Clinical Aspects of MALT

6

Erich Knop, Nadja Knop, Uwe Pleyer

Core Messages

- Mucosa-associated lymphoid tissue (MALT) is an outpost of the immune system located at mucosal surfaces of the body. It can recognize antigens, generate specific effector cells and provide the mucosal organs with such cells
- MALT also occurs at the normal human ocular surface and appendage (lacrimal gland, conjunctiva and lacrimal drainage system), which together form an eye-associated lymphoid tissue (EALT)
- EALT makes an important contribution to the homeostasis of the ocular surface by maintaining the equilibrium between inflammatory immune reactions against pathogens and immune tolerance against non-pathogenic antigens
- Dendritic antigen presenting cells (DC) are a key regulator of the immune response by the promotion of different subpopulations of T-lymphocytes that act via specific sets of cytokines
- Various diseases of the ocular surface include an immune mediated inflammation with production of cytokines, chemokines, adhesion molecules and action of lymphocytes
- This is conceivably influenced by a dysregulation of EALT and can hence be therapeutically addressed by immune modulatory drugs
- Different types of dry eye disease contain an underlying immune modulated inflammation based on a deregulation of the physiological and normally protective mucosal immune system
- In chronic allergic and vernal keratoconjunctivitis (AKC and VKC) inflammatory cells and lymphocytes are activated by mast cell cytokines and result in an inflammatory infiltrate and corneal destruction
- Local as well as systemic T-cell mediated immune processes are involved in transplant rejection. CD4+ lymphocytes play an essential role in the immune response during corneal graft rejection and are the main target of immunomodulatory therapy

6.1 Introduction

The immune protection at the inner and outer mucosal surfaces of the body, including the ocular surface, is maintained by a part of the immune system termed the "mucosa-associated lymphoid tissue" (MALT). This is found in different mucosal organs where the lymphoid tissue for each is designated separately according to an international nomenclature composed of characteristic acronyms. MALT is most prominent in the gut (termed gut-associated lymphoid tissue or GALT) but is also found in the airways (termed bronchial-associated lymphoid tissue or BALT) or in the genitourinary system. Recently MALT was also described as a regular component of the normal human ocular surface and accordingly termed eye-associated lymphoid tissue (EALT).

One of the main functions of MALT is to establish a balance between immunity and tolerance in order to prevent destruction of the delicate mucosal tissues by constant inflammatory reactions, which applies in particular to the eye. This is maintained by an anti-inflammatory cytokine milieu in mucosal tissues and is most likely regulated by antigen presenting dendritic cells (DC) that act as key regulators of the immune system and normally favour anti-inflammatory T- or B-cell responses in mucosal locations. A major defence mechanism of MALT is the production of secretory immunoglobulins, mainly of the IgA and partly of the IgM isotype by differentiated B cells (plasma cells). In contrast to the IgG isotype that prevails in the blood, IgA has very little complement binding activity and therefore does not initiate inflammatory reactions during host defence.

The lymphoid cells of MALT migrate in a regulated fashion, guided by specialized vessels, adhesion molecules and soluble chemotactic factors. They migrate between the different mucosal organs, which are hence assumed to constitute a functionally interrelated mucosal immune system. By these migration pathways, MALT is also connected to the central immune system.

Since the mucosal immune system is a prominent source of professional immune regulating cells and soluble mediators that are constitutively present also at the normal human ocular surface, it is also involved in mucosal disease states as will be pointed out in the present paper. In fact research has indicated that deregulation of the physiological mucosal immune system may contribute as a primary or secondary pathogenetic factor to inflammatory diseases such as ocular allergy but also to conditions such as dry eye disease where an underlying inflammatory component is shown. The mucosal immune system is certainly also involved in the course of a corneal transplant and its potential rejection, which represents a T-cell-mediated process. This is regulated to a large extent through the mediation of conjunctival DC and the newly discovered central corneal DC.

Advances in the understanding of the different mechanisms of ocular mucosal immunity, concerning for example immune regulatory cells (e.g. T cells and DC), regulatory molecules or mechanisms of recirculation that guide the influx of cells into the respective tissues, may therefore be applied as future more effective strategies for some of today's not infrequently therapy resistant diseases. The successful application of immunosuppressive agents in certain cases of dry eye disease, for example, has indicated the rationality of this approach.

6.2 Structure and Function of MALT

6.2.1 Structure of MALT

6.2.1.1 Histology of the Mucosa

Mucosal tissues consist of two sheets (Fig. 6.1). The superficial sheet represents a unilayered or, at the ocular surface, a multilayered arrangement of epithelial cells. They usually have a strong mechanical connection by intercellular adherence junctions (e.g. desmosomes and zonulae adherentes) and are sealed by an apical tight junction complex that prevents entrance of foreign materials including potential antigens. Impairment of the epithelial integrity is a major reason for a deregulation of mucosal immunity and is observed in dry eye disease and in allergic eye disease but is also caused by the surgical trauma during corneal transplantation.

The epithelium is separated by a thin basement membrane from the underlying loose connective tissue of the lamina propria. The lamina propria not only has mechanical properties for anchorage of the epithelium but is highly vascularized to serve for metabolic purposes and to provide migratory pathways for lymphoid cells. Lymphoid cells can immigrate in a regulated fashion via specialized postcapillary high endothelial venules (HEV) or flat-lined vessels into the tissue and can leave from the tissue via afferent lymphatic vessels towards the regional lymph nodes and eventually into the blood circulation in order to recirculate (Fig. 6.1). The lamina propria is filled with a

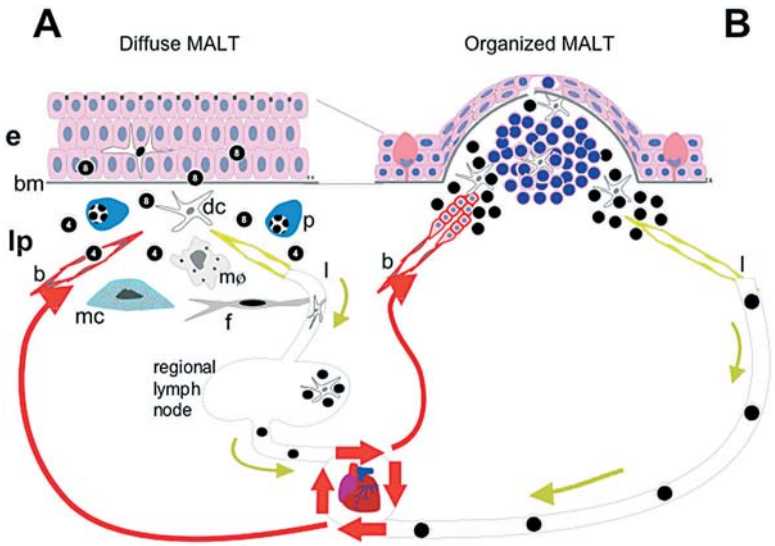

Fig. 6.1 A, B. Structure and function of the mucosal immune system. MALT consists of a diffuse lymphoid tissue (**A**) and of an organized follicular tissue (**B**), shown here at different enlargements. Mucosal tissues in general are composed of two sheets, the luminal epithelium (*e*) with its basement membrane (*bm*) and an underlying lamina propria (*lp*), which both contain lymphocytes. The lamina propria is composed of loose connective tissue with small blood vessels (*b*), afferent lymph vessels (*l*) and numerous cells including lymphoid cells [T-lymphocytes (*black*), B-lymphocytes (*blue*), plasma cells (*p*)]. Accessory cells occur like fibroblasts (*f*), macrophages (*mϕ*), mast cells (*mc*) or dendritic cells (*dc*). Intraepithelial lymphocytes are mainly CD8+ suppressor/cytotoxic T cells whereas in the lamina propria of the diffuse tissue (**A**) they occur in roughly equal numbers together with CD4+ T-helper cells. Follicular lymphoid tissue (**B**) is formed by accumulations of B-lymphocytes with parafollicular T-cell zones, vessels and an overlying specialized follicle-associated epithelium for antigen transport towards the follicle. Naïve lymphocytes enter follicular regions via blood vessels (*b*), come into contact with antigens, antigen-specific lymphocytes proliferate, differentiate and leave via lymphatics (*l*). They finally reach the blood circulation and may later emigrate to populate the same or other mucosal tissues as effector cells (T cells and plasma cells)

large number of different cell types and macromolecules that serve the purpose of nutrition and protection as maintained, e.g. by immunoglobulins and antibacterial peptides. The lamina propria also enables the communication of cells with each other and their extracellular matrix by different types of molecules. Cytokines, chemokines and adhesion molecules transfer (immunoregulatory) information, guide the migration by forming a gradient, act as traffic signals anchored to the extracellular matrix and cells or provide direct cell contacts.

Cells in the lamina propria consist of so-called fixed cells like fibroblasts that are responsible for the production and maintenance of the connective tissue itself and free cells (e.g. lymphocytes, plasma cells, macrophages, DC, eosinophils or mast cells) that can migrate in and partially out of the tissue and mainly have protective tasks. Lymphoid cells occur in both mucosal layers: in the connective tissue as lamina propria lymphocytes (LPL) and plasma cells and inside mainly the basal layers of the epithelium as intraepithelial lymphocytes (IEL). Antigen presenting DC also occur in the epithelium and lamina propria. The other free cell types normally only occur inside the lamina propria.

6.2.1.2 Conformations of MALT

MALT is divided into two forms [29] (Fig. 6.1). In the "organized" lymphoid tissue lymphocytes are organized into lymphoid follicles whereas the "diffuse" lymphoid tissue is composed of diffusely interspersed lymphoid cells along the mucous membranes and their associated glands.

Follicular MALT is regarded as the afferent arm of mucosal immunity where antigens are taken up from the environment by a specialized follicle-associated epithelium (FAE). Antigens can be presented to lymphocytes by antigen presenting cells in the parafollicular T-cell regions around follicles. This leads to lymphocyte activation, proliferation and eventual differentiation into effector cells of the T- or B-lineage. Contact with antigens and proliferation of B cells results in a transformation of the homogeneous primary follicle into a secondary follicle with a bright germinal centre of proliferating B cells. The mucosal antigen presentation to naïve T cells takes place in parafollicular regions in order to produce differentiated effector cells. This can happen in local follicles of MALT but antigens can also be transported by antigen presenting DC via the afferent lymphatic vessels into regional lymph nodes.

