Eye (2003) 17, 949–956

www.nature.com/eye

Immune defense at the ocular surface

EK Akpek and JD Gottsch

Abstract

The ocular surface is constantly exposed to a wide array of microorganisms. The ability of the outer ocular system to recognize pathogens as foreign and eliminate them is critical to retain corneal transparency, hence preservation of sight. Therefore, a combination of mechanical, anatomical, and immunological defense mechanisms has evolved to protect the outer eye. These host defense mechanisms are classified as either a native, nonspecific defense or a specifically acquired immunological defense requiring previous exposure to an antigen and the development of specific immunity. Sight-threatening immunopathology with autologous cell damage also can take place after these reactions. This article discusses the innate and acquired corneal elements of the immune defense at the ocular surface. The relative roles of the various factors contributing to prevention of eye infection remain to be fully defined.
Eye (2003) **17,** 949–956. doi:10.1038/sj.eye.6700617

Keywords: cornea; defensins; calgranulins; neutrophils; herpes; oncocerchiasis

Introduction

The vertebrate cornea has a unique immune defense to protect against foreign material and a number of microbial pathogens. As the epidermis and dermis protect underlying tissues of the body, the cornea must prevent injury to the delicate structures of the eye. However beyond its protective function, the cornea has evolved to be a highly transparent tissue, which refracts light to enable high-resolution vision. In order for the cornea to maximally transmit light, the extracellular matrix of collagens and proteoglycans have become highly ordered, and all extraneous cells and blood vessels have been excluded. The premium of enhancing visual acuity must have provided a selective advantage for early vertebrates. Improved visual acuity would have increased the fitness of these animals and would have outweighed the disadvantage of having local immune cells and blood vessels at a distance where a time delay in addressing a central corneal infection could lead to blindness.

The first vertebrates were jawless fish that were believed to have evolved some 470 million years ago.[1] These creatures had frontal eyes and inhabited the shorelines of ancient oceans. With better vision, these creatures were likely more active and predatory. This advantage along with the later development of jaws enabled bony fish to flourish and establish other habitats. One such habitat was shallow waters where lunged fish made the transition to land several hundred thousand years later.[2] To become established in this terrestrial environment, the new vertebrates had to overcome desiccation and novel pathogens such as viruses, bacteria, fungi, protozoa, and helminths.

The exposure of ocular surface to potentially harmful materials including microbes, and toxic substances found in the environment continually places the ocular surface at risk for immunologic events. There are two approaches to understanding immune defense at the corneal surface. One is to study the cellular and molecular elements present in the human cornea for possible roles in mounting an immune defense. Peripheral dendritic cells or Langerhans cells, for example, have been studied for their global antigen presenting properties or keratocytes for their intrinsic microbial defensive peptides. The other method is to investigate immune defensive pathways in a specific animal model of microbial infection. Well-known examples of this approach are studies performed with murine herpes simplex virus (HSV) and onchocerchiasis. Both approaches provide valuable information, but both have limitations. For ethical reasons, studies of how the native human cornea responds to controlled experimental infections cannot be carried out. However, clinical descriptions and investigation of the progression of culture-proven corneal infections and the response to antimicrobials provide

The Wilmer Eye Institute
The Johns Hopkins University School of Medicine
Baltimore, MA, USA

Correspondence:
JD Gottsch
The Wilmer Eye Institute
600 North Wolfe
St Maumenee # 321
Baltimore
MD 21287-9238, USA
Tel: +1 410 955 7928
Fax: +1 410 614 2816
E-mail: jgottsch@jhmi.edu

Received: 28 February 2003
Accepted in revised form: 28 February 2003

some concepts of how the immune system reacts. For example, *Neiserria gonorrhea* keratitis can proceed rapidly to perforation suggesting that the local immune response is totally inadequate and is overwhelmed. In contrast, helminthic keratitis proceeds slowly with infiltrates and with persistent recurrent disease leading to corneal neovascularization and scarring. In addition, corneas that have been excised for corneal transplantation or biopsy for a particular infection can be valuable for understanding what cells and signaling events are involved, using routine histopathology as well as more sophisticated methods such as immunohistochemistry, polymerase chain reaction, and immunoelectron microscopy. Controlled infections in animal models may provide more detailed analysis of immune pathways, although murine or nonhuman primate experiments may not accurately reflect immune response to human disease. Hence, information from both methods of study is valuable.

Corneal defensive mechanisms

Innate defenses

Innate immunity is the first line of defense against corneal infection.[3] Elements of innate immunity are present at birth and provide a nonspecific surveillance system. Physical barriers, such as the bony orbit and the eyelids, guard against traumatic events, which could comprise the corneal surface. Additionally, there are numerous cellular and molecular elements that constantly protect against inoculation of the corneal surface against microorganisms. These elements include tears, corneal nerves, the epithelium, keratocytes, polymorphonuclear cells, and some cytokines.

