New developments in analysis of ocular surface diseases|Nieuwe ontwikkelingen in analyse van ziekten van het oogoppervlak

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PART III

CORNEAL INFECTIONS

INTRODUCTION

Introduction

The cornea is a highly specialized part of the eye, and its transparency is a requirement for good visual acuity. Corneal infections can damage the integrity of the ocular surface thereby disturbing the transparency of the cornea, leaving scars and decreasing visual acuity. Antimicrobial mechanisms of the eye try to prevent ocular surface infections, whereas anti-inflammatory mechanisms try to limit damage by the immune system. The occurrence of bacterial corneal infections is increasing with the increasing use of contact lenses. Especially bacterial corneal ulcers are a challenge to the ophthalmologist and immediate and strong antibacterial treatment is needed to prevent further visual loss and ocular complications. Another frequent ocular surface infection is the herpetic keratitis, which forms a serious ophthalmologic problem. Because of its latency and recurrences, the herpes simplex virus (HSV) can cause severe damage to the cornea. For a long time, HSV infection was one of the leading causes for corneal transplantation, which decreased after the introduction of strong antiviral medications.[1]

Corneal ulcerations

Infectious corneal ulcers have the potential to perforation the cornea and are therefore a serious threat for the eye.[2,3] In the Western World the incidence of infectious corneal ulcers is about 11 cases per 100.000 inhabitants;[4] however, in other parts of the world like for instance Southern India the incidence can be up to 113 cases per 100.000 inhabitants.[5] In Western Europe, the major causes of infectious corneal ulcers are contact-lens wear, trauma, and ocular surface diseases such as chronic blepharitis, dry eye syndrome, and eyelid pathology. Ocular trauma is the cause of corneal ulceration in 20% of the cases, in 30% the corneal ulcer is contact-lens related.[6] Extended-wear contact lenses are associated with a higher chance to de-

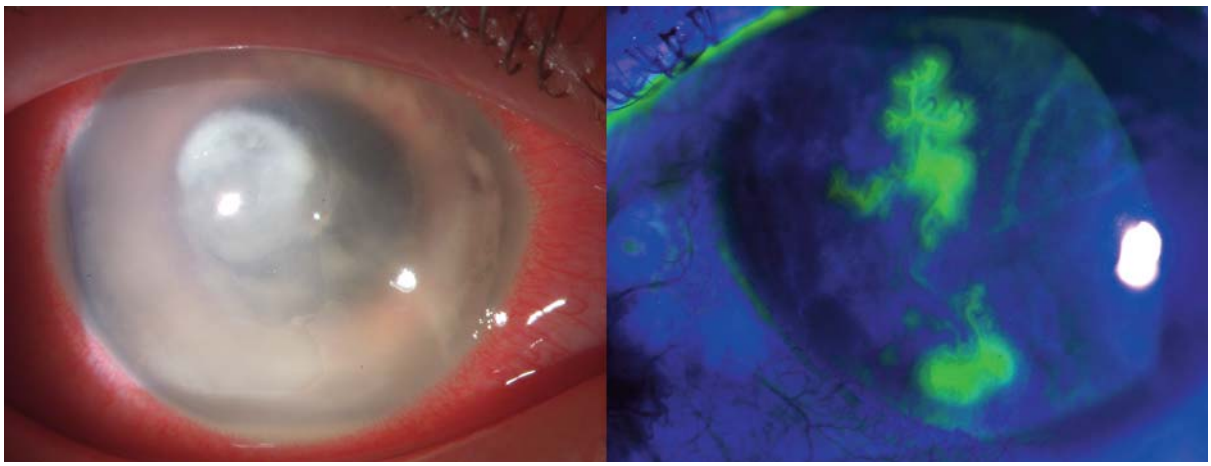
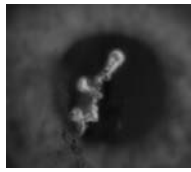


Figure 1. On the left a bacterial corneal ulcer with severe anterior chamber reaction. On the right a herpes simplex keratitis staining with fluorescein with characteristic dendritic shape.



velop corneal ulcers than daily-wear contact lenses (20.9% versus 4.1% corneal ulcers per 10.000 contact lens wearers).[7] Recently, a decrease in the incidence of contact-lens related corneal ulcers was seen, despite an increase of the contact-lens wearing population and is probably due to the shift from extended-wear contact lenses to daily-wear contact lenses.[8] Few reports exist about the incidence of corneal ulcers in patients with daily disposable contact lenses, but it seems that the chance is very low.[8] Other risk factors for corneal ulcers in individuals with contact lenses are overnight wear and improper lens care.[8,7,9]

Culturing the pathogens that cause the corneal ulcer is important for diagnosis and subsequent treatment. However, in 30% to 50% of the cases the bacterial cultures taken in academic centres are negative,[10,11] probably because antibiotic therapy had already been started by the general practitioner. Most commonly found bacteria are *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*.[6,10] Acanthamoeba can also be found; this is an opportunistic parasite that has the ability to transform from trophozoites to cysts and vice versa. The cysts can survive under extreme conditions such as high temperature, high osmolarity, and nutrition-poor environments; the cysts are also resistant to many antimicrobial agents.

