

Microbial infections of the eye

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Introduction

The outcome of microbial infections depends on both the virulence of the infecting organism and the veracity of the host response. The balance of these two entities will determine the severity of disease and the longevity of infection. While in some infections tissue damage is a direct result of cytotoxic activity of microbial products such as exotoxins, the host inflammatory response causes collateral tissue damage associated with antimicrobial activity. Microbial virulence may then be defined as the ability of the organisms to survive in the presence of the host immune response. This chapter will focus on infections of the anterior eye based on exposure to the ocular surface, but will also discuss infections of the posterior eye, including endophthalmitis and ocular toxoplasmosis.

Microbes in the environment

Most of the organisms that cause severe ocular infections are either ubiquitous in the environment or are part of our normal body flora. These microbes are opportunistic pathogens and require breach of the physical barriers of the eye. For example, *Pseudomonas aeruginosa* and *Acanthamoeba* are normal freshwater organisms present in ponds, lakes and

household water supplies, including showerheads. Similarly, *Aspergillus* and *Fusarium* moulds, which are plant saprophytes and pathogens, are ubiquitous in the air we breathe, although spore counts are higher in hot and humid areas of the world, and in agricultural regions, especially during harvest seasons. *Aspergillus* is among the most widely distributed organisms worldwide and, except in extremely cold environments, most individuals inhale *Aspergillus* spores on a daily basis. In humid conditions *Aspergillus* is the cause of the green mould found on bread and other foods, and routine examination of the average bathroom will detect *Aspergillus* and *Fusarium* spores, *Pseudomonas*, *Serratia* and *Acanthamoeba* (which feeds on bacteria and fungi). The number of organisms in the environment is generally higher in warmer climates and during the summer in temperate climates.

In addition to the external environment, several organisms are present in the normal body flora of the human conjunctiva (adenovirus), the skin (staphylococci) and the nasopharynx (streptococcus pneumoniae, candida albicans). Most individuals also harbour herpes simplex virus (HSV) and herpes zoster as latent forms in the trigeminal ganglia. HSV is the most common cause of corneal infections in the USA and other industrialized countries and causes resurgent keratitis following viral exit from latency. *Toxoplasma gondii* is also ubiquitous in the environment and humans are infected following ingestion of cat faecal material or infected meat.

The exceptions to pathogens with near ubiquitous distribution are those that are very restricted geographically or which thrive under conditions of poor hygiene. The bacterium that causes trachoma (*Chlamydia trachomatis*) is the major example of an organism that thrives in unsanitary environments, as

it is readily transmitted by flies that breed on human waste. *Onchocerca volvulus*, the cause of river blindness, and *Loa loa* (eyeworm) are highly adapted human parasites that require an intermediate insect vector, and are found primarily in Africa. These will be discussed later in this chapter. Table 8-1 lists many of the causes of ocular infection.

Host defences at the ocular surface

PHYSICAL BARRIERS

Blinking is a very effective cleansing mechanism and eyelashes can trap microbes, preventing access to the

TABLE 8-1 Microbial pathogens of the eye*		
Group/phylum	Genus, species	Major site of infection
Viruses	Adenovirus	Conjunctiva
	Herpes simplex	Cornea
	Herpes zoster	Cornea
	CMV	Retina
Obligate intracellular	<i>Chlamydia</i>	Conjunctiva, cornea (trachoma)
Gram-negative bacteria	PA	Cornea
	Sm	Cornea
Gram-positive bacteria	SP	Conjunctiva, cornea, vitreous
	Sa	Cornea
	<i>Bacillus cereus</i>	Vitreous
Yeast Mould	<i>Candida</i>	Cornea
	<i>Fusarium</i>	
	<i>Aspergillus</i>	
	<i>Toxoplasma</i>	Cornea
Protozoa	<i>Acanthamoeba</i>	
	<i>Microsporidia</i>	
	<i>Toxocara canis</i>	Retina
Helminths Filarial	<i>Onchocerca volvulus</i> (river blindness)	Cornea, retina
	<i>Loa loa</i> (eye-worm)	Conjunctiva

*Although a search of case reports will produce a much longer list of organisms, the table includes only the most common causes of ocular infection.

CMV, cytomegalovirus; PA, *Pseudomonas aeruginosa*; Sm, *Serratia marcescens*; SP, *Streptococcus pneumoniae*; Sa, *Staphylococcus aureus*.

globe. In addition, the eyelids contain sebaceous glands that secrete lactic acid and fatty acids in a low pH environment, which has a direct inhibitory effect on bacterial replication. Tears also contain antimicrobial compounds including lacritin, lactoferrin, lipocalin lysozyme, β -defensins and other antimicrobial peptides. The tear film also contains secretory immunoglobulin A (IgA), IgG and complement that contribute to protection against bacterial invasion (see Ch. 7). Protection is also afforded by neutrophils in the tears, which increase in numbers following sleep when the eyelids are closed.

EPITHELIUM

The corneal epithelium has a glycocalyx on the apical surface composed of large proteoglycan mucins including MUC1, MUC4 and MUC16 (see Ch. 4). This mucin layer provides a physical barrier that restricts bacterial adherence to corneal epithelial cells in addition to releasing mucins into the tear film (Fig. 8-1). Epithelial cells also form tight junctions that are a very effective barrier against subsequent penetration to the corneal stroma; infections therefore generally occur as a consequence of traumatic injury or alteration of the corneal surface microenvironment. Epithelial cells also secrete antimicrobial peptides including β -defensins, cathelicidin (LL-37) and calprotectin (S100A8/A9), and by degradation of cytokeratin-6A in corneal epithelial cells. These keratin-derived

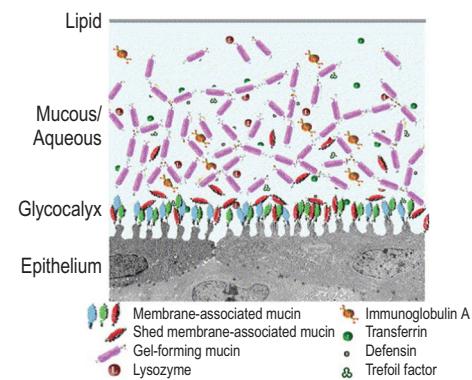


FIGURE 8-1 Ocular surface mucins. The aqueous phase of the tears contains MUC5AC (purple) and other tear components. The glycocalyx has extended membrane-associated MUC1 (blue), MUC4 (green) and MUC16 (red). Extracellular domains are released into the tear film. (From Gipson, 2004.)

antimicrobial peptides (KDAMPs) have a distinct secondary structure and exhibit broad antimicrobial activity.

RESIDENT MACROPHAGES AND DENDRITIC CELLS IN THE CORNEA

The normal mammalian cornea was generally thought to be devoid of immune cells; however, with the advent of improved immunostaining methods, together with examining whole-mount corneas rather than histological sections, an entire network of macrophages and dendritic cells was revealed. Most bone marrow-derived cells in the normal corneal stroma have characteristics of macrophages, with dendritic cells more prominent in the peripheral limbal region of the cornea and in the basement membrane of the epithelium (Bowman's membrane). These cells extend pseudopodia ('periscopes') through to the apical surface, presumably to detect microbes or microbial products (Fig. 8-2A–D). Nanotubes that appear to connect distant cells can also be detected in the corneal stroma during inflammation (Fig. 8-2D).

PATHOGEN RECOGNITION RECEPTORS AND RECRUITMENT OF NEUTROPHILS

Most nucleated cells are able to recognize and respond to microbial products, although macrophages and dendritic cells are specifically adapted for this purpose. These cells express multiple copies of surface receptors that recognize bacterial and fungal proteins, carbohydrates, lipids and DNA and RNA, and are termed pathogen recognition receptors (PRRs) (see Ch. 7, p. 379). Ligand binding initiates intracellular signalling events that result in production of pro-inflammatory and chemotactic cytokines. These are invariant receptors encoded in the germ line genes, which is in contrast to T and B cells, where receptors are generated following gene rearrangement. Further, whereas mature T and B cells primarily recognize specific peptides (and in some cases well-defined carbohydrates), the invariant receptors associated with innate immunity recognize conserved proteins, lipids and nucleic acids primarily associated with microbes. Though not a focus of the current chapter, these receptors can also recognize endogenous self-antigens, danger-associated molecular patterns (DAMPs), which include silica, uric acid or asbestos crystals, and

advanced glycation end products. Among the best characterized receptors are cell surface and endosomal Toll-like receptors, cell surface C-type lectins, and intracellular NOD-like receptors (NLRs).

TOLL-LIKE RECEPTORS (TLR)

TLR family members are single transmembrane receptors that recognize structurally conserved microbial products; further, activation of these receptors leads to production of pro-inflammatory and chemotactic cytokines that mediate recruitment of neutrophils, macrophages and lymphocytes to the site of infection. As shown in Figure 8-3, TLRs are located in cholesterol rich regions of the plasma cell membrane (lipid rafts) and in endosomes, and can recognize lipids, proteins or nucleic acids. Lipid-binding TLRs include TLR2, which forms heterodimers with TLR1 or TLR6 to bind lipopeptides, and TLR4/MD-2, which recognize the lipid A moiety of lipopolysaccharide (LPS). TLR5 and TLR11 recognize proteins, and are activated by bacterial flagellin (TLR5) or uropathogenic *E. coli* or *Toxoplasma gondii* profilin (TLR11). TLR3, TLR7, TLR8 and TLR9 are located on endosomal membranes and bind viral and bacterial nucleic acids. With the exception of TLR3, all TLRs stimulate the cells through the MyD88 common adaptor molecule, leading to NF- κ B translocation to the nucleus and expression of genes encoding pro-inflammatory cytokines and chemotactic cytokines (chemokines). TLR3 and TLR4 activate the TRIF pathway, which induces IRF3 transcription and production of type I interferons that mediate antiviral responses. TLR4 activation involves accessory molecules, including lipopolysaccharide (LPS)-binding protein and CD14, which combine to extract single endotoxin molecules from the outer membrane and form monomeric endotoxin, and MD-2, which is the receptor for the lipid A moiety of LPS. CD14 also chaperones TLR4 from the plasma membrane to endosomes in order to activate the TRIF pathway.

NOD-LIKE RECEPTORS

NOD-like receptors (NLRs) comprise an intracellular family of pathogen recognition molecules which also activate the NF- κ B complex, leading to expression of pro-inflammatory and chemotactic cytokines. NOD2 has been well characterized and shown to recognize

