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Regulation of the Inflammatory Component in Chronic Dry Eye Disease by the Eye-Associated Lymphoid Tissue (EALT)

Nadja Knop[a] · Erich Knop[b]

[a]Department of Cell Biology in Anatomy, Hannover Medical School, Hannover, and
[b]Research Laboratory, Department of Ophthalmology CVK, Charité – Universitätsmedizin Berlin, Berlin, Germany

Abstract

Purpose: The physiologically protective mucosal immune system of the ocular surface consists of lymphocytes, accessory leukocytes and soluble immune modulators. Their involvement has also been observed in inflammatory ocular surface diseases, including dry eye syndrome, and we have attempted here to describe their interaction. **Methods:** Our own results regarding the mucosal immune system of the human ocular surface are discussed together with the available literature on mucosal immunity and inflammatory ocular surface disease. **Results:** The mucosa of the ocular surface proper (conjunctiva and cornea) is anatomically continuous with its mucosal adnexa (the lacrimal gland and lacrimal drainage system) and contains a mucosal immune system termed 'eye-associated lymphoid tissue' (EALT). This extends from the periacinar lacrimal-gland-associated lymphoid tissue along the excretory ducts into the conjunctiva-associated lymphoid tissue (CALT) and further into the lacrimal drainage-associated lymphoid tissue (LDALT). EALT consists of continuous diffuse lymphoid effector tissue and of interspersed follicles for effector cell generation in CALT and LDALT. Typical events in ocular surface disease include alteration and activation of epithelial cells with loss of epithelial integrity, production of inflammatory cytokines, and potential presentation of non-pathogenic and self-antigens – leading to a loss of immune tolerance. Events in the deregulation of physiologically protective EALT, resulting vicious circles, and eventual self-propagating immunomodulated inflammatory disease processes are explained, discussed and visualized by schematic drawings. **Conclusion:** Deregulation of EALT can orchestrate a self-propagating inflammatory mucosal disease process if the capacity of natural compensatory factors is overridden and if the disease is not limited by timely diagnosis and therapy.

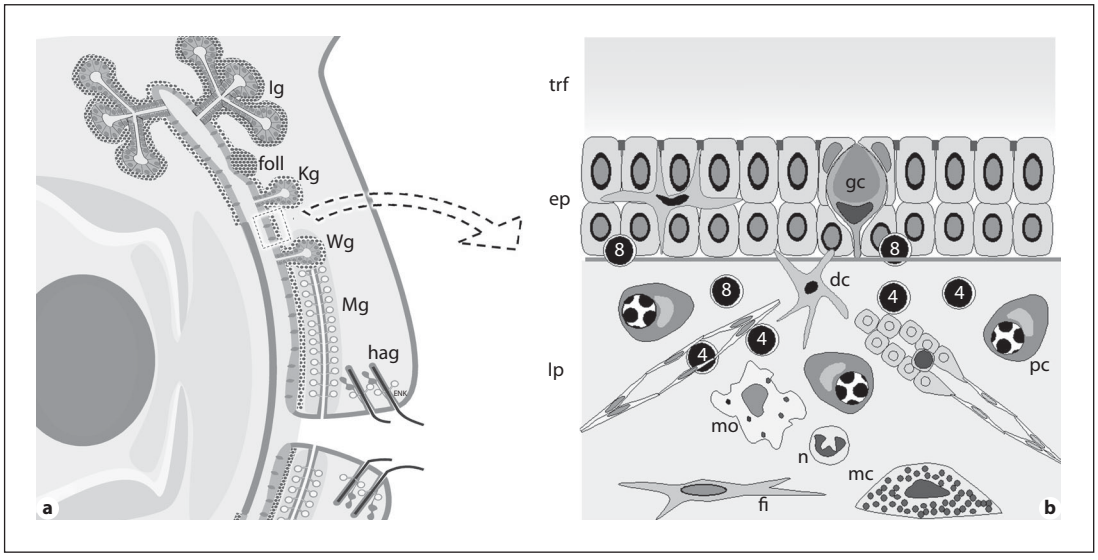

Fig. 1. Topography of eye-associated lymphoid tissue (EALT) at the human ocular surface. **a** EALT extends all along the mucosal surface of the conjunctiva into the periacinar tissue of the lacrimal gland (lg) and the conjunctival accessory lacrimal glands of Krause (Kg) and Wolfring (Wg). Organized accumulations of lymphocytes into lymphoid follicles (foll) also occur. Mg = Meibomian gland; hag = hair associated glands. **b** Diffuse lymphoid tissue can be seen in the lamina propria (lp) and epithelium (ep). Single interspersed lymphocytes and dendritic cells also occur inside the epithelium. In the lamina propria, there are lymphocytes of different subtypes (CD4 and CD8) and plasma cells (pc) together with accessory leukocyte populations, such as macrophages (mo), mast cells (mc), occasional neutrophilic granulocytes (n) and stromal cells (fibroblasts; fi). The lamina propria has vessels of a different kind, and the epithelium is covered by the tear film (trf). gc = goblet cell.

Eye-Associated Lymphoid Tissue (EALT): The Physiological Mucosal Immune System of the Ocular Surface

Ocular Surface

The mucosa of the ocular surface proper (conjunctiva and cornea) is anatomically continuous with its mucosal adnexa, i.e. with the lacrimal and accessory lacrimal glands through the excretory ducts and with the lacrimal drainage system through the lacrimal canaliculi [1]. Together, all 3 organs unite the source of the tears [2] upstream of the conjunctiva, their presumed main target at the ocular surface proper, and their eventual drainage downstream [3] (fig. 1). Hence, this extended ocular surface forms a unit with several functions [for reviews, see 1, 4, 5], e.g. wetting [6], nutrition, cell differentiation and also mucosal immune protection (and its potential deregulation) as considered here.