Treatment with antibiotics must be started as soon as possible, since the internal eye is an ideal environment for bacterial growth and since *Pseudomonas aeruginosa* and *Staphylococcus aureus* are able to perforate the cornea within 24 hours.[12] In the absence of the outcome of positive cultures initial therapy must be with broad spectrum antibiotics. A combination of polymyxin B, neomycin, and gramicidin,[10] or cefazolin and gentamicin[13] is effective. In cases of positive cultures the antibiotics must be aimed at the pathogen found. In case of acanthamoeba keratitis, treatment consists of chlorhexidine, polyhexamethylene biguanide, neomycine, and propamidine isethionate (Brolene).[14]

Risk factors that are associated with penetrating keratoplasty, evisceration or enucleation are: older age, delay in referral to ophthalmologist, larger size of ulcer, central location of the ulcer, topical steroid treatment, prior ocular surgery, and poor vision at presentation.[15,16]

Herpes simplex keratitis

Herpes simplex virus (HSV) type 1 mostly causes facial infection, while HSV type 2 is primarily situated in the genital area. Although the site of the infection is different, the clinical signs and symptoms overlap. The HSV viruses are known for their latency and frequent recurrences. In primary infections, the HSV virus infects epithelial cells and spreads further through the tissue; some HSV particles enter the axons of sensory neurons. Via retrograde transport, HSV migrates to the neuronal cell body, where it can remain latent for a long time. The ability of the HSV virus to evade the immune system is the cause of its latency. By infecting the nervous system (ganglia), the HSV has gained access to a tissue that is relatively inaccessible for the immune system because of the low expression of Major Histocompatibility Complex (MHC) on neuronal cell surfaces. During the latency period in the ganglia, the viral metabolism is shut down and viral protein expression on the cell surface is down-regulated. The HSV-1 virus usually acquires latency in the trigeminal ganglion and can be found there in almost 100% of individuals above the age of 60.[17] Serum antibodies can be found in 45% to 88% of a population; a positive serology is influenced by sex, age

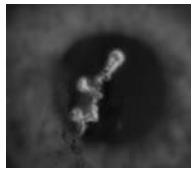
and social economic status.[18] The best known recurrent HSV-1 infection is herpes labialis; about 17-30 % of the western population suffer from this dissease.[19,20,21] Ocular HSV-1 infection has a lower prevalence of about 0.15%,[22,23] but can have a greater social-economic impact, since recurrent infections can cause blindness.[22] Reactivation of ocular HSV from its latent state in the trigeminal ganglion can be caused by a variety of stimuli such as local trauma or tissue damage, stress, ultraviolet light exposure, illness, or hyperthermia.[24,25,26,27] Transmission of HSV virus is usually caused by close contact with mucosal tissue; even corneal transplants can be a source of ocular HSV infection transmission,[28,29,30,31] and it has been suggested that the cornea is an extraneural site for HSV latency.[32,33] Primary ocular infections result in conjunctivitis, blepharitis with vesicles, and epithelial keratitis. Herpetic epithelial keratitis can recur and occurs as a dendritic or geographical shape. A more severe recurrent disease is herpetic stromal keratitis, which is mainly an immunological reaction. Besides infecting the ocular surface HSV is capable of causing trabeculitis, uveitis, and acute retinal necrosis.

The current treatment for herpetic epithelial keratitis is local treatment with aciclovir, a purine-nucleoside analogue that is able to disrupt viral DNA replication. In patients with herpetic stromal keratitis topical prednisolon is used to downregulate the immune system. For severe recurrent disease prophylactic treatment with oral valaciclovir (Zelitrex) is used to reduce the frequency of recurrences. Since the availability of aciclovir, the number of corneal transplants for HSV infections has decreased.[1]

Protection of the eye

The eye is protected against infections by a combination of anatomical, mechanical, antimicrobial, and immunological factors. The intact conjunctival and corneal epithelium forms a barrier against pathogens; once the integrity is broken by trauma or contact-lens wear, pathogens have a chance to infect the deeper layers. Mechanical factors include eyelid blinking and the continuous flow of tears, thereby diluting and removing pathogens such as bacteria, viruses, and parasites from the anterior eye surface. Tears transport metabolic products like oxygen and carbon dioxide, and allow passage of leucocytes after injury. Besides the mechanical function, tears have antimicrobial capabilities. Lysozyme and lactoferrin are tear proteins that are part of the innate immune system and form the first line of defence. Both proteins are known to inhibit growth of bacteria. The adaptive immune system is formed by plasma cells secreting immunoglobulin A or G in the lacrimal gland and the T-cell system. The IgA and IgG secreting plasma cells are probably former B-cells that underwent antigen sensitisation in the gut-associated lymphoid tissue and bronchus-associated lymphoid tissue.[34,35] The conjunctiva but not the cornea is provided with blood vessels and lymphatic vessels, through which T cells can be transported. Blood vessels will invade the cornea in cases of more serious corneal infections, bringing the immune system closer to the site of infection. However, the immune response can seriously damage the cornea, and can even be more destructive than the infection itself.