Tears The main function of tears is to prevent drying of the cornea. In addition, tears flush foreign particles from the ocular surface, and transport antimicrobial proteins (lactoferrin, lysozyme, lipocalin, and beta-lysin) and immunoglobulins to the ocular surface to prevent infections.[4] Chief among the immunoglobulins in tears and at much higher concentrations than serum is immunoglobulin (Ig) A. Secretory IgA binds to bacteria and prevents bacterial adherence to epithelium. Tear IgG as well as IgA can neutralize some viruses and bind bacteria and hence play a role in corneal defense.

Epithelial cells Corneal epithelial cells actively and passively participate in protecting the ocular surface. These cells are capable of secreting cytokines to activate immune defenses to protect against microbial invasion. The cytokine interleukin (IL)-1α is stored in epithelial cells where it is passively released when the cell membrane is ruptured by infectious agents or trauma.[5] This capacity to secrete IL-1α is also shared by stromal keratocytes. Chronic IL-1α secretion would lead to enhanced immune invasion, neovascularization and hence destruction of cornea. Interestingly, the corneal epithelium, but not the keratocytes, is also capable of secreting the soluble and membrane-bound forms of the IL-1α receptor (IL-1RII),[6] a natural IL-1α antagonist. It appears that corneal epithelial cells evolved the ability to modulate the effects of IL-1α secretion by synthesizing IL-1RII. IL-1 R antagonist gene therapy, in experimental models, showed a decreased leucocyte infiltration, selectively altered the cytokine profile, and suppressed corneal neovascularization.[7] Thus, the capacity of the epithelium to modulate the effects of IL-1α on the cornea appears to have important vision-preservation consequences.

Keratocytes Keratocytes also have a defensive capacity during microbial invasion. Under the influence of IL-1α and tumour necrosis factor (TNF)- α, keratocytes synthesize IL-6 and -defensins.[8,9] IL-6 interacts synergistically with IL-1 and perhaps TNF-α.

Defensins hold therapeutic potential in ocular infections as they have a broad spectrum of antimicrobial activity (against bacteria, fungi, and viruses) and accelerate epithelial healing.[10] Defensins have also been identified in neutrophils located in conjunctiva.[11]

In HSV keratitis, IL-8 gene expression, a potent chemoattractant for neutrophils, by keratocytes has also been demonstrated.[12] Il-8 was not found to be expressed in corneal epithelial cells infected with herpes.

Corneal nerves Corneal nerves are important in the innate defense of the cornea by relaying sensory information leading to reflexive movements to protect the eye.[13] Sensations of discomfort and pain may also release neuropeptides that have the capability to induce cytokine activity**.** Two neuropeptides, calcitonin gene-related peptide and substance P, are released from the termini of corneal sensory neurons in response to pain.[14,15] Both can bind to human corneal epithelial cells and induce IL-8 synthesis with a resultant neutrophil influx.

Complement Complement comprises a series of effector and regulatory proteins that activate each other in an orderly fashion to generate biologically active molecules, such as enzymes, opsonins, anaphylotoxins, and chemotaxins.[16]

All seven complement components are more concentrated in the peripheral cornea as compared to the central cornea. This distribution may result from the

diffusion of complement components from limbal vessels into the cornea.[17] Activation of complement by either the classical or alternate pathway can be involved in corneal inflammation.

Interferons Interferons (IFN) are a group of proteins made by cells in response to viral infection, which induce a generalized antiviral state in the surrounding cells.[18,19] IFN-α is secreted by leucocytes, IFN-β by fibroblasts, and IFN-γ by T cells and natural killer (NK) cells. INFs also stimulate production of major histocompatibility complex (MHC) class I molecules and proteins that enhance the ability of virally infected cells to present viral proteins to T cells. IFN-α and IFN-β activate NK cells in order that virus-infected host cells can be targeted and eliminated.

Cells of innate immunity Neutrophils are important in protecting the corneal epithelium from invasion by many microorganisms. Normally found in the cornea, neutrophils move through endothelial cells of the limbal vasculature by adhesion to receptors on vascular endothelial cells, a process called diapedesis. The neutrophil is a critical effector cell in innate immunity and plays vital roles in phagocytosis and microbial killing.[20]

Eosinophils possess surface receptors for IgE and complement components.[21] Activation of eosinophils can occur with IL-3, IL-5, and granulocyte colony-stimulating factor. A number of eosinophil granule proteins, such as major basic protein and cationic protein, have been identified which are presumed to play a role in parasitic toxicity.[21]

