A FUNCTIONAL UNIT FOR OCULAR SURFACE IMMUNE DEFENSE FORMED BY THE LACRIMAL GLAND, CONJUNCTIVAAND LACRIMAL DRAINAGE SYSTEM

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1. INTRODUCTION

Mucosal organs represent a special moist compartment of the body's surface that is equipped with a diverse array of defense mechanisms to avoid microbial colonization. Besides an innate defense, the relevance of lymphoid cells that form a "mucosa-associated lymphoid tissue" (MALT) in these organs is increasingly recognized as an important factor for the preservation of mucosal integrity. The mucosal lymphoid tissue of the different organs together constitutes a "common mucosal immune system".[1] The components of this system interact through migrating lymphoid cells that are primed for antigens in follicular sites and later populate the same or similar tissues as effector cells.

The morphology and function of the mucosal immune system is distinct in that it consists, besides follicular "organized" lymphoid tissue (O-MALT), mainly of a "diffuse" lymphoid tissue (D-MALT) composed of lymphocytes and plasma cells. The latter exclude antigens from the mucosa mainly by the production of secretory IgA (sIgA)(Fig. 1A – C), and these tissues hence together constitute the so-called "secretory immune system".[2] Another important mucosal immune function is the generation of tolerance[3] which is of interest because these tissues are exposed to a variety of non-pathogenic ubiquitous antigens. Secretory immunity and mucosal tolerance are both important to avoid potentially destructive inflammatory reactions that endanger the tissue integrity. This is especially important for the delicate ocular tissues.

MALT represents an accepted component in organs like the intestine, respiratory system or genital tract. However, its presence at the normal human ocular surface is not

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fully recognized as yet, and the supply of the ocular surface with protective immunoglobulins is usually attributed to the lacrimal gland.[4] Our investigations focus on the presence and organization of lymphoid tissue at the ocular surface and appendage, it probable interaction and its undefined role up to date in ocular surface health and disease.

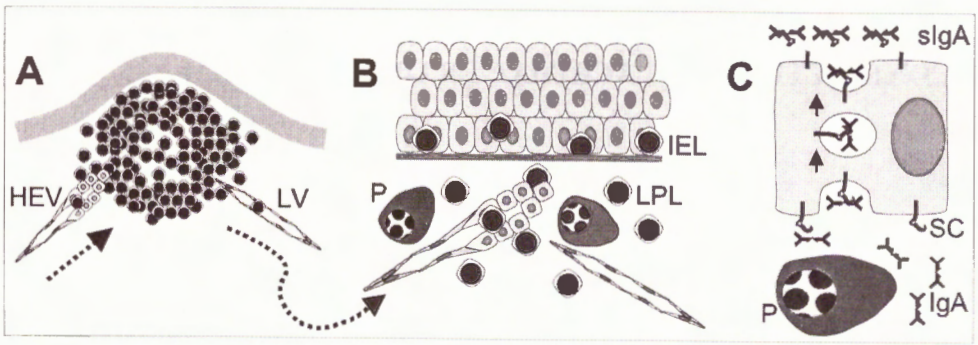

Figure 1. Mucosa-associated lymphoid tissue (MALT) consists of two types: An organized type (**A**) is composed of roundish lymphoid follicles with ordinary vessels and high endothelial venules (HEV) for cell immigration and lymph vessels (LV) for emigration. A diffuse type (**B**) is formed by plasma cells (P), primed at follicular sites, and lymphocytes in the lamina propria (LPL) together with intraepithelial lymphocytes (IEL) all immigrated after recirculation. One of the main functions (**C**) of the diffuse lymphoid tissue is the production of dimeric IgA by the plasma cells and its transcytosis by the epithelial molecule secretory component (SC) to build up a protective coat of secretory IgA (sIgA) at the mucosa.