Under normal circumstances the conjunctiva is densely populated with bacteria; it has been suggested that the normal conjunctival flora has an inhibitory effect on more pathogenic bacteria.[36,37] However, some of these bacteria can be cultured from corneal ulcers or from



infections after surgery.[38,10]

Ocular immune privilege

To protect the micro-anatomical structures of the eye against a potential devastating immune response, the eye is an immune-privileged site. The cornea lacks blood and lymph vessels, which are usually used by the immune system to gain access to an infectious site. Intraocular immune privilege exists in the anterior chamber and is known as the Anterior Chamber Associated Immune Deviation (ACAID), that is able to suppress delayed type hypersensitivity and complement-fixing antibody reactions against anterior chamber antigens. Soluble factors in the anterior chamber (TGF-beta2, alpha-MSH, MIF, IL-10) can suppress T cells or NK cell activities.[39] Furthermore, eye tissues express Fas-ligand (CD95L) that is able to induce apoptosis in activated T-cells.[40,41]

Innate immune system

The innate immune system consists of soluble factors and leucocytes excluding the T and B cells, and forms the non-specific first line of defence. Invasion of tissue by either bacteria or viruses will activate the complement system, macrophages, neutrophils, and NK cells, which are all components of the innate immune system since they use "broad-spectrum" mechanisms and not antigen-specific mechanisms as the T and B cells to cope with infection. Macrophages play a role in the phagocytosis of bacteria or virus particles, thereby processing the pathogen and activating the immune system. In bacterial infections most bacteria are cleared by phagocytes like macrophages and neutrophils. Natural killer (NK) cells are primarily involved in intracellular infections like HSV,[42] as can be illustrated by the occurrence of more severe HSV infections in patients with genetic defects in NK cell function.[43] NK cells recognise infected cells through opsonization of target cells with virus-specific antibodies in an MHC-independent way. Furthermore, HSV induces MHC class I down-regulation in the infected cell thereby making the cells more prone to NK-cell mediated killing.[44] The soluble factors of the innate immune system include lactoferrin, lysozyme, the complement system, interferons (IFNs), and other cytokines or chemokines. Lactoferrin has both antiviral and antibacterial properties and is discussed later in this chapter. Lysozyme is effective against bacteria by perforating the bacterial surface. The complement system can cause direct killing of bacteria, opsonization of bacteria or infected cells, and can act as a chemotactic factor. IFNs (alpha and beta) are cytokines that are able to increase the resistance of cells to viral infections and are produced by infected cells.

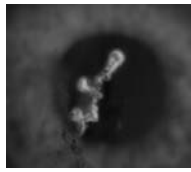
Adaptive immune system

The adaptive immune system uses antigen-specific recognition to clear bacterial or viral infections, and can be divided in a humoral response with antibodies (B cells) or a cellular response by T cells. In viral infections, antibodies against the virus can bind to the infected cell and cause cell death through either formation of a membrane-attack complex with complement or through NK-cell killing by recognition of the antibody by the NK cell. The latter is also called antibody-dependent cell-mediated cytotoxicity (ADCC), which is a critical mech-

anism in the antiviral defence against the HSV virus.[45] In bacterial infections, antibodies can interfere with the motility of bacteria and can block bacterial toxins. The most important effect of antibodies against bacteria is its ability to increase the effectiveness of complement targeting, and to act as an opsonin to make phagocytosis more effective.
The T-cell system consist of CD4- and CD8-positive T cells, which recognise viral antigen in association with major histocompatibility complex (MHC) molecules II and I, respectively. In infected cells, viral particles are degraded and processed on MHC class I on the cells surface for recognition by CD8 positive T cells. MHC class II cells are usually antigen-presenting cells such as macrophages and dendritic cells which present phagocytosed particles in an MHC class II-restricted manner for communication with CD4-positive T cells. CD4-positive T cells act as helper cells that help to induce CD8-positive T cell clones and recruit macrophages. The CD8-positive T cells are cytotoxic T cells, that can cause MHC class I-restricted apoptosis of infected cells. When a CD8-positive T cell recognizes an antigen on the surface of a cell in combination with MHC class I than the target cell will be programmed for apoptosis. For viral infections, the adaptive immune system is crucial for clearance of the virus. For HSV infections both the CD4-positive and the CD8-positive cells are crucial in the clearance of the virus,[46,47] which is further supported by the occurrence of more severe herpes infections in patients with AIDS.[48]