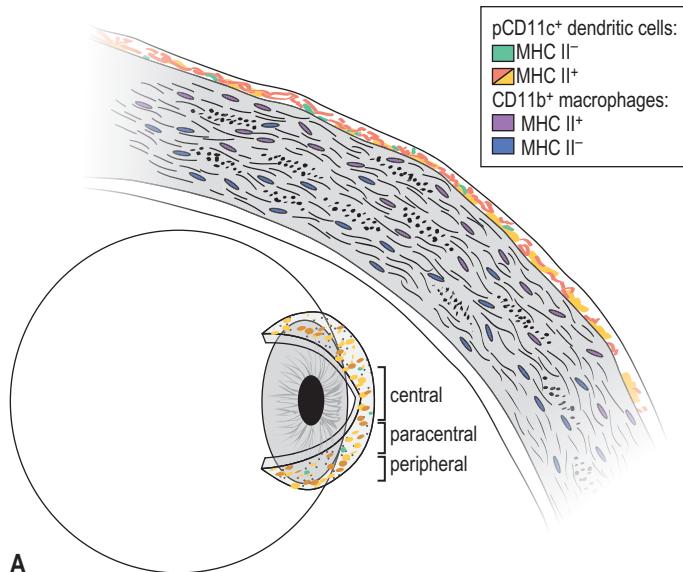
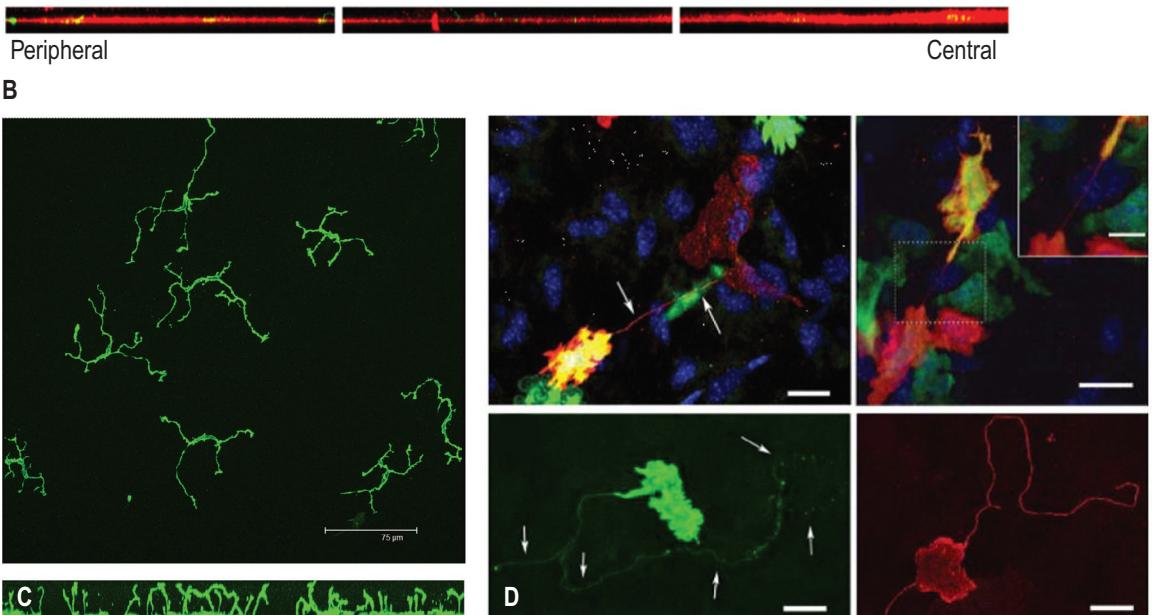
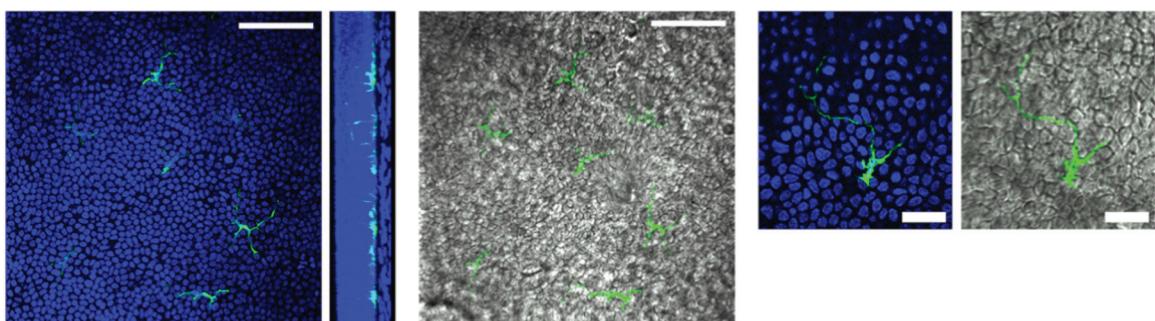


FIGURE 8-2 (A). Diagram and representative images of resident myeloid cells in the normal human cornea, including dendritic cells on the epithelial cell surface (sitting on the basement membrane). (From Hendricks, 2009.) **(B,C).** Dendritic cells in the corneal epithelium of a normal mouse cornea. **(D).** Representative images of MHC class II +ve cells (red) expressing fine, nanotubes (arrows) that appear to connect to other cells. These are chimeric mice receiving bone marrow (green cells). Yellow cells are donor, MHC class II positive. (From Chinnery, 2008.)



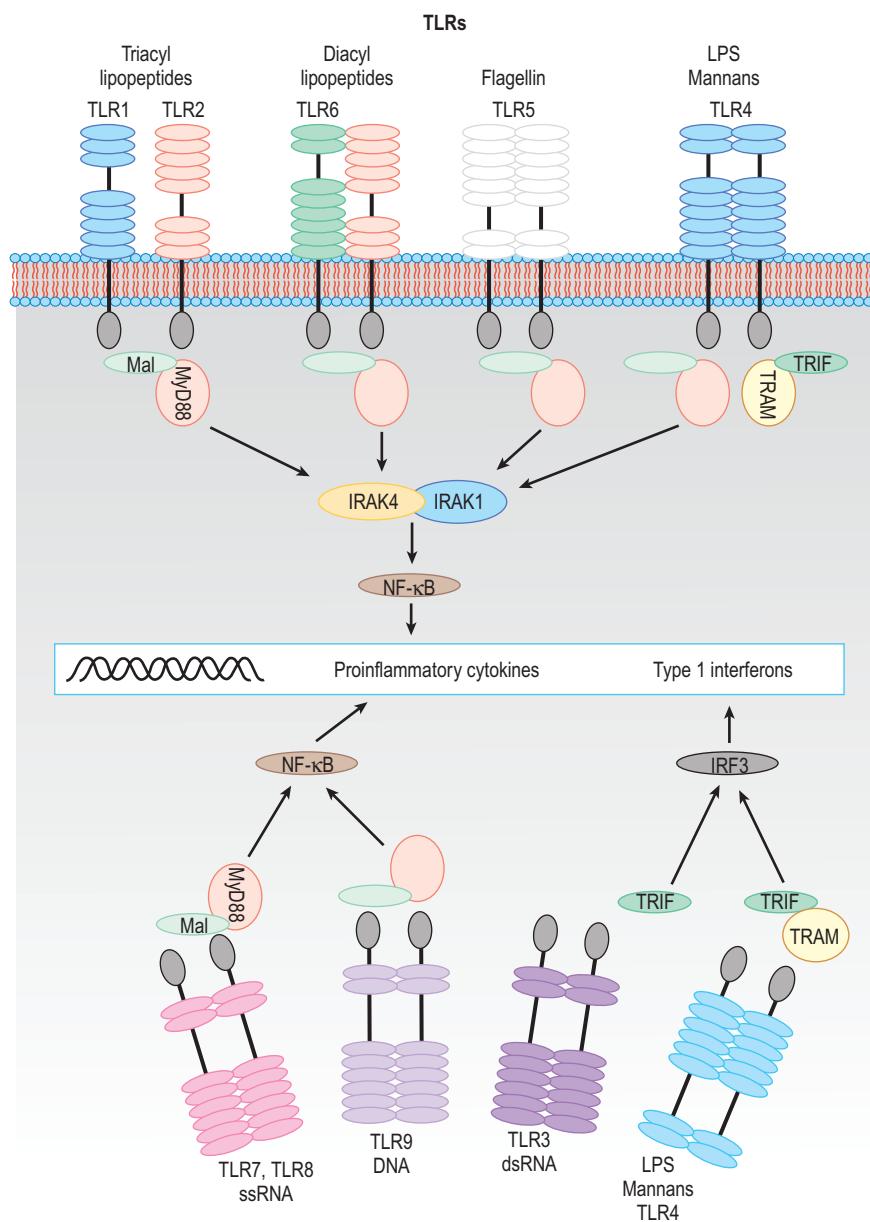


FIGURE 8-3 Toll-like receptors (TLR) respond to cell surface and endosomal microbial products: ss, single stranded; ds, double stranded. (From O'Neill et al., 2013.)

muramyl dipeptide on degraded bacterial cell wall peptidoglycan, and can therefore respond to invading Gram-negative and Gram-positive bacteria (Box 8-1). However, as Gram-positive bacteria have more peptidoglycan in the cell wall, NOD2 activation occurs following infection by staphylococci or streptococci.

NLRs include NLRP3, which recognizes bacterial toxins and crystals, and NLRC4, which recognizes flagellin of Gram-negative bacteria such as *Pseudomonas aeruginosa* (Fig. 8-4). Once activated, these NLRs form a large, multi-protein complex called an inflammasome, which activates caspase-1 and

cleaves IL-1 β , IL-18 and IL-33 from the inactive pro-form, to the bioactive, mature form of these cytokines. Prolonged activation of inflammasomes also leads to caspase-1-mediated cell death, termed pyroptosis.

BOX 8-1 NOD2 AND INFLAMMATION

Mutations in NOD2 are associated with susceptibility to autoimmune diseases that include Crohn's disease, which is a common and painful form of inflammatory bowel disease, and Blau's syndrome, which is manifest by multiple autoimmune disorders, including a severe form of uveitis. Although not completely understood, some of the polymorphisms of NOD2 result in hyperresponsiveness to MDP from otherwise harmless commensal bacteria in the intestine and skin.

C-TYPE LECTINS

In addition to bacterial products, host cells recognize fungal cell wall components by activation of C-type lectins on the cell surface (Fig. 8-5). Dectin-1 recognizes β -glucan, whereas Dectin-2 and Dectin-3 recognize α -mannans, and are activated after either clustering of Dectin-1 or heterodimerization of Dectin-2 and Dectin-3, which leads to production of pro-inflammatory and chemotactic cytokines.

NEUTROPHILS

Neutrophils are the first cells to respond to invading microbes. They are the most abundant leucocytes in the blood of normal individuals, comprising ~25% total white cells. Neutrophils in the blood constitutively express receptors for chemokines, specifically

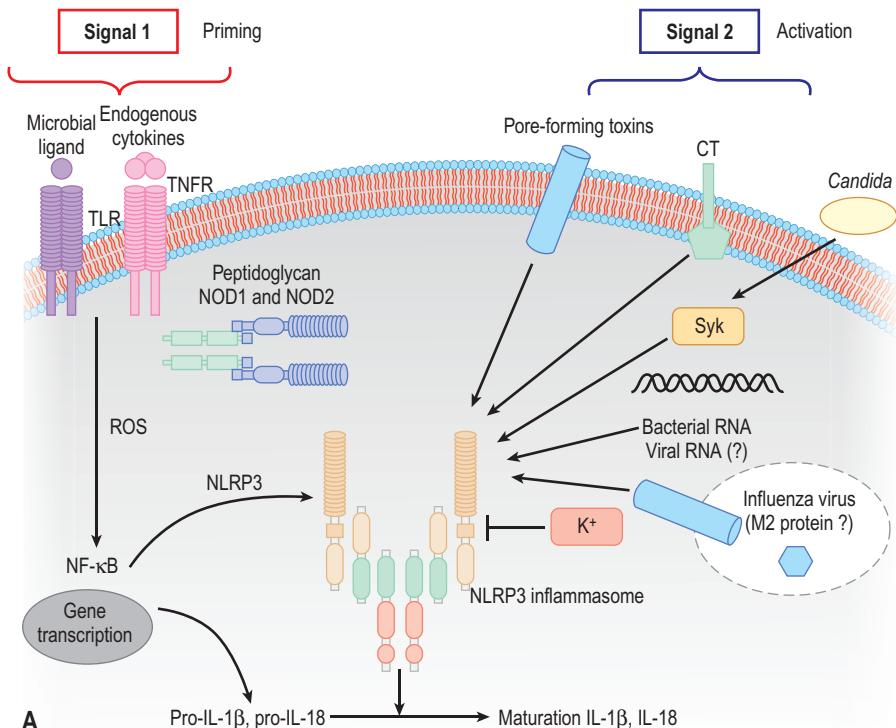


FIGURE 8-4 Nod-like receptors NLRP3 (A) and NLRC4 (B) activation of IL-1 β . Signal 1 TLR activation leads to gene expression of the IL-1 pro-form through NF- κ B. Signal 2 activates the NLRP3 or NLRC4 inflammasome complexes that activate caspase 1 and cleavage of IL-1 β to the bioactive 17 kDa form that is secreted from the cells. (From Nunez, Nat Imm Rev 2012.)