2. DIFFUSE LYMPHIOD TISSUE AND THE SECRETORY IMMUNE SYSTEM

In the normal human conjunctiva[5,6] and lacrimal drainage system[7] there is an associated lymphoid tissue termed CALT[5] and LDALT[7] respectively. It consists, besides follicles, mainly of a diffuse type similar to other mucosal organs[8] that is composed of lymphocytes and plasma cells, spread diffusely in the subepithelial lamina propria, and of intraepithelial lymphocytes (IEL) in the basal epithelial layers. T-lymphocytes prevail (Fig. 2), IEL are mostly CD8-positive suppressor/cytotoxic cells, and lymphocytes expressing the human mucosa lymphocyte antigen (HML-1) regularly occur in CALT[9] and in LDALT[7] (Fig. 2D). This characterizes them as mucosa-specific and the respective tissues as a part of the MALT system. Most of the plasma cells stain positive for IgA (Fig. 3A) and a minority for IgM. In the overlying epithelium of the conjunctiva and lacrimal drainage system, the IgA- and IgM-transporter molecule poly-Ig-receptor,[3,4] the extracellular part of which is termed secretory component (SC),[1,2] is strongly expressed (Fig. 1C,3B), and IgA is present here as deposits or diffuse surface staining. Our findings also show that a diffuse lymphoid tissue with plasma cells and strong expression of SC in the epithelium is continuous from the acinar tissue of the lacrimal gland, along the excretory lacrimal ducts into the conjunctiva (Fig. 4) and from there, further along the lacrimal drainage system. This physical continuity of

lymphoid tissue is another indication to assume that what is present in the lacrimal gland can also be present at the ocular surface.

The presence of plasma cells, however, was controversial in the human conjunctiva[4,5] and lacrimal drainage system. They were reported dating back to the nineteenth century although their normality was under discussion then and later.[10] In a histological investigation,[11] the consistent presence of plasma cells was shown. Their number in the conjunctiva accounted for about two thirds of that in the lacrimal gland. However, in immunofluorescence studies by the same group, IgA could not be located in normal conjunctival plasma cells and SC was not consistently found in the epithelium. Although the absence of IgA was confirmed in biopsies from only few presumably normal controls, some doubt persisted regarding the presence of plasma cells at the ocular surface.

In our histological, electron microscopical and immunohistochemical studies on a large number of complete and normal human tissues, numerous plasma cells were consistently identified in the conjunctiva[5] (except bulbar) and lacrimal drainage system.[7] IgA was found in the plasma cells and in deposits in the epithelium, together with a strong expression of SC (Fig. 3) similar to the lacrimal gland. The identity of IgA staining was verified by preadsorption of the antibody with commercial IgA. Differences to previous studies are probably related to different antibodies, staining techniques, tissue preparation or biopsy locations used.

Further indication for an immunological ability of the conjunctiva may be derived from embryological development. The lacrimal gland arises from an epithelial bud of the conjunctiva, similar to the accessory glands that remain in closer topographical contact with the ocular surface. Both can hence, systematically, be considered as part of the gland-associated lymphoid tissue of the conjunctiva. The presence of plasma cells in the glandular tissues of the eye is commonly accepted.[4] Our findings therefore indicate that the lacrimal gland represents not the only source for immunoglobulins at the ocular surface and that the conjunctiva and lacrimal drainage system are able to contribute to ocular surface immune defense by production of secretory IgA. This may be required early, because after birth the lacrimal gland is not fully functional but the ocular surface needs immunological protection. The observation that the normal conjunctiva and drainage system, unlike the