Lactoferrin

Lactoferrin is an important protein in the non-specific defence,[49,50] and can be found in tear fluid, saliva, milk, airway surface liquid, and in intestinal and vaginal secretions.[49,50,51] The main lacrimal gland produces the lactoferrin that can be found in tears. Granules of neutrophils also release lactoferrin during an inflammatory response.[52] Human lactoferrin contains 692 amino acids, and is folded into two symmetrical lobes (C and N terminal lobe).[53] It is active against many pathogens like gram-negative and positive bacteria, viruses, and fungi.[54,55] Besides the antimicrobial effects, lactoferrin also has immuno-modulatory and anti-inflammatory characteristics.[56] The bacteriostatic effect of lactoferrin is partly explained by its iron-binding capacity,[57,58,59] decreasing the local iron concentration, which is essential for bacterial growth. Lactoferrin also has non-iron-dependent antibacterial, antifungal, and antiviral capabilities.[60,49,50,61] It has been shown that the N-terminal cationic domains of lactoferrin have anti-bacterial activity through depolarization of bacterial membranes and by increasing bacterial membrane permeability.[62] Residues 1-47 of the N-terminus are responsible for the antibacterial effect.[63,64]
The antiviral effect of lactoferrin is primarily due to the interference of the lactoferrin protein with the binding of the virus to the cell surface.[65] The lactoferrin protein has a high affinity to heparan sulfate, which is a key glycosaminoglycan for the herpes virus to enter the cell.[66,67,68] The affinity of lactoferrin to bind to heparan sulfate is influenced by size and charge of the lactoferrin protein. Furthermore, in vitro, lactoferrin is able to inhibit infections with human herpes viruses, such as human cytomegalovirus and HSV-1.[69,70] Lactoferrin also suppresses HSV infection on the mouse cornea when applied prior to virus inoculation.[69] Lactoferrin has the largest effect in the initial stages of virus infection, and seems to prevent the herpes simplex virus to enter the cell. The N-terminal lobe of the lactoferirn protein

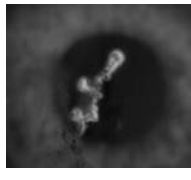


plays a pivotal role in the inhibition of viral infections.[71] High concentrations of lactoferrin, around 1 mg/ml, are needed to efficiently suppress viral infection, which is the case in human tears (about 2 mg/ml).[72]
Lactoferrin polymorphisms encoding amino acids at positions 29 and 561 of the N-terminal alpha-helical region have already been reported.[73,74] In Chapter 8 and 9 we report about a single nucleotide polymorphism encoding amino acid at position 11 of the lactoferrin gene.
The polymorphic forms of the human lactoferrin are defined as Ala11Thr, Lys29Arg, and Asp561Glu, in which Ala = alanine, Thr = threonine, Lys = lysine, Arg = arginine, Asp = aspartic acid, and Glu = glutamic acid. Moreover, it has been reported that lactoferrin Lys29Arg polymorphisms exerts different antibacterial and transcriptional activation activities.[74,75] Chapter 8 and 9 of this thesis investigates the potential role of the three lactoferrin single nucleotide polymorphisms in the development of corneal ulcers or HSV keratitis.

IL-10

IL-10 is a multi-functional cytokine with strong anti-inflammatory and immunosuppressive properties.[76] Cells that are capable of producing IL-10 include macrophages, Th2 cells, neutrophil, and resident corneal cells.[77,78,79]
Especially in HSV infections, the effect of IL-10 has been wel established. In animal models, IL-10 knock-out or IL-10 depletion by antibodies is associated with an increase in HSV corneal disease severity.[79] Similarly, topical IL-10 treatment significantly decreases corneal pathology induced by primary infection with HSV.[80,81,82] Besides the primary infections, IL-10 also seems to play a protective role in recurrent HSV infections in animal models.[83] IL-10 exerts its protective effect by downregulating pro-inflammatory cytokines and thereby decreasing the number of destructive lymphocytes during a stromal HSV infection, i.e. CD4-positive T cells and neutrophils.[83,84,80,81,79]
In infectious corneal ulcers IL-10 seems to have a protective effect for both gram positive and negative bacterial corneal ulcers.[85,86] It can especially prevent corneal perforation in animal models, indicating that the immune system itself also contributes to the corneal damage.
Many single nucleotide polymorphisms (SNP) exist in the promoter region of the IL-10 gene and some are associated with different IL-10 expression levels,[87,88,89] and these IL-10 promotor SNPs are involved in numerous types of infections.[90-98] Regarding HSV infections, people homozygous for the ATA IL-10 haplotype seem to be more resistant to HSV based on serum antibodies.[94] In Chapter 10, we have investigated four key SNPs of the IL-10 promotor gene region, -C819T, -G1082A, -A2763C, and -A2849G, in patients with infectious corneal ulcers.
Combinations of different single nucleotide polymorphisms can form haplotypes. Of the four above mentions SNPs five frequently occuring haplotypes can be inferred; haplotype IL10.1 CGAA, haplotype IL10.2 CACG, haplotype IL10.3 CGAG, haplotype IL10.4 TACG, and haplotype IL10.5 CGCG. These IL-10 haplotypes are associated with IL-10 production in vitro. Haplotypes IL10.1 and IL10.3 are associated with lower IL-10 production, where IL10.2 and IL10.5 are associated with higher IL-10 production. Haplotype IL10.4 is both associated with high and low IL-10 production.[99] Whether the IL-10 haplotypes also influ-