Dry eye disease represents a widespread disease condition [7–9] that was long misunderstood as an ocular variant of feeling unwell, but which can have a serious

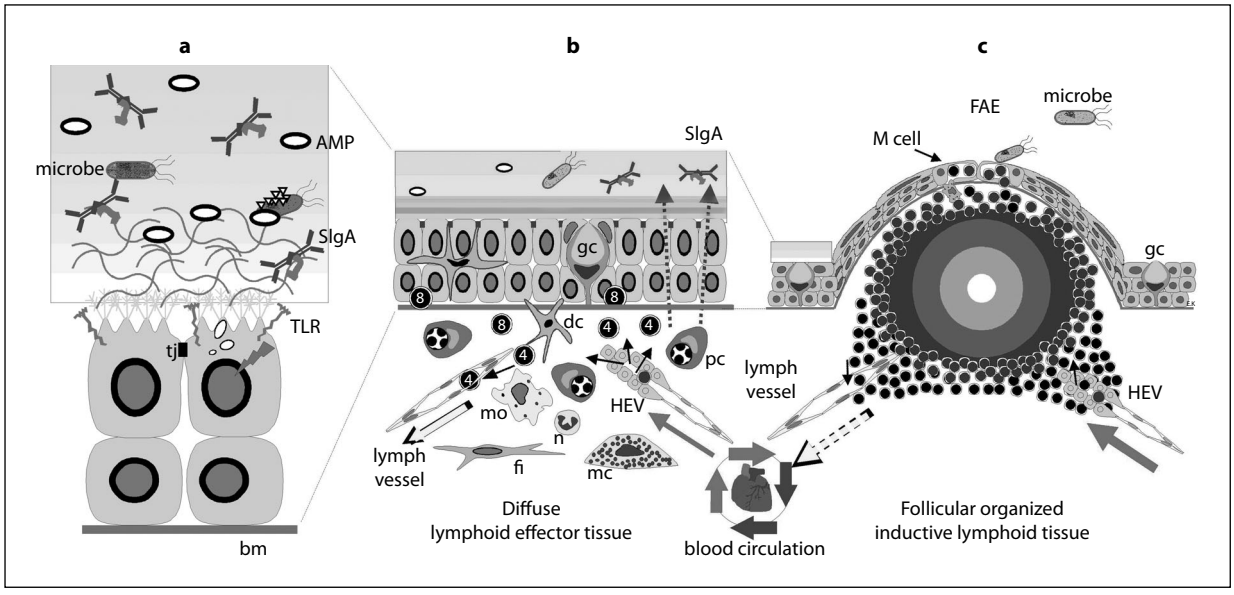

Fig. 2. Arrangement of human conjunctiva-associated lymphoid tissue (CALT). **a** The conjunctiva has several defense systems composed of the integrity of the surface epithelium with the mucin layer, which is enforced by adhering protective molecules (e.g. antimicrobial peptides, AMP) and specific secretory IgA within the tissue and the overlying tear film. SIgA = secretory IgA; TLR = Toll like receptors; bm = basement membrane. **b** CALT consists of a diffuse lymphoid layer composed of mainly effector lymphocytes and also innate effector cells, such as macrophages, mast cells, granulocytes and dendritic cells. This system is supplemented by the lymphoid cells of the specific adaptive immune system, which consists of intraepithelial lymphocytes and lamina propria lymphocytes, such as T cells of different subtypes (CD4+ helper and CD8+ suppressor/cytotoxic cells) and of differentiated B cells (plasma cells) that mainly produce secretory IgA. pc = Plasma cells; dc = dendritic cells; HEV = high endothelial venules; mc = mast cells; gc = goblet cells. **c** Lymphoid follicles and their associated T cell regions, provided with HEV, generate these effector cells. Lymphocytes enter lymphatic tissues preferably via HEV in a regulated fashion and leave via lymphatic vessels, from which they eventually reenter the blood circulation ('recirculation'). Antigen uptake is maintained via a specialized follicle-associated epithelium with antigen-transporting M cells in order to allow ports of regulated antigen entry through the otherwise sealed epithelial surface for the information of the mucosal immune system about the luminal antigen status. Reprinted with permission from Knop E and Knop N, *Encyclopedia of the Eye*, Elsevier, 2010.

impact on ocular surface integrity and may include serious inflammatory reactions [10–13]. In the future, this disease will receive increasing attention in societies where the proportion of the elderly population is growing.

Cell Types in Mucosal Lymphoid Tissue

Mucosa-associated lymphoid tissue (MALT) consists of two sheets: the epithelium and the underlying loose connective tissue of the lamina propria (fig. 2). The epithelium

is sealed by apical tight junctions to prevent the entry of foreign materials, including antigens, and constitutes a mechanical barrier that is further enforced by the adhesive properties of a superficial mucin layer [14] and associated antimicrobial peptides and proteins. Hence, impairments in epithelial integrity are a major reason for alterations in mucosal immunity in general, and are also observed in dry eye disease. The epithelium contains leukocytic cells, such as dendritic cells (for antigen-uptake and subsequent presentation) as well as effector T cells, that are mainly located in the basal epithelial layer and are termed, intraepithelial lymphocytes [15].

