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Mucosal Immunity in the Ocular System

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I. Introduction

Acquired immunity at the ocular surface is mediated primarily by the mucosal immune system. The linkage of ocular subcompartments to the mucosal immune network is now well established (Mestecky *et al.*, 1978; Montgomery *et al.*, 1983, 1985; Gregory and Allansmith, 1986). Since no vaccines currently use the mucosal immune system to specifically target ocular diseases, this chapter will review ocular mucosal immunobiology, examine approaches for inducing ocular mucosal immune responses, and identify target organisms for use in potential vaccination against selected ocular infections. Additional information on ocular mucosal immunity, including the impact of ocular infection on the structure and function of this system, is contained in an excellent review (Sullivan, 1994). It is important to note that other aspects of ocular immunobiology do not involve the mucosal immune system. Descriptions of anterior chamber-associated immune deviation and retinal immunology are provided in a number of other reviews (Gery *et al.*, 1986; Streilein, 1987, 1990, 1993; Rocha *et al.*, 1992; Niederkorn and Ferguson, 1996).

II. Ocular Mucosal Immunobiology

A. Tissues and Cells

The conjunctiva and lacrimal gland are the primary ocular tissues that are considered part of the mucosal immune system. The conjunctiva is a mucous membrane consisting of an outer squamous epithelial cell layer (two to five cells thick) interspersed with goblet cells, a basement membrane, and the underlying connective tissue of the substantia propria (stroma). Figure 1 diagrams the major features of the human conjunctiva. Bulbar conjunctiva overlies the sclera between the corneal limbus and the conjunctival fornix. Multiple sebaceous glands within the deeper stroma contribute to the corneal tear film and the lubrication of the conjunctiva (Fine and Yanoff, 1979). Mast cells have been identified in the conjunctiva of humans, other primates, and several rodents (Allansmith *et al.*, 1977; Fine and Yanoff, 1979). These are particularly prominent in trauma-induced and allergic conjunctivitis. There are no microfold (M) cells in the conjunctiva and the normal conjunctiva is relatively free of lymphocytes. However, similar to gastrointestinal-associated lymphoid tissue (GALT), intraepithelial lymphocytes (IEL) have been identified in human and mouse conjunctiva (Dua *et al.*, 1994; J. A. Whittum-Hudson, unpublished observations). Human conjunctival IEL are T cells and express the mucosal lymphocyte antigen HML-1 and CD8 (Dua *et al.*, 1994). Another study found that the majority of conjunctival T cells expressed $\alpha\beta$ TCR (Soukiasian *et al.*, 1992). The murine equivalent IEL is $CD3^+$, $CD8^+$ and TCR $\gamma\delta^+$ (J. A. Whittum-Hudson, unpublished observations). Few T cells and essentially no B cells are found in the substantia propria of normal human, monkey, or mouse conjunctiva (Reacher *et al.*, 1991; Soukiasian *et al.*, 1992; Dua *et al.*, 1994; Whittum-Hudson *et al.*, 1995a). The majority of the substantia propria T lymphocytes are $CD8^+$ cells in man and monkey and lie subepithelially. In the lower fornix of human conjunctiva, clusters of unorganized (nonfollicular) lym-

MUCOSAL VACCINES
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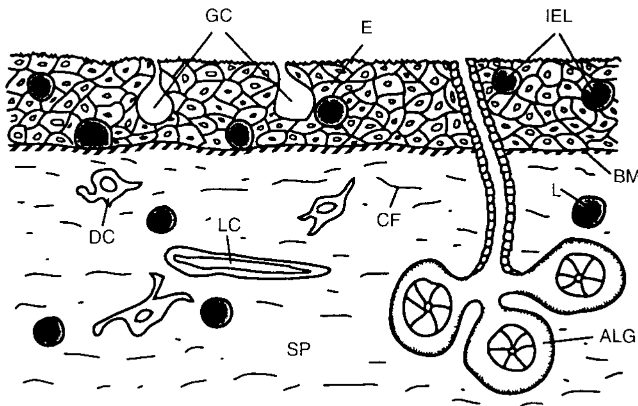

Figure 1. Diagrammatic representation of human conjunctiva showing the conjunctival stratified columnar epithelium (E), intraepithelial lymphocytes (IEL), mucous-producing goblet cells (GC), basement membrane (BM), an accessory lacrimal gland of Krause (ALG), as well as lymphatic channels (LC), collagen fibrils (CF), lymphocytes (L), and dendritic cells (DC) within the substantia propria (SP). (This figure has been adapted from Sacks *et al.*, 1986.)

phocytes may be found (Fine and Yanoff, 1979). Some species such as rabbit and guinea pigs may exhibit true follicles in the lower fornices (Shimada and Silverstein, 1975; Chandler and Gillette, 1983; Sullivan, 1994). These may develop due to persistent exposure to exogenous antigens, or reflect a nonspecific response to the environment.

Lacrimal glands also play a major role in ocular mucosal defense. In humans, lacrimal glands are found in the anterior, superolateral area of the orbit. Smaller accessory lacrimal glands (see Fig. 1) also may be found in the upper and lower conjunctiva. Ducts from the major lacrimal gland drain into the superotemporal conjunctival cul-de-sac, while ducts from accessory glands drain directly through the epithelium of the conjunctiva adjacent to their location. Lacrimal glands of other species are found in different areas both within and outside the orbit. Histological examination shows that the glands are made up of acinar units which are interconnected by ductules and that these interconnected acinar units form the lobes of the gland. Each acinar unit consists of secretory acinar epithelial cells, which are surrounded by a basement membrane, and the glandular ducts are lined with pseudostratified epithelium. Lymphatic channels also are present in the gland, and these drain into the cervical and preauricular lymph nodes. In addition to epithelial cells, a variety of other cell types such as plasma cells, B and T lymphocytes, macrophages, and dendritic cells have been identified in the interstitial connective tissue between the acinar units and ducts. In human, rabbit, rat, and mouse lacrimal glands, IgA plasma cells predominate, although IgG and IgM plasma cells are present and, in human, IgD and IgE plasma cells have been found (Franklin *et al.*, 1973; 1979; Shimada and Silverstein, 1975; Allansmith *et al.*, 1976; Brandtzaeg *et al.*, 1979; 1987; Gillette *et al.*, 1980; Crago *et al.*, 1984; Damato *et al.*, 1984; McGee and Franklin, 1984; Gudmundsson *et al.*, 1984; Brandtzaeg, 1985; Kett *et al.*, 1986; Wieczorek *et al.*, 1988; Ebersole *et al.*, 1988; Pappo *et al.*, 1988b; Hann *et al.*, 1988; Montgomery *et al.*, 1989). B cells bearing various surface immunoglobulin isotypes also have been found in smaller numbers in humans and rodents (McGee and Franklin, 1984; Pappo *et al.*, 1988b; Wieczorek *et al.*, 1988; Montgomery *et al.*, 1989, 1990; Pepose *et al.*, 1990). Both $CD4^+$ and $CD8^+$ T cells also have been identified in human and rat (Ebersole *et al.*, 1988; Gudmundsson *et al.*, 1988; Wieczorek *et al.*, 1988; Pappo *et al.*, 1988b; Pepose *et al.*, 1990; Montgomery *et al.*, 1989, 1990). Of the $CD3^+$ T cells isolated from rat lacrimal gland, 89% bear the $\alpha\beta$ and 7% express $\gamma\delta$ TCR (C. A. Skandera and P. C. Montgomery, unpublished observations). Macrophages have been demonstrated in both human and rat (Wieczorek *et al.*, 1988; Pappo *et al.*, 1988a; Montgomery *et al.*, 1989; Pepose *et al.*, 1990) and Langerhans-type dendritic cells identified in human lacrimal gland (Wieczorek *et al.*, 1988; Pepose *et al.*, 1990). These various cell types contribute a number of biologically active molecules which are important for defense at the ocular surface. The acinar epithelial cells produce lysozyme and lactoferrin which are naturally occurring tear components with bactericidal properties (Franklin *et al.*, 1973; Gillette and Allansmith, 1980; Gillette *et al.*, 1981; McGill *et al.*, 1984). In addition, the plasma cells are the main source of tear S-IgA antibodies (Sullivan and Allansmith, 1984; Peppard and Montgomery, 1987; Franklin, 1989; Sullivan, 1994) which are produced in response to antigenic challenge and are major effector molecules in mucosal defense (Mestecky and McGhee, 1987; Childers *et al.*, 1989; McGhee and Mestecky, 1990).