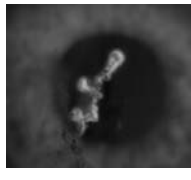
ence the continuous production of IL-10 in resident corneal cells[79] and thereby corneal disease is not known. These IL-10 haplotypes are also investigated in the infectious corneal ulcers patients group in Chapter 10.



References

1. Al Yousuf N, Mavrikakis I, Mavrikakis E, Daya SM. Penetrating keratoplasty: indications over a 10 year period. *Br J Ophthalmol*. 2004;88:998-1001.
2. Jones DB. Early diagnosis and therapy of bacterial corneal ulcers. *Int Ophthalmol Clin*. 1973;13:1-29.
3. Laibson PR. Cornea and sclera. *Arch Ophthalmol*. 1972;88:553-574.
4. Erie JC, Nevitt MP, Hodge DO, Ballard DJ. Incidence of ulcerative keratitis in a defined population from 1950 through 1988. *Arch Ophthalmol*. 1993;111:1665-1671.
5. Gonzales CA, Srinivasan M, Whitcher JP, Smolin G. Incidence of corneal ulceration in Madurai district, South India. *Ophthalmic Epidemiol*. 1996;3:159-166.
6. Schaefer F, Bruttin O, Zografos L, Guex-Crosier Y. Bacterial keratitis: a prospective clinical and microbiological study. *Br J Ophthalmol*. 2001;85:842-847.
7. Poggio EC, Glynn RJ, Schein OD et al. The incidence of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. *N Engl J Med*. 1989;321:779-783.
8. Mah-Sadorra JH, Yavuz SG, Najjar DM et al. Trends in contact lens-related corneal ulcers. *Cornea*. 2005;24:51-58.
9. Schein OD, Glynn RJ, Poggio EC, Seddon JM, Kenyon KR. The relative risk of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. A case-control study. Microbial Keratitis Study Group. *N Engl J Med*. 1989;321:773-778.
10. Bosscha MI, Van Dissel JT, Kuijper EJ, Swart W, Jager MJ. The efficacy and safety of topical polymyxin B, neomycin and gramicidin for treatment of presumed bacterial corneal ulceration. *Br J Ophthalmol*. 2004;88:25-28.
11. Waxman E, Chechelnitsky M, Mannis MJ, Schwab IR. Single culture media in infectious keratitis. *Cornea*. 1999;18:257-261.
12. Ostler H. Disease of the cornea. In: Mitchell C. eds. *Disease of the external eye and adnexa*. Baltimore: Williams and Wilkins; 1993:137-252.
13. Chaudhuri PR, Godfrey B. Treatment of bacterial corneal ulcers with concentrated antibiotic eye drops. *Trans Ophthalmol Soc U K*. 1982;102 (Pt 1):11-14.
14. Khan NA. Pathogenesis of Acanthamoeba infections. *Microb Pathog*. 2003;34:277-285.
15. Cruz CS, Cohen EJ, Rapuano CJ, Laibson PR. Microbial keratitis resulting in loss of the eye. *Ophthalmic Surg Lasers*. 1998;29:803-807.
16. Miedziak AI, Miller MR, Rapuano CJ, Laibson PR, Cohen EJ. Risk factors in microbial keratitis leading to penetrating keratoplasty. *Ophthalmology*. 1999;106:1166-1170.
17. Liedtke W, Opalka B, Zimmermann CW, Lignitz E. Age distribution of latent herpes simplex virus 1 and varicella-zoster virus genome in human nervous tissue. *J Neurol Sci*. 1993;116:6-11.
18. Whitley RJ, Roizman B. Herpes simplex virus infections. *Lancet*. 2001;357:1513-1518.
19. Axell T, Liedholm R. Occurrence of recurrent herpes labialis in an adult Swedish population. *Acta Odontol Scand*. 1990;48:119-123.
20. Lowhagen GB, Bonde E, Eriksson B et al. Self-reported herpes labialis in a Swedish population. *Scand J Infect Dis*. 2002;34:664-667.
21. Young TB, Rimm EB, D'Alessio DJ. Cross-sectional study of recurrent herpes labialis. Prevalence and risk factors. *Am J Epidemiol*. 1988;127:612-625.
22. Liesegang TJ, Melton LJ, III, Daly PJ, Ilstrup DM. Epidemiology of ocular herpes simplex. Incidence in Rochester, Minn, 1950 through 1982. *Arch Ophthalmol*. 1989;107:1155-1159.
23. Liesegang TJ. Herpes simplex virus epidemiology and ocular importance. *Cornea*. 2001;20:1-13.
24. Dhaliwal DK, Romanowski EG, Yates KA et al. Experimental laser-assisted in situ keratomileusis induces the reactivation of latent herpes simplex virus. *Am J Ophthalmol*. 2001;131:506-507.
25. Binder PS. Herpes simplex keratitis. *Surv Ophthalmol*. 1977;21:313-331.
26. Dawson CR, Togni B. Herpes simplex eye infections: clinical manifestations, pathogenesis and management. *Surv Ophthalmol*. 1976;21:121-135.
27. Psychological stress and other potential triggers for recurrences of herpes simplex virus eye infections. Herpetic Eye Disease Study Group. *Arch Ophthalmol*. 2000;118:1617-1625.
28. Beyer CF, Hill JM, Byrd TJ, Kaufman HE. Herpes simplex dendritic keratitis after keratoplasty. *Am J Oph-*