The lamina propria constitutes a lose collagen meshwork into which the majority of the mucosal leukocytes are embedded (fig. 2b). Stromal fibrocytes produce the collagen fibers and serve for the physical maintenance. In addition, there are bone-marrow-derived cells that have migrated into the tissue from the blood stream [16]. These consist of different cell types, such as lymphocytes of various subtypes (including plasma cells), which are termed 'lamina propria lymphocytes'. In addition, there are accessory leukocytes, such as dendritic cells, macrophages (for phagocytosis of antigens and antigen-presentation), mast cells (that have a role in host defense [17], but are better known for their stimulating action in allergic reactions [18]) and occasional neutrophilic granulocytes. From a wider perspective, the overlying epithelial cells communicate with the leukocytes and have a role in immune reactions [19]. In addition, a large number of macromolecules are present, which are either derived from the bloodstream for nutrition and cell metabolism or produced by the local cells and assist in cell communication (e.g. cytokines and chemokines) [20–22] and protection (e.g. immunoglobulins and antibacterial peptides (AMP)) [23, 24]. Their production is regulated by receptors on the epithelial cells, such as Toll-like receptors (TLR) [25] (fig. 2a).

Arrangement and Function of Mucosal Lymphoid Tissue

The lymphoid cells together with the accessory cells form so-called mucosa-associated lymphoid tissue (MALT), of which two types can be differentiated [15] (fig. 2). The main extension of the mucosal surface is provided with lymphocytes that are diffusely interspersed into the tissue and hence constitute a so-called 'diffuse lymphoid tissue'. T lymphocytes that have differentiated into CD8+ suppressor/cytotoxic cells directly act against antigens, whereas CD4+ T-helper (Th) cells regulate immune response [26]. B cells differentiate into immunoglobulin-producing plasma cells and act indirectly via secreted immunoglobulins, mainly mucosal IgA. Diffuse MALT is mainly populated by differentiated effector cells that can act against antigens; it is therefore termed the 'efferent' arm of mucosal immunity [15] (fig. 2b).

As opposed to the diffuse lymphoid tissue, lymphocytes also form accumulations that are organized into specific functional domains, and are hence termed 'organized lymphoid tissue'. Organized MALT consists of lymphoid follicles composed

of mainly B lymphocytes and has parafollicular T cell zones. In organized MALT, naïve lymphocytes that are mature but have not been in contact with antigens are primed by the process of antigen presentation via professional antigen-presenting cells (APC; dendritic cells, macrophages, B cells) and differentiate into effector cells that can act against antigens in different ways. In the follicles, B cells differentiate into antibody-secreting plasma cell precursors, whereas in the parafollicular regions T cells differentiate into the various T cell subtypes. At follicular sites, antigens are taken up from the environment by a specialized follicle-associated epithelium that covers the follicles towards the luminal surface, and hence organized MALT represents the 'afferent' inductive arm of mucosal immunity in a functional sense (fig. 2c), although antigens can also be taken up and later presented by the APC in the diffuse lymphoid tissue.

Differentiated B cells and T cells can act locally inside the tissue but are also eventually distributed (after emigration through lymphatic vessels) throughout the body via the blood stream, and can migrate into the same or other lymphoid organs. This process is known as 'recirculation', and it allows the population of ocular lymphoid tissues with naïve lymphocytes and the exchange of effector cells among different lymphoid organs [27].

Lymphoid Cells at the Human Ocular Surface and Mucosal Adnexa Constitute Eye-Associated Lymphoid Tissue (EALT)

The presence of lymphoid cells as a normal physiological finding in the tissues of the human ocular surface and mucosal adnexa had been under debate until recently, even though they had been mentioned for a long time [28, 29]. This was mainly due to scant knowledge of the mucosal immune system making their functional significance unclear. Understanding was further complicated by the historic misconception that lymphoid cells were, together with the other leukocytes, simply considered as 'inflammatory cells' even though they were observed in every normal tissue [30] and accepted as a normal component, e.g. in the lacrimal and accessory lacrimal glands that are in continuity with the conjunctiva [31, 32].

Later similar relations of lymphoid cell types as in other mucosal organs with a diffuse MALT were found in immunohistological studies of human conjunctiva biopsies [33–35], including the regular presence of mucosa-specific lymphocytes [34, 35]. However, there were different and partly conflicting reports concerning the presence of cell types and the amount location of lymphoid cells, which were most likely based on problems resulting from the minute size of biopsies used and from difficulty in determining their exact location. Also, data from animal tissues was transferred to the human situation [36].

This was only resolved when it was shown in studies on human whole-mount tissues that conjunctiva-associated lymphoid tissue (CALT) [37, 38] and lacrimal

drainage-associated lymphoid tissue (LDALT) [3, 39–41] exist, similar to other mucosal organs. The ocular mucosal tissues are termed CALT and LDALT according to the international nomenclature of mucosal immunology [16].

CALT and LDALT form, together with the gland-associated lymphoid tissue of the lacrimal gland, the eye-associated lymphoid tissue (EALT) [1, 3, 4, 42] as a functional unit for ocular surface immune protection and as a new component of the mucosal immune system in the body. This is in line with (for example) gut-associated lymphoid tissue of the intestine or bronchus-associated lymphoid tissue in the airways. Several studies have meanwhile convinced the scientific community that lymphoid cells are a normal and noninflammatory component of the ocular surface [for a review, see 4]. Therefore, lymphoid cells are: (1) resident at the normal human ocular surface; (2) physiologically noninflammatory; (3) continuously involved in the maintenance of protective mucosal immune regulation; (4) do not need acute immigration in order to interact in local immunological processes [1]. These are important differences in contrast to the previous perception that lymphoid cells migrate as 'inflammatory cells' into a primarily lymphocyte-free ocular surface as a secondary or tertiary event in inflammatory disease processes.