Diffuse lymphoid tissue is populated by the arising effector cells and represents the efferent arm of mucosal immunity. T-lymphocytes [61] that have differentiated into CD8-positive suppressor/cytotoxic cells either directly act against antigens and provide the cellular T-cell immune response or support immunosuppression. B cells in contrast that differentiate into immunoglobulin producing plasma cells, act indirectly by secreted immunoglobulins. In contrast to systemic immunity, plasma cells in mucosal tissues contribute to secretory immunity by the production of polymeric immunoglobulins that are transported through the overlying epithelium with the help of an epithelial transporter molecule (secretory component, SC) and build up a protective layer at the mucosal surface [4].

6.2.1.3 Lymphocytes and Accessory Cells

6.2.1.3.1 Lymphocytes

B-lymphocytes represent the majority of lymphocytes in the follicular zones whereas in the diffuse lymphoid tissue B cells are rare and T cells predominate together with various other cell types. CD8-positive suppressor/cytotoxic cells are usually more frequent in the mucosa than CD4-positive T-helper cells (CD8+) that regulate the differentiation of T- and B-lymphocytes. CD8+ cells strongly dominate in the epithelium and frequently bear the human mucosa lymphocyte antigen (HML-1), whereas in the lamina propria both populations occur in roughly equal amounts. Since there was indication that CD8+ suppressor/cytotoxic T cells may primarily be involved in immune suppression, it was supposed that their presence characterizes the ocular surface, similar to other parts of the mucosal immune system, as a highly immune regulated tissue that favours immune suppression rather than inflammation [6, 53]. However, T cells can also mediate inflammatory immune responses that represent basic pathological mechanisms in the types of ocular surface diseases considered in the present paper.

6.2.1.3.2 Antigen Presenting Cells

Antigen presentation to naïve T cells is performed by so-called professional antigen presenting cells (APC) that are composed of macrophages, B cells and dendritic cells, all of which occur in ocular MALT. Only the bone marrow derived dendritic cells (DC) can directly stimulate T-lymphocytes and are therefore the most important APC also at mucosal surfaces [2] for the initiation of a T-cell-mediated immune response. T-cell-mediated responses are important for most kinds of immunological reactions, including the humoral immunity by production of soluble antibodies which is influenced by T-helper cells, and they also determine the clinical conditions considered in this paper.

DC occur in the epithelium and also in the lamina propria of mucosal tissues. Immature DC prevail, which show an active uptake of antigens but a low surface expression of antigen presenting MHC-class-II molecules and co-stimulatory molecules, performing an ineffective presentation of antigens to T cells [2, 37]. They produce preferably anti-inflammatory cytokines such as interleukin 10 (IL-10) [19]. If these DC come into contact with T cells, they tend to inhibit inflammatory T-cell immune reactions and favour the humoral immune answer with the production of soluble immunoglobulins, as in fact mainly found at mucosal surfaces (IgA and IgM) [4], or they may initiate immune tolerance by induction of regulatory T cells [67].

6.2.1.4 Recirculation of Lymphoid Cells

Due to the enormous variety of potential antigens there is only a limited number of lymphocytes available with a given antigen specificity although the total number of lymphocytes is relatively high (for review see [26]). Therefore naïve lymphocytes continuously patrol through the body and stay in the blood only for a short time. They leave in large numbers from the blood into the tissues, and the term "recirculation" refers to the fact that they finally re-enter the blood circulation via lymphatic vessels, lymph nodes and the thoracic duct (Fig. 6.1). In the search for their specific antigen, naïve lymphocytes may follow this pathway several times and after contact with the cognate antigen they can undergo activation, clonal proliferation and differentiation into effector cells in order to mount a specific response. Antigen-specific memory cells await a second contact with the antigen to boost a rapid and forceful secondary immune response [5].

There is no a unified concept for the homing of lymphoid cells into tissues available so far. Several studies seem to show a cell type specific distribution of adhesion molecules (lymphocytes homing receptors) on lymphoid cells and a respective tissue selective distribution of binding molecules (vascular addressins) on the vascular endothelium of tissues in order to guide lymphocyte migration through the body [5, 61]. Together with previous results and other data on the isotype specific distribution of the secretory IgA response [4], this indicates a certain compartmentalization of the mucosal immune response and a certain tissue specificity of migration and homing in the mucosal immune system. Other authors have challenged this concept by lymphocyte migration studies using adoptive lymphocyte transfer in otherwise unmanipulated hosts and have found that events inside the tissue such as local lymphocyte retention, proliferation or apoptosis may contribute equally to the effective accumulation of lymphocytes inside certain tissues or their effective movement through these tissues (reviewed in [63]).

Summary for the Clinician

- **Mucosa-associated lymphoid tissue (MALT) is a part of the immune system that is located at mucosal surfaces of the body**
- **It consists of a diffuse lymphoid tissue populated by effector cells and accessory cells. T-lymphocytes provide cellular defence and differentiated B-lymphocytes (plasma cells) secrete soluble protective IgA immunoglobulins**
- **Interspersed organized lymphoid follicles allow antigen recognition, activation and differentiation of specific effector cells**

6.2.2 Eye-Associated Lymphoid Tissue (EALT)

There has been considerable controversy about the occurrence and normality of lymphoid cells at the ocular surface and appendage. Recent results in whole mounts of complete normal human ocular tissues have shown that lymphoid cells are a normal tissue constituent and in fact form a continuous mucosa-associated lymphoid tissue in the lacrimal gland, conjunctiva and lacrimal drainage system, termed eye-associated lymphoid tissue (EALT) [22, 23, 25, 28] (Fig. 6.2).

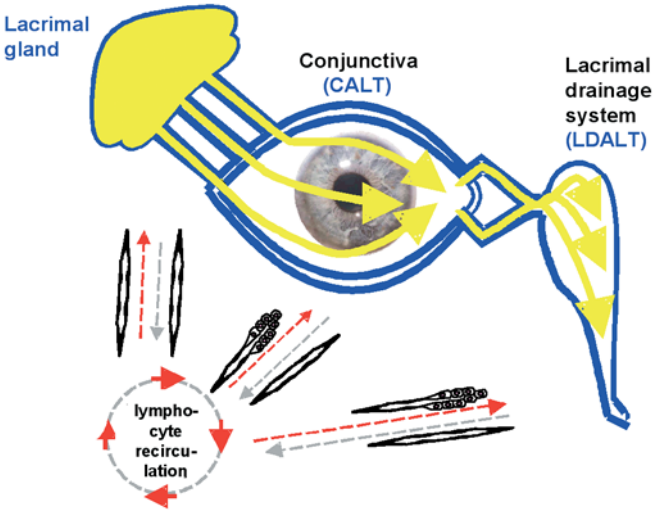

Fig. 6.2. Eye-associated lymphoid tissue (EALT). The ocular surface is an integral part of the mucosal immune system of the body. The diffuse lymphoid tissue with an effector function by lymphocytes and plasma cells is continuous as indicated by blue line from the lacrimal gland along the excretory ducts into the conjunctiva as conjunctiva-associated lymphoid tissue (CALT) and continues through the lacrimal canaliculi inside the lacrimal drainage system as lacrimal drainage-associated lymphoid tissue (LDALT). The lymphoid tissue of these three organs together constitutes EALT. Follicular tissue for the detection of ocular antigens occurs in CALT and LDALT. Effector cells that are primed in follicular tissue against ocular surface antigens can migrate in a regulated fashion via specialized vessels between the organs of EALT and the other parts of the mucosal immune system and can hence provide them with effector cells that are specifically directed against antigens that occur at the ocular surface

6.2.2.1 Organized Lymphoid Tissue in EALT

Organized lymphoid follicles in the human conjunctiva have been reported in different numbers in individuals with a macroscopically normal conjunctiva (as reviewed in [28]). Apart from the fact that most of these studies investigated only small tissue biopsies or selected conjunctival areas which do not represent the whole organ as found later, the amount of follicles also varies with age [44].

Recent results in normal whole-mount tissues of the human conjunctiva have shown that even in an old age population about 60 % of tissues contain organized lymphoid follicles with a distinct topographical distribution. They cumulate in the tarso-orbital zone and have a high (>80 %) bilateral symmetry [28]. In the lacrimal drainage system, a similar lymphoid tissue occurs and was termed, according to the international nomenclature, lacrimal drainage-associated lymphoid tissue (LDALT) [22] with lymphoid follicles in roughly half of the tissues (about 41 % [45], 44 % [22] or up to 56 % of old age body donors [24]).

6.2.2.2 Diffuse Lymphoid Tissue in EALT

Similar relations of lymphoid cell types as in other diffuse MALT were found in immunohistological studies on biopsies of the human conjunctiva [6, 18, 53] including the regular presence of mucosa-specific lymphocytes [6, 18]. However, there were different, partly conflicting, reports concerning the amount and location of lymphoid cells. This is probably due to the topographical distribution of these cells as found in studies on normal human conjunctival whole-mount tissues. Lymphoid cells in the subepithelial lamina propria form a lymphoid layer that can have local inhomogeneities but still shows an overall distinct topographical dis-

tribution with a preference for the tarso-orbital conjunctiva [28].

The components of the secretory immune system (lamina propria plasma cells positive for IgA and its transporter molecule SC in the epithelium) have not been found consistently at the normal human ocular surface except for the lacrimal gland, which therefore appeared as the sole source of specific immune protection, whereas the same plasma cells in the conjunctiva were addressed as inflammatory cells. The universal presence of a secretory immune system that reaches continuously from the lacrimal gland via the conjunctiva into the lacrimal drainage system was only recently verified at the normal human ocular surface by studies that combined the histological and immunohistological investigation of complete tissue whole mounts from normal human body donors [22, 23, 25, 28]. Together with ultrastructural results on the differentiation of the employed cell types and molecular-biological evidence for the presence of the mRNA of IgA and SC [21], this demonstrated the local production of secretory IgA in the conjunctiva and lacrimal drainage system.

6.2.2.3 Dendritic Cells in EALT

At the ocular surface the dendritic Langerhans cells occur as antigen presenting cells similar to the skin [42]. They have been described in a number of animal species and humans for several decades and have frequently been detected with antibodies against some of their antigen presenting surface molecules (e.g. MHC-class-I, MHC-class-II) in isolated epithelial sheets and in histology.