Macrophages are important in the innate immune response against microbial infections as they have phagocytic and antigen presenting capabilities as well as secretion of inflammatory cytokines.[22] Macrophages are believed to have important modulating effects on T-cell immune response. Although macrophages have traditionally been thought to reside in the conjunctiva, resident macrophages have been recently found in the murine corneal stroma and may play a part in host immune responses.[23]

NK cells are large granular lymphocytic cells and have no surface antigen receptors.[3] NK cells recognize MHC class I molecules through surface receptors delivering signals that inhibit, rather than activate, NK cells. As a consequence, NK cells lyse target cells that have lost or express insufficient amounts of MHC class I molecules, as frequently occurs in tumours, cells infected by certain viruses, antibody-coated cells, undifferentiated cells, and tumour cells.[24] Similar to activated macrophages, NK cells secrete TNF-α and IFN-α.

Acquired immunity

When innate immunity fails and the invading microorganism and/or its antigens persist, cell-mediated immunity can help bring microbial replication under control. In some cases, cell-mediated immunity can be out of proportion to the antigenic threat, leading to irreversible tissue destruction. Scarring and disorganization of the extracellular matrix as a result of HSV dermatitis may be inconsequential, whereas the same response in the cornea may lead to a significant permanent visual loss.

Langerhans cells

Langerhans cells are essential sentinel cells that are part of the corneal immune surveillance system.[25–27] Langerhans cells are antigen presenting cells of the cornea that are responsible for the recognition, processing, and presentation of antigens.[25] Langerhans cells have traditionally been noted to have MHC class II antigens and to remain in the periphery of the cornea. However, Langerhans cells have been noted in the central cornea of human infants[27] and, recently, MHC class II-negative Langerhans cells have been demonstrated in the central cornea of BALB/c mice.[28] When Langerhans cells are summoned, as elsewhere in the body, they must recognize a foreign antigen and discriminate between self and nonself. After Langerhans cells recognize an antigen as nonself, the antigen is processed and is transported to the surface by MHC molecules, either class I or II.[29] T cells are activated by the presentation of the antigen on the MHC molecule by binding to the T-cell receptor. The T cells then mature into effector cells, which are CD4+, if the MHC molecules presenting the antigen are class II, or CD8+, if the MHC is class I. The T cells either directly kill foreign invaders (CD8+ cytotoxic cells) or secrete cytokines (CD4+ T helper cells) which bring in other effector cells, mainly macrophages, which are involved in the destruction of pathogens and can activate other inflammatory cells.

Cytokines Two subsets of T helper cells have been described with differential cytokine production profiles.[30–32] Th1 cells secrete IL-2 and IFN-γ whereas Th2 cells secrete IL-4 and IL-5, but not IL-2 or IFN-γ. Furthermore, Th1 cells can be cytolytic and can assist B cells with IgG, IgM, and IgA synthesis but not IgE synthesis. Th2 cells are not cytolytic but can provide help for IgE synthesis as well as IgG, IgM, and IgA syntheses. Selection of CD4 Th1 or CD4 Th2 cells occurs in the case of infection or autoimmune diseases. Th1 cells are preferentially selected as participants in inflammatory

reactions associated with delayed-type hypersensitivity reactions and low antibody production. Th2 cells, on the other hand, are involved in inflammatory reactions associated with persistent antibody production, including allergic responses in which IgE production is predominant. Cytokines produced by Th cells are of critical importance for the outcome of many infectious diseases. The Th1/Th2 distinction has proven useful in the analysis of immune responses to infections. For example in schistosomiasis, a Th1 response is associated with elimination of the parasite, whereas a Th2 response results in extensive disease and granuloma formation.[33] Indeed, the expression of cytokines can dictate the intensity, chronicity, and type of immune/inflammatory response that is produced. These events may be regulated by accumulation of particular cell populations at a site of immune response that can be regulated by the expression of specific chemokines.

Corneal defenses to infection

Immunologic differences exist between the peripheral and central cornea. The peripheral cornea being proximate to the conjunctiva has all of the immunologic machinery necessary to generate an immune response. The peripheral cornea, possessing Langerhans' cells and IgM, also has more C1, the recognition unit of the classic pathway of complement, than the central cornea. Antigen–antibody complexes, whether formed in the cornea itself or whether derived from the tears, aqueous humour, or limbal vessels, may activate complement more effectively in the peripheral than central cornea. To follow are three examples of immune defense at the ocular surface to microbial invasion.