B. Relationship to the Mucosal Network

Although it has been postulated that the conjunctiva functions both as a mucosal inductive and effector site (Chandler and Gillette, 1983; Franklin and Remus, 1984; Franklin, 1989), the precise relationship of the conjunctiva to the mucosal immune network requires further clarification (Sullivan, 1994). With respect to inductive capabilities, the presence of lymphoid follicles containing T and B cells in rabbit (Shimada and Silverstein, 1975; Franklin *et al.*, 1979; Franklin and Remus, 1984) and, in humans, the presence of Langerhans cells, dendritic cells, and macrophages (Sacks *et al.*, 1986), as well as lymphatic channels (Srinivasan *et al.*, 1990), support the potential to process and perhaps respond to antigen. Normal conjunctival epithelial cells do not express MHC class II; however, the presence of class II^+ Langerhans cells in the epithelium and dendri-

tic cells in the stroma suggests that antigen presentation may occur in conjunctiva. Further support for the inductive function comes from studies showing that topical application of antigen to the conjunctiva is an effective means of eliciting tear IgA antibody responses (Montgomery *et al.*, 1984a,b; Peppard *et al.*, 1988). Recent investigations have suggested that nasal-associated lymphoid tissue (NALT) may also function as an inductive site following ocular topical antigen application (Carr *et al.*, submitted; see Section III.A). With the exception of rabbit conjunctiva, which contains substantial numbers of IgA plasma cells and epithelial cells that synthesize secretory component (Franklin *et al.*, 1973, 1979; Liu *et al.*, 1981), the conjunctiva of most species does not display the typical features of a mucosal effector site. However, it should be noted that studies in at least two species, rat and monkey, have documented lymphocyte traffic from gastrointestinal-associated lymphoid tissue (GALT) to conjunctiva (Zhang *et al.*, 1983; Taylor *et al.*, 1985). These data indicate that the functional role of the conjunctiva in the mucosal network may vary significantly between species.

An abundance of evidence now indicates that the lacrimal gland is a functional part of the mucosal immune network. As has been demonstrated for other mucosal effector tissues and described above (see Section II.A), the lacrimal glands of many species contain a predominance of IgA^+ plasma cells. In addition, lacrimal acinar epithelial cells produce secretory component (Franklin *et al.*, 1973; Sullivan *et al.*, 1984b, 1990; Gudmundsson *et al.*, 1985; Hann *et al.*, 1991), which functions as part of the polymeric immunoglobulin receptor to mediate transport of IgA into external secretions (Solari and Kraehenbuhl, 1985). Figure 2 diagrams the biosynthetic events within lacrimal gland that lead to the production and transport of IgA into tears. Linkage of the lacrimal gland to the mucosal network occurs via trafficking lymphocyte populations. While both B and T lymphocytes traffic to mucosal tissues including lacrimal gland, B cells have been studied more extensively than T cells. IgA bearing lymphocytes from GALT (e.g., mesenteric lymph nodes) and glandular mucosal tissues have been shown to seed lacrimal glands (Montgomery *et al.*, 1983, 1985) in a manner similar to other effector sites in the mucosal immune system (Roux *et al.*, 1977; McDermott and Bienenstock, 1979; Weisz-Carrington *et al.*, 1979). Tear IgA antibodies with specificity for oral microbes (Burns *et al.*, 1982; Gregory and Allansmith,

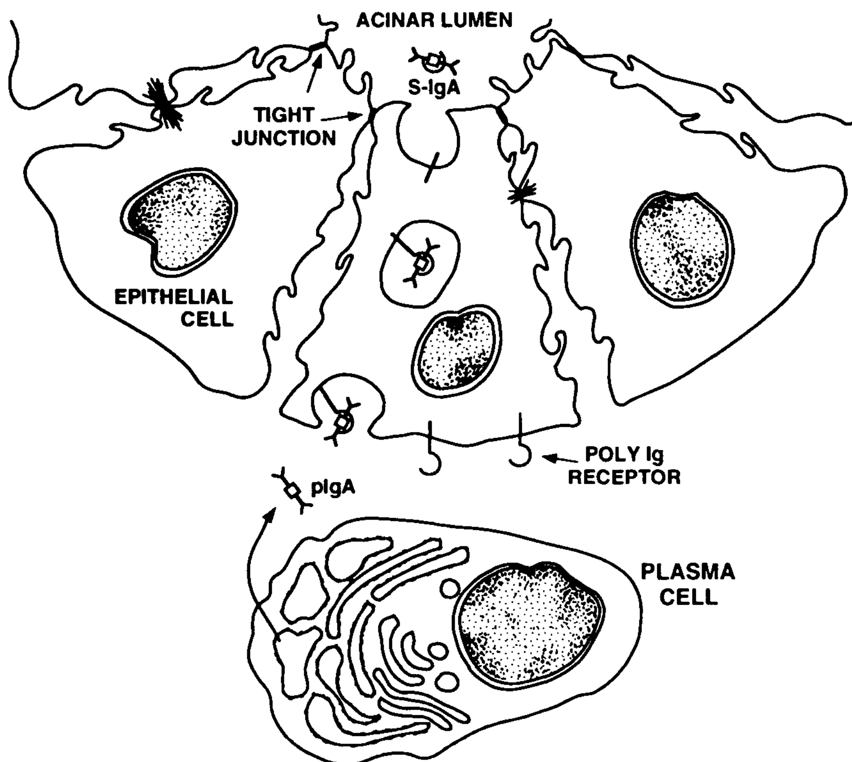

Figure 2. Diagrammatic representation of the lacrimal gland showing S-IgA biosynthesis. Polymeric IgA (pIgA) is synthesized in plasma cells located adjacent to the acinar epithelial cells. pIgA interacts with the polymeric immunoglobulin (poly-Ig) receptor on the basolateral surface of the acinar epithelial cells, is internalized and transported to the apical surface, and is released into the glandular lumen. Prior to release of the S-IgA molecule, the poly-Ig receptor is cleaved, leaving behind the cytoplasmic domain. The remainder of the poly-Ig receptor remains associated with the S-IgA molecule and is designated secretory component (s.c.). (This figure has been reproduced from Franklin and Montgomery (1996) and published courtesy of Mosby.)

1986, 1987) as well as demonstrations that gastrointestinal immunization induced tear IgA antibodies (Mestecky *et al.*, 1978; Montgomery *et al.*, 1983; Gregory and Filler, 1987; Waldman and Bergmann, 1987) have provided further support for this linkage. Figure 3 shows the interrelationship of various compartments of the mucosal network and summarizes events leading to mucosal IgA induction following GALT immunization.