In contrast to the conjunctiva and the marginal cornea that both have relatively frequent MHC-class-II positive DC, it was found that the central cornea is almost free of these cells. Only in inflammatory conditions, for example after vascularization or after experimental wounding or irritation, were appreciable numbers of MHC-class-II positive DC observed in the central cornea. These observations seemed to fit relatively well with the known low percentage of rejection of corneal allografts compared to results with transplantation of other organs, e.g. skin, kidney or heart [50]. Only recently with new antibodies has it been possible to observe that even the central cornea in fact contains numerous DC that are immature and do not express antigen-presenting surface molecules [15] under normal conditions.

6.2.2.4 Recirculation to EALT

In EALT, there is only sparse information so far about homing mechanisms and regulating factors. The presence of high endothelial venules in the normal human conjunctiva has been shown (for review see [26]). The intestinal vascular addressin MAdACAM-1 is not observed on high endothelial venules but other adhesion molecules like VAP-1, ICAM-1, VCAM-1 and E-selectin have been found and showed a weak or sporadic staining. These addressins are thought to be possibly involved in extraintestinal homing but may also indicate an inflammatory response. The presence of ICAM-1 in the normal human conjunctiva was confirmed but ICAM-1, VCAM-1 and E-selectin were found to be inflammation dependent and strongly expressed only under inflammatory allergic conditions [1].

Summary for the Clinician

- **Mucosa-associated lymphoid tissue also occurs at the normal human ocular surface and appendage and is integrated into the mucosal immune system of the body as eye-associated lymphoid tissue (EALT)**
- **It is continuously expressed from the lacrimal gland throughout the conjunctiva (as conjunctiva associated lymphoid tissue, CALT) and inside the lacrimal drainage system (as lacrimal drainage-associated lymphoid tissue, LDALT)**
- **It has the machinery to recognize ocular surface antigens, generate effector cells (T-lymphocytes and plasma cells) that are specifically directed against them and can provide, via lymphocyte recirculation, the ocular effector tissues and other mucosal organs with such cells**

6.2.3 Basic Functions of MALT

MALT modulates between inflammatory immune protection and immune tolerance (Fig. 6.3). There was a historic misunderstanding of the function and significance of lymphoid cells at least at the ocular surface because they were usually considered an indication for an inflammatory infiltration of the mucosa. Consequently lymphocytes and plasma cells were frequently termed "inflammatory cells".

In contrast to this term, lymphoid cells have important functions for the preservation of the tissue integrity. Mucosal immunoglobulins (IgA) from local plasma cells are distinctly anti-inflammatory and perform "immune exclusion", i.e. the inhibition of antigen penetration into and the removal of penetrated antigens from the mucosal tissue [4]. Recent advances in immunology have furthermore shown that T-lymphocytes per se are not inflammatory cells but that there are different types of T-lymphocytes with differential functions (Fig. 6.4). Even those which support a cellular inflammatory immune answer require, in addition to the mere presence of antigen, distinct and highly regulated activation procedures in the context of accessory professional antigen presenting cells together with co-stimulatory signals (Fig. 6.4).

However, if deregulation of the physiological mucosal immune system occurs, lymphoid cells can also be involved as a primary or secondary pathogenetic factor in several forms of ocular surface disease as outlined below for some common ocular diseases.

In contrast to the systemic immunity in the blood and internal organs which favours the destruction of antigens, a main function of mucosal immunity appears to be the generation of immune tolerance (Fig. 6.3) against the multitude of non-pathological antigens at mucosal surfaces that are not supposed to cause constant immune activation [29]. This applies in particular to the ocular surface which is directly exposed to the environment.

6.2.3.1 Immune Regulation at Mucosal Surfaces and the Th1/Th2 Paradigm

To initiate immune responses, antigens must be recognized by naïve T cells in order to activate them to effector cells. According to the present concept, two signals are necessary for the activation of T cells. Besides the correct recognition of a presented antigen (signal 1) by the interaction of the specific T-cell receptor, the peptide antigen and the antigen presenting MHC-class-II molecule on the APC, the presence of co-stimulatory signals (signal 2) such as CD80/86, CD40 or ICAM-1 on the APC is required. This is necessary to initiate the production of a sufficient amount of the cytokine interleukin-2 (IL-2) by the T cell that is required for the autocrine stim-

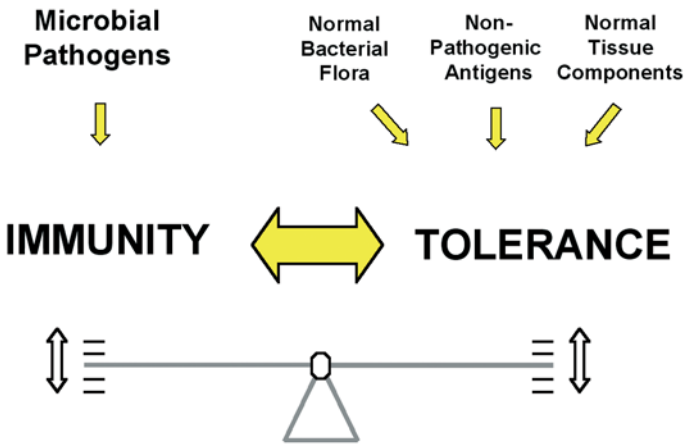

Fig. 6.3. Basic functions of the mucosal immune system. One of the main functions of the mucosal immune system is the maintenance of a fine equilibrium between inflammatory immune protection against microbial infection and the generation of tolerance to the majority of non-pathogenic antigens that occur at mucosal surfaces in order to prevent constant inflammatory reactions that are destructive not only for the antigen but also for the tissue itself

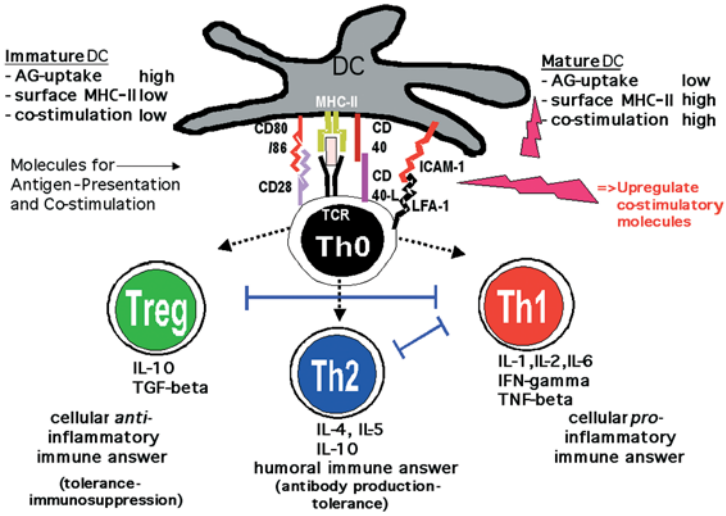

Fig. 6.4. Immune regulation by DC. Bone marrow derived antigen presenting dendritic cells (DC) are key regulators of mucosal immunity by initiating different types of effector T cells that act through different cytokine patterns. Normally DC are in an immature state that cannot efficiently present antigens to naïve T cells (*Th0*). Th0 cells hence develop into inactive anergic T cells or into immunosuppressive anti-inflammatory regulatory T cells (termed Th3 or Treg, according to different nomenclatures) that produce immunosuppressive cytokines (e.g. IL-10, TGF-β) or they support development of Th2 cells which act through promotion of B-cell maturation into immunoglobulin secreting plasma cells. When "danger signals" are introduced by microbial infection or tissue destruction the DC start their maturation, upregulate the antigen presentation molecule MHC-class-II at their surface together with co-stimulatory molecules (e.g. CD80/86, CD40, ICAM-1) and stimulate proinflammatory Th1 cells. Th1 cytokines inhibit Th2 and Treg cells and reverse in order to focus the immune response in a distinct direction

ulation of T-cell proliferation and differentiation. If co-stimulation is missing, non-reactivity (tolerance) is induced by an anergy or deletion of the respective antigen of the specific T-lymphocyte. The requirement of two signals is assumed to represent a control mechanism in peripheral tissues (peripheral tolerance) against accidental activation of autoreactive T cells that may have escaped the mechanisms of central tolerance in primary lymphatic organs (bone marrow and thymus) which produce the naïve lymphocytes.

DC have different functional states in order to modulate the antigen presentation [37]. The type and concentration of local cytokines (cytokine milieu) and other external factors influence the immune regulation by DC and the resulting differentiation of different types of T-helper (Th) cells, which in turn differ in the cytokine signals they produce themselves [40] and the immune reactions they initiate or favour.

In mucosal tissues immature DC prevail that show an active uptake of antigens but a low surface expression of MHC-class-II and co-stimulatory molecules [2] and also produce preferably interleukin 10 (IL-10) [19]. If such immature DC present antigens to T cells, they stimulate the differentiation of T-helper cell type 2 (Th2) that induce immunoglobulin production by plasma cells by their cytokines (IL-4, IL-5 and IL-10). Alternatively, newly (re-)discovered regulatory T cells (termed Treg or Th3) can arise that are more strongly immunosuppressive through the combined production of IL-10 and transforming growth factor β (TGF-β) and can therefore even inhibit the rejection of transplants [67].

Through the contact with maturation signals [37], immature DC differentiate into mature DC. Since the maturation signals are initiated via

unphysiologic events in the context of infection or tissue destruction (e.g. contact with microbial pathogens, inflammatory cytokines, transplant surgery), these signals are also termed "danger signals" [11]. Hereby DC are activated and mature by expression of high levels of surface MHC-class-II and co-stimulatory molecules (CD80, CD86, CD40). This allows an effective antigen presentation that leads to the initiation of a cellular proinflammatory immune answer by T-helper cells type 1 (Th1) and their cytokines (IL-2, IFN-γ, TNF-α).

Th1- and Th2-lymphocytes tend to inhibit each other by shifting the cytokine milieu in opposing directions and this Th1/Th2 paradigm is frequently used to explain the course of immune reactions. However, this paradigm may be oversimplified because other Th subtypes exist and because findings from the ocular surface, e.g. in allergy [38] and in dry eye disease (M.E. Stern, S.C. Pflugfelder, personal communication), indicate that both subtypes can be involved in inflammatory processes as shown below.