thalmol. 1991;112:355-356.
29. Salisbury JD, Berkowitz RA, Gebhardt BM, Kaufman HE. Herpesvirus infection of cornea allografts. *Ophthalmic Surg*. 1984;15:406-408.
30. Remeijer L, Doornenbal P, Geerards AJ, Rijneveld WA, Beekhuis WH. Newly acquired herpes simplex virus keratitis after penetrating keratoplasty. *Ophthalmology*. 1997;104:648-652.
31. Remeijer L, Maertzdorf J, Doornenbal P, Verjans GM, Osterhaus AD. Herpes simplex virus 1 transmission through corneal transplantation. *Lancet*. 2001;357:442.
32. Pavan-Langston D, Rong BL, Dunkel EC. Extraneuronal herpetic latency: animal and human corneal studies. *Acta Ophthalmol Suppl*. 1989;192:135-141.
33. Rong BL, Pavan-Langston D, Weng QP et al. Detection of herpes simplex virus thymidine kinase and latency-associated transcript gene sequences in human herpetic corneas by polymerase chain reaction amplification. *Invest Ophthalmol Vis Sci*. 1991;32:1808-1815.
34. Franklin RM, McGee DW, Shepard KF. Lacrimal gland-directed B cell responses. *J Immunol*. 1985;135:95-99.
35. McClellan KA. Mucosal defense of the outer eye. *Surv Ophthalmol*. 1997;42:233-246.
36. Halbert SP, SWICK LS. Antibiotic-producing bacteria of the ocular flora. *Am J Ophthalmol*. 1952;35:73-81.
37. Morse SA, Vaughan P, Johnson D, Iglewski BH. Inhibition of Neisseria gonorrhoeae by a bacteriocin from Pseudomonas aeruginosa. *Antimicrob Agents Chemother*. 1976;10:354-362.
38. Ashkenazi I, Melamed S, Avni I, Bartov E, Blumenthal M. Risk factors associated with late infection of filtering blebs and endophthalmitis. *Ophthalmic Surg*. 1991;22:570-574.
39. Wilbanks GA, Mammolenti M, Streilein JW. Studies on the induction of anterior chamber-associated immune deviation (ACAID). III. Induction of ACAID depends upon intraocular transforming growth factor-beta. *Eur J Immunol*. 1992;22:165-173.
40. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science*. 1995;270:1189-1192.
41. Stuart PM, Griffith TS, Usui N et al. CD95 ligand (FasL)-induced apoptosis is necessary for corneal allograft survival. *J Clin Invest*. 1997;99:396-402.
42. Habu S, Akamatsu K, Tamaoki N, Okumura K. In vivo significance of NK cell on resistance against virus (HSV-1) infections in mice. *J Immunol*. 1984;133:2743-2747.
43. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol*. 1999;17:189-220.
44. Lanier LL. Natural killer cell receptors and MHC class I interactions. *Curr Opin Immunol*. 1997;9:126-131.
45. Kohl S. Role of antibody-dependent cellular cytotoxicity in defense against herpes simplex virus infections. *Rev Infect Dis*. 1991;13:108-114.
46. Schmid DS, Mawle AC. T cell responses to herpes simplex viruses in humans. *Rev Infect Dis*. 1991;13 Suppl 11:S946-S949.
47. Mester JC, Rouse BT. The mouse model and understanding immunity to herpes simplex virus. *Rev Infect Dis*. 1991;13 Suppl 11:S935-S945.
48. Schmid DS, Rouse BT. The role of T cell immunity in control of herpes simplex virus. *Curr Top Microbiol Immunol*. 1992;179:57-74.
49. Levay PF, Viljoen M. Lactoferrin: a general review. *Haematologica*. 1995;80:252-267.
50. Lonnerdal B, Iyer S. Lactoferrin: molecular structure and biological function. *Annu Rev Nutr*. 1995;15:93-110.
51. Masson PL, Heremans JF, Prignot JJ, Wauters G. Immunohistochemical localization and bacteriostatic properties of an iron-binding protein from bronchial mucus. *Thorax*. 1966;21:538-544.
52. Brock J. Lactoferrin: a multifunctional immunoregulatory protein? *Immunol Today*. 1995;16:417-419.
53. Metz-Boutigue MH, Jolles J, Mazurier J et al. Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. *Eur J Biochem*. 1984;145:659-676.
54. Sanchez L, Calvo M, Brock JH. Biological role of lactoferrin. *Arch Dis Child*. 1992;67:657-661.
55. van der Strate BW, Beljaars L, Molema G, Harmsen MC, Meijer DK. Antiviral activities of lactoferrin. *Antiviral Res*. 2001;52:225-239.
56. Ward PP, Uribe-Luna S, Conneely OM. Lactoferrin and host defense. *Biochem Cell Biol*. 2002;80:95-102.
57. Bullen JJ. The significance of iron in infection. *Rev Infect Dis*. 1981;3:1127-1138.