The mechanism accounting for the preferential localization or retention of specific lymphoid populations in ocular mucosal tissues is an issue of central importance in understanding the regulation of ocular mucosal immunity. Lymphocyte–high endothelial venule (HEV) interactions control lymphocyte trafficking into organized lymphoid tissue and lymph nodes (Butcher, 1986; Woodruff *et al.*, 1987, Stoolman, 1989; Chin *et al.*, 1991b; Picker and Butcher, 1992; Shimizu *et al.*, 1992; Bevilacqua, 1993) as well as into organized mucosal lymphoid tissues (Jalkanen *et al.*, 1989; Chin *et al.*, 1991a). In general, these interactions involve lymphocyte homing receptors which interact with vascular addressins on HEVs and other specialized endothelial cells. Although little is known regarding the mechanisms involved in cell trafficking to conjunctival tissue, the parameters regulating lymphocyte localization within the lacrimal gland are beginning to be defined. With respect to glandular structures, the control of lymphocyte traffic could occur on two levels: (1) at exit from the vasculature, or (2) within the stroma of the glandular tissue. There are no HEVs in lacrimal gland and no selective interactions with glandular endothelium have yet been demonstrated. Therefore, it appears that lymphocyte entry into lacrimal gland is random (McGee and Franklin, 1984). Current evidence suggests that lymphocyte populations are selectively retained based on interactions that occur within the lacrimal tissue microenvironment. Early studies indicated that B-cell retention, particularly those committed to IgA production, resulted from a direct interaction with T cells located in lacrimal tissue (Franklin and Shepard, 1990). Recently, an *in vitro* binding assay has been used to show that circulating lymphocyte populations preferentially adhere to lacrimal gland acinar epithelium (O'Sullivan and Montgomery, 1990). Both B and T lymphocytes participate in this interaction with B cells binding in greater numbers (O'Sullivan *et al.*, 1994a). Studies with biochemical inhibitors and antibodies directed against various adhesion molecules initially suggested the involvement of L-selectin (O'Sullivan *et al.*, 1994a,b). Other data indicated that pretreatment of lymphocytes with certain cytokines (IL-4 and TGFβ) reduced the capacity of those cells to interact with lacrimal gland but not lymph node or Peyer's patches (Elfaki *et al.*, 1994). These latter data provided the initial support for the involvement of a receptor distinct from those mediating interactions with lymph node and Peyer's patch HEVs. The characterization of the lymphocyte receptor is still ongoing, but an 85-kDa factor has been identified which specifically inhibits lymphocyte interactions with lacrimal and salivary gland tissues (Montgomery and Liberati, 1995). Current studies are focused on defining the relationship of this molecule to other lymphocyte surface determinants and adhesion molecules, as well as on the identification of the acinar epithelial cell ligand or counter receptor. With respect to the ligand, recent studies using cultured rat lacrimal gland acinar cells suggest that this molecule is shed into the culture medium (O'Sullivan *et al.*, 1995) and has led to the speculation that trafficking lymphocytes may also interact with secreted ligand accessing the interstitial glandular spaces. Although the ligand or counter receptor has not yet been isolated, it appears to be a 20- or 21-kDa glycoprotein (R. Raja and P. C. Montgomery, unpublished observations).

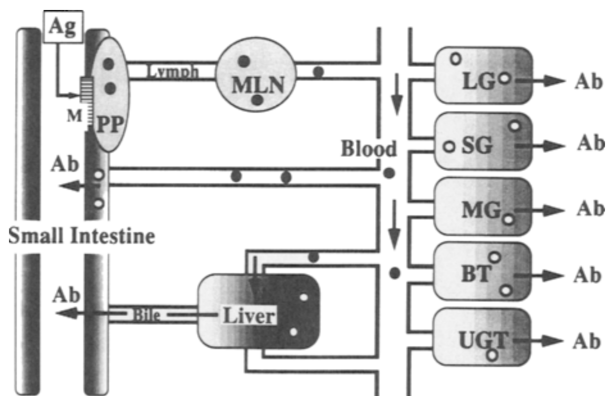

Figure 3. Schematic representation of the major features of the mucosal immune network as they relate to gastrointestinal-associated lymphoid tissue (GALT). Intestinal antigens (Ag) are taken up by microfold (M) cells overlying Peyer's patches (PP) and are delivered to lymphoid cells in the PP. T cells and IgA committed B cells (●) migrate to the mesenteric lymph nodes (MLN), enter the circulation, and traffic to the lacrimal (LG), salivary (SG), mammary (MG) glands as well as to the lamina propria of the small intestine, the bronchial (BT) and urogenital (UGT) tracts, and liver. B lymphocytes lodging in mucosal tissues differentiate into IgA secreting plasma cells (○), producing pIgA which is transported into external secretions as S-IgA antibody (Ab). In some species the liver directly transports significant quantities of pIgA from the circulation into bile. (This figure has been reproduced from Montgomery *et al.* (1994) and published courtesy of Plenum.)

III. Induction of Ocular Mucosal Immune Responses

Regulatory events leading to the induction and expression of ocular mucosal immune responses are not completely defined. However, it is clear that many factors governing immune responses at other mucosal sites (Kiyono *et al.*, 1992; Walker, 1994) have general appli-

cability to mucosal immune responses at the ocular surface. The immunization route is of primary importance with local (e.g., topical application to the eye) and remote site (e.g., oral or gastrointestinal) routes generating tear IgA antibody responses. Combinations of routes and their sequence of administration can also affect antibody induction in tears. Not surprisingly for replicating microbes, tissue tropism and invasive properties influence the outcome of mucosal responses. When dealing with nonreplicating immunogens, particulate antigens are more effective at inducing ocular mucosal immune responses than soluble antigens. Thus far, traditional mucosal adjuvants such as cholera toxin, delivered topically to the eye with antigen, do not appear to enhance ocular antibody responses (Peppard and Montgomery, 1990). Helper T (Th) cells and T-cell products such as cytokines appear to play key roles in regulating ocular mucosal IgA responses, but the direct role of cytotoxic T (Tc) cells in ocular mucosal immunity is currently not clear.

As noted above (see Section II.B), lymphocyte traffic from mucosal inductive to effector sites is a central feature of the ocular mucosal system. Antigen-presenting cells (e.g., macrophages and dendritic cells) are also required, but their participation in ocular mucosal responses has yet to be delineated. The neuroendocrine system also plays a role in regulating ocular mucosal responses affecting both secretory component synthesis *in vivo* (Sullivan *et al.*, 1984a) and cultured lacrimal gland acinar cells (Kelleher *et al.*, 1991) as well as tear IgA production (Sullivan and Allansmith, 1985; Sullivan, 1988). Neuroendocrine regulation is reviewed in detail elsewhere (Stanisz *et al.*, 1989; Sullivan, 1990, 1994). Although these as well as other factors yet to be identified impact ocular mucosal immune responses, our focus will be on selected parameters relevant to the development of ocular vaccination strategies. Therefore, the effects of immunization route, immune potentiators and delivery vehicles on the induction of ocular mucosal responses, in particular IgA antibody production, will be examined in detail.

