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Anatomy and Immunology of the Ocular Surface

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Abstract

The ocular surface, in a strict sense, consists of the cornea and its major support tissue, the conjunctiva. In a wider anatomical, embryological, and also functional sense, the ocular mucosal adnexa (i.e. the lacrimal gland and the lacrimal drainage system) also belong to the ocular surface. This definition includes the source and the eventual drainage of the tears that are of utmost importance to ocular surface integrity. The ocular surface is directly exposed to the external environment, and therefore is endangered by a multitude of antigens and pathogenic microorganisms. As a mucosa, it is protected by the mucosal immune system that uses innate and adaptive effector mechanisms present in the tissue and tear film. Immune protection has two partly opposing tasks: the destruction of invading pathogens is counterbalanced by the limitation of inflammatory events that could be deleterious to the subtle structure of the eye. The immune system of the ocular surface forms an eye-associated lymphoid tissue (EALT) that is recognized as a new component of the mucosal immune system. The latter consists of the mucosa-associated lymphoid tissues in different organs of the body. Mucosa- and hence eye-associated lymphoid tissues have certain characteristics that discriminate them from the central immune system. The mechanisms applied are immunological ignorance, tolerance, or an immunosuppressive local microenvironment, all of which prefer non-reactivity and anti-inflammatory immunological responses. The interaction of these mechanisms results in immune privilege of the ocular surface. During eye closure, the ocular surface appears to have different requirements that make an innate pro-inflammatory environment more attractive for immune defense. The structural and functional components that contribute to this special immune regulation will be the focus of this chapter.

Anatomy of the Immune System at the Ocular Surface and Adnexa

Cornea

The cornea consists of a transparent connective tissue (stroma) covered by epithelia on both sides. The endothelium that lines the anterior chamber is a monolayer and the outer border of the cornea is a stratified non-keratinized squamous epithelium that is 5–7 cells thick [1]. It seals the stroma from the external environment by luminal junctions and forms a physical barrier against external antigens. This is supplemented by a physicochemical barrier of the epithelial-derived mucin layer that protects against the adhesion and entrance of antigens and by mechanical washing effects of the tear fluid and lid wiping combined with the action of protective proteins [2].

In the normal cornea, very few cells can assist in immune defense. Lymphoid cells do not occur under physiological conditions. The central cornea is avascular because blood and lymph vessels end in the limbal zone [3] and hence prevent an access of the vast majority of immunologically relevant cells. Major histocompatibility complex (MHC) class II-positive dendritic antigen-presenting Langerhans cells are present in the epithelium of the peripheral cornea and their absence from the central cornea was assumed to be a major reason for corneal immune privilege. Other dendritic cells (DCs) that are negative for markers of cell activation were recently observed in the central cornea of mice [4]. Further bone marrow-derived DC precursors or macrophage-like cells were reported in the anterior stroma and in the posterior stroma.

Conjunctiva

Morphology

The conjunctiva consists of an epithelium and an underlying loose connective tissue, known as the lamina propria; both are separated by the epithelial basement membrane. The epithelial histology is stratified non-squamous and consists of two-to-three cell layers having cuboidal morphology in most parts. The lamina propria is rich in bone marrow-derived cells that form a mucosal immune system known as the conjunctiva-associated lymphoid tissue (CALT) and of blood vessels of different kinds. Apart from capillaries and lymph vessels, specialized high endothelial venules [5] for the regulated migration of lymphoid cells are present in the conjunctiva [6]. They are a normal component of ocular lymphoid tissue, have a characteristic ultrastructure as in other lymphoid tissues, and express cell adhesion molecules.

Diffuse Leukocyte Subpopulations

Over the last decades, evidence has accumulated that leukocytes, including lymphoid cells, are normal, non-inflammatory components of the ocular surface [for review see ref. 7].