58. Ellison RT, III, Giehl TJ, LaForce FM. Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin. *Infect Immun*. 1988;56:2774-2781.
59. Stuart J, Norrell S, Harrington JP. Kinetic effect of human lactoferrin on the growth of Escherichia coli 0111. *Int J Biochem*. 1984;16:1043-1047.
60. Bellamy W, Wakabayashi H, Takase M et al. Killing of Candida albicans by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. *Med Microbiol Immunol (Berl)*. 1993;182:97-105.
61. Wakabayashi H, Abe S, Okutomi T et al. Cooperative anti-Candida effects of lactoferrin or its peptides in combination with azole antifungal agents. *Microbiol Immunol*. 1996;40:821-825.
62. Chapple DS, Mason DJ, Joannou CL et al. Structure-function relationship of antibacterial synthetic peptides homologous to a helical surface region on human lactoferrin against Escherichia coli serotype O111. *Infect Immun*. 1998;66:2434-2440.
63. Bellamy W, Takase M, Yamauchi K et al. Identification of the bactericidal domain of lactoferrin. *Biochim Biophys Acta*. 1992;1121:130-136.
64. Yamauchi K, Tomita M, Giehl TJ, Ellison RT, III. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infect Immun*. 1993;61:719-728.
65. WuDunn D, Spear PG. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J Virol*. 1989;63:52-58.
66. Andersen JH, Jenssen H, Sandvik K, Gutteberg TJ. Anti-HSV activity of lactoferrin and lactoferricin is dependent on the presence of heparan sulphate at the cell surface. *J Med Virol*. 2004;74:262-271.
67. Marchetti M, Trybala E, Superti F, Johansson M, Bergstrom T. Inhibition of herpes simplex virus infection by lactoferrin is dependent on interference with the virus binding to glycosaminoglycans. *Virology*. 2004;318:405-413.
68. Jenssen H, Andersen JH, Uhlin-Hansen L, Gutteberg TJ, Rekdal O. Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. *Antiviral Res*. 2004;61:101-109.
69. Fujihara T, Hayashi K. Lactoferrin inhibits herpes simplex virus type-1 (HSV-1) infection to mouse cornea. *Arch Virol*. 1995;140:1469-1472.
70. Hasegawa K, Motsuchi W, Tanaka S, Dosako S. Inhibition with lactoferrin of in vitro infection with human herpes virus. *Jpn J Med Sci Biol*. 1994;47:73-85.
71. Seganti L, Di Biase AM, Rega B et al. Involvement of bovine lactoferrin moieties in the inhibition of herpes simplex virus type 1 infection. *Int J Immunopathol Pharmacol*. 2001;14:71-79.
72. Kijlstra A, Jeurissen SH, Koning KM. Lactoferrin levels in normal human tears. *Br J Ophthalmol*. 1983;67:199-202.
73. Araki-Sasaki K, Ando Y, Nakamura M et al. Lactoferrin Glu561Asp facilitates secondary amyloidosis in the cornea. *Br J Ophthalmol*. 2005;89:684-688.
74. Velliyagounder K, Kaplan JB, Furgang D et al. One of two human lactoferrin variants exhibits increased antibacterial and transcriptional activation activities and is associated with localized juvenile periodontitis. *Infect Immun*. 2003;71:6141-6147.
75. Lee TH, Shimazaki K, Yu SL et al. Polymorphic sequence of Korean Native goat lactoferrin exhibiting greater antibacterial activity. *Anim Genet*. 1997;28:367-369.
76. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy—review of a new approach. *Pharmacol Rev*. 2003;55:241-269.
77. Cole N, Krockenberger M, Stapleton F et al. Experimental Pseudomonas aeruginosa keratitis in interleukin-10 gene knockout mice. *Infect Immun*. 2003;71:1328-1336.
78. Romani L, Mencacci A, Cenci E et al. An immunoregulatory role for neutrophils in CD4+ T helper subset selection in mice with candidiasis. *J Immunol*. 1997;158:2356-2362.
79. 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 Leukoc Biol*. 2001;69:149-157.
80. Tumpey TM, Elner VM, Chen SH, Oakes JE, Lausch RN. Interleukin-10 treatment can suppress stromal keratitis induced by herpes simplex virus type 1. *J Immunol*. 1994;153:2258-2265.
81. 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.
82. Daheshia M, Kuklin N, Kanangat S, Manickan E, Rouse BT. Suppression of ongoing ocular inflammatory disease by topical administration of plasmid DNA encoding IL-10. *J Immunol*. 1997;159:1945-1952.