A. Immunization Routes

In humans, most investigations have focused on use of the oral or gastrointestinal (GI) immunization route. Ingestion of heat-killed *Streptococcus mutans* elicited IgA antibodies in tears and saliva, but not serum antibodies (Mestecky *et al.*, 1978). In addition, a second oral immunization markedly increased tear and salivary IgA responses. Other studies using inactivated *S. mutans* have generally confirmed these initial observations (Gregory *et al.*, 1984; Gregory and Filler, 1987; Czerkinsky *et al.*, 1987). Human tear IgA antibody responses have also been noted following oral immunization with influenza vaccine (Bergmann *et al.*, 1986, 1987; Waldman and Bergmann, 1987) as well as following intranasal immunization with live rhinovirus (Douglas *et al.*, 1967; Knopf *et al.*, 1970).

The rat model has been employed to directly compare the effectiveness of antigen application to the conjunctiva (ocular topical, ot) and administration by the oral or gastrointestinal (GI) route using the nonreplicating particulate antigen, dinitrophenylated type III pneumococcus (DNP-Pn). These studies have shown: (1) both the immunization route and the sequence of stimulation play a role in tear antibody expression (Montgomery *et al.*, 1984b); (2) long-term repeated ot stimulation leads to an eventual downregulation of tear IgA antibody levels (Montgomery *et al.*, 1984a,b); (3) the ot route generally yields higher tear IgA antibody levels than GI immunization (Peppard *et al.*, 1988); and (4) coadministration of antigen and cytokines by the ot route enhances tear IgA antibody levels (Pockley and Montgomery, 1991; see Section III.B). The rat model also has been used to study the effect of immunization route on the kinetics of serum and tear antibody responses to live *Chlamydia trachomatis* (Davidson *et al.*, 1993). This study compared the effectiveness of ot, GI, subconjunctival, and intraperitoneal immunization and showed that ot application was the most effective route for eliciting IgA antibody response in tears. Interestingly, subconjunctival injection of chlamydia yielded a vigorous serum IgG response and minimal tear IgA antibodies, which is consistent with responses to other antigens delivered by this route (Cousins *et al.*, 1991). The explanation for this latter finding is not clear, but subconjunctival delivery may bypass an essential ocular mucosal processing step during antigen uptake or allow live organisms to gain access to the circulation.

Although the precise mechanism by which ot immunization elicits tear IgA antibody responses is not known, it appears that the inductive process could involve at least two pathways: (1) antigen uptake, processing, and triggering of B cells in the conjunctival substantia propria or (2) antigen drainage *via* the nasolacrimal canal, uptake by NALT M cells, processing and triggering of resident B cells in NALT (Carr *et al.*, submitted). The schematic details of these two pathways are shown in Fig. 4. Not shown in this figure is the option for antigen drainage *via* the nasolacrimal canal to access GALT or other alternatives which may be applicable to primates. In humans and monkeys, antigen may reach the accessory lacrimal glands directly or gain access to potential ductal associated lymphoid tissue as has been shown for minor salivary glands (Nair and Schroeder, 1986). However, since it is not clear how antigen would be processed within the acinar units of the accessory lacrimal glands and ductal-associated lymphoid tissue has not been identified for these glandular structures, these latter alternatives appear unlikely.

Several animal models have also been used to eval-

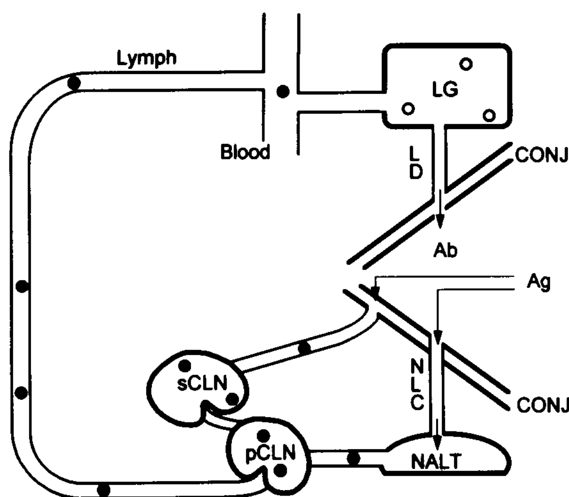

Figure 4. Schematic representation of the major components of the ocular mucosal immune system showing possible pathways for antigenic stimulation at the ocular surface. 1. Antigen may gain access to the conjunctival (CONJ) substantia propria where dendritic cells would present it to resident lymphocytes (see Fig. 1). Once triggered, B cells (●) would traffic to the superior cervical lymph node (sCLN) and gain access to lymph. 2. Ag may drain *via* the nasolacrimal canal (NLC), be taken up by M cells, and gain access to the nasal-associated lymphoid tissue (NALT) where presentation and B-cell triggering would occur. B cells (●) would traffic to the posterior cervical lymph node (pCLN) and gain access to lymph. B cells reaching lymph from either pathway would enter the circulation, traffic to the major lacrimal gland (LG), and differentiate into IgA antibody-secreting plasma cells (○). The IgA would be processed as shown in Fig. 2 and reach the ocular surface *via* the lacrimal duct (LD).

uate tear antibody responses after immunization or infection at ocular surfaces with chlamydia and other pathogens. In guinea pigs, only ocular immunization with live *Chlamydia psittaci* (GPIC) induced tear IgA (and in some cases IgG) antibodies (Murray *et al.*, 1973; Watson *et al.*, 1977; Rank and Whittum-Hudson, 1994). Killed organisms administered *via* ocular topical (Murray *et al.*, 1973) or intraperitoneal routes (Malaty *et al.*, 1981) failed to induce tear antibodies. However, similar studies with human biovars of *C. trachomatis* in owl monkeys suggested that inactivated chlamydia could indeed induce tear antibodies (MacDonald *et al.*, 1984). In the cynomolgus monkey model, ocular infection with the chlamydial organism induced vigorous IgA, IgG, and IgM responses in both serum and tears (reviewed by Taylor, 1990). Only oral immunization with live L_2 biovar of chlamydia, but not ocular serotypes, successfully induced mucosal and systemic immunity, which partially protected the host from subsequent infectious challenge (Taylor *et al.*, 1987b).

Cynomolgus monkeys develop conjunctival disease similar to that observed in humans (Whittum-Hudson and Taylor, 1984). An intense mononuclear infiltrate appears in infected conjunctiva (Whittum-Hudson *et al.*, 1986a,b; Whittum-Hudson and Taylor, 1989) and is composed of high frequencies of chlamydia-specific T and B lymphocytes (Pal *et al.*, 1992). Supporting the importance of regional draining cervical lymph nodes in conjunctival responses to pathogens, essentially identical frequencies of antigen-specific cells were detected in these lymph nodes and the conjunctivae after infection, and in "ocular-immune" animals which were topically challenged with the chlamydial hsp60 antigen (Pal *et al.*, 1990b, 1992). Serologic responses to chlamydial hsp60 and hsp70 are vigorous during chronic chlamydial infections in man and animals (Newhall *et al.*, 1982; Pal *et al.*, 1990a), although neither of these proteins delivered by the ot route immunized naive monkeys (Taylor *et al.*, 1987b, 1990). In contrast, ot boosting after systemic or oral immunization with purified or recombinant chlamydial antigens induced vigorous tear IgA and IgG and serum antibodies (Campos *et al.*, 1995; O'Brien *et al.*, 1994). It remains unclear whether the initial dosage of some antigens is too small to induce typical primary secretory immune responses when presented to the mucosal immune system by ot delivery, or if an antigen must replicate and/or be invasive to adequately prime experimental animals. Nevertheless, ocular boosting with purified antigens or infectious ocular challenge of systemically primed animals has demonstrated that anamnestic tear antibody responses occur rapidly for chlamydia (Campos *et al.*, 1995). Combined oral and subcutaneous priming with a chlamydial fusion protein of a major outer membrane protein (MOMP) variable domain incorporated into liposomes also primed for mucosal immune responses (O'Brien *et al.*, 1994).