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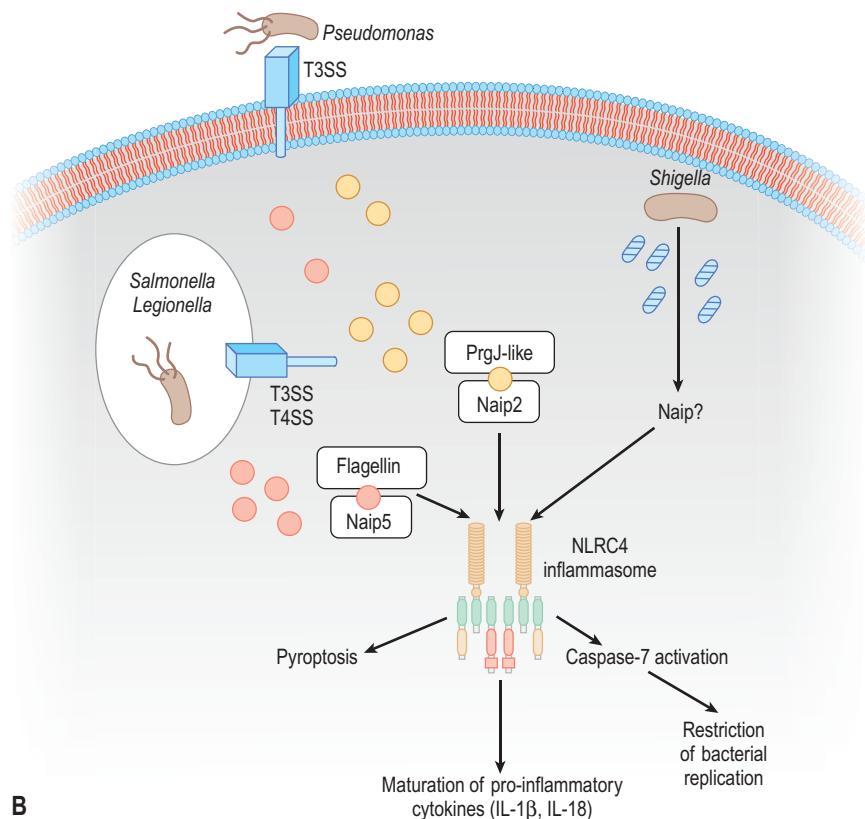


FIGURE 8-4, cont'd

CXCR1, which binds IL-8/CXCL8, and CXCR2, which binds CXCL1, CXCL2 and CXCL5 (although murine neutrophils express CXCR1, they do not produce IL-8 and primarily respond to CXCL1, 2 and 5 through CXCR2). Neutrophils also constitutively express adhesion molecules that bind to receptors on vascular endothelial cells (Box 8-2).

Neutrophils (and other leucocytes) are recruited from capillaries (including limbal blood vessels) to infected tissues through a sequence of events in which pro-inflammatory cytokines produced at the site of infection induce expression of adhesion molecules on vascular endothelial cells. Selectins mediate tethering, intracellular adhesion molecules (ICAM-1, -2 and VCAM-1) bind to integrins on the neutrophils, and chemokines stimulate their transmigration across the vascular endothelium (Fig. 8-6A). Once in the tissue, migration of neutrophils to the site of infection is also dependent on a chemokine gradient, especially in an avascular tissue such as the cornea,

BOX 8-2 CHEMOTACTIC CYTOKINES (CHEMOKINES)

CXC chemokines such as IL-8 are directly (and specifically) chemotactic for neutrophils, whereas pro-inflammatory cytokines such as IL-1 α , IL-1 β and TNF- α can induce increased expression of vascular cell adhesion molecules on capillary endothelial cells in the limbus. Expression of these adhesion molecules has a critical role in tethering, binding and facilitating neutrophil transmigration into the corneal stroma (Fig. 8-6). This is a general mechanism for extravasation of leucocytes such as lymphocytes, where expression of specific adhesion molecules and chemokines on vascular endothelial cells are recognized by specific receptors on different cell types, thereby coordinating the cellular recruitment to the tissue.

where cells need to migrate from peripheral limbal vessels.

The response of neutrophils to bacteria and yeasts involves phagocytosis, degranulation and neutrophil extracellular trap formation (NETs, Fig. 8-6B). If they

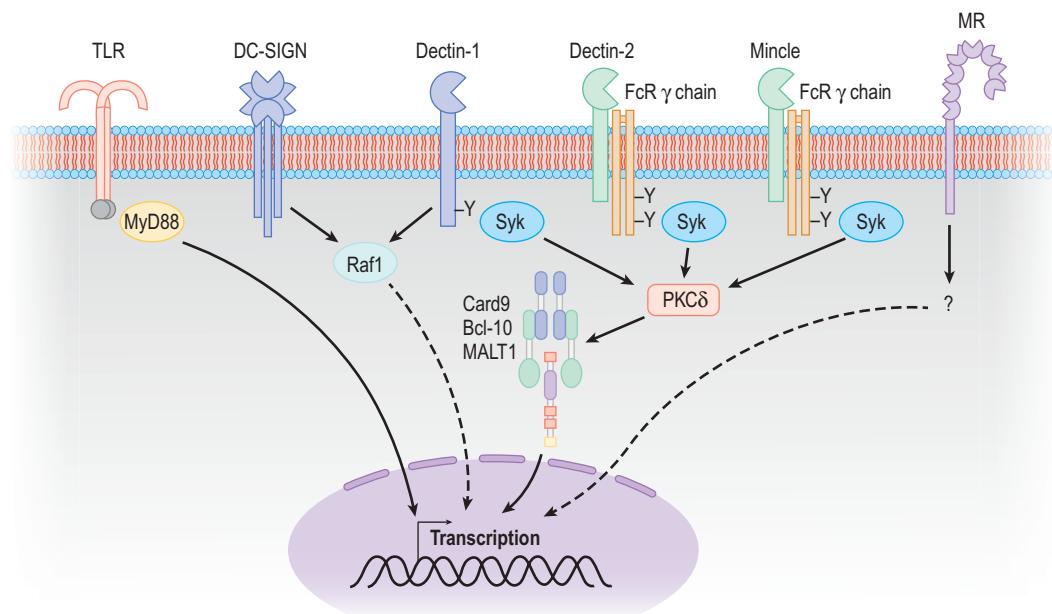


FIGURE 8-5 C-type lectins recognize fungal cell wall carbohydrates and mediate antifungal immunity, including phagocytosis and cell signalling. (From Brown, Nat Imm 2012.)

are unable to ingest the much larger fungal hyphae or *Acanthamoeba*, they can bind to the pathogen surface and release cytotoxic components, including reactive oxygen and nitrogen species, antimicrobial peptides, serine proteases and matrix metalloproteinases. They can also undergo NETosis, releasing these cytotoxic components in the context of a DNA/histone-rich net. Neutrophils also express pathogen recognition molecules and ligand activation stimulates production of pro-inflammatory and chemotactic cytokines that exacerbate neutrophil infiltration and recruit other cells such as macrophages and T cells. The role of macrophages and dendritic cells in the retina and uveal tract is discussed in Chapters 1 and 2. However, these mediators are also cytotoxic, and can contribute to loss of epithelial cells and keratocytes. Further matrix metalloproteinases can degrade the stromal collagen resulting in visual impairment and corneal scarring.

Adaptive immunity to microbial infection

Although most bacterial infections are extracellular and are dealt with rapidly by innate immunity, intracellular pathogens such as mycobacteria, protozoa and especially viruses survive longer and can induce an adaptive immune response. Thus they

stimulate T- and B-cell responses which control infection and regulate the severity of infection. For example, CD4 cells play an important regulatory role in herpes simplex keratitis, and CD8 cells regulate herpes latency in trigeminal ganglia. Also, long-term exposure to airborne fungal spores induces systemic T-cell responses that likely regulate the severity of subsequent corneal infection. Toxoplasmosis and onchocerciasis are examples of chronic infections in which adaptive immunity plays an important role in determining disease severity and outcome. Other factors that affect adaptive immunity and increased susceptibility to microbial infections include immunosuppressive drugs and HIV infection (see also Ch. 7, p. 437).

Ocular infections worldwide

CONTACT LENSES

Contact lenses are a major risk factor for microbial keratitis. Approximately 134 million people worldwide wear contact lenses, and lens wear is the most common risk factor for corneal infections in the industrialized world. Long-term contact lens wear inhibits epithelial cell proliferation and migration and suppresses limbal stem cell production of basal corneal

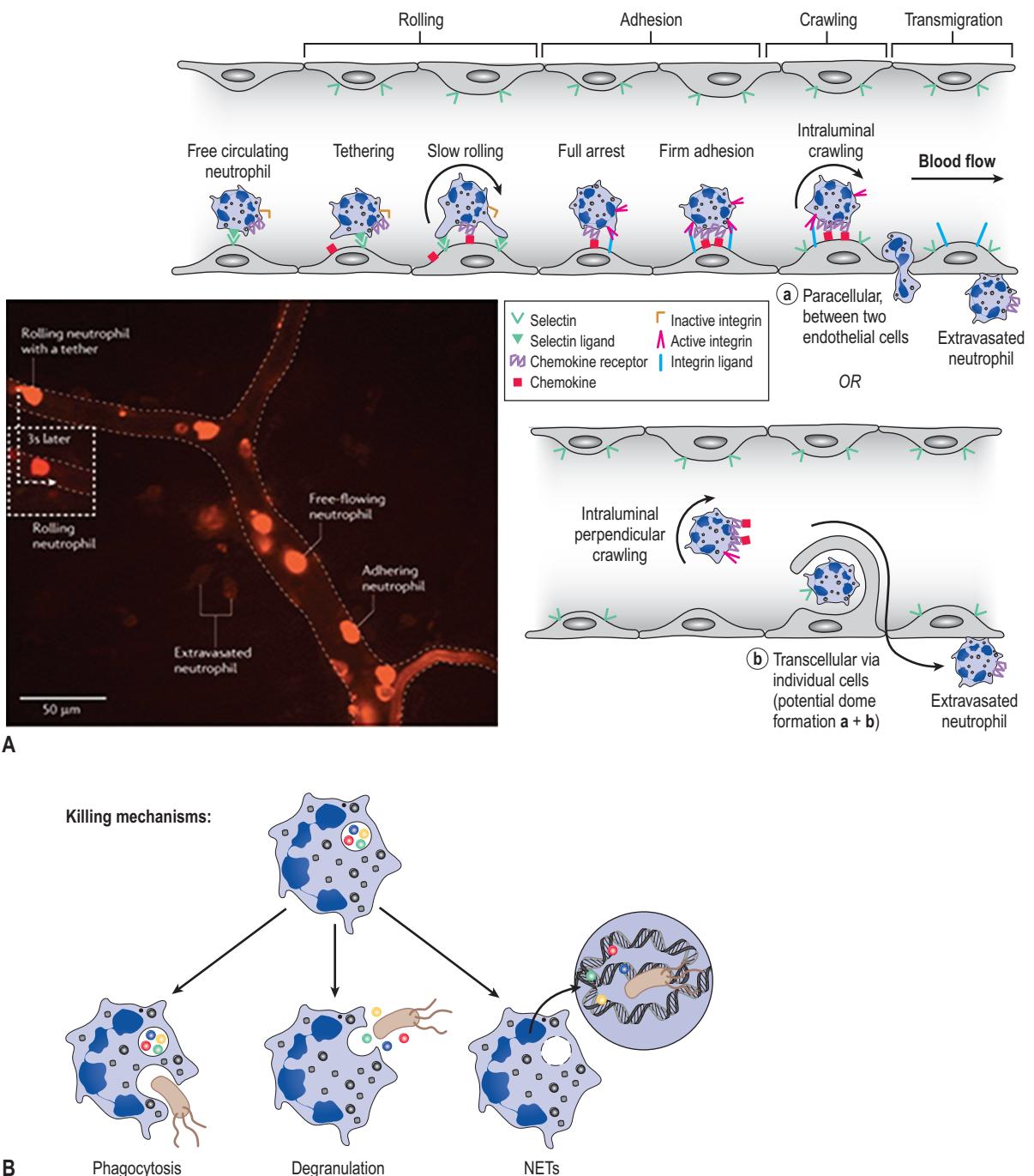


FIGURE 8-6 Neutrophil infiltration and activation. (A) Multistep process of neutrophil migration from capillaries to sites of infection. (B) Antimicrobial activity of neutrophils occurs: phagocytosis, degranulation and release of reactive oxygen and proteolytic enzymes. A third mode of killing is formation of neutrophil extracellular traps (NETs) where neutrophils release DNA, histones and other microbialic proteins as they undergo necrosis. (From Kubes, Nat Rev Immunol 2013.)

epithelial cells. Soft contact lenses, especially extended wear lenses, alter the microenvironment of the ocular surface by reducing the flow and effectiveness of tears, and trapping microbes at the cell surface. Further, poor hygiene in relation to lens cases and lens care solutions facilitates growth of bacteria and fungi, often forming multi-organism biofilms which are more resistant to antibiotics and lens care solutions. In this outbreak, one Lens care solution was found to be ineffective killing clinical and environmental isolates of *Fusarium*, resulting in over 300 cases of keratitis in the USA, Europe and Singapore in 2005/2006. Similarly, the increased incidence of *Acanthamoeba* keratitis in Chicago in 2007 was initially thought to be due to a lens care solution, resulting in withdrawal of the product; however, later findings showed the outbreak was due instead to reduced chlorination of the Chicago river.