83. Keadle TL, Stuart PM. Interleukin-10 (IL-10) ameliorates corneal disease in a mouse model of recurrent herpetic keratitis. *Microb Pathog*. 2005;38:13-21.
84. Minagawa H, Sakai Y, Li Y et al. Suppression of infectious virus spread and corneal opacification by the combined use of recombinant interferon beta and interleukin-10 following corneal infection with herpes simplex virus-1 in mice. *Antiviral Res*. 1997;36:99-105.
85. Hazlett LD, McClellan SA, Barrett RP et al. Spantide I decreases type I cytokines, enhances IL-10, and reduces corneal perforation in susceptible mice after Pseudomonas aeruginosa infection. *Invest Ophthalmol Vis Sci.* 2007;48:797-807.
86. Hume EB, Cole N, Khan S et al. A Staphylococcus aureus mouse keratitis topical infection model: cytokine balance in different strains of mice. *Immunol Cell Biol*. 2005;83:294-300.
87. Gibson AW, Edberg JC, Wu J et al. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J Immunol*. 2001;166:3915-3922.
88. Turner DM, Williams DM, Sankaran D et al. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*. 1997;24:1-8.
89. Westendorp RG, van Dunne FM, Kirkwood TB, Helmerhorst FM, Huizinga TW. Optimizing human fertility and survival. *Nat Med*. 2001;7:873.
90. Gallagher PM, Lowe G, Fitzgerald T et al. Association of IL-10 polymorphism with severity of illness in community acquired pneumonia. *Thorax*. 2003;58:154-156.
91. Haanpaa M, Nurmikko T, Hurme M. Polymorphism of the IL-10 gene is associated with susceptibility to herpes zoster. *Scand J Infect Dis*. 2002;34:112-114.
92. Helminen M, Lahdenpohja N, Hurme M. Polymorphism of the interleukin-10 gene is associated with susceptibility to Epstein-Barr virus infection. *J Infect Dis*. 1999;180:496-499.
93. Helminen ME, Kilpinen S, Virta M, Hurme M. Susceptibility to primary Epstein-Barr virus infection is associated with interleukin-10 gene promoter polymorphism. *J Infect Dis*. 2001;184:777-780.
94. Hurme M, Haanpaa M, Nurmikko T et al. IL-10 gene polymorphism and herpesvirus infections. *J Med Virol*. 2003;70 Suppl 1:S48-S50.
95. Miyazoe S, Hamasaki K, Nakata K et al. Influence of interleukin-10 gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus. *Am J Gastroenterol*. 2002;97:2086-2092.
96. Schaaf BM, Boehmke F, Esnaashari H et al. Pneumococcal septic shock is associated with the interleukin-10-1082 gene promoter polymorphism. *Am J Respir Crit Care Med*. 2003;168:476-480.
97. Shin HD, Winkler C, Stephens JC et al. Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. *Proc Natl Acad Sci U S A*. 2000;97:14467-14472.
98. Smolnikova MV, Konenkov VI. Association of IL2, TNFA, IL4 and IL10 Promoter Gene Polymorphisms with the Rate of Progression of the HIV Infection. *Russ J Immunol*. 2002;7:349-356.
99. Kurreeman FA, Schonkeren JJ, Heijmans BT, Toes RE, Huizinga TW. Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. *Hum Mol Genet.* 2004;13:1755-1762.