The rabbit model has also been used to test ocular immune responses to other organisms. Live herpes simplex virus type 1 administered by the ocular route elicited tear IgA, but no IgG or IgM antibodies (Willey *et al.*, 1985). In contrast, application to scarified cornea induced tear IgA, IgG, and IgM responses (Centifanto *et al.*, 1989). Whether these differing results relate to virus strain invasiveness or access to underlying dendritic cells is unclear. Live *Staphylococcus aureus* administered ot elicited tear IgA and IgG antibodies as did a *S. aureus* subunit (peptidoglycan-ribitol teichoic acid) vaccine administered by the intradermal or subconjunctival routes with complete Freund's adjuvant (Mondino *et al.*, 1987a,b, 1991). However, given the severe inflammatory properties of Freund's adjuvant and the restrictions on its use, this latter approach is impractical as a vaccination strategy.

B. Immune Potentiators

Immune potentiators, or adjuvants, have been used to enhance antibody responses in both humans and animal models. Although the mechanisms responsible for response enhancement vary and are often not well de-

fined, adjuvants appear to function by one or a combination of mechanisms: (1) increasing the influx of inflammatory cells; (2) promotion of antigen presentation; and/or (3) enhancing antigen uptake. Consideration of immune potentiators to enhance ocular mucosal responses requires selection of compounds which will not promote ocular inflammation and pathology.

The most widely studied mucosal adjuvant has been cholera toxin (Lycke and Holmgren, 1968) although its effects on ocular immunity are less defined. Enhancement of mucosal responses depends on the binding of the cholera toxin B subunit to the G_{M1} ganglioside displayed on the luminal surface of enterocytes such as M cells as well as potential modulatory effects of the toxin on the B, T, and antigen-presenting cells (Dertzbaugh and Elson, 1990; Elson and Dertzbaugh, 1994). Cholera toxin enhances antibody responses when coadministered with many antigens, whereas the B subunit apparently requires conjugation to antigen to function optimally. Oral immunization with cholera toxin induced the appearance of toxin-specific antibody-forming cells in rat conjunctiva (Zhang *et al.*, 1983) and toxin-specific antibodies in tears of monkeys (Campos *et al.*, 1995). However, ot administration of cholera toxin with the DNP-Pn antigen did not enhance tear IgA antibody responses in the rat model (Peppard and Montgomery, 1990), but the effects of oral or ocular administration of antigens conjugated to the B subunit on tear IgA responses have not been fully investigated. Based on recent data suggesting that nasal associated lymphoid tissue can serve as an inductive site for tear IgA antibody responses (Carr *et al.*, submitted) and the ability of the B subunit to serve as an adjuvant when coupled to antigens administered by the intranasal route (Bessen and Fischetti, 1990; Wu and Russell, 1993), this immunization protocol requires rigorous testing. Two other mucosal adjuvants, avridine and muramyl tripeptide, administered topically by the ocular route with DNP-Pn, did not enhance tear IgA antibody responses (Peppard *et al.*, 1988).

Cytokines also appear to exert important regulatory effects on mucosal IgA responses including those at ocular sites. Interleukin (IL)-6 knock-out mice have greatly reduced numbers of IgA producing cells at mucosae and exhibit grossly deficient local antibody responses to mucosal challenge (Ramsay *et al.*, 1994). In addition, transforming growth factor-β (TGFβ), IL-5, and IL-6 have been shown to enhance *in vitro* IgA responses in murine B-cell cultures. TGFβ appears to increase IgA synthesis by inducing B cells to switch to IgA production (Coffman *et al.*, 1989; Ehrhardt *et al.*, 1992), whereas IL-5 and IL-6 induce IgA^+ B cells to terminally differentiate into IgA producing plasma cells (Coffman *et al.*, 1987; Beagley *et al.*, 1988, 1989; Harriman *et al.*, 1988). IL-5, IL-6, and TGFβ have been shown to specifically enhance IgA production *in vitro* in a rat lacrimal gland tissue fragment culture system (Pockley and Montgomery, 1990; Rafferty and Montgomery, 1993). Further, IL-5 and IL-6 in combination with antigen have been shown to enhance specific tear IgA antibody responses in rats following ot administration without affecting serum IgG antibody levels (Pockley and Montgomery, 1991). Recent studies indicate that these enhanced tear IgA antibody responses persist following restimulation with antigen in the absence of IL-5 and IL-6 (Montgomery *et al.*, 1994). Although the mechanism(s) underlying this *in vivo* cytokine-mediated enhancement is not yet known, it is clear that the regulatory roles and therapeutic potential of these and other cytokines to modulate ocular mucosal antibody responses require further investigation.

C. Delivery Vehicles

Although the oral or GI immunization route triggers IgA antibody responses at both intestinal and distal mucosal sites and represents a safe, convenient alternative for vaccine administration, it is often difficult to present intact antigen to GALT-inductive sites. Particulate antigens, including viable organisms, generally appear to be the most effective immunogens, probably due to their ability to survive the pH extremes and enzymatic degradation that occur along the alimentary tract. Since many potential vaccine candidates are microbial components or subunits and are not viable organisms or particulate immunogens, considerable effort has focused on the development of delivery systems. Such delivery systems include liposomes and biodegradable microparticles, as well as recombinant bacterial and viral vectors. Strategies for vehicle development have included not only delivery of intact immunogens and genes that code for relevant antigens, but also systems that promote uptake by relevant cells (e.g., M cells) at GALT inductive sites. Detailed information on antigen delivery systems can be found in several recent reviews (Eldridge *et al.*, 1993; Michalek *et al.*, 1994, 1995). Although many of these delivery systems may be relevant to the induction of mucosal antibody responses owing to their ability to induce vigorous responses at GALT effector sites, information is more limited on the potential to employ these vehicles to induce ocular mucosal immune responses. Based on recent observations indicating that microparticle-encapsulated antigens evoke ocular mucosal responses, discussion will be confined to this delivery vehicle.

Poly(lactide-*co*-glycolide) (PLG) microparticles are biodegradable under physiological conditions and represent an important candidate vehicle for delivery and controlled release of vaccine components (O'Hagan *et al.*, 1991; O'Hagan, 1994). PLG has been used for suture material (Wise *et al.*, 1979) and as a delivery system for drugs (Hutchinson and Furr, 1985). PLG does not

evoke a tissue response (Visscher *et al.*, 1987) and has been certified by the FDA for human use. Recently, PLG microparticles have been used for ocular drug delivery without provoking histological or electrophysiological changes to the eye (Moritera *et al.*, 1992). In addition, PLG encapsulated antigens induce sustained systemic and secretory immune responses following oral administration (Challacombe *et al.*, 1992; Maloy *et al.*, 1994). Based on these data as well as observations that the cytokines IL-5 and IL-6 enhanced tear IgA antibody responses following topical application to the eye (Pockley and Montgomery, 1991; see Section III.B), PLG microparticles appear to be an appropriate vehicle to deliver antigens and immune potentiators to ocular mucosal inductive sites. Studies indicate that DNP–bovine serum albumin as well as IL-5 and IL-6 can be encapsulated in PLG microparticles without significant loss of activity. In addition, ot delivery of encapsulated antigen and cytokines evokes long-term mucosal antibody responses in tears and distal secretions as well as circulating antibodies in the rat model (Rafferty *et al.*, 1996). Further, oral delivery of a poly(lactide) encapsulated chlamydia candidate vaccine in mice induced antigen specific serologic responses and conferred partial protection against ocular challenge (Whittum-Hudson *et al.*, 1995b; see Section IV.A). While further studies are required to define the regulatory parameters and events leading to these responses, microparticles clearly are promising candidate delivery vehicles to elicit ocular mucosal immune responses.