VIRAL INFECTIONS OF THE EYE

There are several viruses that infect the eye, including adenovirus, which causes epidemic keratoconjunctivitis (serotypes 3, 7, 8 and 19), and pharyngoconjunctival fever (serotypes 1, 2, 3, 5, 7 and 14). Human papillomavirus causes epithelial proliferation resulting in formation of benign papilloma (warts) on the lids and conjunctiva. Herpes zoster ophthalmicus causes an extremely painful corneal infection as well as extensive involvement of the skin in the same dermatome served by the ophthalmic division of the trigeminal nerve (see Ch. 1, p. 74) and occurs following resurgence of latency from the nerve. However, globally, herpes simplex virus 1 is the most common cause of ocular viral infections.

Herpes stromal keratitis

Herpes simplex virus 1 (HSV-1) is among the most common causes of ocular infections worldwide, as evidenced by high seroprevalence rates in industrialized and developing countries. Infection is most often asymptomatic, but oral and genital lesions are common manifestations of infection. However, HSV-1 can also cause herpes stromal keratitis (HSK) and can infect the eyelids, conjunctiva, cornea, uveal tract and retina. As with oral and genital infection, HSK can occur repeatedly and cause progressive corneal scar formation.

Following primary infection of the corneal epithelium, the virus enters corneal neurones and migrates to the trigeminal ganglia, which provides sensory innervation to the cornea (see Ch. 1, p. 14). HSV-1 then enter a latent state where viral DNA is present in neurones but no infectious virus is produced. Following exposure to ultraviolet light, the virus can be reactivated and axonally transported into the corneal epithelium, which is highly innervated. Immune suppression also leads to reactivation, indicating an essential role of the host immune response to maintain latency (discussed below).

Primary infection. TLR9 expression on corneal epithelial cells is important in the initial activation by HSV-1 infections and can be induced by HSV-1 DNA alone. These cells produce type 1 interferons (IFN- α/β), which inhibit viral replication. Natural killer cells are also recruited to the corneal stroma and produce IFN- γ and tumour necrosis factor α (TNF- α), which activate macrophages. CD4 $^+$ Th1, Th17 and T regulatory (Treg) cells also play an important role in limiting the primary response, with the suppressive activity of Tregs balancing the pro-inflammatory and pro-angiogenic activity of Th17 cells.

Angiogenesis and lymphangiogenesis. Blood and lymph vessel formation of the normally avascular cornea is a characteristic feature of HSK, and is important to initiate an adaptive immune response to the virus as lymphatic vessels transport viral antigens to draining nodes, and blood vessels transport mature HSV-1-specific T cells to the cornea. However, angiogenesis also impairs visual acuity, and neovascularization is tightly regulated by selective production of vascular endothelial cell growth factors and receptor antagonists.

Latency. In the course of primary corneal infection, the virus enters the axons of sensory neurones and is transported in a retrograde manner to the cell bodies, which are located in the trigeminal ganglia, where viral DNA is inserted into the nucleus. This episomal stage of the virus is maintained by expression of HSV-1 latency-associated transcripts. HSV-1 specific CD8 $^+$ T cells are found in close association with infected neurones and can form immunological synapses in which the T-cell receptor and CD8 molecules are in the same

proximity as class I receptors on the neurones (Fig. 8-7). Although IFN- γ is important, release of perforin in CD8 $^{+}$ cell lytic granules is essential to maintain latency, which occurs without killing the neurones.

Viral retinitis

Viral retinitis is an important cause of blindness and visual impairment, especially in AIDS patients. The most common causes of retinitis include herpes simplex virus, varicella zoster virus and human cytomegalovirus (CMV). Less common but significant causes include the lymphocytic choriomeningitis virus

(LCMV), Epstein–Barr virus, rubella, measles, West Nile virus, dengue and the chikungunya virus, which is considered an emerging pathogen.

CMV is a double-stranded DNA virus in the herpes family that infects an estimated 40–70% of the world's population. Primary CMV infection is controlled by CD8 $^{+}$ T cells, and as with all herpes viruses, the virus remains persistent over the lifetime of the host. CMV is mostly pathogenic following congenital infection, and can cause chorioretinitis, hydrocephalus and microcephaly. LCMV is a single-stranded RNA arenavirus, and humans are infected

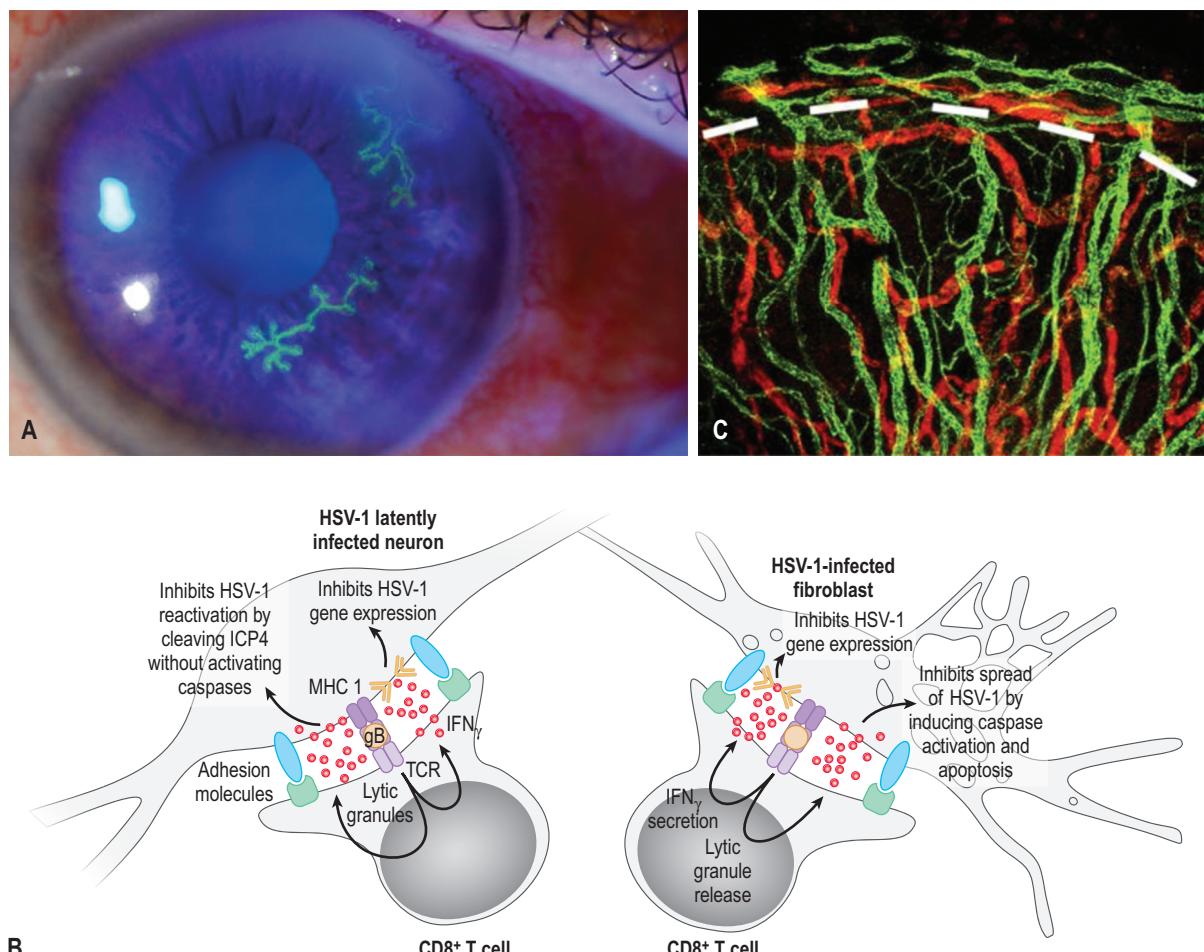


FIGURE 8-7 Herpes simplex keratitis. (A) Herpes simplex keratitis (HSK) showing characteristic dendritic-shaped lesion. HSK is also characterized by pronounced corneal angiogenesis. (B) HSV-specific CD8 $^{+}$ T cells, interactions with HSV-1 latently infected neurones and lytically infected fibroblasts. IFN- γ and lytic granules inhibit HSV-1 reactivation from latency through a non-lytic mechanism; therefore virus emerges only when T-cell function is compromised. However, lytic granules induce apoptotic cell death of infected fibroblasts. (From Hendricks, Science 2008.). (C) Confocal image of representative cornea from animal latently infected with HSV-1 showing LYVE-1+ lymphatic vessels (red) and CD31+ blood vessels (green) at day 30 post-infection. Limbus is above and cornea is below the dashed line; Bar, 500 μ m. (From Wuest and Carr, J Exp Med 2010)

by inhalation or ingestion of particles contaminated with mouse faeces, urine or saliva. Experimental models of CMV and LCMV retinitis in which the virus is injected into the vitreous show rapid dissemination to the lymph nodes and spleen, production of virus-specific T cells and invasion of the retina by cytotoxic T cells, which can cause significant retinal disease.

HIV patients are at higher risk for CMV, and the advent of highly active antiretroviral therapy (HAART) has reduced the incidence of CMV retinitis; however, a long-term study of CMV retinitis patients with AIDS taking HAART showed that these patients still have an increased risk for progression of retinitis with complications and visual loss.

BACTERIAL INFECTIONS OF THE EYE

Ocular surface

Bacteria causing conjunctivitis and blepharitis include *Streptococcus pneumoniae* and *Staphylococcus aureus*. These infections cause inflammation and irritation but do not affect vision and are relatively easily treated. In contrast, bacterial infections of the cornea are a major cause of blindness and visual impairment worldwide. Once bacteria gain entry to the corneal stroma they can replicate quickly and induce a pronounced neutrophil infiltrate that contributes to vision loss.

Pseudomonas aeruginosa virulence factors. *P. aeruginosa* is the most common cause of bacterial keratitis worldwide. These Gram-negative bacteria are ubiquitous in fresh water, and contact lens wear and corneal injury are the major risk factors. Although *P. aeruginosa* induces a rapid host response in the cornea, most clinical isolates express virulence factors that counter this response and facilitate bacterial survival. Although these bacteria have a broad spectrum of virulence factors, *P. aeruginosa* produces exotoxins as part of the type III secretion system (T3SS) where they play an essential role in bacterial survival *in vivo*. As shown in Figure 8-8, the T3SS includes a needle-like organelle that injects exotoxins directly into host cells. The needle structure protrudes from the surface of the bacterium and forms a pore in the host cell membrane through which ExoS, ExoT or ExoU are injected. ExoU causes rapid cell lysis and severe corneal disease due to its phospholipase activity, whereas ExoS and ExoT are related proteins that have ADP ribosyl

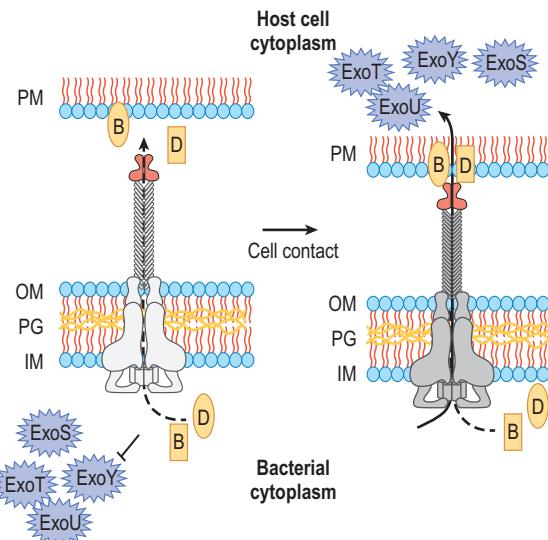


FIGURE 8-8 *Pseudomonas aeruginosa* type III secretion system (T3SS). The T3SS spans the bacterial cell envelope including the inner membrane, (IM), outer membrane (OM) and peptidoglycan layer (PG). Following contact with the host cell plasma membrane (PM), the translocator proteins PopB (B) and PopD (D) form a pore-forming complex with the needle tip (PcrV, red), and the effector proteins are exported through the bacterial cell envelope and the needle complex, and into the cytosol of the targeted cell. To date, four effector proteins have been described in *P. aeruginosa*, ExoS, ExoT, ExoY, and ExoU, although the exact complement of effectors varies among strains and clinical and environmental isolates. Effector proteins are either directly cytolytic (ExoU) or modulate cell function to facilitate bacterial survival in the host cell. (Diagram by A. Rietch, from Pearlman et al., 2013.)

transferase and GTPase activating protein (GAP) enzymatic activity that inhibit cell migration and phagocytosis by blocking cytoskeleton activity. Animal models show that expression of specific T3SS exotoxins and the needle structure is essential for bacterial survival and replication in the cornea. Although almost all clinical and environmental isolates express either ExoS or ExoU, they do not produce both exotoxins. Strains expressing ExoU but not ExoS have been designated cytotoxic strains, whereas ExoS-expressing strains are considered invasive as they can replicate in corneal epithelial cells without causing rapid lysis. ExoS and ExoT facilitate bacterial survival in neutrophils by inhibiting production of reactive oxygen species.