Lymphocytes and plasma cells are the main populations of leukocytes [8] and form a diffuse lymphoid tissue throughout all conjunctival zones, with predominant expression in the tarso-orbital conjunctiva [6]. Lymphocytes occur in the basal layer of the epithelium as intraepithelial lymphocytes (IEL) and more frequently in the lamina propria [8, 9] as lamina propria lymphocytes. Several lines of evidence indicate that the ocular surface has a mucosal immune system with common characteristics: CD8+ suppressor/cytotoxic T cells dominate over CD4+ T helper (Th) cells in IEL and a reverse distribution occurs in lamina propria lymphocytes [9, 10]. It is assumed that most of the CD8+ cells act in the suppressor mode and hence provide an immunosuppressive environment [9]. Conjunctival lymphocytes are activated cells (CD45Ro+ and CD25+) and express human mucosal lymphocyte antigen-1 [10, 11]. Local plasma cells regularly occur in the lamina propria [6, 11–13]. They mainly produce IgA and the joining molecule (J chain) that forms the dimeric type of IgA. Its transepithelial transporter molecule secretory component (SC) is found in the epithelium, as verified by immunohistochemistry [6] and molecular biology (RT-PCR) [14]. The conjunctiva hence produces secretory SIgA on its surface and constitutes a secretory immune system [15]. Interspersed B lymphocytes are rarely found as they are restricted to organized lymphoid follicles [6, 9, 11].

Other bone marrow-derived accessory leukocyte subpopulations exist in the conjunctiva and mainly act for the innate immune system. Macrophages enable the engulfment and destruction of pathogens and remnants of dead cells, and their potential antigen presentation to lymphocytes. They are frequent in the lamina propria but difficult to detect in conventional histological specimens. An immunohistological study reported CD68+ macrophages as the second most frequent leukocyte population in the conjunctiva [11]. Dendritic Langerhans cells, which aid in the uptake and professional presentation of antigens to lymphocytes, are regularly found [16]. They express activation markers such as MHC class II or ATPase. Depending on their maturation and migratory behavior, they are critical regulators of immunity and link innate and adaptive immune effector mechanisms [17]. Mast cells are resident accessory leukocytes in the lamina propria [18]. They produce several factors, including cytokines, which recruit other leukocytes and orchestrate inflammatory reactions for the destruction of pathogens. Although their role in physiological host defense is poorly understood, they are potentially useful cells. They are mainly known, however, for their deleterious inflammatory activity during IgE-mediated allergic disease [19]. Granulocytes of different subtypes (neutrophils, basophils, and

eosinophils) emigrate from the blood circulation only if recruited. Neutrophils are occasionally observed in minor amounts or as single cells in the normal human conjunctiva [6, 8]. Eosinophils are normally lacking in the absence of inflammatory conditions such as ocular allergy [8].

Follicles

Lymphoid follicles involved in the production of lymphoid effector cells are regularly observed on normal human whole-mount conjunctivas [6, 12, 13], and in several other species [20], being mostly secondary follicles [12]. Their frequency is age dependent [13]; increased levels are noted before onset of puberty which decrease with age. About 60% of individuals in their mid-70s still have follicles in the conjunctiva, with an average number of 10 follicles per conjunctival sac [6]. Follicles show typical mucosal characteristics: they consist of B cells with parafollicular T cells and associated high endothelial venules and have an apical follicle-associated epithelium. It is thin, highly permeated by lymphocytes, and includes M cells for antigen uptake in several species [21], but lacks the IgA transporter SC.

Lacrimal Gland

The human lacrimal gland is anatomically continuous with the conjunctiva via 10–12 lacrimal excretory ducts. It is a tubulo-acinar gland with short-branched tubules that end in secretory acini [1]. Between the secretory acini is a loose connective tissue resembling that of the conjunctiva and, in fact, continuous with it along the excretory ducts. Plasma cells are more frequent than lymphocytes, IEL are fewer, and CD8+ suppressor/cytotoxic T lymphocytes are generally more frequent than CD4+ Th cells in the gland in contrast to the conjunctiva [22]. Plasma cells are mainly positive for IgA, and the acinar epithelium expresses the IgA transporter SC [23]. Therefore, the lacrimal gland is an established component of the secretory immune system and was until recently considered as the only source of IgA proteins present in the tear film [9, 24]. T cells are reported to form groups around intralobular ducts [22] but ordinary lymphoid follicles are very rarely observed and may not be physiologically relevant.