IV. Targets for Vaccine Development

The conjunctiva is the most commonly infected tissue of the eye (Syed and Hyndiuk, 1992; Tabbara and Hyndiuk, 1995). Infection may be limited to the conjunctiva only or may be part of a lid or corneal infection. Most conjunctival infections are not vision-threatening, but most notable exceptions are *Neisseria gonorrheae* and herpes simplex virus. Conjunctivitis associated with tularemia (adults) (Knorr and Weber, 1994) and haemophilus infections (infants) (Syed and Hyndiuk, 1992) are potentially fatal because of systemic dissemination. The profile of pathogens isolated from normal and diseased human conjunctiva may vary depending upon geographic locale (Syed and Hyndiuk, 1992). Similarly, some veterinary pathogens of the conjunctiva/lacrimal gland are geographically confined (Lepper *et al.*, 1993; Rogers *et al.*, 1993). Chronic inflammation of the conjunctiva may lead to scar formation and is termed chronic progressive conjunctival cicatrisation (Bernauer *et al.*, 1993). Cicatricial diseases often progress to involve the lacrimal and meibomian glands. The combination of altered tear film, scar contraction, and corneal trauma from trichiasis may lead to blindness. Although there are noninfectious etiologies for many cicatrical disorders including pemphigoid, chlamydial infections of the conjunctiva are a classic example of the blinding sequelae of infection.

As discussed above and elsewhere (Sullivan, 1994), there are physical and biochemical barriers to help prevent ocular infection. For extracellular ocular bacterial infections, antibodies are a major component of protective immunization. Both antibody and cell-mediated immunity are believed to be important in protection against intracellular pathogens including viruses, chlamydia, and mycobacteria. Antibodies would block infection and clear free organisms, whereas $CD8^+$ cytotoxic T cells would clear infected cells and prevent full differentiation and spread from lysed host cells. There is little *in vivo* evidence from ocular sites that cytotoxic T lymphocytes are important in clearance of infectious pathogens. However, CD4 T cells serve as antigen presenting cells for B-cell responses and both CD4 and CD8 T cells produce cytokines that facilitate B- and T-cell responses. Many bacteria, viruses, and parasites are targets for vaccine development to prevent respiratory, genital tract, or other infections. If protective mucosal immunization is induced at one or more of these latter sites, it is probable that the ocular mucosal surfaces would also be protected.

A. Bacterial Infections

A variety of bacteria can infect ocular tissues. Table I lists some of the bacterial pathogens that are known to cause conjunctivitis. Bacterial conjunctivitis is most commonly caused by *S. aureus. Streptococcus* sp. are common conjunctival pathogens, except for β-hemolytic streptococci (*S. pyogenes*), while other members of this group are part of the normal conjunctival flora. These bacteria and *Haemophilus influenzae* are the most common agents causing dacryocystitis (lacrimal gland infection). *Corynebacterium diphtheriae* is a rare ocular pathogen, but can cause conjunctival scarring and associated problems. For the most part, bacterial infections of the conjunctiva are self-limiting and nonvision threatening. *Neisseria gonorrhoeae* is an exception, and this organism is the leading cause of hyperacute conjunctivitis particularly in developing countries (Syed and Hyndiuk, 1992). *N. meningitis* conjunctivitis, which cannot be distinguished clinically from *N. gonorrhoeae,* also may be associated with systemic disease. *H. influenzae* conjunctivitis occurs in children as well as adults, and one could predict a decline in the incidence of this ocular disease now that the Hib vaccine is available. Anaerobic organisms compose another part of the normal flora of the conjunctival sac. As for other infectious pathogens, the incidence of conjunctival infections with abnormal anaerobic organisms is increased in patients

TABLE I
Major Bacterial Pathogens Causing Conjunctivitis

Organism	References[a]
Staphylococcus aureus	Holzberg *et al.*, 1992
Neisseria gonorrheae	Syed and Hyndiuk, 1992
Streptococcus sp.	Murphy *et al.*, 1991
Haemophilus influenzae, H. meningitidis	Syed and Hyndiuk, 1992
Borellia burgdorferi	Aaberg, 1989; Zaidman, 1993
Francisella tularensis	Knorr and Weber, 1994
Chlamydia trachomatis	Wills *et al.*, 1987; Taylor *et al.*, 1992; Heggie and Lass, 1994; Brunham and Peeling, 1994;
C. psittaci	Dhingra and Mahajan, 1991
Pseudomonas aeruginosa[b]	King *et al.*, 1988; Brook and Hulburd, 1993
Shigella flexneri, S. sonnei[c]	Linde *et al.*, 1993
Brucella melitensis[d]	Young, 1983; Zundel *et al.*, 1992

[a]Selected primary articles and/or reviews of bacterial ocular pathogens.

[b]May cause conjunctivitis, but usually is associated with corneal infections following abrasion or tissue injury (Hazlett *et al.*, 1976; Ramphal *et al.*, 1981).

[c]Guinea pig ocular challenge model is used to test for protective immunity induced by anti-Shigella vaccine, because corneal/conjunctival epithelium is similar to intestinal epithelium in susceptibility to invasion by *Shigella*.

[d]Infects conjunctival mucosa, but is more of a problem for abortion in ewes and goats; however, vaccination *via* the conjunctiva reduced spontaneous abortions compared to systemic immunization.

with AIDS (Dugel and Rao, 1993; Campos *et al.*, 1994). Similarly, some cases of microsporidial conjunctivitis have been reported in AIDS patients (Lacey *et al.*, 1992; Lowder, 1993; Weber *et al.*, 1993).

Chlamydia trachomatis, an obligate intracellular bacterium, has received a great deal of attention with respect to vaccine development. While chlamydia has gained notoriety in recent years as a major sexually transmitted pathogen, it is, in fact, the leading cause of preventable blindness. Trachoma, resulting from chronic chlamydial infection of the conjunctiva, has been known since ancient times. Studies of patients in several endemic areas worldwide yielded valuable information regarding the epidemiologic, microbiologic, and serologic characteristics of trachoma (Nichols *et al.*, 1973; Grayston *et al.*, 1985; Dawson *et al.*, 1989; Mabey *et al.*, 1992; Taylor *et al.*, 1992). Two major animals models have been used for immunologic studies of chlamydial ocular infections: a guinea pig model which can be infected with *C. psittaci* causing guinea pig inclusion conjunctivitis (GPIC), and subhuman primates, including owl monkeys and cynomolgus monkeys, which can be infected with several biovars of *C. trachomatis,* the usual human ocular pathogen (Patton, 1990; Rank and Whittum-Hudson, 1994). More recently, a mouse model of ocular infection was developed using a human ocular biovar (Whittum-Hudson *et al.*, 1995a); this model should aid in immunologic studies which have not been possible with the other outbred species.