The host response to *P. aeruginosa* in the cornea has been extensively studied using murine models, and although adaptive immunity can be demonstrated at later stages of infection, innate immune responses are

essential to regulate the rapid growth of these bacteria in the cornea. Experimental evidence supports the concept that *P. aeruginosa* LPS and flagellin activate TLR4 and TLR5 on resident macrophages, which produce CXC chemokines and pro-inflammatory cytokines that mediate neutrophil recruitment from limbal vessels to the corneal stroma. Inhibition or blockade of TLR4/5, intracellular signalling pathways, IL-1R, or neutralization of IL-1 α/β or CXC chemokines impairs neutrophil infiltration and function, allowing the bacteria to survive, which results in more severe corneal disease. Expression of these mediators is also elevated in corneal exudates from infected individuals. Suppressor molecules such as members of the TLR family, neuropeptides such as vasoactive intestinal protein (VIP) and even high concentrations of flagellin also have a suppressive effect on the host inflammatory response, although the mechanisms have yet to be fully characterized. Ultimately, a therapeutic approach involving targeted anti-inflammatory rather than corticosteroids may have potential application when given together with antibiotics.

Streptococcus pneumoniae. *Streptococcus pneumoniae* is a common cause of bacterial keratitis, especially in developing countries. As a normal commensal in the lungs and upper respiratory tract, *S. pneumoniae* is frequently isolated from the conjunctival sac of healthy individuals, and corneal infection is likely associated with traumatic injury. *S. pneumoniae* produces several exotoxins; however, the most potent is pneumolysin, which can directly form pores in the host cell membrane, leading to rapid leakage and cell death. Pneumolysin is released as a monomer but forms a multimer on the host cell membrane, leading to pore formation and rapid cell death. Although some serotypes are more commonly found in clinical isolates, there is no apparent correlation between the serotype expressed and severity of infection.

Staphylococcus aureus. *Staphylococcus aureus* causes less severe disease than *Streptococcus* or *Pseudomonas*, and is associated with peripheral ulcer formation. However, antibiotic-resistant isolates, including meticillin-resistant *Staphylococcus aureus* (MRSA), make treating this infection more challenging. *S. aureus* also produces several virulence factors,

including haemolysins α , β and γ , which facilitate bacterial survival *in vivo*.

Contact lens-related corneal inflammation. Other organisms, including *Serratia marcescens* and coagulase-negative staphylococci such as *S. epidermidis*, can cause a mild corneal inflammation associated with contact lens wear, although secretion of the serralysin toxin may also contribute to disease severity. In many cases of contact lens-related inflammation such as contact lens-associated red eye (CLARE) and contact lens peripheral ulcers (CLPU) bacteria cannot be cultured, although corneal infiltrates that are most likely neutrophil-rich are present. As CLARE and CLPU can be replicated in experimental models using TLR agonists such as LPS, it is likely that products of dead and degenerating bacteria activate TLRs on resident corneal cells, leading to neutrophil recruitment to the stroma.

Bacterial endophthalmitis

Bacterial endophthalmitis is characterized by a pronounced inflammatory response in the vitreous, resulting in extensive tissue damage and vision loss. Bacteria can invade the posterior segment of the eye from exogenous infection, following ocular surgery (most commonly cataract) or after traumatic or penetrating injury. Endophthalmitis can also be initiated haematogenously via the retinal vasculature from infection elsewhere in the body. Recent studies also indicate that diabetes mellitus can increase the likelihood of bacterial endophthalmitis due to increased permeability of the blood-retinal barrier.

Coagulase-negative staphylococci including *S. epidermidis* cause the majority of postoperative endophthalmitis, although streptococci, *S. aureus* and enterococci such as *Enterococcus faecalis* and *Propionibacterium acnes* can also cause infection. Staphylococci, streptococci, *E. coli* and *Klebsiella pneumoniae* are among the most common causes of exogenous endophthalmitis, whereas *Bacillus cereus* is more likely to be isolated from cases of post-traumatic endophthalmitis. Animal models show studies indicate that bacterial cell wall components activate TLRs on Müller cells, microglia and macrophages in the neural retina and the inner retinal layer, in addition to hyalocytes in the vitreous. Although most infections respond to antibiotics, retinal damage and visual impairment

occur as a result of bacterial toxin production and cellular infiltration.

FUNGAL INFECTIONS OF THE EYE

Yeast and moulds can infect the cornea, causing pronounced long-lasting infections that are very difficult to treat. *Candida albicans* is the most common yeast causing corneal infections, most often following cataract surgery or corneal and endothelial transplants. *Candida* is also associated with therapeutic contact lenses, steroid use or immunosuppressive disease and corneal surgery. In these dimorphic organisms it is the yeast stage that infects the cornea and then germinates to form pseudohyphae in the corneal stroma.

However, worldwide, it is moulds rather than yeast that are the predominant cause of fungal keratitis, with ocular trauma being the major risk factor. As moulds such as *Aspergillus* and *Fusarium* species are plant saprophytes and grow abundantly on crops, the highest incidence and prevalence of fungal keratitis is in agricultural regions, especially during harvest season when there is an abundance of airborne spores. On a global scale, fungal keratitis accounts for ~65% of all corneal ulcers and fungi are more common than bacteria as the cause of ocular infections. *Fusarium solani* and *F. oxysporum* are the main aetiological agents of fungal keratitis followed by *Aspergillus* species (*A. flavus*, *A. fumigatus*), while *Curvularia*, *Alternaria* and *Penicillium* species are less common causes.

Corneal infection with *Aspergillus* and *Fusarium*

Spores, or conidia, are produced in large numbers on conidiophores attached by stalks to live or decaying plants (Fig. 8-9). Conidia are dispersed by wind, and corneal injury by airborne plant material or by insects carrying plant material can introduce multiple spores into the corneal stroma (in rural India, whiplash injury by a cow tail is a not uncommon cause of infection). Once in the corneal stroma, conidia germinate and hyphae can penetrate throughout the corneal stroma. The hyphal tips contain multiple proteases, including collagenases that facilitate migration of the hyphae throughout the corneal stroma. If not treated in time, the hyphae will also penetrate into the anterior chamber, where they stimulate a pronounced neutrophil infiltrate seen clinically as a hypopyon, which limits further penetration to the

posterior segment. Although rare, fungal endophthalmitis is very difficult to treat and often requires removal of the entire globe.

Contact lens-associated fungal keratitis. In contrast to rural agricultural areas, contact lens wear is the primary risk factor in industrialized countries. The impact of this disease on the industrialized world was illustrated by several hundred cases in the USA, Western Europe and Singapore during the 2005/2006 outbreak of contact lens-associated fungal keratitis. *Fusarium solani* and *F. oxysporum* were the cause of this outbreak, which was resolved in large part by full-thickness keratoplasty, and occasionally by removal of the entire globe. In contrast to trauma-induced fungal keratitis in which conidia directly cause infection, contact lens-associated fungal keratitis is more likely initiated by hyphae. Lenses and lens cases tested during this outbreak not only carried fungal spores but also contained *Fusarium* in the form of biofilms. Fungal biofilms are complexes of hyphae and extracellular carbohydrates that have increased resistance to antimicrobial agents and are an important cause of infection associated with implants and catheters. Following contact with the ocular surface, hyphae in the biofilm can penetrate into the cornea stroma through minor epithelial abrasions where they establish infection.

Treatment of fungal keratitis. In contrast to antibiotics used to treat bacterial keratitis, antimycotic agents are much less effective as hyphae can penetrate into the deeper stroma, thereby limiting accessibility. Topical natamycin or voriconazole are effective if given early after infection, but in general fungal keratitis is notoriously difficult to treat, especially after the hyphae penetrate deeper stromal layers. Therefore, full-thickness keratoplasty is required; however, transplant failure is not uncommon if the fungus has not been completely cleared (high-risk grafts, see Ch. 4). Even in milder cases that respond to antifungal treatment, resolution is associated with fibrosis, resulting in corneal opacity and visual impairment.

Pathogenesis of fungal keratitis. Although *Aspergillus* and *Fusarium* produce mycotoxins that are important in plant disease, there is no direct evidence that they contribute to the pathogenesis of keratitis. Most

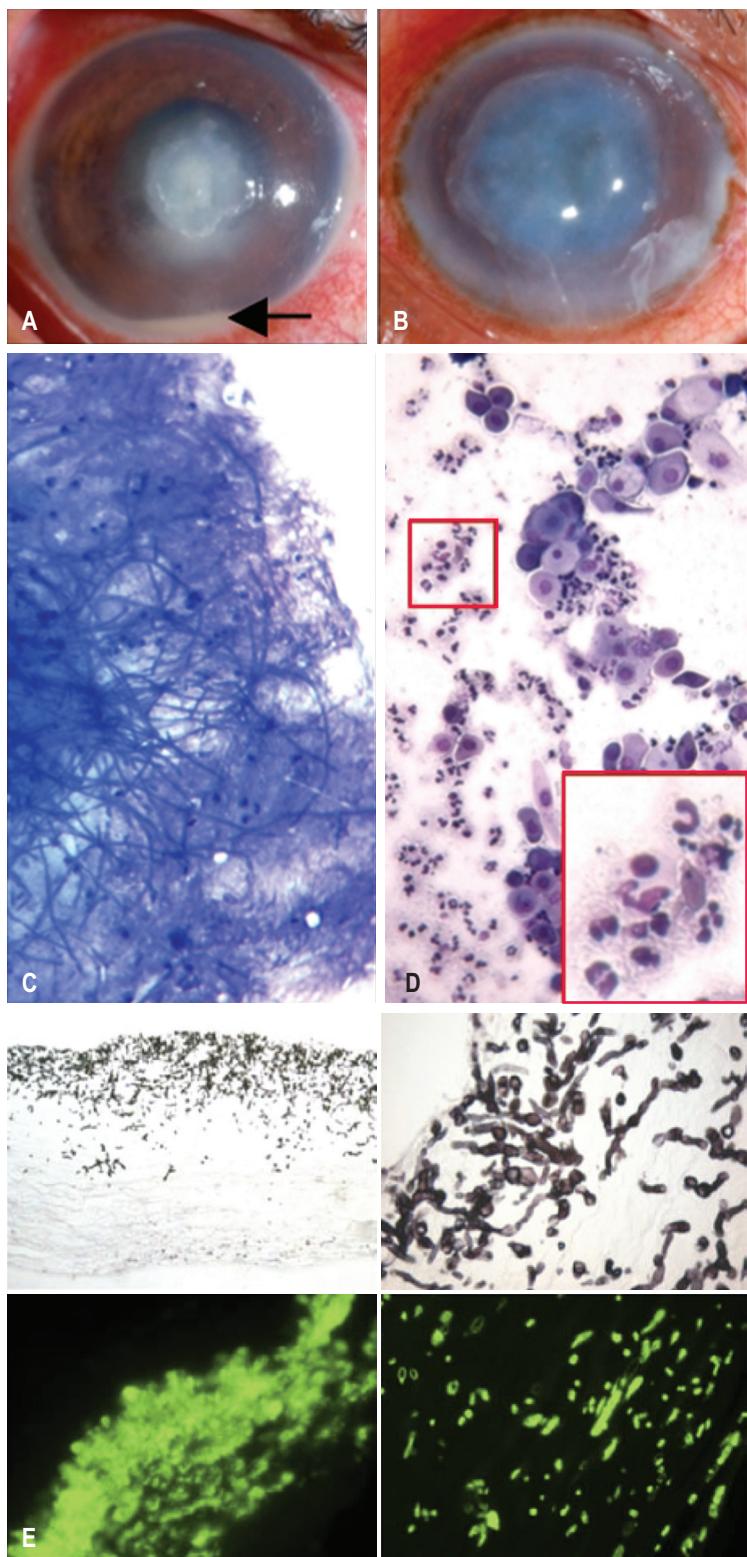


FIGURE 8-9 Fungal keratitis caused by *Fusarium* and *Aspergillus* moulds. *Aspergillus flavus* (A) and *Fusarium solani* (B) conidiophores, which contain multiple conidia (spores) on their stalks, can penetrate the corneal stroma following ocular trauma with plant material. Conidia germinate and form hyphae that can be detected in corneal ulcer smears after Giemsa staining (C). *Aspergillus*. Neutrophils are the predominant cell type in corneal ulcers (D). Hyphae penetrate the corneal stroma and express the cell wall component β -glucan (E) upper panels: silver stain of *Aspergillus*-infected cornea post-transplant. Lower panels stained with antibody to β -glucan. (Parts C–E from Karthikeyan et al., 2011.)