EALT Unites the Ocular Surface and Its Mucosal Adnexa

The parts of EALT are connected on different levels (fig. 3): (1) The diffuse lymphoid tissue is physically continuous from the lacrimal gland and accessory lacrimal glands along the conjunctiva into the lacrimal drainage system. (2) The whole ocular mucosal surface shares the same luminal fluid, represented by the tears, and hence shares the same soluble protective factors but conceivably also the same pathogenic repertoire. The latter applies at least to CALT and LDALT due to the fact that the majority of ocular pathogens enter through the open palpebral fissure and float downstream. This may not equally apply to the lacrimal gland because the upstream travel of ocular surface pathogens is conceivably prevented by the tear flow, and this may explain why lymphoid follicles for antigen uptake and effector cell generation are not normally found in the lacrimal gland. (3) The ocular mucosal tissues are also connected by the regulated migration of lymphoid cells ('recirculation') via the numerous small vessels including specialized high endothelial venules [43, 44] that have specific adhesion molecules on the surface of the vascular endothelial cells [45].

Analysis of the topographical distribution of EALT in the conjunctiva shows that it matches the position of the cornea [46] and is in direct contact with the corneal surface when the eye is closed, at night and during blinking. This topography, the fact that the cornea does not contain its own lymphoid cells, and the fact that with the eyes closed during the night corneal integrity is protected by a massive influx of leukocytes and their secretory products [17] strongly suggest that EALT has an important role in the protection of the corneal integrity and health [4].

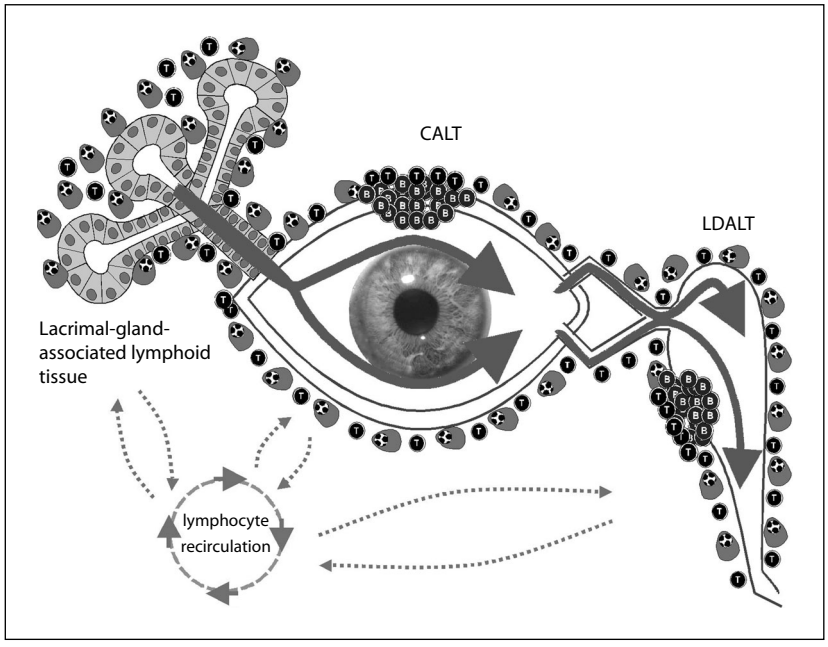

Fig. 3. Eye-associated lymphoid tissue (EALT) consists of CALT at the ocular surface proper and is continuous with the lacrimal and accessory lacrimal glands through their excretory ducts (upstream). Downstream it continues via the lacrimal canaliculi along the lacrimal drainage route as LDALT. A diffuse lymphoid tissue of T lymphocytes (dark round cells) and interspersed IgA-secreting plasma cells (including accessory leukocyte populations; not indicated) occurs along the whole mucosa, whereas lymphoid follicles are physiologically only found in CALT and LDALT. The diffuse lymphoid tissue in EALT is physically continuous and the organs of EALT are also connected via the flow of tears (large arrows) by which they share protective factors as well as pathogens. The organs are furthermore connected by lymphocyte recirculation via specialized vessels (dotted arrows at the bottom; compare with figure 2) with each other and with the other organs of the immune system. Reprinted with permission from Knop E and Knop N, *Immune Response and the Eye*, Karger, 2007.

The main function of ocular surface lymphocytes is (in contrast to previous assumptions) not to generate inflammation, but rather the reverse, i.e. to protect the ocular surface against inflammatory processes that could lead to tissue destruction. Therefore, the ocular mucosal immune system maintains the delicate balance between the tolerance to ubiquitous nonpathogenic antigens and adverse immune reactions against pathogenic microorganisms that involve inflammation and hence endanger ocular surface integrity (fig. 4).

Different Subtypes of Lymphocytes Occur in EALT

Conjunctival lymphocytes in the diffuse lymphoid tissue are mainly CD3+ T cells, whereas most of the CD20+ B cells are restricted to the solitary lymphoid follicles [47].