Each organism provokes a different defensive response, but each has components of innate and acquired immunity that play a defensive role. There are two defensive stages for each, an early and a late corresponding to innate and acquired immune responses, respectively. The first stage is an immediate response within minutes to several hours to microbial invasion. A bacterium can replicate in an hour, and after 24 h, 2 million organisms can exist establishing an infection that may overwhelm immune defenses. The innate immune system is charged with this initial defensive response, which always involves a neutrophilic infiltrate. If the initial defensive effort fails, adaptive immunity is called upon where Langerhans cells identify the foreign antigen in the cornea. An acquired response may occur within 24–48 h. The full adaptive immune response, however, may take days.

Pseudomonas aeruginosa Many bacterial and fungal organisms have components of their cell membrane and walls that play a vital role in the maintaining viability and have been retained over millions of years. These conserved molecular signatures have been recognized by host immune systems as a sign of incipient microbial invasion and have evolved to elicit an immediate and intense neutrophilic hypersensitivity response.[3] Phylectenular corneal disease is an example of a hypersensitivity response most commonly noted with staphylococcal antigens and has been reproduced in animal models by intrastromal injection of ribitol teichoic acid, a staphylococcal protein.[34]

P. aeruginosa is a common cause of corneal infection, particularly among users of soft contact lenses. Lipopolysaccharide (LPS) is another conserved molecule, a glycolipid in the outer membrane of Gram-negative bacteria including *P. aeruginosa*.[3] Corneal ulcers can be induced by intrastromal delivery of LPS to rabbit cornea, where histopathological examination demonstrates an intense infiltrate predominantly composed of neutrophils,[35] in the absence of any live bacteria.

The recruitment of neutrophils is mediated by an ancient 'toll receptor', first found in *Drosophila*, which was found to regulate the dorsal–ventral orientation in embryologic development.[3] The toll receptor also upregulated defensin expression in fungally infected Drosophila. A toll-like receptor, TLR4, has been identified in corneal epithelial cells.[36] TLR4 was shown to regulate endotoxin-induced keratitis by increased expression of platelet adhesion molecule (PECAM)-1 and macrophage inflammatory factor (MIP)-2, a homologue of IL-8.[37] PECAM is necessary to allow the egress or diapedesis of neutrophils from blood vessels into the extracellular matrix. Thus, the immune recognition of LPS as a conserved signature of Gram-negative bacterial invasion allows for the rapid influx of neutrophils from the perilimbal vessels.

Recruitment of neutrophils by MIP-2 in *Pseudomonas*-infected corneas has been demonstrated in susceptible mice.[38] The mechanism by which MIP-2 is upregulated appears to be related to the increase in IL-1 that is noted to occur in infected mice.[39] Thus, a corneal infection with *Pseudomonas* may have a positive feedback loop with increasing IL-1 secretion leading to a sustained neutrophilic infiltrate that is characteristic for the disease.

Contact lens wear has been shown to be a risk factor for the development of *Pseudomonas* keratitis. An acquired immune defensive component may be a possible explanation for this risk factor as experimental contact lens wear can cause migration of Langerhans cells into the central cornea.[40] With Langerhans cells present in the central cornea, an acquired immune response could proceed more rapidly leading to cytokine release and further influx of inflammatory cells.[41] Indeed,

CD4+ Th1 cells have been shown to increase in mice corneas susceptible to perforation. Further influx of CD4+ positive cells, responsible for persistence of a neutrophilic infiltrate in *Pseudomonas* keratitis, may be a result of unregulated MIP-1 which is produced by Langerhans cells, activated T cells, and macrophages.[41]

Herpes simplex virus *Herpes simplex* keratitis (HSK) has many different clinical presentations. Replication of the virus in corneal epithelium produces a classic dendrite, while immune reactions to the virus in the stroma and endothelium can produce infiltration, ulceration, vascularization, or oedema. Peripheral ulcerative keratitis can also seen with the disease. The relation of the immune surface defenses and HSV is complex. An intense and/or prolonged immune response to the virus can lead to corneal blindness. There appears to be a balance of ameliorating and exacerbating immune factors to keep viral replication and release of proinflammatory cytokines in check without incurring sight-threatening corneal damage.

Important in the defense against herpetic and other viral infections are mechanisms involved in innate immunity leading to the nonspecific clearing of viral particles. Neutrophils play a central role in the suppression of HSV viral replication in the murine model.[42] IFNα also plays a role in viral suppression as mice treated with neutralizing antibody to interferon have elevated viral titres and corneal disease.[43] IL-1α and TNF-α can induce corneal epithelial cells and keratocytes to secrete IL-6, which in turn has been found to induce corneal cells to produce MIP-1α and MIP-2.[44,45] MIP is known to be a potent chemoattractant for neutrophils and T cells. NK cells also play a role as they indirectly influence the severity of HSK in a mouse model by affecting neutrophil migration into the cornea.[46] Macrophages are required for the development of an acquired immune response, presumably by functioning in antigen processing and presentation.[47] IFN-α1, similarly, has a protective role, which is dependent upon the local or distal participation of CD4+ and CD8+ T lymphocytes early in the course of the infection.[48]