evidence points to the role of the inflammatory response as a major factor in causing tissue damage. The host response to fungal pathogens involves recognition of cell wall components, including β -glucan and α -mannose, which bind to the C-type lectins Dectin-1 and Dectin-2 on the host cell membrane (β -glucan also binds to CD18 on neutrophils). Figure 8-9 shows β -glucan expression in an infected post-transplant cornea. Activation leads, through a distinct signalling pathway, to the production of pro-inflammatory and chemotactic cytokines. However, these responses also activate IFN- γ and IL-17 producing T cells (Th1 and Th17 cells), in addition to IL-17 production by neutrophils. IL-17 activates receptors on epithelial cells and fibroblasts to produce cytokines and exacerbates the host response.

Corticosteroids. Treatment options for inflammation are limited to corticosteroids and non-steroidal anti-inflammatory agents such as ciclosporin. Steroid use is particularly risky, because if given before the infection is controlled, there will be unrestricted hyphal growth and tissue destruction. Thus, it will be important to develop more targeted and effective anti-inflammatory agents. Other approaches will be to target essential pathways of survival such as antioxidant and iron scavenging pathways, which are effective in controlling disease in animal models of disease.

PROTOZOAN CAUSES OF OCULAR DISEASE

Acanthamoeba keratitis

Acanthamoeba castellani and other species is an emerging cause of corneal infections, causing severe pain and visual impairment and forming a characteristic lesion (Fig. 8-10). Infection is often associated with contact lens wear and occurs when the cornea is exposed to the relatively high concentration of free-living amoebae that are present in water supplies. The increased incidence of keratitis was noted in relation to changes in water treatment such as in an outbreak in Chicago in 2007. The US Centers for Disease Control and Prevention (CDC) continues to monitor cases of *Acanthamoeba* keratitis.

Acanthamoeba is a free-living protozoan that exhibits a biphasic life cycle as a vegetative trophozoite and a physiologically inert cyst form. Trophozoites are the active stage that exist as free-living amoebae and feed

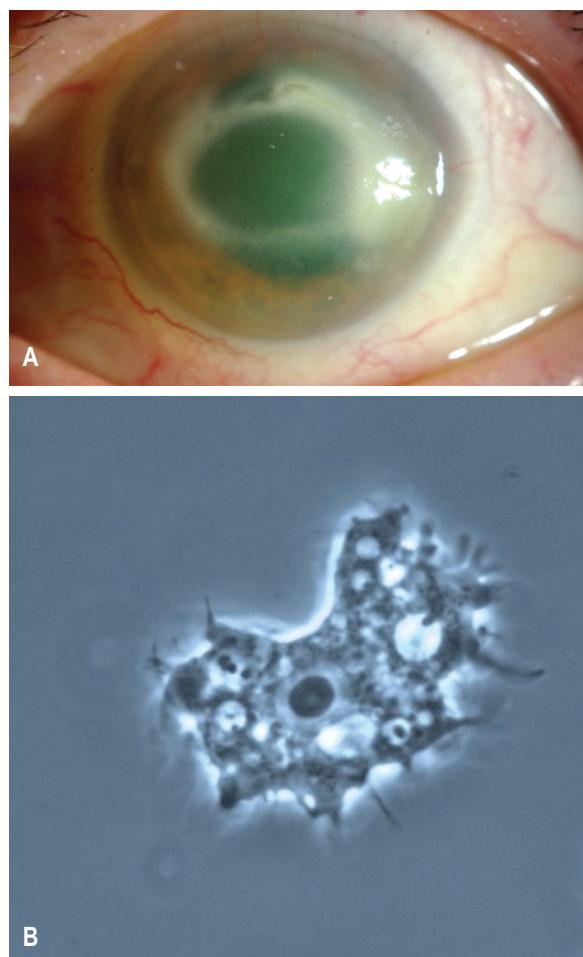


FIGURE 8-10 (A) *Acanthamoeba* keratitis showing characteristic radial neuropathy. (B) *Acanthamoeba* trophozoite. (Part A from Tu and Joslin, 2012.)

on bacteria and fungi. As noted below, some species of ingested bacteria survive, notably *Legionella* species, and replicate in the trophozoites, which can behave as 'Trojan horses' when they infect the cornea, releasing bacteria into the tissue. Under adverse physiological conditions, the trophozoites encyst and the cyst wall is extremely resistant to environmental agents, irradiation and the host immune system. These infections are therefore extremely difficult to treat and require the use of highly cytotoxic drugs such as biguanides and chlorhexidine (propamidine isothionate, chlorhexidine digluconate and polyhexamethyl biguanide) given over months. These drugs are extremely

cytotoxic, and can cause severe ocular surface damage. Also, they are not always effective and the patients still require corneal transplantation. *Acanthamoeba* keratitis is most prevalent among contact lens wearers, although it can also be introduced into the corneal stroma by ocular trauma. *Acanthamoeba* has been recovered from soil, air, chlorinated swimming pools, hot tubs, tap water and contact lens solutions. Wearing contact lenses when showering or swimming in fresh water ponds increases the risk of infection. Although the underlying conditions for infection are not known, it likely involves a combination of having trophozoites trapped under the contact lens.

***Acanthamoeba* virulence factors.** The first step in the pathogenesis of infection is adhesion to the ocular surface, which is mediated by carbohydrate–lectin interactions. *Acanthamoeba* expresses a 400 kDa transmembrane mannose-binding protein (MBP) composed of 130 kDa subunits and has characteristics typical of a cell surface receptor. MBP is associated with virulence as it is expressed at higher levels by pathogenic strains, and immunization of experimental animals results in protection from infection. A second virulence factor is the mannose-induced protein (MIP) 133, which is a serine protease with collagenase activity and cytopathic effect on corneal epithelial cells.

Host response to *Acanthamoeba*. There are very few studies on the host response to this pathogen; serum antibodies to *Acanthamoeba* antigens are commonly detected in uninfected individuals, which is consistent with environmental exposure by inhalation or ingestion. In animal models IgA in the tears is protective and trophozoites can be killed *in vitro* by neutrophils or macrophages. Both cell types have been detected by analysis of post-transplant corneas; however, as these corneas are examined only after long-term treatment prior to keratoplasty, it is difficult to determine which cells are killing the trophozoites during infection. It seems reasonable to postulate that activation of pathogen recognition receptors stimulates infiltration of these cells to the cornea. These have not been identified, but could be a result of collagen breakdown products such as Pro-Gly-Pro tripeptides, which activate neutrophil chemokine receptors directly. In addition, release of endogenous bacteria

could trigger this pathway and induce an inflammatory response. This concept does not address the chronic infection seen in this disease, which most likely relates to the inability to kill *Acanthamoeba* cysts.

***Acanthamoeba* as a ‘Trojan horse’.** As some bacteria are not killed by *Acanthamoeba*, trophozoites inadvertently act as a host of intracellular bacteria that are also associated with human disease. For example, *Acanthamoeba* is a natural host of *Legionella* spp. (mostly *L. pneumophila*), which is associated with legionnaires disease; however, *Acanthamoeba* keratitis clinical isolates also harbour *Pseudomonas* (mostly *P. aeruginosa*), mycobacteria and *Chlamydia*, and *L. pneumophila* and *P. aeruginosa* survival in *Acanthamoeba* is dependent on expression of bacterial virulence factors.

Toxoplasmosis

Toxoplasma gondii is the most common cause of infectious retinochoroiditis worldwide, causing up to 50% of all posterior uveitis cases in some countries in the industrialized world, with higher levels in developing countries. *T. gondii* is an obligate intracellular protozoan parasite and is among the most ubiquitous pathogens worldwide, with >70% of the global population seropositive. This parasite has been found in almost all mammals and can cause infections in domesticated farm animals (especially pigs) and in pets. Cats are the definitive hosts and are the only mammals that support the sexual stage of this parasite (Fig. 8-11). Transmission therefore occurs by ingesting the oocysts in food that has been contaminated with cat faeces, although individuals can also be infected following ingestion of tissue cysts (bradyzoites) in undercooked meat or by maternal transmission during pregnancy (often in the third trimester). In humans and other intermediate hosts, the tachyzoite form can invade and undergo rapid asexual replication in any nucleated cell. Following oral infection, this occurs in enterocytes, where they rapidly replicate and cause cell death. *T. fondii* can cross the epithelial barrier to infect circulating monocytes, and are disseminated to multiple organs including the brain and the retina. Despite the presence of a functional innate immune response, the parasites evade the immune system in these immune-privileged sites but remain semi-dormant as viable bradyzoites within sequestered tissue cysts. However,