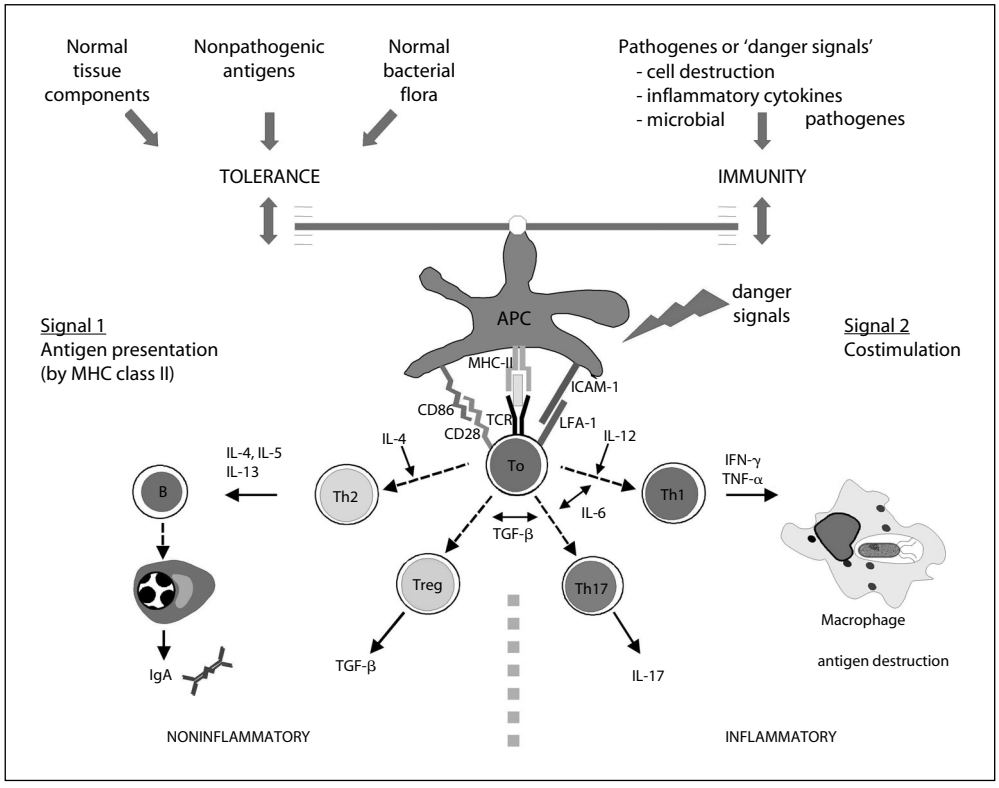

Fig. 4. Ocular surface immune regulation. Immune tolerance to host tissue constituents, nonpathogenic antigens and physiological commensal bacterial flora is essential for tissue integrity in order to avoid unnecessary destructive immune reactions. This must however be in equilibrium with the detection of and defense against pathogens. The fine equilibrium between these 2 important functions is critical for mucosal immunity and the balance is maintained by the mode of antigen-presentation exerted by APC via MHC-class II to naïve (To) lymphocytes that results in the generation of different subtypes of Th cells which produce characteristic cytokine patterns of interleukins (IL). The presentation of an antigen (signal 1) in the context of danger signals as represented by cell destruction, inflammatory cytokines or microbial pathogens leads to the expression of costimulatory molecules (signal 2) and results in inflammatory immune responses by e.g. Th1 or Th17 cells. Regulatory T cells (Treg) suppress inflammatory responses and the normal mucosal noninflammatory IgA response is produced by the differentiation of B cells into plasma cells inside the lymphoid follicles.

They are activated (CD45Ro+, CD25+) [35], and express the human mucosa lymphocyte antigen (HML-1, CD103, integrin αEβ7) [34]. CD8+ cytotoxic/effector lymphocytes are prevalent in the epithelium, and have been proposed to act in a suppressor mode [33] that provides a component of the immune tolerance at the ocular surface. In contrast to the intraepithelial lymphocytes, the lamina propria lymphocytes (fig. 2b) consist predominantly of CD4+ Th cells [34, 35]. Plasma cells account for about one fifth of the conjunctival leukocytes [30], but in absolute numbers they are quite substantial and equivalent to two thirds of those in the lacrimal gland (which had been regarded

as the sole source of tear film secretory IgA; SIgA). Therefore, the conjunctiva is able to contribute considerably to its own defense [23] and is not dependent on a passive supply of IgA from the lacrimal gland. Since the number of normal conjunctival lymphocytes is at least three times that of the plasma cells [30], as shown in histology, the number of T cells is therefore considerable (as also verified in immunohistochemistry) [34, 35].

Subtypes and Functions of Conjunctival Lymphocytes Are Determined by the Mode of Antigen Presentation

The generation and modulation of the mucosal immune response is regulated through the process of antigen presentation [48] by APC. These are mainly dendritic cells [49], including Langerhans cells, but also macrophages and B cells. APC present antigens via their MHC class II receptor (MHC-II) to the T cell receptor on naïve T cells. Apart from this signal (signal 1), accessory signals (co-stimulation, signal 2) [50] such as e.g. CD86 or intercellular adhesion molecule 1 (ICAM-1) are also necessary to mount an efficient T cell response. Pathogenic molecular danger signals in the tissue environment – such as molecules from destroyed cells, inflammatory cytokines (e.g. IL-6, TNF-α, INF-γ) or bacterial molecules (e.g. lipopolysaccharide) signal the presence of a nonphysiological and hence dangerous environment to the immune cells and lead to the initiation of an inflammatory immune response. If inflammatory cytokines and other 'danger signals' [50] occur in the tissue, they can bind to (for example) Toll-like receptors and mediate (via intracellular signaling pathways, e.g. NF-kB or protein kinases [51]) the secretion of inflammatory cytokines that skew EALT towards an inflammatory immune response.