HSV replication in corneal epithelium does not always incite stromal disease. Resistance to HSV stromal keratitis in mice is associated with allotypic variation in immunoglobulin genes.[49] Cross-tolerization of T cells specific for corneal tissue autoantigens by the circulating Ig-derived peptides was suggested as the possible mechanism. Further studies demonstrated that HSV stromal keratitis was mediated by CD4+ T-cell clones specific for corneal self-antigens, which also recognize an allotype-bearing peptide derived from IgG2a,[50] and UL6 peptide of HSV cross-reacts with the IgG2a(b) isotype. Exposure of susceptible mice to a soluble form of this peptide was thought to confer resistance to keratitis. However, other work has cast doubt on this ''molecular mimicry'' hypothesis as an explanation for the pathogenesis of herpetic stromal keratitis.[51]

The influx of neutrophils and T cells producing HSV keratitis is greatly influenced by the cytokine MIP-1α.[52] In addition, knockout mice for MIP-1α have decreased inflammatory infiltrates with HSV infections. HSK has been found to have an increased expression of the Th1 cytokines IL-2 and IFN gamma,[53] and expression of IL-10, which suppresses the expression of these cytokines, ameliorates the development of HSK.[54,55]

Helminthic keratitis Parasitic filarial nematodes infect more than 200 million individuals worldwide, causing debilitating inflammatory diseases such as river blindness and lymphatic filariasis.[56] The ocular disease is thought to result from microfilariae that migrate from the conjunctiva into the cornea, become immotile and die. An intense immune response to dead microfilaria ensues which upon repeated infestation results in sclerosing keratitis and loss of vision. The mechanism by which the microfilaria become immobile and die in the cornea is unknown. However, a neutrophilic protein, calgraulin C, has been identified on the surface of worms isolated from onchoceral nodules.[57] Experiments have shown that calgranulin C can be synthesized by keratocytes and is filariastatic and filaracidal for the helminth *Brugia malayi*.[58] A parasitic muscle protein, paramyosin, is a binding protein for calgranulin C, resulting in a dose-dependent immobilization and at higher concentration killing of worms.[59] The binding complex of paramyosin–calgranulin C is highly antigenic and capable of inducing a stromal keratitis mimicking the human onchocercal human corneal disease.[59]

In human onchoceral conjunctival biopsies, $CD4^+$ cells are prominent with predominant IL-4 expression suggesting a Th2 immune response.[60,61] In animal models of onchocerciasis, the clinical disease could be reproduced by the injection of onchocercal extracts.[62] The early inflammatory cellular infiltrate is composed of neutrophils, however; eosinophils are the most prominent inflammatory cells when inflammation peaks.[63,64] In addition, recruitment of neutrophils and eosinophils to the cornea is antibody dependent, with PECAM-1 and ICAM-1 playing important roles.[65] Also, sensitized CD4+ T cells expressing IL-4 and IL-5 and but not IFN-γ cells have been shown to play a role in the keratitis noted after injection with *O. volvulus* antigen.[66] Modulation of cytokines has been shown to affect the severity of keratitis in animal models. By using an IL-4 gene knockout model, a reduced inflammatory infiltrate

with eosinophils was noted. Collectively, these studies demonstrate that a predominantly Th2 immunity contributes to the inflammatory response in onchocercal keratitis.

However, recently, using a murine model for river blindness in which soluble extracts of filarial nematodes were injected into the corneal stroma, the predominant inflammatory response in the cornea was demonstrated to be because of a species of endosymbiotic Wolbachia bacteria.[67] In addition, the inflammatory response induced by these bacteria was dependent on expression of functional TLR4 on host cells, providing further evidence of how the innate immune system plays a crucial role in the initial inflammatory response to onchocercal infection.

Summary

The ocular surface has many elements to help defend against microbial pathogens. Innate and acquired immune defenses can be both intensely involved in microbial keratitis. However, the elimination of replicating organisms in the early stages of infection is the most desirable, as the least amount of scarring and the greatest amount of vision results. Prolonged infections that ultimately activate the acquired immune system, in general, have the poorest prognosis.

