation. In the airways, IgA-producing plasma blast implant by binding to the CCR10 chemokine receptor on its surface and the CCL28 chemokine secreted from the respiratory epithelium [1, 5].

10. Mucosal vaccination

By administering one or more oral doses of mucosal vaccine, in addition to producing sIgA on mucosal surfaces, it also stimulates cellular and systemic immune responses. With the entry of pathogens into O-MALT, the process of production and maintenance of memory lymphocyte population is established. In addition to the characteristics of injectable vaccines, oral vaccines must be able to pass through the stomach, intestines, and be resistant to bacterial enzymes and low pH.

Also, oral vaccines must be able to escape clearance mechanisms such as being trapped in mucus and be able to reach specific areas of the FAE-covered mucosa.

Furthermore, in addition, these vaccines need to compete by binding to the inner membrane to penetrate M cell vesicles. Immunological epitopes should be able to maintain their immunogenicity after crossing the epithelial barrier and penetrating the vesicles and be available to antigen-presenting cells for processing [2].

10.1 How vaccines get access to O-MALT

10.1.1 Inert particulate carriers

Vaccine access to Peyer's patches depends on the ability of M cells to transmit adherent multivalent macromolecules. One of the strongest products that have been

proven to be effective in the form of systemic vaccines is the Immune stimulating complex (ISCOM).

ISCOMs are particles 35 nm in diameter that are formed by the accumulation of protein antigens, such as the surface proteins of viruses, in a specific pattern. It should be noted that this form of immunogen was created for the proper and immunological present of viral surface proteins [70].

Immunization by ISCOMs leads to IgG production and cellular immune response against other viruses such as measles as well as inhibition of TH cells [70]. Intranasal immunization with ISCOM and influenza hemagglutinin leads to a local increase in anti-influenza cytotoxicity [71]. As a result, ISCOMs, as mucosal antigens, can be thought to produce IgA. In other words, ISCOMs are useful for mucosal use and are resistant to salt and bile acids.

Oral immunization in multiple doses with ISCOM containing ovalbumin or bacterial proteins results in the production of sIgA, systemic IgA, and cellular immunity [72].

They can also be used to immunize viral proteins that are naturally resistant to digestive proteases. Because they may not be resistant in the gut unless they are inside the capsule. Today, with the help of small hydroxyapatite crystals, effective solutions for particle penetration have been developed.

Crystals of 0.1 to 0.5 microns attach to M1 cells and are efficiently transported to intraepithelial envelopes. Because hydroxyapatite is a non-immunogenic and non-toxic component of bone structure, these antigen-coated crystals can be consumed in large quantities. These compounds should be used in capsule coatings [2].

10.1.2 Live vaccine vectors

The best way to stimulate mucosal immunity is to insert antigens into living microorganisms that can attach to M cells and settle and multiply in Peyer's patches and mucous membranes. Because living microorganisms elicit a strong and long-lasting immune response, a large number of viral and bacterial carriers are considered for this purpose. Given that living carriers can produce antigens for a long time and cause the production of antibodies as well as the development of cellular immune responses, the possibility of their use as a vaccine is being strongly considered.

The vaccinia virus recombinant has been tested as an oral vaccine [73]. But the mechanism of its absorption and transfer to Peyer's patches is still unknown. This method can probably be a safe and effective method of mucosal immunization. Because infection of mucosal cells with the recombinant virus can cause the presence of antigens on the cell surface. The vaccinia virus recombinant is used as a mucosal vaccine to enhance the capacity of bacterial carriers for foreign DNA [74]. Because viral carriers have limited replication and are unable to germinate the virus, the infection may be transient, with limited antigen present and the carrier cannot spread well in the mucosa of Peyer's patches.

Different species of bacteria can settle in Peyer's patches, including the live Attenuated strains of Salmonella and BCG [75, 76]. BCG is an effective adjuvant whose systemic immunization is safe. Once given at the time of birth, this vaccine provides long-term safety. BCG is also considered an oral vaccine [77] and is effective in transmitting O-MALT through M cells [78].

By orally administering recombinant Salmonella, laboratory animals have been vaccinated against a range of foreign antigens, including the heat-stable *E. Coli* enterotoxin [79], the streptococcal adhesin [80], and the malaria circumsporozoite protein [81]. In general, Salmonella is considered a strong mucosal immunogen. However, this limits the use of these carriers for repeated immunizations. Because

the anti-secretory immune response prevents re-absorption of oral doses of the carrier that deliver this antigen or other recombinant antigens.

IgA secretion of the superficial salmonella typhoid epitope of Morium can favorably prevent the penetration of these microorganisms into the mucosa [82].

However, applying effective methods to various events, such as immunogen retention in the gut, the ability of immunogen to bind to the surface of M cells, effective interaction with antigen-supplying cells, or facilitating its detection by M cells, can enhance mucosal immunity.

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Conflict of interest

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