Host tissue antigens (self-antigens) are constitutively taken up and presented by APC that are in an immature state, which leads to anergy or deletion of a respective cognate T cell. T cells that detect self-antigens are mainly destroyed in the process of central tolerance inside the primary lymphoid organ (thymus). Since not all antigens of the body are available in the thymus, some autoreactive T cells escape deletion and must be silenced inside the peripheral mucosal organs (peripheral tolerance) in order to avoid autoimmune reactions [52]. Since the presence of danger signals leads to the maturation of dendritic cells with the upregulation of costimulatory molecules on their surface, independent of the nature of the antigen presented on their MHC class II, the presence of chronic inflammation increases the risk that the presentation of autoantigens may lead to the activation (priming) of potential autoreactive T cells. The mode of antigen presentation by APC decides about the subtypes of lymphocytes that are generated from naïve T cells in the priming process (fig. 4).

An important function of mucosal immune regulation is the generation of plasma cells that produce the noninflammatory IgA, which is achieved when antigen presentation occurs under the influence of cytokines, such as IL-4, and results in type 2 Th cells. These interact with B cells and produce cytokines (e.g. IL-4, IL-5, IL-13) that promote the isotype class switch to IgA and the differentiation into IgA-secreting plasma

cell precursors [53]. Regulatory T cells (Treg) are generated under the influence of the anti-inflammatory cytokine TGF-β and in turn produce further TGF-β which, together with IL-10, generates a tolerogenic milieu within EALT and prevents inflammation [54, 55]. Interestingly, if the inflammatory cytokine IL-6 is present in addition to TGF-β, this leads – through a switch in signaling pathways – to the preferred generation of inflammatory Th17 cells [56]. These produce the inflammatory IL-17 and other cytokines that are capable of inducing a heavy inflammatory burst against extracellular antigens. Hence, IL-6 appears to be a crucial factor in mucosal immune regulation. If only IL-6 is present, the well-known inflammatory Th1 cells are formed, and these produce inflammatory cytokines [57] (such as IFN-γ and TNF-α) which have the physiological function to activate cells, in particular phagocytes, to destroy intracellular pathogens.

Immune-Mediated Ocular Surface Inflammation Is a Key Event in Advanced Dry Eye Disease

Deregulation of EALT

Epithelial alteration and cell activation due to various stress factors is a central component of dry eye disease, and is certainly well known to every clinician. Intense investigations over recent years have revealed a large body of information on the underlying factors and pathways that link epithelial alteration to inflammatory processes and immune-mediated inflammation.

Various stress mechanisms [51] – e.g. mechanical alteration by increased sheer forces due to tear film defects [58], hyperosmolar stress [59] or exposure to inflammatory cytokines [60] – can activate the ocular surface epithelium. The epithelial cells interpret these events as a threat to tissue integrity, and hence launch mechanisms for immune defense and tissue repair in order to cure this threat. Therefore, they respond by secreting inflammatory cytokines in order to alarm the mucosal immune system, upregulate surface markers (such as MHC-II and ICAM-1 [61]) in order to allow presentation of pathological antigens, and activate proteases (such as matrix-metalloproteinase [60]) in order to digest cell debris and to provide space for the necessarily increased amounts of leukocytes (which is later followed by the onset of repair mechanisms).

If these basically protective mechanisms are constantly overexpressed through continuous stimulation (e.g. by chronic mechanical irritation) as occurs in dry eye disease, this results in a number of unfortunate consequences. The natural tolerogenic bias can be lost and the mucosal immune system becomes deregulated. This conceivably represents the underlying reason for the observed self-perpetuating inflammatory process at the ocular surface and its associated glands [62–64]. This promotes an inflammatory process in which epithelial cells, via MHC-II on their surface, acquire the potential for presentation of antigens, including self-antigens, to