Lacrimal Drainage System

The lacrimal drainage system is continuous with the conjunctiva via the lacrimal puncta and canaliculi into the lacrimal sac and through the nasolacrimal duct into the nose. Like the conjunctiva, it represents a moist mucous membrane. The epithelium is a stratified squamous non-keratinized layer inside the canaliculi

and transforms into a pseudostratified epithelium with columnar ciliated cells in the lacrimal sac and nasolacrimal duct [25]. The mucosa contains diffuse lymphoid tissue [26] that contributes to the secretory immune system, and also follicles similar to the conjunctiva [25, 27]. Its mucosa-associated lymphoid tissue was accordingly integrated as a lacrimal drainage-associated lymphoid tissue (LDALT) into the mucosal immune system [25]. The reported frequency of organized lymphoid follicles with typical morphology varies from 41 [27] to 56% in old age human populations [28].

Tear Film and Integrated Proteins

The tear film is an important functional component of immune defense in the ocular mucosal surface. Apart from a cleansing effect induced by lid wiping, it contains specific IgA antibodies that are secreted by the lacrimal gland and by the ocular mucosal surfaces. In addition, there is an ever-increasing number of reported peptides and proteins of the immune system [29]. Some of them have a direct antimicrobial effect whereas others (e.g. chemokines and cytokines) recruit and activate leukocytes, including lymphoid cells.

Historically, and due to their relative concentration, three secreted antimicrobial proteins are most important. Lysozyme destroys the bacterial cell wall, lactoferrin binds iron, and tear-specific prealbumin (lipocalin) acts as a scavenger of bacterial products; complement occurs as a transudate from the serum. Angiogenin is a newly described tear protein found at high concentrations in virtually all tear samples [29]. It appears to have primarily an antimicrobial effect within the tear film. Other multifunctional antimicrobial molecules are predominant in the closed eye during sleep, e.g. specific leukocyte protease inhibitor, elafin, and neutrophil gelatinase-associated lipocalin. CXC and CC chemokines, such as interleukin (IL)-8, epithelial neutrophil-activating peptide 78, interferon- γ -inducible protein-10, growth-regulated oncogene or macrophage chemoattractant protein-1 and macrophage inhibitory protein-1 β are able to recruit leukocytes into the tear film. Inflammatory cytokines such as IL-6 and macrophage colony-stimulating factor appear to occur in every normal tear film [29]. Most of these tear proteins show an inverse correlation with the amount of aqueous tear secretion and their concentration strongly increases in the closed-eye tear film, when lacrimal secretion has almost ceased.

Mucosal Immune Defense Mechanisms at the Ocular Surface

The anatomy and leukocyte cell types clearly show that a mucosal immune system is maintained at the normal human ocular surface and mucosal adnexa.