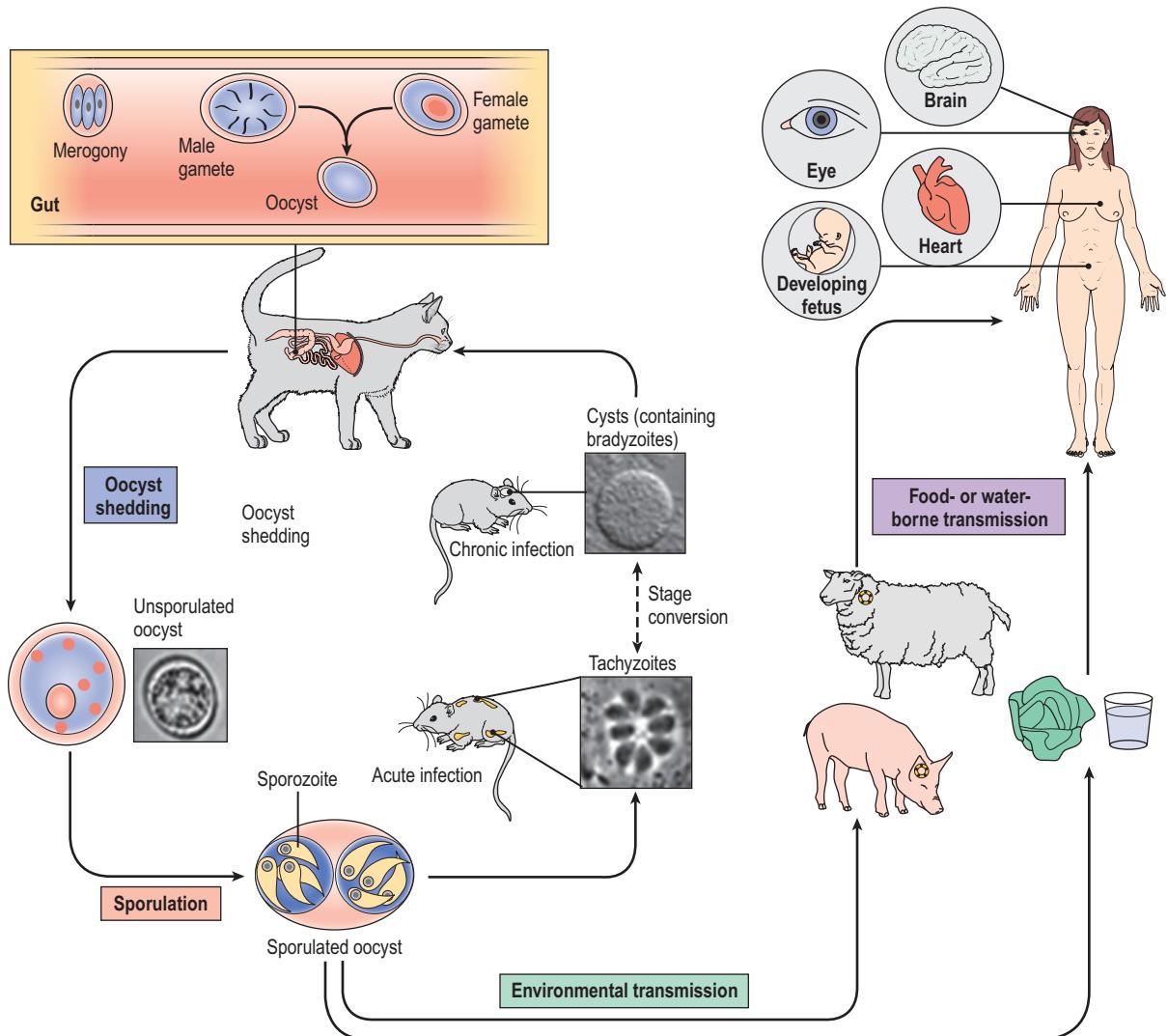


FIGURE 8-11 The complex life cycle of *Toxoplasma gondii*. Humans are accidental hosts and represent a dead end to continuing the life cycle. Cats are the only definitive host permissive for sexual replication in which male and female gametes are formed in the cat enterocytes. After fusion of the *T. gondii* gametes to form oocysts, these survive in the environment and are ingested by intermediate hosts, including rodents, sheep, pigs and humans. Fast-replicating tachyzoites and slow-replicating bradyzoites are generated in epithelial cells, and can cause inflammation and tissue damage, including in the eye. (From Hunter and Sibley, 2011.)

in immune compromised individuals the parasites can again replicate and cause multifocal brain and retinal lesions.

Host response. Most of our current understanding of the control of *T. gondii* infection comes from murine models. *T. gondii* proteins activate TLR2, TLR4, and

TLR11 on monocytes and dendritic cells to produce IL-12, which stimulate natural killer (NK) cells to produce IFN- γ , resulting in activation of CD4 $^{+}$ and CD8 $^{+}$ T cells. However, there is also an IFN- γ -independent mechanism of parasite killing which is mediated by CD40–CD40L-dependent autophagy (see Ch. 7).

Ocular toxoplasmosis. *T. gondii* induces an inflammatory response in the uveal tract and in the retina, causing uveitis and retinochoroiditis, although specific mediators of inflammation have yet to be identified. *In vitro*, the parasites can replicate in Müller cells, retinal epithelial cells and retinal endothelial cells. These cells then produce pro-inflammatory and chemotactic cytokines that mediate recruitment of macrophages and lymphocytes to the retina. Animal models of toxoplasmosis have shown that depletion of both CD4⁺ and CD8⁺ T cell subsets, but not either one alone, results in recurrence of the disease, implying that both cell types are required.

In immunocompetent individuals, *Toxoplasma* uveitis is self-limiting; however, recurrent lesions are associated with development of retinochoroiditis and loss of vision. It is likely that reactivation of tissue cysts is the basis for recurrence, with the stimulus likely to lead to the failure of the host response to contain the infection.

HELMINTH CAUSES OF OCULAR INFECTION

Helminths are a rare but well recognized cause of ocular disease, the most ubiquitous being toxocariasis. Other less well recognized or unidentified helminths may cause an unusual retinal infection known as diffuse unilateral subacute neuroretinitis in which the worms are found in the fundus and in the subretinal space, causing a pigmentary retinopathy and low-grade retinal inflammation with progressive loss of visual acuity.

TOXOCARIASIS

Toxocara canis and *Toxocara cati* are nematodes transmitted by dogs and cats, respectively, where the adult worms are found in the intestines. Eggs are secreted in the faeces and are ingested by the secondary hosts, which include pigs and rodents. Larvae migrate through the intestine and can be carried to any part of the body via the bloodstream. The cycle is completed when the dog or cat eats the secondary host. Human infection occurs following ingestion of contaminated food, although humans are a dead end in terms of the worm's life cycle. However, the larvae still migrate through the intestine and are disseminated, to multiple organs including the eye.

Toxocara that appears as a large inferior white intraocular vitreoretinal mass, although the live worm

can also migrate to the anterior chamber. Treatment of ocular disease is with oral tiabendazole or albendazole.

Ocular infections in developing countries

ONCHOCERCIASIS (RIVER BLINDNESS)

The filarial nematode *Onchocerca volvulus* is the causative organism of onchocerciasis, also known as river blindness, which has caused devastating blindness throughout sub-Saharan Africa, Central and South America, and in the Arabian peninsula. The 'river' connection relates to the *Simulium* blackfly vector that breeds in fast-flowing rivers. Although extensive control efforts have been underway since the 1970s, several foci of disease remain, primarily in West and Central Africa. Blindness occurs as a result of anterior and posterior segment ocular inflammation, and manifestations include sclerosing keratitis, chorioretinitis and optic neuritis.

Life cycle and parasite burden

First-stage larvae (microfilariae, L1) in infected individuals are ingested during the female blackfly's blood meal, and migrate through the gut and thorax to the salivary gland, having undergone two moults to the third-stage larvae (L3). During the second blood meal, the L3 enter the bloodstream, develop into stage four larvae (L4), and then into adult males and females that can live for over 10 years. Adult worms are sequestered in collagenous nodules and females produce millions of microfilariae during their lifetime and can survive in the skin for up to 30 months; therefore individuals can be very heavily infected. Microfilariae are readily detected in the anterior chamber by slit lamp microscopy. Fundus examination can also detect microfilariae in the retina.

Pathogenesis

Given the large parasite burden in infected individuals, a strong immune response might be expected; however, live microfilariae appear to cause minimal tissue damage either to the skin or the eyes (Fig. 8-12). As with other chronic parasitic infections in which the host and parasite have co-evolved, the infected host generates a predominantly immunosuppressive response that involves T regulatory cells, IL-10 and TGF-β, which facilitates the longevity of adult

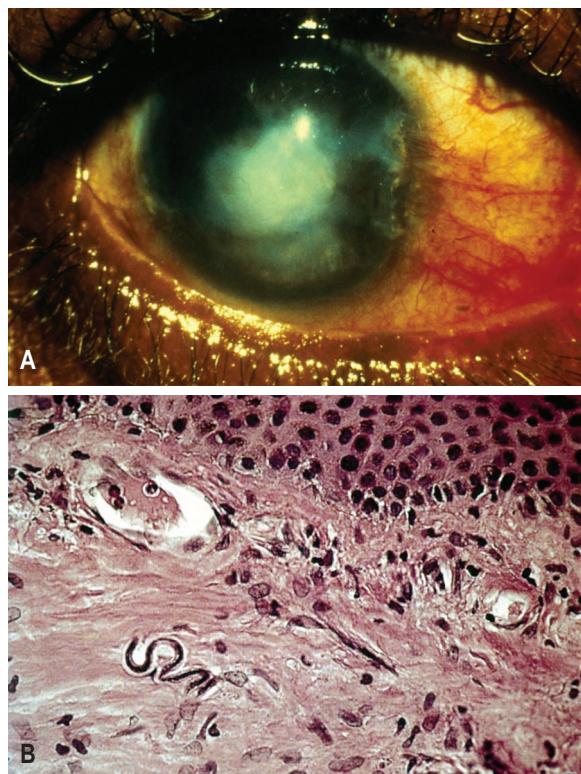


FIGURE 8-12 (A) Late stages of ocular onchocerciasis (river blindness) caused by the parasitic nematode *Onchocerca volvulus*. (B) A microfilaria in the corneal stroma not associated with an inflammatory response, whereas cellular infiltrates and corneal blood vessels are present at other sites. (From Taylor and Pearlman (Duane's Ophthalmology).)

and larval worms and continuation of the life cycle. In marked contrast to the suppressive response to live *O. volvulus*, when the larvae die either by natural attrition or following chemotherapy, infected individuals experience tissue damage, which manifests as onchodermatitis and stromal keratitis. Epidemiological and experimental data indicate that almost all immunopathology associated with onchocerciasis is directly or indirectly related to the local death of microfilariae.

Endosymbiotic *Wolbachia* bacteria

Although immunopathology has long been associated with dead and degenerating microfilariae, the finding that *O. volvulus* and other filariae harbour intracellular Rickettsia-like bacteria led to an increased understanding of the disease. *Wolbachia* are ubiquitous in the ovaries of insects and other arthropods, where they are

transmitted transovarially. As filariae have obligate developmental stages in insects (*O. volvulus* in blackflies, *Wuchereria* and *Briugia* species that cause lymphatic filariasis in mosquitoes) this likely explains why they are the only group of nematodes that harbour *Wolbachia*. Using antibodies to the major *Wolbachia* surface protein (WSP), *Wolbachia* were found to be abundant in the nematode hypodermis in the gravid uterus (Fig. 8-13) and also in microfilariae. Thus, larvae migrating through the skin and the eye also harbour *Wolbachia*, which are released following microfilaria death. Several lines of evidence support the concept that *Wolbachia* mediate the immunopathology associated with ocular onchocerciasis: (1) *Wolbachia* organisms and DNA are found in the blood of filarial infected individuals following chemotherapy; (2) *O. volvulus* from individuals depleted of *Wolbachia* by antibiotic treatment do not induce corneal inflammation; and (3) isolated *Wolbachia* induce corneal inflammation through TLR2 activation, which is the same as soluble extracts from the whole worms. *Wolbachia* are also detected in microfilariae in the skin of infected individuals. Analysis of the *Wolbachia* genome showed they do not have the LPS synthase gene, and therefore cannot produce LPS. A synthetic *Wolbachia* lipopeptide was able to induce corneal inflammation that is dependent on TLR2 and TLR6. These findings therefore indicate that the early inflammatory response in river blindness is mediated by activation of TLR2/6 by *Wolbachia* lipopeptides.

Onchocerciasis control

In 1974 the World Health Organization launched the Onchocerciasis Control Programme (OCP), which was based on aerial spraying of insecticides combined with chemotherapy. Although diethylcarbamazine had serious side-effects due to rapid death of the worms, ivermectin (Mectizan®) was found to kill the microfilariae (though not the adults). After repeated annual treatments, transmission was significantly reduced in the initial target area in West Africa, and as ivermectin was provided free by Merck, Inc. Onchocerciasis control was expanded as the African Programme for Onchocerciasis Control (APOC) and a similar ivermectin distribution programme was initiated in the Americas and in the Arabian peninsula. These programmes have met with considerable success and improved the lives of millions of individuals.

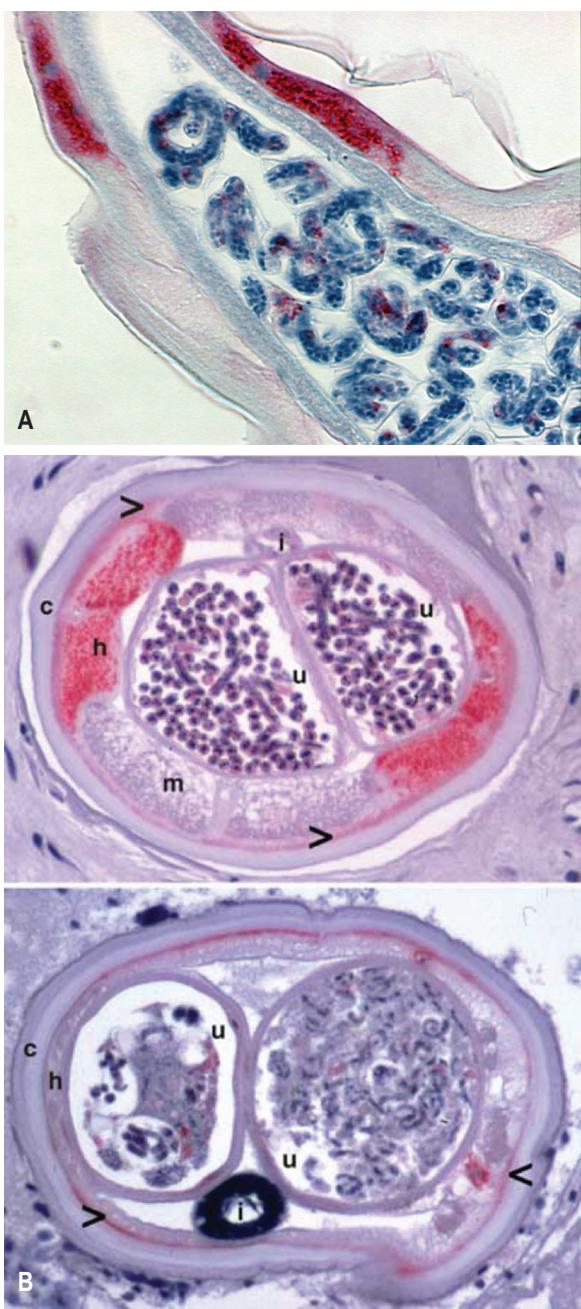


FIGURE 8-13 (A) Endosymbiotic *Wolbachia* bacteria in the hypodermis and embryos of an adult female worm (sections immunostained with antibody to the *Wolbachia* surface protein. (B) Cross-section of adult female worms following treatment with ivermectin alone (upper panel) or with doxycycline (lower panel). (Part A Photomicrograph by A. Hise, from Pearlman et al., Science 2002. Part B from Hoerauf, Lancet, 2001.)