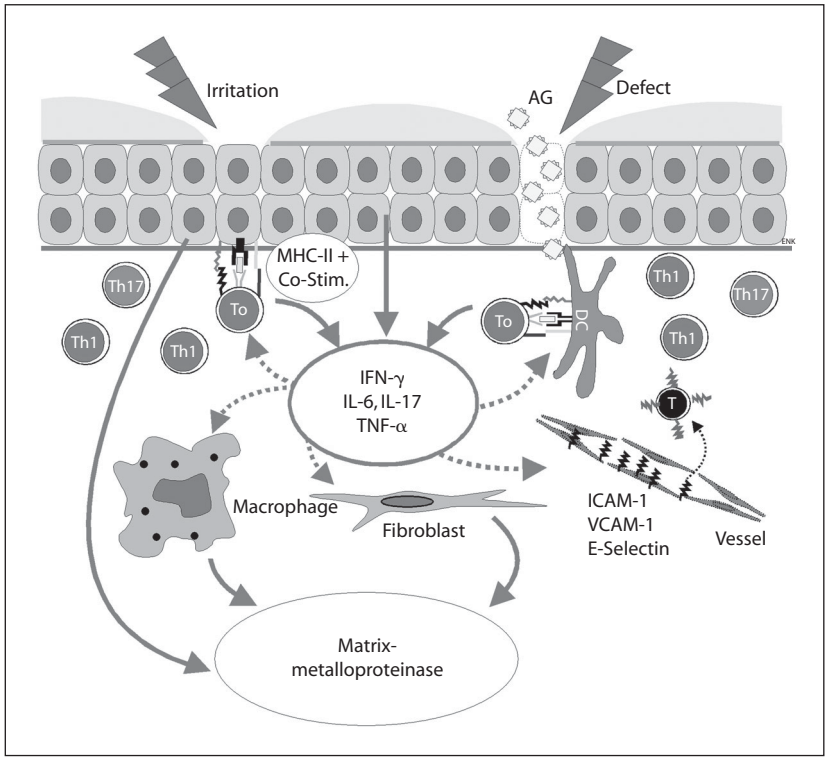

Fig. 5. Deregulation of EALT in ocular surface inflammation. Persistent irritation of the ocular surface epithelium can lead to events that result in a deregulation of EALT. A starting point is (e.g.) tear film deficiency, as found in dry eye disease, that leads to epithelial wounding with eventual activation of the epithelial cells. These respond by secretion of inflammatory cytokines and by expression of the antigen-presenting molecule MHC-II, and they can hence present antigens (including self-antigens) to the resident conjunctival T cells in an inflammatory context. Epithelial defects that breach the physical barrier can lead to the entry of pathogens and nonpathogenic antigens into the tissue, and their subsequent presentation by dendritic cells (DC), also in an inflammatory context, as observed in ocular allergy for example. This contributes to the further accumulation of inflammatory cytokines in the tissue, which is a crucial factor in inflammatory ocular surface disease, and to the generation of inflammatory, potentially autoreactive, types of T cells. Downstream effects are the activation of blood vessel endothelial cells that upregulate adhesion molecules with subsequent recruitment of further leukocytes from the vascular compartment and the activation of bystander cells including stromal cells (fibroblasts and macrophages). These contribute to the activation of matrix metalloproteinases that lead to tissue degradation. All together this constitutes an immune-mediated conjunctival mucosal inflammatory process that is based on deregulation of the physiologically protective EALT. Reprinted with permission from Knop E and Knop N, *The Ocular Surface*, 2005.

resident conjunctival T cells (fig. 5), that may lead to a loss of natural ocular surface immune tolerance [1, 12, 64]. In fact, in experimental inflammatory ocular surface disease, autoreactive T cells are generated that are specific to ocular surface tissue [65] and respective regulatory T cells can prevent the tissue destruction [55].

Increased levels of inflammatory cytokines are associated with further unfortunate consequences, such as: (1) the upregulation of adhesion molecules (like ICAM-1, VCAM-1 or E-selectin) on vascular endothelial cells [61] that results in the recruitment of leucocytes, including further lymphocytes which contribute to the disease [66], from the vascular compartment into the ocular tissues (fig. 5); (2) the increased proliferation of epithelial cells that remain in an immature state and hence lead to the development of squamous metaplasia [67] (fig. 6); (3) impairment of the afferent sensory neural signaling from the ocular surface that results, via a blockade of the lacrimal functional unit [68], in decreased tear production by the lacrimal gland [69].

All together, these events represent an immune-mediated inflammatory episode, orchestrated by deregulation of the resident lymphoid cells of EALT. This is an important common factor in the advanced stages of ocular surface inflammation, including dry eye disease, and can lead to several vicious circles (fig. 6) that propagate the disease [1, 4, 11, 64, 70]. This inflammation is at first subclinical, but tends to amplify if it is not limited in the early stages.

Important starting points for tear film deficiency and consequent ocular surface alteration are a primary deficiency of tear production by the lacrimal gland, which is relatively rare, or a deficiency in the lipid phase of the tear film, which results in increased evaporation of the aqueous tears and hence in an evaporative dry eye (fig. 6). Evaporative dry eye appears more frequently, since a lipid deficiency is reported in about 75% of dry eye patients [71] and in 65% of these it is caused by obstructive meibomian gland dysfunction (MGD) [72]. MGD is mainly based on an obstructive process [73, 74] of the excretory duct and orifice of the meibomian glands [75] that secrete their oils onto the posterior lid margin (see another contribution to this volume) from which they glide onto the tear film as the most superficial phase and limit evaporation [76, 77]. MGD often occurs with minor symptoms and represents an underdiagnosed and underestimated reason for dry eye disease [78, 79]. As judged from its epidemiology and pathophysiology [80], obstructive MGD appears as an important factor for the onset of dry eye (fig. 6) because it: (1) induces epithelial alterations via a hyperevaporative tear deficiency; (2) simultaneously results in hyperosmolarity of the tears, which represents a stress to the ocular surface epithelium, and hence directly activates the epithelial cells to produce inflammatory cytokines [59]; (3) leads to decreased tear clearance and hence an increase in the concentration of inflammatory cytokines in the remaining tears [81] with the results explained above. It can be assumed that an impairment in neural innervation may also apply to the meibomian glands since they share important similarities with the innervation pattern of the lacrimal gland [75]. Therefore, immunomodulated inflammatory processes with all the downstream results may also contribute to the understanding of why the late stages of aqueous deficient and hyperevaporative dry eye share similarities [82].