Summary for the Clinician

- **A basic function of MALT is the immune regulation at mucosal surfaces by balancing between an inflammatory immune defence of pathogens and a tolerance of the ubiquitous non-pathogenic antigens**
- **The preference is for a generation of tolerance mechanisms in order to avoid constant inflammatory destruction of the delicate mucosal surface**
- **Immune regulation is mainly performed by a special class of professional antigen presenting cells, dendritic cells (DC)**
- **Depending on external influences in the tissue (cytokine milieu, microbes, cell wounding, etc.), the function of DC is biased and leads to the stimulation of different types of T-helper (Th) cells that govern different directions of immune response by differential patterns of secreted immunomodulatory mediators (cytokines)**
- **Th1-lymphocytes maintain inflammatory defence, Th2 cells stimulate the mainly anti-inflammatory immunoglobulin production and Th3 (or regulatory T cells) act immunosuppressively**

6.3 Dry Eye Disease

6.3.1 Introduction

Recent findings have shown that dry eye disease (keratoconjunctivitis sicca, KCS), similar to other types of ocular surface disease, frequently contains an inflammatory component. This is regulated by immune modulators (e.g. cytokines and chemokines), can affect other parts of the integrating functional anatomy of the ocular surface [27] and eventually leads to a vicious circle of degenerative remodelling of the ocular surface. In other mucosal organs of the body it was shown that inflammatory mucosal disease is initiated by destruction of the surface epithelium which allows uncontrolled influx of antigens and leads to a deregulation of the cells of the mucosal immune system. This is characterized by a shift of the effector T cells in the direction of Th1. The main aspects of this pathogenesis have also been verified at the ocular surface in dry eye. Consequently, immunosuppressive therapy has proven to be effective in moderate to severe cases of dry eye disease.

6.3.2 Epidemiology, Definition and Characteristics of Dry Eye

Dry eye disease is a widespread disruption of the normal homeostasis of the ocular surface that affects, depending on the tests applied for diagnosis in various studies, up to 10–30% of the population [56]. It is not homogeneously distributed in the population but more likely affects elderly people and preferentially women, which may point to certain risk factors such as age or hormonal status. It is caused, according to a definition of the American National Eye Institute (NEI) [30], by an alteration of the tear film either due to aqueous deficiency or to increased evaporation. This condition leads to a disruption of the cellular and morphological integrity of the ocular surface and eventually

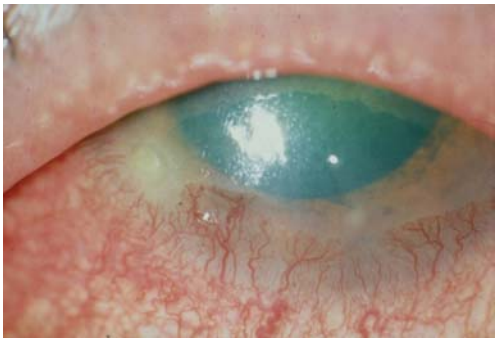

Fig. 6.5. Clinical photo of severe dry eye disease. A severe dry eye shows dryness of the ocular surface with epithelial staining (if different kinds of vital stains such as fluorescein or rose bengal are applied). The corneal reflex is disturbed and the transparency of the cornea is decreasing. The eye is severely inflamed and shows neovascularization with new vessels that grow from the limbus onto the cornea

causes the symptoms that are presented to the ophthalmologist [48]. Symptoms can range over a wide spectrum from mild discomfort and increased fatigue of the eye to redness, itching, burning and stinging sensations (Fig. 6.5). It can be associated with usually minor alterations of visual acuity but may in severe cases lead to severe inflammation, scarring and blinding.

The dry eye syndrome appears as a complex deregulation of the functional anatomy of the ocular surface [27] and deficiencies of the tear film can originate, for example, from alterations of the lid shape, blinking mechanism or innervation, from alterations of the endocrine network, from the presence of meibomian gland disease or from chronic mechanical irritation of any kind (e.g. contact lenses).

In recent years evidence has accumulated from intensive investigations that various forms of dry eye disease are associated with inflammatory alterations [41, 48, 58] of the ocular surface and appendage that are associated with inflammatory factors inside the tissue and tear film.

6.3.2.1 Lacrimal Gland Contribution to Dry Eye Disease – Sjögren's Syndrome

The lacrimal gland is an associated gland of the ocular surface that functionally and embryologically constitutes an integral part of the ocular surface. Similarly, from the viewpoint of mucosal immunology, it is an integral part of the ocular mucosal immune system (EALT) [23] together with the conjunctiva-associated lymphoid tissue (CALT) and the lacrimal drainage-associated lymphoid tissue (LDALT). It contains similar cell populations of T- and B-lymphocytes and DC [64].

T-cell-mediated inflammatory alterations of the lacrimal gland have been known for a long time; they appear to be associated with an impairment of the innervation that triggers the final release of aqueous secretion, and Sjögren's syndrome is a major cause for tear deficiency [7]. Alterations of B-lymphocytes are also described in Sjögren's syndrome. The aetiology of the disease is unknown, but it may originate from activation of the acinar epithelial cells due to viral infection by Epstein-Barr [52] or other viruses which stimulate a production of inflammatory cytokines and can lead to presentation of epithelial autoantigens by upregulated MHC-class-II and ICAM-1 production and expression at the epithelial surface. A number of respective autoantigens are characteristic for Sjögren's syndrome and can be used to support the diagnosis (e.g. SS-A, SS-B, α- and β-fodrin, M3 receptor). A distinct repertoire of antigen receptors was found on T and B cells in Sjögren's patients. This can lead to a breakdown of the physiological peripheral self-tolerance and results in an activation of lymphocytes that carry receptors for self antigens and happen to have escaped the central tolerance mechanisms in primary lymphoid tissues. An accumulation of mainly CD4+ T-helper cells, DC and smaller amounts of B cells in the salivary and lacrimal glands has been reported [46]. This results in the destruction of acinar epithelial tissue by binding of the self-intolerant cytotoxic T cells to acinar cells and lymphocyte induction of acinar apoptosis via release of cytotoxic molecules. The lymphocytes, and to a certain extent also

epithelial cells, produce a large amount of mainly inflammatory Th1-type cytokines (IL-2, IL-6, IFN-γ, TNF-α). Th2 cytokines (IL-4, IL-5, IL-10) are produced in smaller amounts and preferably in areas of occasional B-cell accumulations [43]. The presence of inflammatory cytokines induces a further influx of more lymphocytic cells by upregulation of adhesion molecules on glandular vessels and leads to an activation of stromal cells with release of matrix metalloproteinases that cause a degenerative remodelling of the extracellular matrix around the epithelial acini.

Since only about half of the secretory acinar cells are destroyed by this process, it appears likely that the remaining intact acinar cells are inhibited from secretion by negative interference with innervation [7, 8]. Suggested mechanisms include an observed reduction of density of innervating nerve fibres, the inhibition of release of neurotransmitters by inflammatory cytokines or the blockade of innervation effects in the epithelial cells by autoantibodies against their muscarinic M3 receptor [7].

Androgen deficiency is shown as an important predisposing factor for the initiation of inflammatory reactions as well as alterations of the secretion of the lacrimal and meibomian glands resulting in tear deficiency [60].

6.3.2.2 Conjunctival Contribution to Dry Eye Disease – Non-Sjögren's Dry Eye

Inflammatory affections in dry eye disease are not only found in the lacrimal gland. Even in the clinically inflammation free and primary tear deficient non-Sjögren's dry eye an elevation of inflammatory cytokines (IL1α, IL6, IL8, TNFα) is found in the tear film and inside the tissue of the conjunctiva [48]. The ability of conjunctival epithelial cells to release inflammatory cytokines has been reported [12]. This indicates a shift of the cellular immune response into the direction of an inflammatory Th1 response similar to the inflammatory affections in the lacrimal gland and may similarly lead to a destruction of the epithelium and the underlying extracellular matrix [48, 58]. The primary affection seems to lie in the epithelial cells, similarly to the lacrimal gland, but also in the conjunctiva an upregulation of inflammatory markers that indicate an activation of the mucosal lymphocytes was recently described [59].

Protective factors such as growth factors (EGF, HGF) that are responsible for the proliferation but even more for the mature differentiation of the tissue may at the same time be downregulated [48]. Consequently in dry eye syndromes a hyperproliferation of the conjunctival epithelium is observed combined with impaired differentiation. This is conceivably driven by the presence of inflammatory cytokines and the relative inhibition of cell differentiation is due to diminished growth factors. The conjunctival epithelium in dry eye shows an immature phenotype of the apical cells with a basal cell type cytokeratin pattern and an absence of integral epithelial surface mucins [48, 49], which in turn diminishes the adherence of the tear film to the ocular surface and hence reduces the tear film stability.

Elevated inflammatory cytokines further induce an upregulation of proteases (matrix metalloproteinases) in the tissue and tearfilm, which indicates additional degenerative remodelling of the connective tissue of the mucosal lamina propria at the ocular surface [13, 31, 36]. Inflammatory cytokines can also impair ocular surface innervation in the sense that they inhibit sensory information about ocular dryness to reach the central nervous system in order to elicit efferent secretomotor impulses in glandular tissue. The neural reflex arc is thereby interrupted, leading to a further decrease of secretion and potentially inducing neurogenic inflammation of the lacrimal gland also in primary non-Sjögren's dry eyes [58].