References

1 Allman J. *Evolving Brains*. Scientific American Library: New York, 2000.
2 Strickberger MW. *Evolution*. Jones and Bartlett: Boston, MA, 2000.
3 Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. *The Adaptive Immune System in Molecular Biology of the Cell*, 4th ed. Garland Science: New York, 2002.
4 Mannis MJ, Smolin G. Natural defense mechanisms of the ocular surface. In: Pepose JS, Holland GN, Wilhelmeus KR (eds). *Ocular Infection and Immunity*. Mosby: St Louis, MO, 1996, pp 185–190.
5 Niederkorn JY, Peeler JS, Mellon J. Phagocytosis of particulate antigens by corneal epithelial cells stimulates interleukin-1 secretion and migration of Langerhans cells into the central cornea. *Reg Immunol* 1989; **2**: 83–90.
6 Cubitt CL, Lausch RN, Oakes JE. Synthesis of type II interleukin-1 receptors by human corneal epithelial cells but not by keratocytes. *Invest Ophthalmol Vis Sci* 2001; **42**: 701–704.
7 Moore JE, McMullen TC, Campbell IL, Rohan R, Kaji Y, Afshari NA *et al*. The inflammatory milieu associated with conjunctivalized cornea and its alteration with IL-1 RA gene therapy. *Invest Ophthalmol Vis Sci* 2002; **43**: 2905–2915.
8 Cubitt CL, Lausch RN, Oakes JE. Differences in interleukin-6 gene expression between cultured human corneal epithelial cells and keratocytes. *Invest Ophthalmol Vis Sci* 1995; **36**: 330–336.
9 Gottsch JD, Li Q, Ashraf MF, O'Brien TP, Stark WJ, Liu SH. Defensin gene expression in the cornea. *Curr Eye Res* 1998; **17**: 1082–1086.
10 Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF *et al*. Defensins. Natural peptide antibiotics of human neutrophils. *J Clin Invest* 1985; **76**: 1427–1435.
11 Haynes RJ, Tighe PJ, Dua HS. Antimicrobial defensin peptides of the human ocular surface. *Br J Ophthalmol* 1999; **83**: 737–741.
12 Oakes JE, Monteiro CA, Cubitt CL, Lausch RN. Induction of interleukin–8 gene expression is associated with herpes simplex virus infection of human corneal keratocytes but not human cornea epithelial cells. *J Virol* 1993; **67**: 4777–4784.
13 Muller LJ, Pels L, Vrensen GF. Ultrastructural organization of human corneal nerves. *Invest Ophthalmol Vis Sci* 1996; **37**: 476–488.
14 Tran MT, Ritchie MH, Lausch RN, Oakes JE. Calcitonin gene-related peptide induces IL-8 synthesis in human corneal epithelial cells. *J Immunol* 2000; **164**: 4307–4312.
15 Tran MT, Lausch RN, Oakes JE. Substance P differentially stimulates IL-8 synthesis in human cornea epithelial cells. *Invest Ophthalmol Vis Sci* 2000; **41**: 3871–3877.
16 Law SKA, Reid KBM. Complement. In: Rickwood D, Male D (eds). *Focus Series*, 2nd ed. Oxford University Press: Oxford, 1995.
17 Mondino BJ, Brady KJ. Distribution of hemolytic complement in the normal cornea. *Arch Ophthalmol* 1981; **99**: 1430–1433.
18 Su YH, Oakes JE, Lausch RN. Ocular avirulence of a herpes simplex virus type 1 strain is associated with heightened sensitivity of alpha/beta interferon. *J Virol* 1990; **64**: 2187–2192.
19 Thacore HR, Mount DT, Chadha KC. Interferon system of human cornea cells: interferon production, characterization, and development of antiviral state. *J Interferon Res* 1982; **2**: 401–408.
20 Burg ND, Pillinger MH. The neutrophil: function and regulation in innate and humoral immunity. *Clin Immunol* 2001; **99**: 7–17.
21 Trocme SD, Aldave AJ. The eye and the eosinophil. *Surv Ophthalmol* 1994; **39**: 241–252.
22 Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 2002; **20**: 825–852.
23 Brissette-Storkus CS, Reynolds SM, Lepisto AJ, Hendricks RL. Identification of a novel macrophage population in the normal mouse corneal stroma. *Invest Ophthalmol Vis Sci* 2002; **43**: 2264–2271.
24 Moretta L, Bottino C, Pende D, Mingari MC, Biassoni R, Moretta A. Human natural killer cells: their origin, receptors and function. *Eur J Immunol* 2002; **32**: 1205–1211.
25 Gillette TE, Chandler JW, Greiner JV. Langerhans cells of the ocular surface. *Ophthalmology* 1982; **89**: 700–711.
26 Garcia-Olivares E, Carreras B, Gallardo JM. Presence of Langerhans cells in the cornea of Klebsiella keratoconjunctivitis mice. *Invest Ophthalmol Vis Sci* 1988; **29**: 108–111.
27 Chandler JW, Cummings M, Gillette TE. Presence of Langerhans cells in the central corneas of normal human infants. *Invest Ophthalmol Vis Sci* 1985; **26**: 113–116.
28 Hamrah P, Zhang Q, Liu Y, Dana MR. Novel characterization of MHC Class II-negative population of resident corneal Langerhans cell-type dendritic cells. *Invest Ophthalmol Vis Sci* 2002; **43**: 639–646.