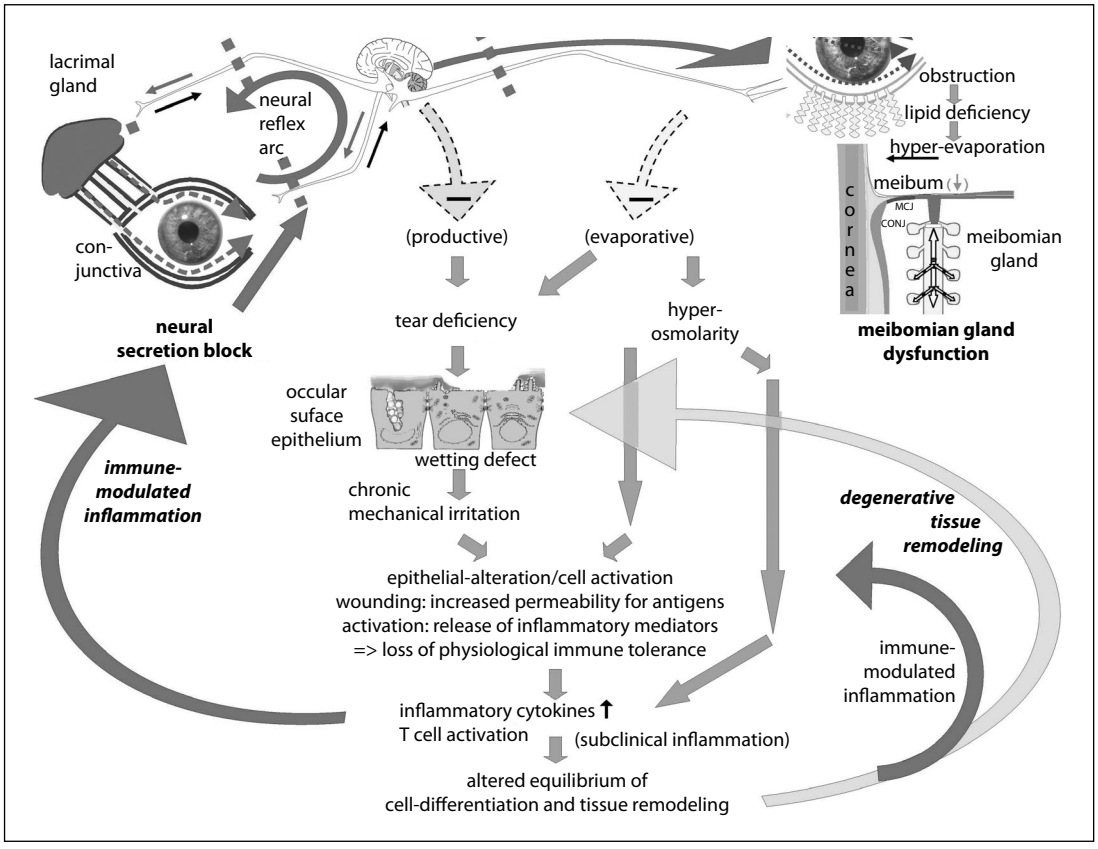

Fig. 6. Vicious circles lead to self-propagation in chronic dry eye disease. The main uniting factor in different forms of ocular surface inflammation, including severe stages of dry eye disease, is the presence of epithelial defects and cell activation with a buildup of inflammatory cytokines and a subclinical inflammation with potential downstream T cell activation and deregulation of the resident mucosal immune system. Different events at the ocular surface can lead to epithelial alterations. These are a secretory or an evaporative tear deficiency that results in chronic mechanical irritation. Evaporative tear deficiency is mainly due to a deficiency in the lipid phase of the tear film due to meibomian gland dysfunction and results, besides a decreased tear volume, in tear hyperosmolarity that directly exerts cell activation and in decreased tear clearance with a buildup of inflammatory cytokines. Persistent subclinical inflammation, modulated by a deregulation of EALT (on the basis of the events explained in figure 5) leads to an altered equilibrium of cell differentiation and tissue remodeling that typically results in squamous metaplasia of the ocular surface. Several vicious circles that originate from these events can negatively reinforce starting points in the sense of vicious circles of immune-modulated inflammation. This aggravates the initial subclinical ocular surface inflammation into an overt form if protective factors are overridden or if this is not limited by timely diagnosis and therapy. An impairment of afferent sensory innervation from the ocular surface due to inflammatory cytokines results in a blockade of the efferent secretory stimuli to the glands and hence aggravates the tear film deficiency. Deterioration of the epithelial differentiation can on the one hand increase the entry of luminal antigens and release host tissue antigens that can be presented in an inflammatory context and hence reinforce immunological deregulation of the mucosal immune system; on the other hand, the lubrication of the surface is negatively influenced.

The question arises: why do not all patients that have experienced a mild or incipient dry eye condition due to (for example) environmental factors such as low humidity, increased air flow or decreased blinking rate (which frequently occur in ordinary office work at video display terminals in air-conditioned environments) develop severe immune-mediated ocular surface inflammation? The answer is probably quite straightforward, i.e. because in most of us there is a sufficient amount of physiological compensatory factors, such as: (1) the availability of excess tear production capacity in the aqueous, lipid and mucous glands (fig. 1); (2) a sufficient level of androgens that maintain an anti-inflammatory environment at the ocular surface and glands [83] and positively influence the function of the meibomian and other glands [9, 84]; (3) a sufficient regenerative capacity of the tissue; (4) sufficient availability of antigen-neutralizing IgA antibodies by the secretory mucosal immune system [23, 85].

However, if such physiological compensatory factors are reduced (e.g. due to advanced age or hormonal imbalances; in particular, decreased levels of androgen action, increased lid margin degeneration [86] or constant severe environmental desiccating stress [78]), the likeliness of a self-enforcing progressive course of dry eye disease will increase. This may explain why the prevalence of dry eye is generally increased in the aged population, and in particular in females [8].

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Dr. Nadja Knop
Department of Cell Biology in Anatomy, Hannover Medical School
Carl-Neuberg-Strasse 1
DE–30625 Hannover (Germany)
E-Mail knopnadja@aol.com