6.3.2.3 Common Mechanisms in Immune Mediated Dry Eye Disease

The starting point of immune mediated inflammation in the conjunctiva, similar to events in the lacrimal gland in Sjögren's syndrome, may lie in an alteration of the epithelial cells. In the case of the conjunctiva, this is caused by destructions that are observed in all kinds of dry eye due to mechanical abrasion via increased

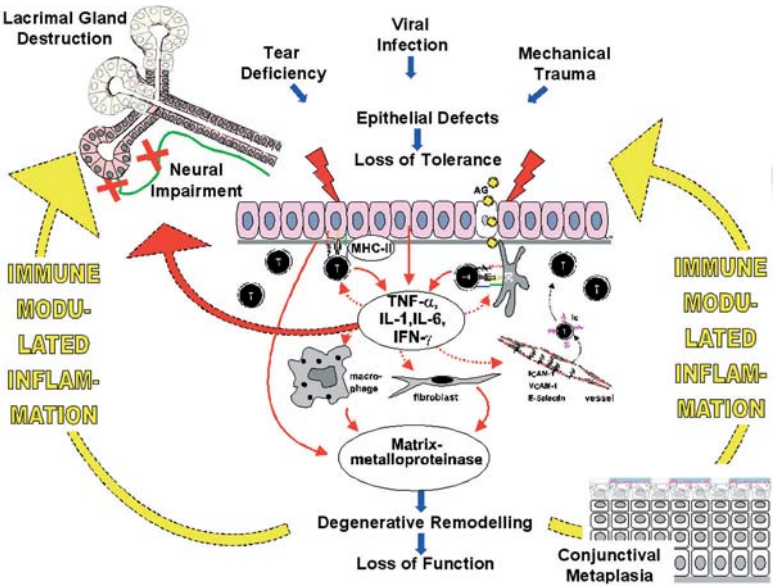

Fig. 6.6. Common mechanisms in immune mediated dry eye disease. Different types of dry eye disease share an immune modulated inflammatory process that can similarly occur in the lacrimal gland (e.g. in Sjögren's syndrome) and at the ocular surface. It appears to start from epithelial defects resulting in a loss of immunological tolerance. Epithelial cells produce inflammatory cytokines (e.g. TNF-α, IL-1, IL-6, IFN-γ), upregulate MHC class II and co-stimulatory molecules on their surface and allow uncontrolled antigen (*AG*) influx through defects. Together this leads to an uncontrolled activation of normal resident mucosal T cells into the inflammatory Th1 type that also produces inflammatory cytokines and hence amplifies the inflammatory cytokine milieu in the tissue. Further events include an impairment of innervation with a decrease of glandular secretion, and an activation of matrix metalloproteinases that results in a degenerative remodelling of the tissue with loss of function (e.g. destruction of secretory acini in the lacrimal gland or squamous metaplasia in the conjunctiva) and the risk of further epithelial defects. The events involved here can hence result in a vicious circle of tissue destruction (*solid arrows* indicate production of molecules; *interrupted arrows* indicate action of molecules or movement of cells)

friction of the eyelids on the ocular surface (Fig. 6.6). In addition, cell damage can also be caused by hyperosmolarity of the tear film [14]. In the case of the lacrimal gland, epithelial alterations may arise after viral infection [52].

If alteration of the epithelial barrier occurs due to such damage, antigens can achieve uncontrolled access to the tissue, which may be the dominating effect in the conjunctiva; or epithelial cells gain the ability to present autoantigens as observed in the lacrimal gland. In both affections the mucosal immune tolerance is likely to fail. Resident T-helper cells of the physiological mucosal immune system in the subepithelial connective tissue can then be activated and immunological reactions shifted towards inflammation [59], resulting in the further elevation of proinflammatory cytokines. Additional new T-cell can immigrated via an upregulation of adhesion molecule on the vascular endothelium. Similar events have been shown in inflammatory bowel disease (IBD) [34], which represents an inflammatory mucosal condition of the intestine where 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. This may be due to the fact that the presence of a resident population of lymphocytes and plasma cells constituting a physiologic mucosal immune system at the normal ocular surface (EALT) was unknown until recently because lymphoid cells in general

were erroneously believed to be "inflammatory". However, in the intestine where the presence of a physiologic mucosal immune system has been accepted for a longer time, it has been verified that TNFα and IL-1β are also secreted by activated lamina propria lymphocytes promoting an inflammatory reaction and resulting in the production of matrix metalloproteinases by stromal cells [34]. A shift of the cytokine profile towards a TH-1 response has been reported in several inflammatory ocular surface diseases, as similarly found in IBD, and both disorders are reported to respond to immunosuppressive treatment.

6.3.3 Novel Therapeutic Approaches to Dry Eye Disease

Combining these results, it can be noted that the widespread dry eye syndrome is increasingly being recognized to include an inflammatory component [47, 59] and it thus resembles disorders in other mucosal organs which are governed by lymphocytes of the mucosal immune system [34]. Hence, the resident lymphatic population localized in the eye-associated lymphoid tissue of the ocular surface (EALT), which represents a potent source of professional cytokine producing cells, may also act as an important regulator of inflammatory ocular surface disease. Consequently the activation of T cells, which can be inhibited by different immunosuppressive strategies, is an interesting target for new therapeutic approaches [47].

Some compounds that interfere with the process of lymphocyte activation (Fig. 6.4), and hence act more specifically than for example glucocorticoids, are known from immunosuppression after transplantation of solid organs where they are administered systemically. An important step in lymphocyte activation is the production of IL-2 in T cells that is necessary for full activation. Cyclosporin A (CsA), like another agent (tacrolimus also known as FK506), prevents the transcription of the IL-2 gene by binding to the transcription factor calcineurin. Another compound, rapamycin, acts later in the activation cascade and blocks IL-2 peptide after production in the lymphocytes. Therefore CsA or FK506 can act synergistically in combination with rapamycin.

In inflammatory ocular surface disease, topical administration of immunosuppressive drugs has been attempted in order to achieve a high local concentration and to avoid systemic side effects. CsA has been the focus of interest in recent years. A CsA ophthalmic oil-in-water emulsion that was previously only available for veterinary use is now also approved for human therapy and has been tested in multicentre studies. It proved to be significantly better than placebo in reducing objective findings (corneal staining, Schirmer's test) and subjective symptoms when it was applied twice daily over a period of 3–6 months, with the best results at a concentration of 0.05 % CsA [54].

Other approaches successfully improved the underlying androgen deficiency, which increases susceptibility to ocular inflammation and negatively affects the secretion of ocular glands, with topical androgen therapy in animal [60] and human trials [65].

Summary for the Clinician

- **Different types of dry eye disease all contain an underlying immune modulated inflammatory component that is mediated by a deregulation of the physiological and normally protective mucosal immune system**
- **New causative topical treatment options are now available for a therapeutic approach to the immune mediated inflammation in moderate to severe dry eye disease**
- **Immunosuppression with CsA 0.05–0.1 % eyedrops, given twice daily for several months, is an effective treatment as tested in multicentre studies**
- **Topical androgen acts as a trophic and anti-inflammatory factor and normalizes glandular function. It was successfully used in animal models and tested in a case report on a human patient**

6.4 Ocular Allergy

6.4.1 Introduction

Allergy is characterized by an increased sensitivity against external factors that act as allergens and usually reach the ocular surface through the air. Characteristic is an IgE mediated degranulation of mast cells which leads to inflammatory events characterized by vasodilatation, edema and itching. Allergy is a hypersensitivity reaction and occurs in different forms. Seasonal and perennial allergic disease (SAC and PAC) are acute forms whereas vernal and atopic keratoconjunctivitis (VKC and AKC) are more severe and chronic. An iatrogenic form, giant papillary conjunctivitis (GPC), is caused by the introduction of artificial materials such as contact lenses or ocular prostheses onto the ocular surface.

Allergic eye disease shows signs of inflammation and appears to be modulated, apart from mast cells, by other inflammatory cells, such as eosinophils, and by T-lymphocytes. In contrast to inflammation in dry eye disease, effector T cells in allergy show a bias in the direction of either a Th2 or a Th1 immune answer, depending on the subtype of allergy. The mast cells and eosinophils can also produce both types of cytokines. Apart from substances that prevent the release or action of bioactive mediators from mast cells, a modulation of the mucosal immune system in the sense of application of antibodies or antimetabolites that interfere with cell migration, antigen presentation or T-cell activation has proven helpful in treating ocular allergy.

6.4.2 Epidemiology, Definition and Characteristics of Allergic Eye Disease

Ocular allergy is a widespread inflammatory process at the ocular surface that affects about 15–30 % of the population, with a higher incidence in industrialized countries. It is caused by an inappropriate reaction to external allergens that are able to crosslink IgE bound to the high affinity IgE receptor on mast cells. It leads to the release of bioactive substances from the mast cells and other cells and results in edema and inflammatory leukocyte infiltration [20, 57] (Fig. 6.7). Since the access of external antigens to intraconjunctival mast cells is normally prevented by an intact epithelial barrier at the healthy ocular surface, it has been assumed that patients suffering from allergic eye disease may have an underlying impairment of the epithelial integrity, and a respective increased uptake of fluorescein was found in patients [66].

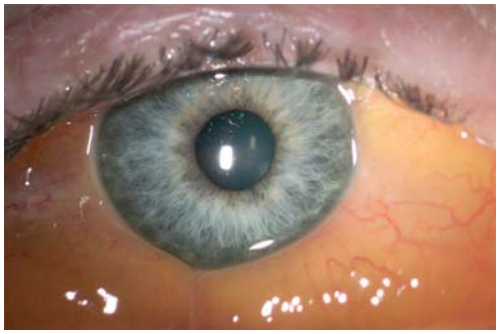

Fig. 6.7. Clinical photo of allergic eye disease. Acute allergic eye disease, as seen here, shows a distinct edema and hyperaemia of the conjunctiva due to degranulation of conjunctival mast cells with release of vasoactive substances (e.g. histamine). The cornea usually remains clear in acute allergic eye disease, and there is no ingrowth of vessels from the limbus. Chronic allergic eye disease, however, includes production of inflammatory cytokines and matrix metalloproteinases together with an inflammatory cell infiltrate. This causes a keratoconjunctivitis with corneal destruction and the beginning of impairment of vision

6.4.2.1 Mast Cells

Mast cells are mesenchymal cells that occur in the connective tissue of most organs and serve as host defence [62]. They have a varying shape that is influenced by the tissue microenvironment but generally shows prominent granules inside the cytoplasm on histological and electron microscopic examination. The granules

contain preformed bioactive agents, e.g. histamine and the enzymes tryptase and/or chymase. According to the content of tryptase and/or chymase, mast cells are divided into a connective tissue type containing tryptase and chymase (M_{TC}) and a mucosal type that contains only tryptase (M_T). In the normal human conjunctiva mast cells only occur in the lamina propria and their majority (95%) are positive for tryptase and chymase. In allergic eye disease the number of M_T mast cells increases and they can occur inside the epithelium and tear film [39]. They produce further signalling molecules such as cytokines [10] of the Th1 and Th2 type that act in an immune modulatory way on various cell types including leukocytes and epithelial cells and influence the course of ocular allergy.