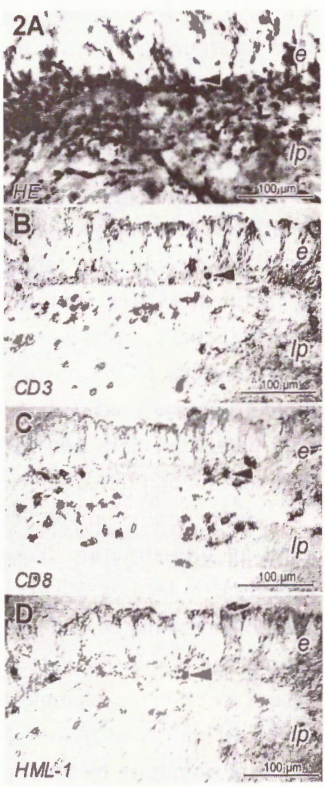

Figure 2. T-cells represent the majority of lymphocytes in the diffuse lymphoid tissue of the lamina propria (lp) and epithelium (e). **(A)** They stain positive for CD3 **(B)**, CD8 **(C)** and HML-1 **(D)**; IEL are indicated by arrowheads. Sections from the human lacrimal sac; staining and

lacrimal gland, can have follicles, known to be most frequent before puberty,[12] may indicate that during an active learning phase, that later declines or transforms into a steady state, these tissues may also have the function of antigen uptake and plasma cell priming to provide the lacrimal gland with appropriate plasma cells.

3. FOLLICLES ARE A NORMAL COMPONENT OF CALT AND LDALT

Organized lymphoid follicles were reported in different amounts in the normal human conjunctiva. However, there are natural factors that influence their number because this declines with increasing age[12] and it also depends on the investigated location[5] which is important if the prevailing studies that use biopsies or incomplete conjunctival tissue are considered. In conjunctival wholemounts of an elderly human population follicles were shown to occur in 60% of cases, to be most frequent in the tarso-orbital conjunctiva and to have a bilateral symmetry[5]. This underlines their normal character even though they may not be found consistently in every single specimen. Such follicles similarly occur in the lacrimal drainage system,[7] have a complementary composition of B- and T-cells (Fig. 5) and represent the afferent limb of mucosal immunity where antigens are transferred into the tissue for antigen presentation to lymphocytes and their subsequent activation and proliferation.[8] S pecialized epithelial cells (M-cells) that allow antigen transfer through the intact epithelium into the lymphoid tissue are described for follicles of the intestine.[13] There is indication for cells with a similar morphology also in the conjunctiva, where they are also located at the apices of follicles with an epithelium free of SC and IgA to allow attachment of antigens.[5] Ultra-structural characteristics o f M-cells containing lymphocytes in intraepithelial pockets were observed in the lacrimal drainage system.[7] A germinal center (that contains CD68-positive macrophages–see another contribution to this book) and typical zones around (Fig. 5A) in some of these follicles verifies that antigen had indeed been presented here and indicates that eye-associated lymphoid tissue can perform an afferent immune function.

High endothelial venules (HEV) are consistently present at the ocular surface.[14] They have an ultrastructure like those found in other lymphoid tissues[15] and express cell adhesion molecules.[16] This shows that the eye-associated lymphoid tissue is an integral component of the MALT system and shares receptor molecules with other mucosal sites, connecting it to a regulated traffic of lymphocytes known as homing.[17] Thereby, ocular tissues are able, for example, to receive and distribute primed plasma cell precursors, a role which is mainly attributed to intestinal lymphoid tissue,[3,8] and may thus contribute to mucosal immunity by local priming of B-cells for antigens that are first taken up via the ocular tissues. In this way, the follicles of the conjunctiva and lacrimal drainage system are able to perform an afferent immune function. This is a strong indication that the human ocular tissues may thus serve, within the system of the eye-associated lymphoid tissue, for the population of the lacrimal gland with plasma cells that produce antibodies with specificities relevant for ocular surface needs. Data on recirculation of such cells in ocular