29 Unanue ER. Perspective on antigen processing and presentation. *Immunol Rev* 2002; **185**: 86–102.
30 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; **136**: 2348–2357.
31 Cher DJ, Mosmann TR. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by TH1 clones. *J Immunol* 1987; **138**: 3688–3694.
32 Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med* 1987; **166**: 1229–1244.
33 Infante-Duarte C, Kamradt T. Th1/Th2 balance in infection. *Springer Semin Immunopathol* 1999; **21**: 317–338.
34 Mondino BJ, Adamu SA, Pitchekian-Halabi H. Antibody studies in a rabbit model of corneal phlyctenulosis and catarrhal infiltrates related to *Staphylococcus aureus*. *Invest Ophthalmol Vis Sci* 1991; **32**: 1854–1863.
35 Schultz CL, Morck DW, McKay SG, Olson ME, Buret A. Lipopolysaccharide induced acute red eye and corneal ulcers. *Exp Eye Res* 1997; **64**: 3–9.
36 Song PI, Abraham TA, Park Y, Zivony AS, Harten B, Edelhauser HF *et al*. The expression of function LPS receptor proteins CD14 and toll-like receptor 4 in human corneal cells. *Invest Ophthalmol Vis Sci* 2001; **42**: 2867–2877.
37 Khatri S, Lass JH, Heinzel FP, Petroll WM, Gomez J, Diaconu E *et al*. Regulation of endotoxin-induced keratitis by PECAM-1, MIP-2, and toll-like receptor 4. *Invest Ophthalmol Vis Sci* 2002; **43**: 2278–2284.
38 Kernacki KA, Barrett RP, Hobden JA, Hazlett LD. MIP-2 is a mediator of PMN influx in ocular bacterial infection. *J Immunol* 200; **164**: 1037–1045.
39 Rudner XL, Kernacki KA, Barrett RP, Hazlett LD. Prolonged elevation of IL-1 in *Pseudomonas aeruginosa* ocular infection regulates macrophage-inflammatory protein-2 production, polymorphonuclear neutrophil persistence, and corneal perforation. *J Immunol* 2000; **164**: 6576–6582.
40 Hazlett LD, McClellan SM, Hume EB, Dajcs JJ, O'Callaghan RJ, Willcox MD. Extended contact lens usage induces Langerhans cell migration into cornea. *Exp Eye Res* 1999; **69**: 575–577.
41 Hazlett LD. Pathogenic mechanisms of *P. aeruginosa* keratitis: a review of the role of T cells, Langerhans cells, PMN, and cytokines. *DNA Cell Biol* 2002; **21**: 383–390.
42 Tumpey TM, Chen SH, Oakes JE, Lausch RN. Neutrophil-mediated suppression of virus replication after herpes simplex virus type 1 infection of the murine cornea. *J Virol* 1996; **70**: 898–904.
43 Hendricks RL, Weber PC, Taylor JL, Koumbis A, Tumpey TM, Glorioso JC. Endogenously produced interferon alpha protects mice from herpes simplex virus type 1 corneal disease. *J Gen Virol* 1991; **72**: 1601–1610.
44 Cubitt CL, Lausch RN, Oakes JE. Differences in interleukin-6 gene expression between cultured human corneal epithelial cells and keratocytes. *Invest Ophthalmol Vis Sci* 1995; **36**: 330–336.
45 Fenton RR, Molesworth-Kenyon S, Oakes JE, Lausch RN. Linkage of IL-6 with neutrophil chemoattractant expression in virus-induced ocular inflammation. *Invest Ophthalmol Vis Sci* 2002; **43**: 737–743.
46 Bouley DM, Kanangat S, Rouse BT. The role of the innate immune system in the reconstituted SCID mouse model of herpetic stromal keratitis. *Clin Immunol Immunopathol* 1996; **80**: 23–30.
47 Cheng H, Tumpey TM, Staats HF, van Rooijen N, Oakes JE, Lausch RN. Role of macrophages in restricting herpes simplex virus type 1 growth after ocular infection. *Invest Ophthalmol Vis Sci* 2000; **41**: 1402–1409.
48 Noisakran S, Carr DJ. Plasmid DNA encoding IFN-alpha 1 antagonizes herpes simplex virus type 1 ocular infection through CD4+ and CD8+ T lymphocytes. *J Immunol* 2000; **164**: 6435–6443.
49 Foster CS, Opremcak EM, Rice B, Wells P, Chung H, Thompson P *et al*. Clinical, pathologic, and immunopathologic characteristics of experimental murine herpes simplex virus stromal keratitis and uveitis is controlled by gene products from the Igh-1 locus on chromosome 12. *Trans Am Ophthalmol Soc* 1987; **85**: 293–311.
50 Avery AC, Zhao ZS, Rodriguez A, Bikoff EK, Soheilian M, Foster CS *et al*. Resistance to herpes stromal keratitis conferred by an IgG2a-derived peptide. *Nature* 1995; **376**: 431–434.
51 Deshpande SP, Lee S, Zheng M, Song B, Knipe D, Kapp JA *et al*. Herpes simplex virus-induced keratitis: evaluation of the role of molecular mimicry in lesion pathogenesis. *J Virol* 2001; **75**: 3077–3088.
52 Tumpey TM, Cheng H, Cook DN, Smithies O, Oakes JE, Lausch RN. Absence of macrophage inflammatory protein-1alpha prevents the development of blinding herpes stromal keratitis. *J Virol* 1998; **72**: 3705–3710.
53 Niemialtowski MG, Rouse BT. Predominance of Th1 cells in ocular tissues during herpetic stromal keratitis. *J Immunol* 1992; **149**: 3035–3039.
54 Yan XT, Zhuang M, Oakes JE, Lausch RN. Autocrine action of IL-10 suppresses proinflammatory mediators and inflammation in the HSV-1-infected cornea. *J Leukocyte Bio* 2001; **69**: 149–157.
55 Tumpey TM, Cheng H, Yan XT, Oakes JE, Lausch RN. Chemokine synthesis in the HSV-1-infected cornea and its suppression by interleukin-10. *J Leukoc Biol* 1998; **63**: 486–492.
56 Taylor HR, Nutman TB. Onchocerciasis. In: Pepose JS, Holland GN, Wilhelmeus KR (eds). *Ocular Infection and Immunity*. Mosby: St Louis, MO, 1996, pp 1481–1504.
57 Marti T, Erttmann KD, Gallin MY. Host–parasite interaction in human onchocerciasis: identification and sequence analysis of a novel human calgranulin. *Biochem Biophys Res Commun* 1996; **221**: 454–458.
58 Gottsch JD, Eisinger SW, Liu SH, Scott AL. Calgranulin C has filariacidal and filariastatic activity. *Infect Immun* 1999; **61**: 6631–6636.
59 Akpek EK, Liu SH, Thompson R, Gottsch JD. Identification of paramyosin as a binding protein for calgranulin C in experimental helminthic keratitis. *Invest Ophthalmol Vis Sci* 2002; **43**: 2677–2684.
60 Chan CC, Li Q, Brezin AP, Whitcup SM, Egwuagu C, Otteson EA *et al*. Immunopathology of onchocerciasis. Th-2 helper T cells in the conjunctiva. *Ocular Immunol Inflammation* 1993; **1**: 71–77.
61 Chan CC, Ottesen EA, Awadzi K, Badu R, Nussenblatt RB. Immunopathology of ocular onchocerciasis. I. Inflammatory of cells infiltrating the anterior segment. *Clin Exp Immunol* 1989; **77**: 367–372.