6.4.2.2 Allergic Edema is Caused by Vasoactive Substances

The initial reaction of mast cells upon IgE mediated stimulation is the degranulation of the preformed substances like histamine, tryptase and chymase that leads to dilatation and increased permeability of vessels and results in connective tissue edema (Fig. 6.8). Cytokines of the Th2 type (IL-4, IL-5) and of the inflammatory Th1 type (IL-6, TNF-α) are also produced by mast cells and released as an answer to stimulation. Other secondary agents (e.g. leukotrienes, prostaglandins) are produced within several hours after stimulation [62]. Together these mediators initiate an inflammatory cascade the next step of which is the recruitment of inflammatory leukocytes (eosinophilic and basophilic granulocytes) and of T-lymphocytes into the edematous area.

6.4.2.3 Inflammatory Cytokines Induce a Leukocyte Infiltrate

The leukocyte immigration is mediated by chemoattractants such as the leukotrienes released from mast cells but also by chemotactic cytokines (chemokines) produced in epithelial cells and stromal fibroblasts after stimulation by mast cell cytokines, namely by the inflammatory cytokine TNF-α. TNF-α also initiates an upregulation of the cell adhesion molecule ICAM-1 on vascular endothelial cells and on epithelial cells. VCAM-1 and E-selectin were also found to be inflammation dependent and strongly expressed under allergic conditions [1].

Together this increases the local adhesion of intravascular leukocytes to endothelial cells and their immigration into the tissue as well as their chemotactic migration within the lamina propria and later adhesion inside the epithelium. Stromal fibroblasts activated by inflammatory cytokines appear to produce the chemokine eotaxin, which acts chemotactically on eosinophils and attracts them to the tissue.

After TNF-α stimulation epithelial cells produce a variety of chemokines (MCP, MIP-1, RANTES, IL-8) [9] that act as chemoattractants and conceivably regulate the further migration of immigrated leukocytes from the conjunctival lamina propria into the epithelium and eventually into the preocular tear film. Stimulated fibroblasts secrete Eotaxin. Mast cell derived TNF-α also leads to production of inflammatory cytokines and chemokines (IL-6, IL-8, TNF-α, GM-CSF), which are detected in the tissue and tear film, by epithelial cells and eosinophils [12]. Epithelial cells therefore reinforce the inflammatory pathomechanism and become an active player in allergic eye disease [17].

6.4.2.4 Activation of T-Lymphocytes by Cytokines

TNF-α upregulates ICAM-1, which is known to be an important factor in lymphocyte adhesion and can provide co-stimulation during their activation, not only on endothelial and epithelial cells but also on eosinophils. The activated lymphocytes and other cell types in turn produce further cytokines. Thereby mast cell derived cytokines interrelate the innate immune response with a specific T-cell-mediated immune answer. Since it is shown that the conjunctiva contains a population of resident lymphocytes [28] that belong to the physiological mucosal immune system, an activation of lymphocytes is possible without the previous necessity of lym-

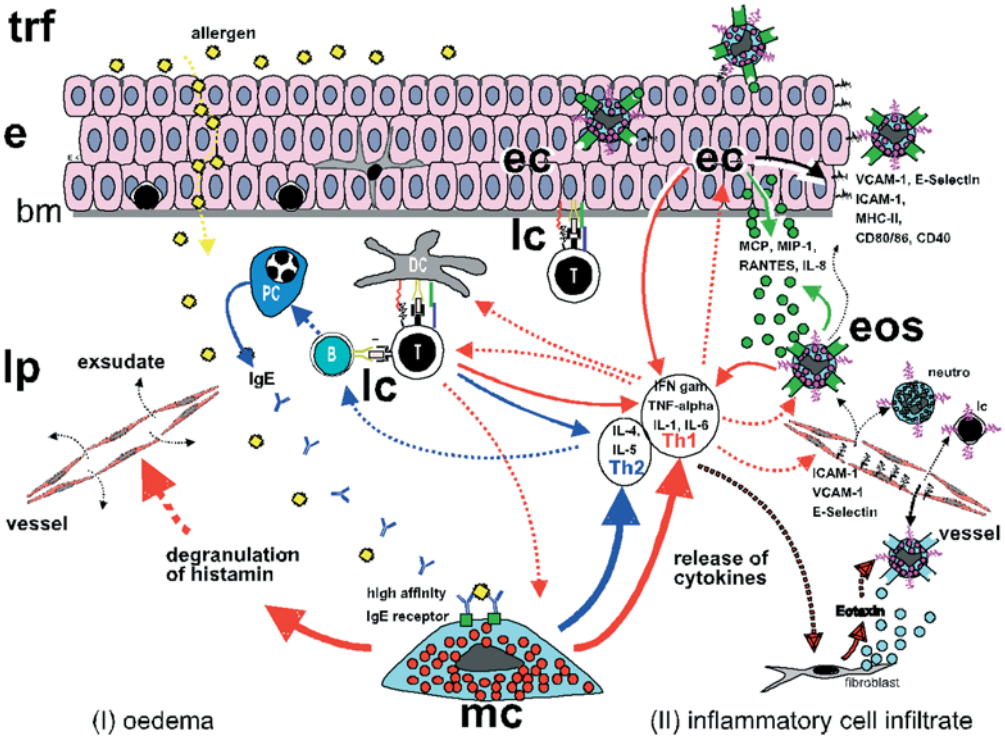

Fig. 6.8. Pathophysiological events in allergic eye disease. Allergic eye disease is an inflammatory process that starts with the activation of mast cells (*mc*) by allergens crosslinking IgE bound to the high affinity IgE receptor on mc. This initially leads to degranulation of mc with release of vasoactive substances resulting in vascular exudation and edema. In the chronic forms this is accompanied by a release of Th1 and Th2 cytokines by mc. These cytokines activate several other cell types such as (counterclockwise in the figure) stromal fibroblasts, vascular endothelial cells, eosinophils (*eos*), conjunctival epithelial cells (*ec*), dendritic cells (*dc*) and lymphocytes (*lc*). The activated cells in turn produce further mediators that reinforce the inflammatory process. Activated cells produce adhesion molecules (dendriform lines) like ICAM-1, VCAM-1, E-selectin and chemokines (small circles) and/or their receptors (dendriform lines and excavated squares) that allow binding and directed migration of cells. Endothelial adhesion molecules permit vascular arrest of leukocytes [eosinophils, neutrophils (*neutro*) and lymphocytes] and their immigration from the vessels into the tissue leading to the characteristic inflammatory cell infiltrate. Secreted chemokines produced by fibroblasts (e.g. eotaxin) and by the epithelium and eosinophils (e.g. MCP, MIP-1, RANTES, IL-8) guide the immigrated leukocytes with respective upregulated cell surface receptors into the lamina propria (*lp*) and from there into the epithelium (*e*) and tear film (*trf*). Upregulation of co-stimulatory molecules like ICAM-1, CD80/86, CD40 and the antigen presenting molecule MHC class II enables epithelial cells to potentially present antigens to T cells resulting in uncontrolled immune reactions (*solid arrows* indicate production of molecules, *interrupted arrows* indicate action of molecules or movement of cells)

phocyte immigration. If the inflammation process proceeds, an additional influx of lymphatic cells into the conjunctival tissue conceivably occurs. Besides the usual process of antigen presentation via DC, epithelial cells that have upregulated MHC-class-II and co-stimulatory ICAM-1 may be able to present antigens to T cells and reinforce the inflammatory process [17]. Investigation of T-lymphocyte cytokines in allergic ocular disease surprisingly showed that Th2-like cytokines prevail in some types such as VKC and partially GPC whereas AKC appears to have a predominant Th1 response [38]. Th2 cytokines act as stimulators of immunoglobulin

production by plasma cells and may therefore be involved in the upregulation of IgE that is observed in allergy. This indicates that inflammatory reactions are not solely mediated by Th1-type lymphocytes and questions the Th1/Th2 paradigm to some extent.

6.4.3 Course and Therapy Options in Allergic Ocular Disease

The inflammatory events that occur in allergic eye disease are moderate in the acute seasonal and perennial allergic disease, SAC and PAC, and mainly lead to edema, redness and itching, whereas the immigration of inflammatory cells is limited. However, in the more chronic allergic diseases such as vernal and atopic keratoconjunctivitis (VKC and AKC) there is a more pronounced immigration of inflammatory cells. In chronic allergic eye disease but not, or only weakly, in acute ocular allergy, activated matrix metalloproteinases occur in the tissue and tear film and may explain the occurrence of corneal destruction in the chronic forms. There the inflammatory process can lead to scarring and can have sight threatening complications, especially in AKC. The giant papillary type GPC causes tarsal conjunctival thickening and is of intermediate severity [20, 57].

Due to the multistep cascade with various involved factors from the bioactive content of mast cell granules to leukocyte immigration and T-cell-mediated immune processes there are a number of different therapeutic strategies. Apart from prevention of mast cell degranulation, the use of antihistamines and potential application of blocking antibodies to chemokines and cytokines is possible [3, 16]. Topical treatment with a 2% CsA solution over a period of 3 months has proven to have beneficial effects on the chronic AKC by reducing the number and activation of T cells and their production of the inflammatory Th1 cytokines IL-2 and IFN-γ. Although this treatment had no influence on the number of conjunctival mast cells and eosinophils, it is assumed that the immunosuppression may still modulate and normalize their function [16].