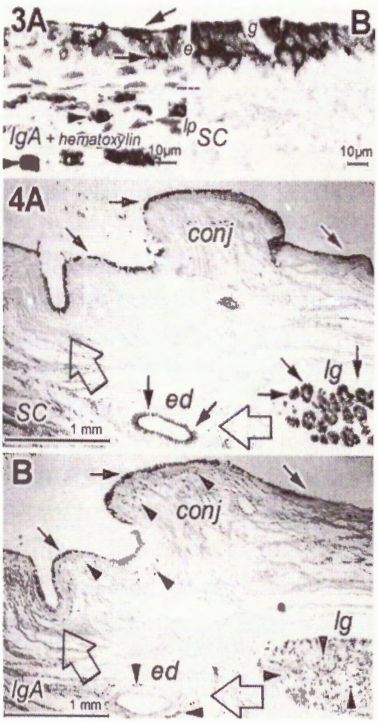

Figure 3. The normal human conjunctiva contains IgA-positive plasma cells (**A**, arrowheads) in the lamina propria (lp). IgA is seen as deposits (arrows) or diffuse staining in the epithelium and its transporter molecule SC (**B**) is in the upper layers of the epithelium (e), except in goblet cells (g). **Figure 4.** SC (**A**, arrows) occurs continuously in epithelial cells from the acini of the palpebral part of the lacrimal gland (lg), along one of its excretory ducts (ed, open arrows) into the conjunctiva (conj). (**B**) IgA is present along the same path in plasma cells (arrowheads) in the lacrimal gland, in the diffuse lymphoid tissue along its duct and in the conjunctiva (arrows).

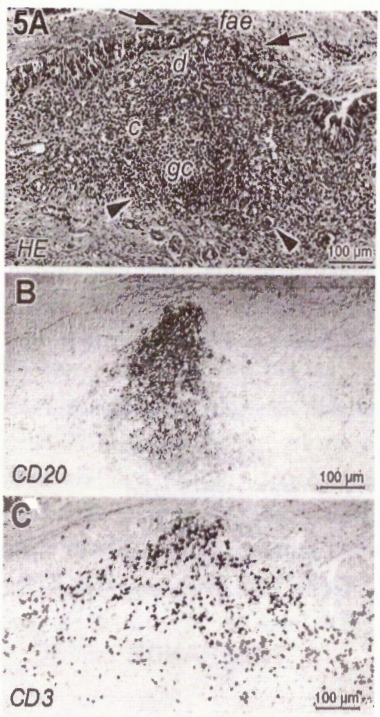

Figure 5. A secondary follicle (**A**) in the human lacrimal sac shows a bright germinal center (gc), dark lymphocyte corona (c), dome-like zone (d) and flat follicle associated epithelium (fae) at the apex (arrows) and numerous parafollicular vessels (arrowheads). It is composed of central B- (**B**) and peripheral T-cells (**C**).

tissues is scarce compared to other organs and therefore requires further studies. The differential importance of the conjunctiva and the lacrimal gland for ocular surface immune defense is not clear as yet, however our findings give substantial indication for a reappraisal of the conjunctiva.

4. LYMPHOID CELLS CAN NOT GENERALLY BE ADDRESSED AS "INFLAMMATORY"

A problem complicating the view on lymphoid cells at the ocular surface is the fact that lymphocytes as well as plasma cells, have a long tradition[10] of being referred to as pathological or distinctly termed "inflammatory cells". This misleading terminology was also applied by authors who found them consistently in their normal tissues.[12,18] However, lymphocytes per se and especially those forming a diffuse lymphoid tissue along mucosal

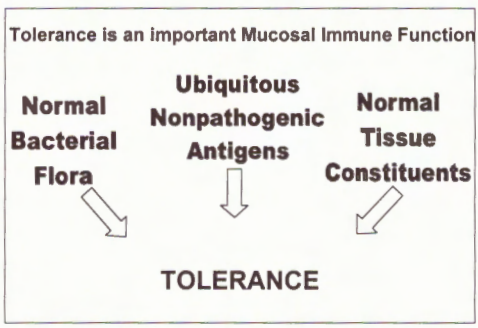

Figure 6. Generation of tolerance (unresponsiveness) is one of the main mucosal immune functions.