Vaccine development for this bacterium has been hampered by several key factors: (1) no protective antigen or epitope(s) has been conclusively identified from the plethora of immunogenic proteins; (2) no chlamydial target antigens have been shown convincingly to be expressed on the surface of infected host cells, and (3) chlamydia-induced disease sequelae are immune-mediated, though only the 57-kDa chlamydial heat-shock protein has been associated with immunopathogenic responses (Watkins *et al.*, 1986; Taylor *et al.*, 1987a; Morrison *et al.*, 1989a,b).

Various permutations of the chlamydial major outer membrane protein (MOMP) have been tested for immunogenicity and their ability to induce protective immunity in experimental animals. Biochemically extracted native MOMP, recombinant MOMP, or its variable domains have been presented by systemic and mucosal routes. In some cases, protection was tested by infectious challenge at the ocular mucosal surface or other sites (genital tract, lung, etc.). Table II lists some of the chlamydial vaccine candidates tested experimentally over the past two decades. In most cases, whole MOMP or subunits were highly immunogenic as measured primarily by serologic responses, but no significant *in vivo* protection against ocular infection was observed after direct immunization. Studies by several groups (Ward *et al.*, 1986; Stephens, 1990; Mabey *et al.*, 1991; Stagg *et al.*, 1993; Murdin *et al.*, 1993, 1995; Tuffrey, 1994) have identified important MOMP peptide epitopes to which immune responses have been induced. None of these have been tested in ocular infection models as yet, but several have been at least partially protective in chlamydial genital infection models (reviewed by Tuffrey, 1994).

Other chlamydial antigens such as the chlamydial lipopolysaccharide (LPS) have been shown to be immunogenic but not protective in an ocular infection model (Taylor and Prendergast, 1987). An additional chlamydial antigen, the exoglycolipid antigen (GLXA) (Stuart and MacDonald, 1989), is also expressed by chlamydia and secreted from infected cell *in vitro* (Stuart *et al.*, 1991, 1994; Wyrick *et al.*, 1994). Monoclonal anti-idiotypic (Id) antibodies to GLXA have been shown to immunize and protect mice against subsequent infectious ocular challenge with a human biovar of chlamydia (Whittum-Hudson *et al.*, 1994). Oral immunization of BALB/c mice with poly(lactide) encapsulated anti-Id antibodies to GLXA was shown recently to be even more protective than the soluble antibody since 25-fold less antibody presented in microspheres induced equivalent or better reduction in microbiologic disease. In both cases, mice developed serum antibodies to the

TABLE II
Potential Chlamydial Vaccine Antigens Tested in Ocular or Extraocular Models

Antigen[a]	Delivery route[b] 1°/2°	Adjuvant/carrier[c]	References
Whole organism	i.n.		Pal *et al.*, 1994
	Oral		Taylor *et al.*, 1987b
Whole organism extracts	i.m. or s.c.	Oil emulsion	Grayston *et al.*, 1962; Nichols *et al.*, 1966, 1969; Soldati *et al.*, 1971; Schachter, 1985
MOMP (TX100)	s.c.	CFA	Taylor *et al.*, 1987a
	Oral		Taylor *et al.*, 1988
MOMP (OGP)	s.c.	CFA	Batteiger *et al.*, 1993; Campos *et al.*, 1995
	Oral/ot	CT	Campos *et al.*, 1995
rMOMP subunit	ot		Wong *et al.*, 1990
	Oral/ot	CT-B conjugate	Taylor *et al.*, 1989
MOMP/rMOMP subunits	s.c./Peyer's patches	Alum	Tuffrey, 1994
rMOMP subunit fusion protein	s.c./s.c. (EB)	Titermax	Allen and Stephens, 1993
rMOMP subunit fusion protein	Oral/oral ot s.c./s.c.	CT/liposomes	O'Brien *et al.*, 1994; Whittum-Hudson *et al.*, 1995a
	i.d.	Alum/*Salmonella typhimurium*	Hayes *et al.*, 1991
rMOMP hybrid	s.c./s.c.	Poliovirus hybrid	Murdin *et al.*, 1993, 1995
hsp 70	s.c. or ot		Taylor *et al.*, 1990
hsp 60	ot		Taylor *et al.*, 1990
	s.c./ot		Rank *et al.*, 1995
Anti-idiotypic antibodies to	s.c./s.c.	Alumina (Maalox)	Whittum-Hudson and Taylor, 1994; Whittum-Hudson *et al.*, 1995a,b
chlamydial exoglycolipid	Oral/oral	Encapsulated (microspheres)	

[a]MOMP, major outer membrane protein; TX100, Triton X-100; OGP, octyl glucosyl pyranoside; rMOMP, recombinant MOMP; hsp, heat shock protein.
[b]s.c., subcutaneous; i.n., intranasal; ot, ocular topical; i.d., intradermal; i.m., intramuscular.
[c]CFA, complete Freund's adjuvant; CT, cholera toxin; CT-B, cholera toxin B subunit; IFA, incomplete Freund's adjuvant; EB, chlamydial elementary bodies.

GLXA and these antibodies were neutralizing for *in vitro* infectivity (Whittum-Hudson *et al.*, 1995b; Whittum-Hudson *et al.*, submitted). This type of vaccine approach has potential value for chlamydial infections at other sites, and against other biovars since the GLXA antigen is genus-specific as opposed to being serovar-specific.

It has been hypothesized by several investigators that protective immunity against chlamydia will require induction of neutralizing antibody in tears and it is assumed that this will be S-IgA. IgA in tears from patients with trachoma passively neutralized chlamydia and reduced disease in eyes of owl monkeys (Nichols *et al.*, 1973; Barenfanger and MacDonald, 1974). This was consistent with observations in guinea pigs ocularly infected with *C. psittaci* (Orenstein *et al.*, 1973; Malaty *et al.*, 1981). The dilemma with the chlamydial pathogen is that individuals who have had a previous ocular (or other site) infection with chlamydia are not significantly protected against subsequent ocular infections. This lack of protective immunity is seen despite evidence of vigorous antibody responses to many chlamydial antigens in tears and serum and *in vitro* lymphocyte proliferative responses to whole organism or purified antigens (Newhall *et al.*, 1982; Ward *et al.*, 1986; Whittum-Hudson and Taylor, 1989; Taylor, 1990). Several clinical studies have shown a poor correlation of acquisition of genital chlamydial infection with the presence of neutralizing serum antibodies (Jones and Van der Pol, 1994).

Cytotoxic T cells have not yet been associated directly with ocular protective immunity, nor with the immunopathogenesis of chlamydia ocular infection. It has been argued that the lack of exposure of chlamydial antigens on the surface of infected host cells would prevent them from serving as immunologic targets and may explain the failure to induce T-cell-mediated immunity. However, recent electron microscopic studies of human endometrial cells have shown that chlamydial MOMP, LPS, and the GLXA (Wyrick *et al.*, 1994) all pass from the inclusion body through the cytoplasm to the cell membrane. LPS are GLXA and both known to exit infected cells (Brade *et al.*, 1986; Stuart *et al.*, 1991;

Lukacova *et al.*, 1994). In addition, recent studies demonstrated CD8 mediated cytotoxic killing by chlamydia-primed T cells *in vitro* and some *in vivo* protection after experimental genital infection (Beatty and Stephens, 1994; Starnbach *et al.*, 1994). Thus, it is possible that chlamydial antigen presentation to $CD8^+$ effector T cells may occur in the context of MHC class I.