Summary for the Clinician

- Due to a pathological sensitivity, non-pathogenic environmental antigens act as allergens. They crosslink IgE that is bound to the mast cell through the high affinity IgE receptor and hence stimulate the cell
- This leads to a release of vasoactive (e.g. histamine) and immunomodulatory (e.g. cytokines and chemokines) substances from mast cells
- In acute (seasonal and perennial) ocular allergy the affection is mainly restricted to a conjunctival edema
- In chronic allergic and vernal keratoconjunctivitis (AKC and VKC) various inflammatory leukocytes (primarily eosinophils) but also lymphocytes of the mucosal immune system are activated by mast cell cytokines. A T-cell-mediated inflammatory processes arises that is associated with an inflammatory cell infiltrate and corneal destruction
- In addition to established antihistaminic therapy options, topical immunosuppression is effective in chronic allergic eye disease
- Two percent CsA eyedrops given over a period of 3 months improve objective findings (corneal destruction) and subjective symptoms in patients with severe AKC

6.5 Keratoplasty

6.5.1 Introduction

Keratoplasty, as an organ transplantation procedure, is enormously dependent on immunological mechanisms. The rejection rate in the otherwise normal and inflammation free ocular surface is relatively low because the normal cornea contains no blood vessels, no lymphocytes and only relatively few cells that express MHC class II. Furthermore, the anterior chamber appears to possess an immune privilege. This anterior chamber associated immune privilege (ACAID) induce stolerance against anti-

Fig. 6.9. Clinical photo of a penetrating keratoplasty with the beginning of rejection. The clinical picture of an eye where a penetrating keratoplasty was performed shows the graft tissue fixed by a continuous suture in the centre of the host cornea. The margin between donor and host tissue is demarcated by a *fine whitish line*. Beginnings of the process of immunological transplant rejection are indicated by a haze in the lower half of the graft tissue and result from swelling of the stroma due to destruction of the underlying corneal endothelium that is attacked by the host T cells

gens that are recognized in the anterior chamber of the eye.

In contrast to previous studies it has been shown in recent years that even the central cornea contains a large number of DC that are in a quiescent immature state but may upregulate MHC class II and respective antigen presentation capacity as soon as they are stimulated by the occurrence of antigen and other factors. DC represent components of the mucosal immune system and are key modulators of antigen presentation also at the ocular surface. They can, via the initiation of different types of T-helper cells, modulate between tolerance or rejection of a corneal transplant and hence determine the fate of a corneal graft (Fig. 6.9). Consequently they also represent an interesting target for future immunomodulatory therapeutic strategies.

6.5.2 Immunological Characteristics of Keratoplasty

In order to undergo corneal graft rejection, three processes have been implicated [50]. The donor antigen has to be released, recognized and transported to lymphoid tissue that is present in the form of the organized follicular conjunctival CALT, on the ocular surface itself and in the draining lymph nodes (afferent arm). Alloantigens have to be processed so that a specific cellular immune response might be generated (central stage). Finally in the efferent arm cellular and humoral effector mechanisms are delivered to the graft and cause its destruction.

6.5.2.1 Antigen Presenting Cells

6.5.2.1.1 Langerhans Cells

With regard to the role of antigen presentation ocular DC, Langerhans cells (LHC), are considered as a "key" element of the "afferent" immune process. These dendritic cells play a dominant role in processing and presentation of antigens and carry MHC-class-II antigens that are important stimulators of T and B cells. The distribution of LHC is compartmentally localized within specific regions of the ocular surface. The central cornea is normally devoid of LHC that are positive of MHC class II, but a number of stimuli may induce their immigration. However, recently it has been shown in the mouse that even the central cornea contains DC in an immature state and their potential precursors [15] that can be activated by the presence of antigens as in corneal transplantation.

6.5.2.1.2 Macrophages

In contrast to LHC that have been extensively studied, the role of macrophages (Mø) is less clear. Mø are a heterogeneous cell population that may exert multiple functions. They regularly occur in the conjunctiva and macrophage-like cells are also described in the cornea [15]. Beside their phagocytic activity they produce a number of highly active mediators such as tumour necrosis factor alpha (TNF-α) and nitric oxide (NO) displaying immunomodulatory functions. It is of interest that Mø are also present intraocularly in high density and provide a close network together with DC in the anterior

chamber, in particular the iris [35]. The potential role of Mø in the process of corneal graft rejection has been demonstrated by depletion studies. Following topical application of clodronate liposomes that selectively eliminate Mø, a highly significant prolonged survival of experimental keratoplasty could be observed. It is very likely that Mø play an as yet underestimated role in the afferent arm of the immune response following corneal transplantation.

6.5.2.2 Antigen Presentation

Two pathways of antigen presentation, either "direct" or "indirect", have been proposed in alloantigen recognition [55]. Donor APC can present antigen to host T cells via the direct pathway, thereby inducing a strong immune response. The emigration of antigen presenting dendritic cells with upregulated MHC-class-II molecules from the donor cornea into the host tissue has recently been shown in the mouse model of corneal transplantation [15, 32]. On the other hand, host APC are able to present corneal alloantigens via the indirect pathway of antigen presentation. The direct pathway is a specific feature of the response towards alloantigens, whereas the indirect pathway represents the "normal" mechanism for the generation of an immune response. Even when the indirect pathway is considered less effective, experimental studies have shown that it may result in prompt corneal graft rejection [33].

6.5.2.3 Allograft Rejection and Immune Modulatory Therapy

The critical role of T cells in allograft rejection is well established. The prevailing view is that a specific T-cell response against HLA antigens is initiated through CD4+ cells. Further potentiation of the reaction then takes place via cytokine-dependent co-stimulation (e.g. by CD80/86) is necessary for full activation of the lymphocyte as indicated by high production of the cytokine IL-2 and its receptor. The respective involved molecules are important targets for immunomodulation and immunosuppression especially after corneal transplantation (indicated by *blue text* and *arrows* for inhibition approaches)

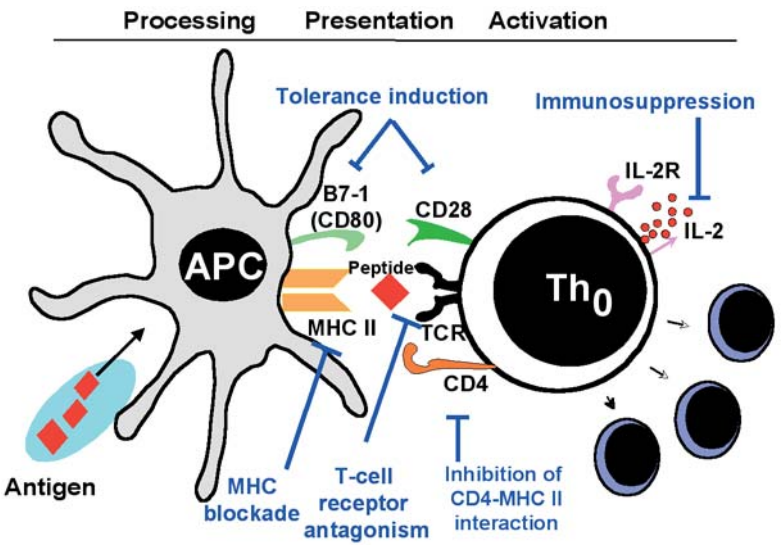

Fig. 6.10. Antigen presentation and potential targets for immune modulation. Antigen presentation is performed by antigen presenting cells (*APC*) after intracellular antigen processing. Processed peptides are loaded on MHC-class-II molecules and presented to the T-cell receptor (*TCR*) on naive T-helper cells. CD4 on the T cell acts as an accessory molecule and addi-

kine release with activation, proliferation and differentiation of other lymphocytes. It is obvious that new methods to prevent allograft rejection must take place in this early phase of graft reaction before activation of the T cell. The respective molecules involved in the processing and presentation of antigens as well as in the activation of lymphocytes represent promising targets for immune modulation and immunosuppression (Fig. 6.10). Much attention has therefore been paid in particular to the recognition behaviour of CD4+ cells (T-cell receptor) and the interaction with the target antigen and cytokines that are responsible for expansion of the immune response.

Cells of the physiological mucosal immune system are involved in the afferent antigen recognition phase (mainly conjunctival and corneal dendritic cells) and also in the efferent effector phase (performed by T cells). Besides systemic immunosuppression as similarly performed in solid organ transplantation, topical immunosuppression is possible after keratoplasty. In addition to immunosuppression with CsA or similar agents that suppress T-cell activation, it is also possible to inhibit mechanisms of antigen presentation (as indicated in Fig. 6.10) before rejection is initiated, for example by immunosuppressive cytokines, antibodies against co-stimulatory molecules (e.g. CD80/86, CD40) or against accessory molecules such as CD4. In addition to potential topical therapy which is possible in keratoplasty, in contrast to the transplantation of solid organs, the protective factors can also be applied by a gene therapeutic approach. In this case the DNA for the respective product is transfected into host cells with the help of different kinds of vectors (e.g. liposomes, viruses) and is then produced directly by the cells of the transplant or by surrounding tissues [51].

Summary for the Clinician

- **Transplant rejection remains the single most important cause of graft failure following penetrating keratoplasty**
- **Local as well as systemic immune processes are involved in transplant rejection and may allow development of more specific preventive and therapeutic options**
- **CD4+ lymphocytes play an essential role in the immune response in corneal graft rejection and are the main target of immunomodulatory therapy**
- **The relative importance of CD4+ subtypes classified as "Th1/Th2" is still a matter of debate but may allow new immunomodulatory strategies, such as gene therapy**

References

1. Abu El-Asrar AM, Geboes K, al-Kharashi S, Tabbara KF, Missotten L, Desmet V (1997) Adhesion molecules in vernal keratoconjunctivitis. Br J Ophthalmol 81:1099–1106
2. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252
3. Bielory L, Mongia A (2002) Current opinion of immunotherapy for ocular allergy. Curr Opin Allergy Clin Immunol 2:447–452
4. Brandtzaeg P, Baekkevold ES, Morton HC (2001) From B to A the mucosal way. Nat Immunol 2: 1093–1094
5. Butcher EC, Picker LJ (1996) Lymphocyte homing and homeostasis. Science 272:60–66
6. Dua HS, Gomes JA, Jindal VK, Appa SN, Schwarting R, Eagle RC Jr, Donoso LA, Laibson PR (1994) Mucosa specific lymphocytes in the human conjunctiva, corneoscleral limbus and lacrimal gland. Curr Eye Res 13:87–93
7. Fox RI (1998) Sjogren's syndrome. Pathogenesis and new approaches to therapy. Adv Exp Med Biol 438:891–902
8. Fox RI, Stern M (2002) Sjogren's syndrome: mechanisms of pathogenesis involve interaction of immune and neurosecretory systems. Scand J Rheumatol Suppl 2002:3–13
9. Fukagawa K, Tsubota K, Simmura S, Saito H, Tachimoto H, Akasawa A, Oguchi Y (1998) Chemokine production in conjunctival epithelial cells. Adv Exp Med Biol 438:471–478
10. Galli SJ, Gordon JR, Wershil BK (1993) Mast cell cytokines in allergy and inflammation. Agents Actions Suppl 43:209–220
11. Gallucci S, Matzinger P (2001) Danger signals: SOS to the immune system. Curr Opin Immunol 13:114–119
12. Gamache DA, Dimitrijevich SD, Weimer LK, Lang LS, Spellman JM, Graff G, Yanni JM (1997) Secretion of proinflammatory cytokines by human conjunctival epithelial cells. Ocul Immunol Inflamm 5:117–128
13. Garrana RM, Zieske JD, Assouline M, Gipson IK (1999) Matrix metalloproteinases in epithelia