62 Gallin MY, Murray D, Lass JH, Grossniklaus HE, Greene BM. Experimental interstitial keratitis induced by *Onchocerca volvulus* antigens. *Arch Ophthalmol* 1988; **106**: 1447–1452.
63 Kaifi JT, Diaconu E, Pearlman E. Distinct roles for PECAM-1, ICAM-1, and VCAM-1 in recruitment of neutrophils and eosinophils to the cornea in ocular onchocerciasis (river blindness). *J Immunol* 2001; **166**: 6795–6801.
64 Pearlman E, Hall LR, Higgins AW, Bardenstein DS, Diaconu E, Hazlett FE *et al*. The role of eosinophils and neutrophils in helminth-induced keratitis. *Invest Ophthalmol Vis Sci* 1988; **39**: 1176–1182.
65 Hall LR, Lass JH, Diaconu E, Strine ER, Pearlman E. An essential role for antibody in neutrophil and eosinophil recruitment to the cornea: B cell-deficient (microMT) mice fail to develop Th2-dependent, helminth-mediated keratitis. *J Immunol* 1999; **163**: 4970–4975.
66 Pearlman E, Lass JH, Bardenstein DS, Kopf M, Hazlett FE Jr Diaconu E *et al*. Interleukin 4 and T helper type 2 cells are required for development of experimental onchocercal keratitis (river blindness). *J Exp Med* 1995; **182**: 931–940.
67 Saint Andre A, Blackwell NM, Hall LR, Hoerauf A, Brattig NW, Volkmann L *et al*. The role of endosymbiotic Wolbachia bacteria in the pathogenesis of river blindness. *Science* 2002; **295**: 1892–1895.