Remaining challenges are to maintain this level of reduced transmission and to examine the effectiveness of additional therapeutic agents such as albendazole and antibiotics that target *Wolbachia*.

In targeting *Wolbachia*, studies showed that doxycycline and related antibiotics reduced not only the microfilarial load but also the adult worm burden, effectively curing the infection. The caveat has been that in contrast to ivermectin, which is given annually, doxycycline requires multiple doses over several weeks. The challenge remains to discover more potent antibiotics that can be administered *en masse*. The Bill and Melinda Gates Foundation has invested in this approach to develop new antibiotics (the Anti-*Wolbachia* Consortium, A-Wol.com), and results from this project are likely to have important implications for this disease.

***Loa loa* (Loiasis, EYEWORM)**

Loa loa is a filarial nematode with a similar life cycle to *O. volvulus* except the adults migrate through subcutaneous tissues and the microfilariae are in the blood. The intermediate vectors are large *Chrysops* flies rather than the skin and transmission is limited to regions of western Africa. Disease manifestations are due to the migrating adults that can live for up to 17 years in a human host and can move through subcutaneous tissues at an estimated 1 cm per minute. Adult worms also migrate through the conjunctiva (not the cornea), where they can be surgically removed (Fig. 8-14). Localized inflammation, known as Calabar swellings, may occur in subcutaneous tissues of the skin but they do not cause major tissue damage. *Loa loa* does not harbour *Wolbachia* and infection is treated with corticosteroids and diethylcarbamazine.

TRACHOMA

Trachoma is the most prevalent microbial cause of blindness worldwide, with ~1.3 million people blind and an estimated 1.8 million with impaired vision. Figure 8-15 shows the global distribution of trachoma, which is endemic in some 50 countries, especially in sub-Saharan Africa. In Ethiopia and Sudan an estimated 50% of children and 9% of adults have active trachoma.

Trachoma is caused by *Chlamydia trachomatis*, specifically subgroups (serovars) A, B, Ba and C. These

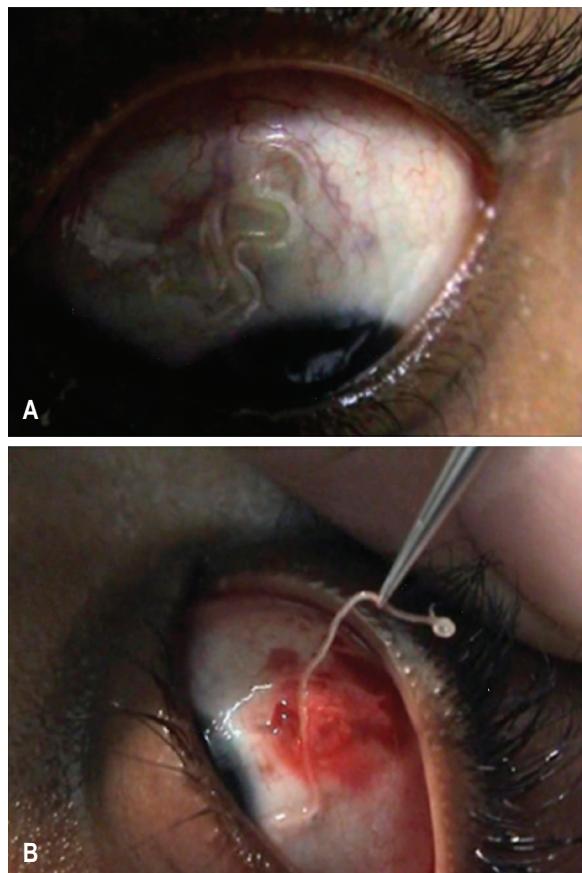


FIGURE 8-14 Loiasis (eyeworm). Adult *Loa loa* migrating across the conjunctiva (A), where they can be surgically removed (B). (From Shah and Saldana, 2010.)

bacteria replicate in conjunctival epithelial cells as reticulate bodies that do not appear to have a cell wall. They also form spore-like elementary bodies which do have cell walls, and this is the stage that is transmitted, either person to person or by flies that are attracted to the eyes.

The predominant disease manifestations are shown in Figure 8-15. Recurrent infection of the conjunctival epithelium results in inflammation and development of characteristic papillary conjunctivitis, which eventually cause conjunctival scarring. Contraction of scar tissue causes entropion (rolling of the eyelids inward). The eyelashes then scratch the cornea (trichiasis), which can result in corneal opacification and blindness. The inflammatory response has not been well

characterized, although animal models show an important role for IFN- γ .

The World Health Organization initiated the Global Alliance for the Elimination of Blinding Trachoma (GET 2020), which is based on the SAFE strategy to control transmission by means of Surgery for inturned eyelids, Antibiotic use, with at least two oral treatments of azithromycin (Zithromax®, donated by Pfizer), facial cleanliness to prevent person-to-person transmission and fly- (*Musca sorbens*) to-person transmission, and, lastly, environmental changes to increase access to clean water and encourage handwashing and the use of latrines, which would also reduce the number of these flies.

Trachoma in Australia

Trachoma has long been prevalent in the indigenous population of Australia, and a 2008 survey reported that children in 60% of outback communities have blinding disease. However, the Australian government has made a major commitment to reduce the prevalence by using the SAFE strategy and making improvements in housing and health care.

Conclusion

In summary, microbial infections of the eye are a major cause of blindness and visual impairment worldwide. Infections can affect the anterior and posterior segments and disease pathogenesis is the net result of both microbial virulence and the host response. Pathogens have evolved multiple strategies for survival in the eye, and the host is not always discriminating in its ability to kill the microbes without causing tissue damage. However, the ocular surface barriers block invasion by environmental microbes under normal physiological conditions, and infection occurs primarily when the ocular surface is compromised by trauma or extended contact lens wear.

Future treatments for these blinding eye infections will be based on an increased understanding of the pathogenesis of disease which will also identify new, more specific targets for immune intervention compared with corticosteroids. Potential strategies include blocking pathogen recognition molecules or neutrophil chemokines which could be given together with effective antibiotics. However, many of these

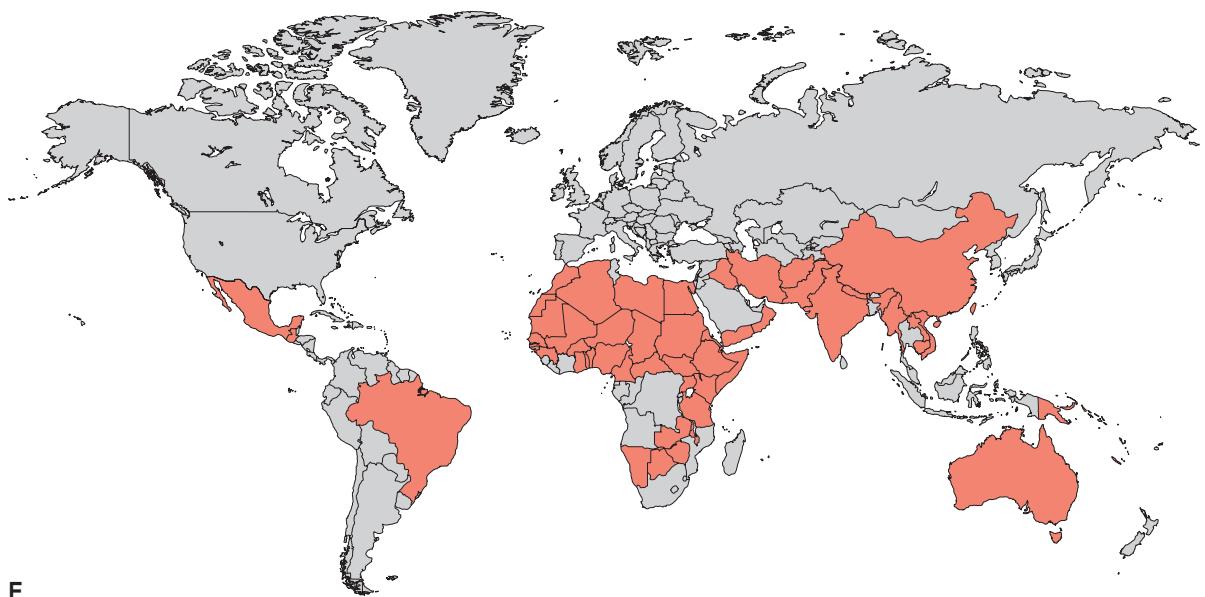
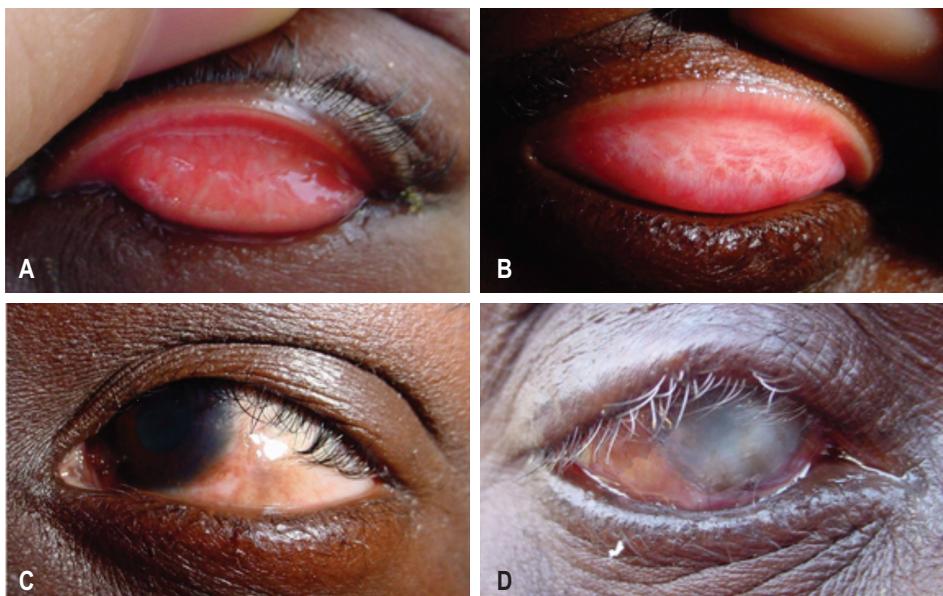


FIGURE 8-15 Clinical features of trachoma: (A) active trachoma in a child, characterized by a mixed papillary (TI) and follicular response (TF); (B) tarsal conjunctival scarring (TS); (C) entropion and trichiasis (TT); and (D) blinding corneal opacity with entropion and trichiasis (TT). (E) Prevalence of trachoma worldwide. (From Burton and Mabey, 2009.)

microbes, bacteria in particular, have become resistant to antibiotics, and there is a pressing need to develop new classes of antimicrobial agents. To this end, antimicrobial peptides have some potential as they are biological rather than pharmacologic agents, their specificity is well understood and they can be readily synthesized as a native sequence or with substituted

amino acids that may improve efficacy. Targeting microbial scavenging for essential metals such as iron and zinc may also prove a feasible approach.

FURTHER READING

A full reading list is available online at <https://expertconsult.inkling.com/>.



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