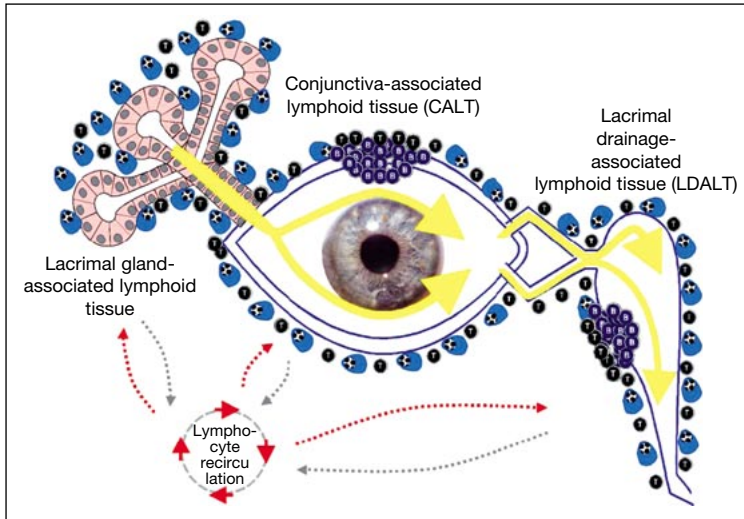


Fig. 1. The eye-associated lymphoid tissue (EALT) is the mucosa-associated lymphoid tissue for immune protection of the ocular surface and its mucosal adnexa. It is anatomically continuous from the lacrimal gland throughout the conjunctiva- and lacrimal drainage-associated lymphoid tissue (i.e. CALT and LDALT, respectively). It consists of a diffuse lymphoid tissue of T lymphocytes and IgA-secreting plasma cells, including accessory leukocyte populations in all organs and of lymphoid follicles in conjunctiva- and lacrimal drainage-associated lymphoid tissue (in the drawing, large blue cells represent plasma cells, small blue cells represent B cells and small black cells represent T cells). Protective as well as aggressive factors inside the tear film, which connects the different parts of the ocular surface and protects it from the external environment, are a major component of ocular surface immunity. The organs are also connected by lymphocyte recirculation via specialized vessels with each other and with the rest of the immune system.

It is termed ‘eye-associated lymphoid tissue’ (EALT) [7, 30] (fig. 1) and is integrated into the mucosal immune system of the body. Therefore, the laws of mucosal immunity apply to the ocular surface. It has certain specializations suggesting immune privilege, as discussed below. Mucosal, like systemic, immunity uses two approaches for defense, the innate and the adaptive immune system. These have almost opposing characteristics (table 1), use different effector mechanisms, and appear unrelated at first glance. Increasing knowledge has indicated, however, that they are complementary and even act in concert [31]. Together they effectively protect against a highly diverse array of non-pathogenic and pathogenic antigens combined with minimal risk of allergic and autoimmunological disease.

Table 1. Characteristics of innate and adaptive immunity

Characteristics	Innate	Adaptive
Repertoire Recognition	preexisting unspecific pattern	acquired specific (to diverse epitopes)
Action Reaction	immediate inflammatory	intermediate modulated (inflammation to tolerance)
Memory	–	+
Transfer	±	+

Innate Immunity at the Ocular Surface

Function of the Innate Immune System

Innate immunity is an evolutionary old system that primarily aims at the detection and destruction of microbial pathogens. To do so effectively, it relies on a limited number of conserved and genetically determined receptors that work alone or in combination with innate effector cells, mainly phagocytes. Pattern recognition receptors are able to bind to pathogen-associated molecular patterns on microbes such as lipopolysaccharides, flagellin, and CpG-DNA etc. and initiate respective immune responses.

Innate Effector Cells at the Ocular Surface

Phagocytes are important innate effector cells that contribute to defense during infection. Macrophages act almost exclusively by phagocytosis (e.g. in *Acanthamoeba* infection), but also perform antigen processing and presentation, which are necessary for the development of an acquired immune response. In dendritic Langerhans cells, as sentinels of the immune system, antigen presentation dominates phagocytosis. Neutrophil granulocytes are more effective in pathogen elimination due to the secretion of toxic mediators such as myeloperoxidase, which is able to kill pathogens such as *Acanthamoeba* cysts. Mast cells orchestrate the inflammation e.g. in *Toxoplasma gondii* infection.

Toll-Like Receptors

Different Toll-like receptors (TLR) are present in the mouse eye and induce the secretion of CXC chemokines which leads to neutrophil recruitment,

a possible mechanism of corneal pathology in early stages of microbial infection [32]. Following bacterial flagellin exposure, TLR5 induces inflammation on human corneal cells. Then, cells of the ocular surface secrete inflammatory cytokines (IL-6 and IL-8) via a nuclear factor- κ B-dependent pathway [33] as shown in other tissues. Other results may point into a different direction because it was found that although TLR2, TLR3 and TLR4 occur in human corneal epithelial cells, they do not induce inflammatory immune responses to lipopolysaccharides [34] as a potential mechanism to prevent constant ocular surface inflammation.

Secreted Antimicrobial Peptides

In addition to the established antimicrobial factors such as lysozyme and lactoferrin, a broad spectrum of antimicrobial peptides was recently observed in the normal human ocular surface. β -Defensin-1 to -4 were found together with liver-expressed antimicrobial peptide-1 and -2, and cathelicidin (LL37) [35]. Also, β -defensin-3 has been found to be upregulated in inflammatory conditions. Collectins, observed in human and mouse tear fluid and corneal epithelia, are able to inhibit invasion by *Pseudomonas aeruginosa* [36]. Trefoil factors TFF1 and TFF3 occur in human conjunctival goblet cells [37]. A broad spectrum of antimicrobial peptides, including different α - and β -defensins, secretory phospholipase, bactericidal permeability-increasing protein, and 37-kDa cationic antimicrobial protein, was observed in human nasolacrimal ducts with an induction of human β -defensin-2 under inflammatory conditions [38].