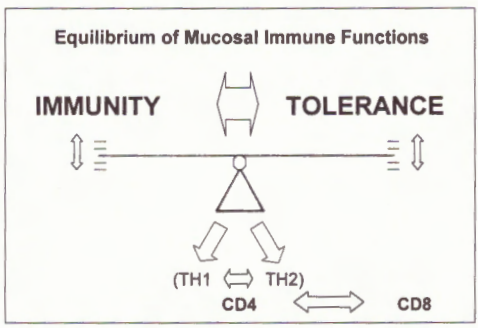

Figure 7. Immunity and tolerance must be kept in an equilibrium in order to maintain ocular surface integrity.

surfaces cannot generally be considered as inflammatory. Recent evidence shows that inflammatory reactions are only induced after appropriate activation of lymphocytes in a specific context as determined, for example, by the prevailing cytokines in the tissue and by the presence of adequate co-stimulation.[19–21] In contrast to the misleading term "inflammatory", a basic function of mucosal lymphocytes is the induction of tolerance[3], i.e., un-responsiveness (Fig. 6). This is directed against ubiquitous nonpathogenic antigens to prevent inflammatory reactions that could be destructive for the mucosal tissue and is, besides by a balance of TH1 and TH2 T-helper cells, also achieved by CD8-positive T-cells.[3,8,19,20] The necessity of tissue integrity especially applies to the delicate ocular surface and the mucosal lymphocytes in CALT are in fact predominantly of the CD8 type,[9,22] similar to the situation in LDALT.[7] However, immunity and tolerance must be kept in equilibrium to preserve ocular surface integrity (Fig. 7). The secretory IgA produced by mucosal plasma cells that excludes antigens from access to the tissue, also prevents inflammation and is hence "anti-inflammatory".[3] These more recent advances in the understanding of the mucosal immune system should lead to a more differentiated view on lymphoid cells also at the ocular surface.

5. LYMPHOID TISSUE MAY BE AN IMPORTANT REGULATOR OF OCULAR SURFACE DISEASE

If impairment of the epithelial barrier occurs due to tissue damage as in dry eye disease and/or antigens achieve uncontrolled access to the tissue, the mucosal immune tolerance fails. Resident T-helper cells in the subepithelial connective tissue of the conjunctiva may then be activated and immunological reactions shifted towards inflammation, resulting in the secretion of proinflammatory cytokines. In dry eye syndromes, a respective elevation of inflammatory cytokines (IL-1α, IL-6, IL-8, TNF-α) is reported in the tear film and inside the tissue,[23] and a hyperproliferation of the conjunctival epithelium is observed combined with impaired differentiation.[24] Similar events occur in the intestinal mucosa during inflammatory bowel disease (IBD)[20] about which a large body of information is already acquired. At the ocular surface, the production of these cytokines is as yet mainly attributed to the epithelial cells. However, in the intestine it is verified that TNFα and IL1β are secreted by activated lamina propria lymphocytes, resulting in the production of epithelial growth factors by stromal cells.[20] The production of growth factors locally at the ocular surface under inflammatory conditions would also represent an explanation for the epithelial hyperproliferation that occurs in dry eye disease despite of the decline in EGF supply from the lacrimal gland.[24] Subsequent alterations include a release of proteases from epithelial[25] or stromal[26] cells that is upregulated by inflammatory cytokines and results in tissue destruction perpetuating the disease (as indicated in Fig. 9).

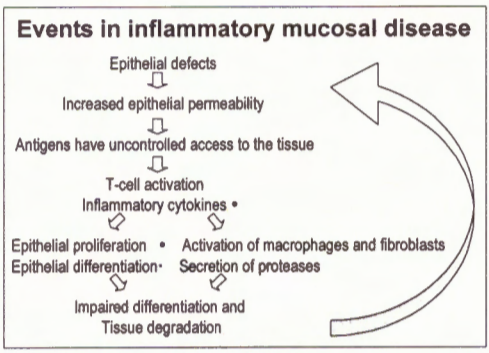

Figure 8. Inflammatory disease of the ocular surface and the bowel shows common events and tends to perpetuate.

