NEW PARADIGMS IN INFECTIOUS EYE DISEASE: ADENOVIRAL KERATOCONJUNCTIVITIS

NUEVOS PARADIGMAS EN LA PATOLOGIÁ OCULAR INFECCIOSA: QUERATOCONJUNTIVITIS POR ADENOVIRUS

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Most ocular adenoviral infections present clinically as either simple follicular conjunctivitis, pharyngoconjunctival fever, or epidemic keratoconjunctivitis. In epidemic keratoconjunctivitis, ocular surface infection by species D adenovirus (Ad) serotypes 8, 19, or 37 induces an intense acute inflammatory response. Clinical signs include epiphora, eyelid edema, conjunctival pseudomembrane formation and hemorrhage, and punctate or geographic epithelial keratitis. In the absence of an effective antiviral therapy, treatment typically includes cool compresses, artificial tears, and in select cases, topical antibiotics and/or corticosteroids. Even with supportive treatment, the intense conjunctival inflammatory response can lead to permanent symblepharon formation and dry eye. In the cornea, multifocal subepithelial infiltrates (SEI) typically develop within 7 to 10 days after onset of the clinical signs of infection and can persist for months to years. SEI are quite literally the *sine qua non* of epidemic keratoconjunctivitis. In a recent case series of patients diagnosed with epidemic keratoconjunctivitis, fully one-third of patients had symptomatic SEI for longer than 45 days from the onset of conjunctivitis (1).

In 1958, Barrie Jones, an ophthalmologist at London's Institute of Ophthalmology and preeminent cornea and external disease specialist, put forth an explanation of SEI formation following ocular adenovirus infection that has since been cited repeatedly and without apparent reservation in the ophthalmic literature. Jones stated that upon adenovirus infection of the ocular surface epithelium, «the corneal stroma acts as an immunological blotter soaking up viral antigen from virus replicating in the overlying epithelium, to react in situ when hypersensitivity develops» (2). Later permutations of this theory focused on a delayed antigen-antibody reaction in the corneal stroma as the cause of SEI. Curiously, Jones' hypothesis has never been demonstrated experimentally or even directly studied, and antigen-antibody reactions in the corneal stroma, now recognized by clinicians as Wessley rings, are not seen in ocular adenovirus infections. Furthermore, proponents of the theory of SEI as antigen-antibody precipitations would seem to view the corneal stroma and its cellular constituents as passive targets of inflammation, rather than active participants in the innate immune response to infection. Emerging information details the central role of connective tissue fibroblasts in the early innate inflammatory response to infection and wounding, and in parallel, recent attention has focused on the role of the keratocytes, the predominant cells in the corneal stroma, as important players in the corneal stromal response to infection (3).

We hypothesized that adenovirus infection of corneal cells induces them to express chemokines, the proteins that induce leukocyte migration into tissues. Chemokines are 8-10 kDa, basic, heparin-binding peptides with a four-cysteine motif that cause leukocyte chemotaxis with a high degree of specificity for cell type. The α or CXC chemokines contain one amino acid between the first and second cysteine, while the β or CC chemokines have adjacent cysteines. Interleukin-8 (IL-8, also

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known as CXCL8) is an α chemokine that strongly and selectively induces chemotaxis and degranulation of neutrophils with a long duration of action. Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), is a β chemokine that induces chemotaxis of monocytes, basophils, CD4+ and CD8+ lymphocytes, and T lymphocytes of the activated memory subset. Ad19 infection of keratocytes induces the expression of large quantities of IL-8 and MCP-1 at very early times —within 30-60 minutes— after infection when adenoviral gene expression has not yet begun in these cells (4). Notably, IL-8 expression by Ad19-infected keratocytes greatly exceeds that of similarly infected corneal epithelial cells. Furthermore, the expression of IL-8 and MCP-1 appears to be mediated by an intracellular signaling cascade initiated by Ad19 binding to surface integrins on the keratocyte, and not by adenoviral replication. The entire signaling cascade appears to be controlled by activation of Src family kinases. More specifically, IL-8 and MCP-1 expression are differentially regulated by the ERK1/2 and JNK mitogen-activated protein kinases (MAPK) (4, 5). Chemical inhibitors of these signaling molecules inhibit chemokine expression in a dose-responsive fashion and as effectively as dexamethasone. We have recently found that a third member of the MAPK family, p38 MAPK, is also activated by Ad19 infection and plays a role in the expression of IL-8 by an indirect pathway (unpublished data). Other revelations about the role of signaling molecules in ocular adenoviral pathogenesis come from studies of phosphoinositide 3-kinase (PI3K), an anti-apoptotic signaling molecule also activated in keratocytes by Ad19 infection. Ad19 infection in the presence of inhibitors of the PI3K signaling cascade induced rapid apoptosis before the virus could replicate (6). These data suggest that inhibitors of PI3K or its downstream targets might act in antiviral fashion by a novel disinhibition of apoptosis in the infected cell.

To test these mechanisms of adenovirus-induced inflammation in the cornea, we have developed 2 new experimental models of infection. In the first, an *in vitro* tissue model is created using cultured keratocytes embedded in 3-dimensional disks of type I collagen. This «corneal facsimile» mimics the normal anatomy of the corneal stroma. By transfection of inducible dominant negative or constitutively active kinases into the keratocytes before embedding in the collagen matrix, we can modify intracellular signaling at any time during the course of subsequent infection. For our second approach, we induce keratitis in the C57BL/6J mouse cornea *in vivo* by the intrastromal injection of high titer Ad37, allowing the application of genetic mouse models deficient in specific signaling molecules or cytokines to these studies.

In summary, recent investigations into the cellular mechanisms that contribute to corneal inflammation in epidemic keratoconjunctivitis have revealed the tremendous capacity of keratocytes to initiate and amplify an innate immune response through receptor-mediated signaling and chemokine expression, and strongly support a role for adenovirus-infected keratocytes in corneal SEI formation. Although induced in lesser quantities after adenovirus infection, corneal epithelial chemokines may also contribute to stromal inflammation after infection. These observations will allow the design of novel therapies for the corneal stromal inflammation in epidemic keratoconjunctivitis, and perhaps other inflammatory corneal disorders.

REFERENCES

1. *Butt AL, Chodosh J. Adenoviral keratoconjunctivitis in a tertiary care eye clinic. Cornea 2006; 25: 199-202.*
2. *Jones BR. The clinical features of viral keratitis and a concept of their pathogenesis. Proc Royal Soc Med 1958; 51: 13-20.*
3. *Kumagai N, Fukuda K, Fujitsu Y, Lu Y, Chikamoto N, Nishida T. Lipopolysaccharide-induced expression of intercellular adhesion molecule-1 and chemokines in cultured human corneal fibroblasts. Invest Ophthalmol Vis Sci 2005; 46: 114-120.*
4. *Natajaran K, Rajala M, Chodosh J. Corneal IL-8 expression following adenovirus infection is mediated by c-Src activation in human corneal fibroblasts. J Immunol 2003; 170: 6234-6243.*
5. *Xiao J, Chodosh J. JNK regulates MCP-1 expression by adenovirus type 19-infected human corneal fibroblasts. Invest Ophthalmol Vis Sci 2005; 46: 3777-3782.*
6. *Rajala MS, Rajala RV, Astley RA, Butt AL, Chodosh J. Corneal cell survival in adenovirus type19 infection requires phosphoinositide 3-kinase/Akt activation. J Virol 2005; 79: 12332-12341.*