Specific Adaptive Immunity at the Ocular Surface

Function of the Adaptive Immune System

Similar to the innate system, the adaptive immune system is divided into cellular defense, which is mediated by direct action of T cells, and humoral defense, which is maintained by soluble antigen receptors (immunoglobulins) secreted by local mucosal plasma cells. In contrast to innate immunity, the adaptive system consists of lymphoid cells, and it offers a higher degree of specificity, variability, and immune regulation. An 'afferent' antigen uptake and processing phase must be differentiated from the 'efferent' distribution and action of effector cells. In between is the recognition of antigens by lymphocytes and their differentiation and proliferation into effector cells. The processing and presentation of antigens by phagocytes to lymphoid cells links innate and adaptive immunity.

Uptake of Antigen at the Ocular Surface

After antigen enters mucosal surfaces, it is transported by antigen presenting cells (APC) to local lymphoid follicles for its presentation to lymphocytes. Antigen can also be transported, either by APC or in a soluble form, by the efferent lymph, to follicles in regional draining lymph nodes [39]. This is shown to be an important route for processing of corneal transplantation antigens [40]. In the FAE overlying CALT and LDALT follicles, specialized M-cells take up antigen [21]. Phagocytosed antigen is degraded into small fragments and loaded onto MHC-class-II antigen presentation molecules for recognition by the cognate T-cell receptor. Ocular antigen presenting Langerhans cells, which are specialized for this purpose, are described in physiological conditions and can be altered in ocular pathology [4, 16, 41].

Immune Regulation in Follicular Lymphoid Tissue

Since lymphocytes have an enormous variety of different antigen receptor specificities, some can detect self antigens of the host, thus raising the risk of autoimmune disease [42] or allergic eye disease [43]. This is the reason that the mere recognition of an antigen by a T cell is not sufficient for its activation [31]. In contrast to lymphoid cells, innate phagocytes have the ability to recognize the microbial origin of antigens. During antigen presentation, they transmit this information by the expression of co-stimulatory molecules [31] (e.g. CD80/86, CD40, ICAM-1) that also interact with complementary lymphocyte receptors in the ocular surface immune system [44]. Additional cytokines influence the activation of Th cells that produce different cytokine profiles and hence support different immune reactions. Antigen presentation without co-stimulation results in anergy or deletion of the reactive T cells or in generation of active immunosuppressive regulatory T cells [45], both leading to non-reactivity, i.e. immune tolerance. Co-stimulation in the presence of IL-4 skews Th cells into the direction of Th2 cells, which support the differentiation of antibody-producing plasma cells that normally produce anti-inflammatory IgA. Co-stimulation in the presence of IL-12 generates Th1 cells that produce inflammatory cytokines e.g. IFN- γ or TNF- α and mount an inflammatory immune response that is detrimental to the ocular surface.

Diffuse Lymphoid Tissue with Effector Cells

After emigrating from follicular regions via the lymph eventually into the blood, effector cells recirculate in the body. They can home via specialized vessels which are also regularly present at the normal human ocular surface and are equipped with adhesion molecules. This serves for a proposed organ specificity for the same or similar tissues and is the basis for the concept of the mucosal immune system [46, 47]. The mucosal lymphoid effector cells mainly constitute the diffuse lymphoid tissue described in all parts of the eye-associated lymphoid tissue.

Table 2. Examples of strategies and mechanisms for immune privilege at the ocular surface

Strategy	Mechanisms
Ignorance	immune exclusion by secretory IgA absence of corneal lymph vessels absence of corneal lymphocytes few MHC class II on epithelium
Tolerance	immature corneal DCs potential innate corneal unresponsiveness
Immunosuppressive environment	Fas ligand on corneal epithelium factors in the tear film CD8+ IEL in the suppressor mode

Defense Strategies: One Does Not Fit for All at the Ocular Surface

The ocular surface is not only a sophisticatedly constructed organ, but it also uses sophisticated mechanisms of immune defense to preserve its integrity. The actual approach used depends on the requirements of the situation and appears to change in a diurnal rhythm that meets the different needs of the usual open eye and of the closed eye condition during sleep.