B. Viral and Parasitic Infections

Table III lists many of the viral and parasitic pathogens known to infect ocular tissues. With respect to viruses, such infections frequently result in conjunctivitis in both children and adults and these highly contagious infections (epidemic keratoconjunctivitis) resolve spontaneously over 2–3 weeks. The most common viral pathogen of the conjunctiva is adenovirus. Several adenovirus types (3, 7, 8, 19) may be associated with conjunctivitis, though corneal involvement is often seen (Murrah, 1988).

Ocular herpesvirus infections, though most often affecting the cornea, may cause conjunctivitis during primary infection. However, conjunctivitis is rare during recurrent infections with herpes simplex virus (Holzberg *et al.*, 1992). Herpes zoster ophthalmicus may develop in patients with latent varicella-zoster virus infections, and the conjunctivia is one of several ocular sites most often affected (Liesegang, 1993). Since at present there is no vaccine for herpesviruses, antiviral drugs remain the therapeutic option. There is a large literature concerning anti-herpesvirus vaccine development strategies, and this information is detailed in several reviews (Rouse and Lopez, 1984; Dix, 1987; Mader and Stulting, 1992; Stanberry, 1994). In addition to the fact that herpesviruses are ubiquitous and that the majority of individuals have been exposed previously, vaccine development strategies for ocular and extraocular herpes virus infections must deal with a number of questions: (1) Can recurrence of latent infections be ablated by vaccination or must primary infection be prevented? (2) Will herpesvirus-induced immunosuppression be overcome by appropriate immunization? (3) Will a combination subunit vaccine be required? and (4) Is systemic rather than mucosal immunization required? Table IV lists some recent herpes simplex virus vaccine candidates. A number of viral glycoproteins have been targeted for vaccines (e.g., Burke *et al.*, 1994), although there are some examples of better protection being conferred by vaccination with live organisms (Ghiasi *et al.*, 1995). Studies which directly tested for protection against ocular herpes simplex virus infections have used various viral constructs ranging from single or multiple glycoprotein constructs and deletion mutants delivered with or without adjuvant. Avirulent constructs have been tested in a variety of experimental models and clinical trials. While most immunizations have been *via* systemic routes (subcutaneous or intraperitoneal), a recent study using the rabbit model of recurrent ocular herpes infection showed that subconjunctival immunization with recombinant gB and gD after establishment of latent infection reduced spontaneous recurrences (Nesburn *et al.*, 1994). Promising results have been

TABLE III
Major Viral and Parasitic Pathogens of Ocular Tissues

Organisms	References[a]
Coronavirus[b]	Wickham *et al.*, 1994
Herpesviruses	Syed and Hyndiuk, 1992; Tabarra and Hyndiuk, 1995
Cytomegalovirus	Huang *et al.*, 1994
Epstein-Barr virus	Pepose, 1994; Jones *et al.*, 1994
Herpes simplex	Holzberg *et al.*, 1992; Pepose, 1994
Adenoviris (Types 3,7,8,19)	Holzberg *et al.*, 1992; Syed and Hyndiuk, 1992
Papillomavirus 16	McDonnell *et al.*, 1992
Picornaviruses (Coxsackie virus A24)	Syed and Hyndiuk, 1992
Simian immunodeficiency virus	Conway *et al.*, 1991
Feline immunodeficiency virus	Callanan *et al.*, 1992
Onchocerca vulva	Taylor, 1994
Rhinosporidium seeberi	Gori and Scasso, 1994

[a]Selected primary articles and/or reviews of nonbacterial ocular pathogens.
[b]Infects and replicates in cultured rat lacrimal gland acinar cells; but its capacity to infect *in vivo* is not known.

TABLE IV
Recent Herpes Simplex Virus Vaccine Candidates

Vaccine	References[a]
Purified or synthetic glycoproteins	
gB, gC	Chan, 1983
gB, gC	Roberts *et al.*, 1985
gD-1, gD-2	Long *et al.*, 1984
gB-1, gD-1,-2	Dix and Mills, 1985
gD	Ishizaka and Mishkin, 1991; Burke *et al.*, 1994
Baculovirus-expressed glycoproteins	
gB, gI, gC, gE, gG, and/or gH	Ghiasi *et al.*, 1995
gB-2, gD-2	Nesburn *et al.*, 1994
Adenovirus-expressed glycoproteins	
gB	Gallichan *et al.*, 1993
Vaccina virus-expressed glycoproteins	Aurelian *et al.*, 1991; Rooney *et al.*, 1991; Banks *et al.*, 1994; Fleck *et al.*, 1994
Deletion mutants (gH)	Farrell *et al.*, 1994
Anti-sense nucleic acids	Cantin *et al.*, 1992

[a]Selected references; see also Rouse and Lopez, 1984; Dix, 1987; Mader and Stulting, 1992; Stanberry, 1994.

shown in extraocular infection models by several groups. These have included strategies of mucosal vaccination with recombinant adenovirus expressing herpes simplex virus gB (Gallichan *et al.*, 1993), subcutaneous immunization with virus constructs missing an essential glycoprotein (e.g., gH; Farrell *et al.*, 1994), and systemic delivery of synthetic peptides. Other approaches have included use of anti-sense nucleic acids (Cantin *et al.*, 1992) and use of other expression systems (e.g., baculovirus and vaccinia). Recent studies of cytokine responses associated with *in vivo* or *in vitro* anti-viral protection have implicated IL-6, IL-10, and TNFα in either recovery from or reduced herpes infection (Chen *et al.*, 1994; Tumpey *et al.*, 1994; Babu *et al.*, 1995). Further investigations are required to distinguish between T-cell-mediated immunopathologic effects (Newell *et al.*, 1989; Hendricks and Tumpey, 1990; Hendricks *et al.*, 1992) and the potential requirement for individual T-cell subsets in vaccine-induced immunity.

Several common parasitic infections may cause conjunctivitis. These include *Mulluscum contagiosum, Onchocerca vulva, Verruca vulgaris,* and *Trypanosoma cruzi.* In some cases, the conjunctival mucosa may be the portal of entry (e.g., *T. cruzi*). The pathologic results of trypanosomiasis include the oculoglandular or ophthalmoganglionar complex composed of lid edema, granulomatous conjunctivitis, follicles, and inflammation of the lacrimal gland. Parinaud's oculoglandular syndrome is rare, and may also be caused by additional viral or bacterial agents (e.g., *F. tularensis, M. tuberculosis*) (Syed and Hynduik, 1992).

Experimental models of conjunctival and lacrimal gland infections have been used for many years both to identify pathogenic and protective immune responses and, more recently, to evaluate vaccine candidates. Recent advances include new rat and rabbit models of adenovirus ocular infections, which should help in evaluation of immune-mediated responses to these pathogens (Tsai *et al.*, 1992; Gordon *et al.*, 1994).

V. Summary

It is clear that events taking place both in conjunctival tissue and lacrimal gland are relevant to ocular mucosal vaccination strategies. While the lacrimal gland functions as a primary effector site for mucosal IgA antibody responses, the precise relationship of the conjunctiva to the mucosal network requires further study. It appears that the conjunctiva plays a role in the induction of mucosal antibody responses as well as serving as an effector site for cell-mediated responses. A variety of factors are involved in the regulation of ocular mucosal antibody responses and many options such as immunization routes, delivery vehicles, and immune potentiators have been investigated. Although the development of appropriate immunization strategies will depend on the properties of each potential vaccine candidate as well as the target ocular pathogen, the appropriate technology is now available to elicit mucosal antibody responses at the ocular surface. The current challenge is to identify and select the vaccine component(s) that can be used to elicit long-term protective responses to ocular pathogens.

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