A shift of the cytokine profile towards a TH-1 response is reported in several inflammatory ocular surface diseases[21], as similarly found in IBD,[20] and both disorders respond to immunosuppressive treatment. The widespread dry eye syndrome that is increasingly recognized to include an inflammatory component thus resembles disorders in other mucosal organs which are governed by lymphocytes. Hence, the resident lymphatic population localized in the mucosa-associated lymphoid tissue of the ocular surface, which

represents a potent source of professional cytokine producing cells, may also be able to act as an important regulator of inflammatory ocular surface disease.

6. CONCLUSION

We found the regular presence of a mucosa-associated lymphoid tissue (MALT) in the conjunctiva (CALT) and the lacrimal drainage system (LDALT). This tissue has all components for a complete immune response. It consists of a diffuse type of lymphocytes and plasma cells that contributes to the secretory immune system and of follicles responsible for antigen uptake and lymphocyte activation. Lymphoid cells are traditionally termed "inflammatory cells" although they occur in every normal tissue. More recent advances in mucosal immunology indicate that mucosal lymphocytes per se do not represent inflammatory cells but rather contribute to mucosal tolerance which preserves tissue integrity. Similarly, the ocular mucosal plasma cells were observed to produce preferably IgA that contributes to secretory immunity by the anti-inflammatory exclusion of antigens from the ocular surface.

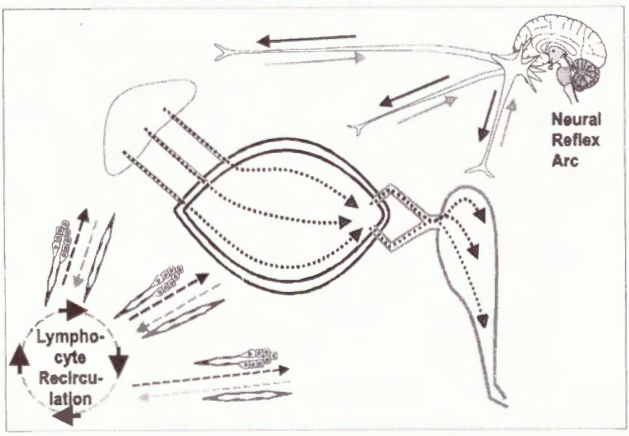

Figure 9. Eye-associated lymphoid tissue (EALT), consisting of the lacrimal gland, conjunctiva and lacrimal drainage system is physically continuous and connected by the flow of tears (dotted arrows), the recirculation of lymphocytes via vessels (interrupted arrows) and probably the neural reflex arc (solid arrows). It forms a functional unit for ocular surface immune defense.

This lymphoid tissue was found to be present from the lacrimal gland along its excretory ducts into the mucosa of the conjunctiva and from there into the lacrimal drainage system (Fig. 9). The ocular mucosal lymphoid tissues are hence not only connected by the flow of tears but also anatomically continuous. They are, furthermore, connected by the regulated traffic of lymphocytes via specialized vessels. Since the mucosa is innervated and neural stimuli influence the preservation of ocular surface integrity, dry eye development[27] and also immunological regulation, these tissues are conceivably also

connected by a neural reflex arc. Altogether this leads us to propose the concept of an "Eye-Associated Lymphoid Tissue" (EALT), consisting of the lacrimal gland, conjunctiva, and lacrimal drainage system, and forming a functional unit for the immune defense of the ocular surface.

If ocular surface defense fails, as occurs in several forms of ocular surface disease, inflammatory reactions can occur with an alteration of the cytokine profile. The lymphoid tissue of the ocular surface, which represents a potent source of these factors, may then also be able to act as an important modulator of ocular surface disease similar to events observed in other mucosal organs.

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