The Immune Privilege Approach

Immune privilege (table 2) represents a state in which innate and adaptive inflammatory immune mechanisms are inhibited [42]. In terms of adaptive immune regulation, the activation of an inflammatory Th1 immune response must be avoided in favor of Th2 cells or regulatory T cells. Different strategies contribute to an immune privilege [42] such as *ignorance*, i.e. presentation of an antigen to the immune system is impeded, or the active generation of *tolerance* by regulatory T cells. Alternatively, an *immunosuppressive microenvironment* achieved by soluble factors such as transforming growth factor- β_2 or by surface-bound FAS ligand (CD95L) that eliminates CD95-positive T effector cells through apoptosis contributes to immune privilege. In the eye, this concept was primarily applied to the anterior chamber in order to explain the observed anterior chamber-associated immune deviation [48].

Some of these mechanisms of immune privilege also apply to the surface of the eye. Its immune protection is governed by the rules of the mucosal

immune system which generally favors the inhibition of inflammation by tolerogenic mechanisms [49]. The generation of secretory IgA is one of the best-characterized mucosal effector mechanisms [15]. IgA is anti-inflammatory since it does not activate complement. It leads to immune exclusion because it is deposited on the ocular surface and in the tear film where it prevents the entrance of pathogens into the body and can even clear the tissue of antigens during its active SC-mediated transepithelial transport. The majority of environmental antigens and pathogens is hence ignorant to T cells. If the mucosal immune system is deregulated and the default IgA response is switched to IgE, the unresponsiveness to non-pathogenic antigens is lost and allergy occurs [43, 50]. A deregulation of the mucosal immune system with loss of physiological tolerance seems to represent a yet underestimated factor in inflammatory ocular surface conditions in general [51]. Furthermore, the central cornea is less immunogenic because the epithelial cells normally express few MHC class II antigens, it has no blood and lymph vessels and contains no resident lymphoid cells. Some characteristics of an immunosuppressive microenvironment are also present at the ocular surface because the corneal epithelium, like the corneal endothelium, expresses CD95L. In the conjunctiva and lacrimal gland, CD8+ IEL are assumed to be in the suppressor mode and may be anti-inflammatory [9, 22]. Immune tolerance is indicated by immature MHC class II-negative DCs [4] in the central cornea that are assumed to induce tolerance to the presented antigen [17]. Innate immune mechanisms may support immune tolerance because the human corneal epithelium shows innate microbial receptors, but does not necessarily show an inflammatory reaction to ubiquitous microbial stimuli such as lipopolysaccharides [34].

The Pro-Inflammatory Approach

At the ocular surface, there seems to be a unique shift of paradigms for optimal immune protection that follows a diurnal cycle [52] because the conditions and hence the requirements change dramatically when the eye is closed for 6–8 h or more overnight. During this time, lacrimal secretion has almost ceased, and entrapped microbes enjoy a ‘moist chamber’ at the ocular surface that is rich in nutrients, provides optimal temperatures and is devoid of its normal main protective lacrimal proteins lysozyme and lactoferrin. Therefore, the closed eye represents a very special condition that appears to be governed in particular by innate defense mechanisms [52].

The defense hence switches into a protective approach dominated by pro-inflammatory factors that are locally produced at the ocular surface. Numerous chemokines, cytokines and growth factors orchestrate a subclinical

inflammatory reaction. In contrast to the open eye, leukocytes, in particular polymorphonuclear neutrophils (PMN), are increasingly recruited into the tear film. Neutrophils produce increased levels of proteases that attack microbes while the epithelial cells of the host are protected by anti-proteases. Leukocyte proteases also modulate the function of other proteins. For example, neutrophil elastase promotes a switch of angiogenin, which is abundantly present in the tear film, from its angiogenic function to an antimicrobial function. Consequently, a new equilibrium of pro- and anti-inflammatory factors is achieved on a higher level to suppress microbial growth. This appears as a more suitable approach in a highly contaminated closed eye environment than the promotion of immune privilege that is successful during daytime in the open eye.

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