from human recurrent corneal erosion. Invest Ophthalmol Vis Sci 40:1266–1270
14. Gilbard J (1985) Tear film osmolarity and keratoconjunctivitis sicca. In: Holly FJ (ed) Proc 1 Int Tear Film Symposium, Lubbock, Texas, pp 127–139
15. Hamrah P, Zhang Q, Liu Y, Dana MR (2002) Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. Invest Ophthalmol Vis Sci 43:639–646
16. Hingorani M, Calder VL, Buckley RJ, Lightman S (1999) The immunomodulatory effect of topical cyclosporin A in atopic keratoconjunctivitis. Invest Ophthalmol Vis Sci 40:392–399
17. Hingorani M, Calder VL, Buckley RJ, Lightman SL (1998) The role of conjunctival epithelial cells in chronic ocular allergic disease. Exp Eye Res 67: 491–500
18. Hingorani M, Metz D, Lightman SL (1997) Characterisation of the normal conjunctival leukocyte population. Exp Eye Res 64:905–912
19. Iwasaki A, Kelsall BL (1999) Mucosal immunity and inflammation. I. Mucosal dendritic cells: their specialized role in initiating T cell responses. Am J Physiol 276:G1074–G1078
20. Katelaris CH (2003) Ocular allergy: implications for the clinical immunologist. Ann Allergy Asthma Immunol 90:23–27
21. Knop E, Claus P, Knop N (2003) Eye-associated lymphoid tissue (EALT): RT-PCR verifies the presence of mRNA for IgA and its transporter (secretory component) in the normal human conjunctiva. Invest Ophthalmol Vis Sci 44:S3801
22. Knop E, Knop N (2001) Lacrimal drainage associated lymphoid tissue (LDALT): a part of the human mucosal immune system. Invest Ophthalmol Vis Sci 2001:566–574
23. Knop E, Knop N (2002) A functional unit for ocular surface immune defense formed by the lacrimal gland, conjunctiva and lacrimal drainage system. Adv Exp Med Biol 506:835–844
24. Knop E, Knop N (2002) Human lacrimal drainage-associated lymphoid tissue (LDALT) belongs to the common mucosal immune system. Adv Exp Med Biol 506:861–866
25. Knop E, Knop N (2003) [Eye-associated lymphoid tissue (EALT) is continuously spread throughout the ocular surface from the lacrimal gland to the lacrimal drainage system]. Ophthalmologe 100: 929–942
26. Knop E, Knop N (2004) Lymphocyte homing in the mucosal immune system to the eye-associated lymphoid tissue (EALT). In: Zierhut M, Sullivan DA, Stern ME (eds) Immunology of the ocular surface and tearfilm. Swets & Zeitlinger, Amsterdam
27. Knop E, Knop N, Brewitt H (2003) [Dry eye disease as a complex dysregulation of the functional anatomy of the ocular surface. New impulses to understanding dry eye disease]. Ophthalmologe 100:917–928
28. Knop N, Knop E (2000) Conjunctiva-associated lymphoid tissue in the human eye. Invest Ophthalmol Vis Sci 41:1270–1279
29. Kraehenbuhl JP, Neutra MR (1992) Molecular and cellular basis of immune protection of mucosal surfaces. Physiol Rev 72:853–879
30. Lemp MA (1995) Report of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes. CLAO J 21:221–232
31. Li DQ, Lokeshwar BL, Solomon A, Monroy D, Ji Z, Pflugfelder SC (2001) Regulation of MMP-9 production by human corneal epithelial cells. Exp Eye Res 73:449–459
32. Liu Y, Hamrah P, Zhang Q, Taylor AW, Dana MR (2002) Draining lymph nodes of corneal transplant hosts exhibit evidence for donor major histocompatibility complex (MHC) class II-positive dendritic cells derived from MHC class II-negative grafts. J Exp Med 195:259–268
33. Liu Z, Sun YK, Xi YP, Maffei A, Reed E, Harris P, Suciu-Foca N (1993) Contribution of direct and indirect recognition pathways to T cell alloreactivity. J Exp Med 177:1643–1650
34. MacDonald TT, Bajaj-Elliott M, Pender SL (1999) T cells orchestrate intestinal mucosal shape and integrity. Immunol Today 20:505–510
35. McMenamin PG, Crewe J, Morrison S, Holt PG (1994) Immunomorphologic studies of macrophages and MHC class II-positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. Invest Ophthalmol Vis Sci 35:3234–3250
36. Meller D, Li DQ, Tseng SC (2000) Regulation of collagenase, stromelysin, and gelatinase B in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1 beta and tumor necrosis factor-alpha. Invest Ophthalmol Vis Sci 41: 2922–2929
37. Mellman I, Steinman RM (2001) Dendritic cells: specialized and regulated antigen processing machines. Cell 106:255–258
38. Metz DP, Hingorani M, Calder VL, Buckley RJ, Lightman SL (1997) T-cell cytokines in chronic allergic eye disease. J Allergy Clin Immunol 100: 817–824
39. Morgan SJ, Williams JH, Walls AF, Church MK, Holgate ST, McGill JI (1991) Mast cell numbers and staining characteristics in the normal and allergic human conjunctiva. J Allergy Clin Immunol 87:111–116

40. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357
41. Nelson JD, Helms H, Fiscella R, Southwell Y, Hirsch JD (2000) A new look at dry eye disease and its treatment. Adv Ther 17:84–93
42. Novak N, Siepmann K, Zierhut M, Bieber T (2003) The good, the bad and the ugly – APCs of the eye. Trends Immunol 24:570–574
43. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, Yamada A, Itoh K, Nomoto K (1996) Cytokine messenger RNA expression in the labial salivary glands of patients with Sjogren's syndrome. Arthritis Rheum 39:1376–1384
44. Osterlind G (1944) An investigation into the presence of lymphatic tissue in the human conjunctiva, and its biological and clinical importance. Acta Ophthalmol Copenh Suppl 23:1–79
45. Paulsen FP, Paulsen JI, Thale AB, Schaudig U, Tillmann BN (2002) Organized mucosa-associated lymphoid tissue in human naso-lacrimal ducts. Adv Exp Med Biol 506:873–876
46. Pepose JS, Akata RF, Pflugfelder SC, Voigt W (1990) Mononuclear cell phenotypes and immunoglobulin gene rearrangements in lacrimal gland biopsies from patients with Sjogren's syndrome. Ophthalmology 97:1599–1605
47. Pflugfelder SC (2004) Antiinflammatory therapy for dry eye. Am J Ophthalmol 137:337–342
48. Pflugfelder SC, Solomon A, Stern ME (2000) The diagnosis and management of dry eye: a twenty-five-year review. Cornea 19:644–649
49. Pflugfelder SC, Tseng SC, Yoshino K, Monroy D, Felix C, Reis BL (1997) Correlation of goblet cell density and mucosal epithelial membrane mucin expression with rose bengal staining in patients with ocular irritation. Ophthalmology 104:223–235
50. Pleyer U, Dannowski H, Volk HD, Ritter T (2001) Corneal allograft rejection: current understanding. 2. Immunobiology and basic mechanisms. Ophthalmologica 215:254–262
51. Pleyer U, Ritter T (2003) Gene therapy in immune-mediated diseases of the eye. Prog Retin Eye Res 22:277–293
52. Rhodus NL (1999) Sjogren's syndrome. Quintessence Int 30:689–699
53. Sacks EH, Wieczorek R, Jakobiec FA, Knowles DM (1986) Lymphocytic subpopulations in the normal human conjunctiva. A monoclonal antibody study. Ophthalmology 93:1276–1283
54. Sall K, Stevenson OD, Mundorf TK, Reis BL (2000) Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. Ophthalmology 107:631–639
55. Sayegh MH, Turka LA (1998) The role of T-cell costimulatory activation pathways in transplant rejection. N Engl J Med 338:1813–1821
56. Schaumberg DA, Sullivan DA, Dana MR (2002) Epidemiology of dry eye syndrome. Adv Exp Med Biol 506:989–998
57. Stahl JL, Cook EB, Barney NP, Graziano FM (2002) Pathophysiology of ocular allergy: the roles of conjunctival mast cells and epithelial cells. Curr Allergy Asthma Rep 2:332–339
58. Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC (1998) The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. Cornea 17:584–589
59. Stern ME, Gao J, Schwalb TA, et al. (2002) Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye. Invest Ophthalmol Vis Sci 43:2609–2614
60. Sullivan DA, Edwards JA (1997) Androgen stimulation of lacrimal gland function in mouse models of Sjogren's syndrome. J Steroid Biochem Mol Biol 60:237–245
61. von Andrian UH, Mackay CR (2000) T-cell function and migration. Two sides of the same coin. N Engl J Med 343:1020–1034
62. Wedemeyer J, Tsai M, Galli SJ (2000) Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 12:624–631
63. Westermann J, Engelhardt B, Hoffmann JC (2001) Migration of T cells in vivo: molecular mechanisms and clinical implications. Ann Intern Med 135:279–295
64. Wieczorek R, Jakobiec FA, Sacks EH, Knowles DM (1988) The immunoarchitecture of the normal human lacrimal gland. Relevancy for understanding pathologic conditions. Ophthalmology 95:100–109
65. Worda C, Nepp J, Huber JC, Sator MO (2001) Treatment of keratoconjunctivitis sicca with topical androgen. Maturitas 37:209–212
66. Yokoi K, Yokoi N, Kinoshita S (1998) Impairment of ocular surface epithelium barrier function in patients with atopic dermatitis. Br J Ophthalmol 82:797–800
67. Zelenika D, Adams E, Humm S, Lin CY, Waldmann H, Cobbold SP (2001) The role of CD4+ T-cell subsets in determining transplantation, rejection or tolerance. Immunol Rev 182:164–179