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Review

Chemokines and ocular pathology caused by corneal infection with herpes simplex virus

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The role played by chemokines in disease process is an active area of research that continues to uncover new players. In this report we discuss the likely role of selected chemokines in the disease herpetic stromal keratitis (HSK). This lesion occurs as a sequel to herpes simplex virus infection and is currently accepted as an immunopathological process which primarily involves $CD4^+$ T lymphocytes. In this review we discuss the events involved in HSK, the chemokine profile associated with this disease, and speculate on cellular activities and molecular events which characterize HSK as an immunopathological disease.

Keywords: chemokines; herpes simplex virus; immunopathology

Introduction

The last decade has seen major advances in our understanding of molecular events that occur during inflammatory reactions. One family of molecules involved was virtually unknown 10 years ago. These are chemokines, structurally related small proteins that include at least 40 named molecules (Luster, 1988). The list is expanding rapidly in large part because new members are being recognized by bioinformatics (Rossi *et al*, 1997) rather than by the old fashioned approach of isolation from biologically active samples. Although initially recognized as molecules produced under pathologic conditions, chemokines also play important housekeeping roles. These include leukocyte development, renewal as well as trafficking to sites such as the secondary lymphoid organs (Baggiolini, 1998). Chemokines are named as such because the proteins recognized initially were involved in attracting leukocytes. Cell chemotaxis is not, however, the only role that chemokines play in the inflammatory process. Recently, for example some chemokines were shown to influence neovascularization (Streiter *et al*, 1995a). Some molecules, such as the CXC chemokine IL-8, may be angiogenic whereas others of quite similar structure can either be either nonangiogenic or even angiostatic (Koch *et al*, 1992). The marked differences in biological activity appear to be mainly dependent on the presence or not of the three amino acid motif glutamine-leucine-arginine (ELR) close to the N terminus of the molecule (Koch *et al*, 1992). Chemokines of the CXC subfamily with ELR are usually angiogenic and those lacking the motif are nonangiogenic or angiostatic (Streiter *et al*, 1995b).

Inflammatory reactions, particularly if they involve significant neovascularization, occurring along the visual axis markedly impair vision. Indeed the eye appears to have adopted several mechanisms that serve to minimize inflammatory events. Examples include the presence of molecules in ocular fluids which are anti-inflammatory such as TGFβ and the expression of ligands such as Fas ligand that induces apoptosis in potentially Fas expressing inflammation mediating T lymphocytes (Ferguson and Griffith 1997; Streilein, 1996). The topic of ocular immune privilege along with its benefits and drawbacks has received some recent excellent reviews (Ferguson and Griffith, 1997; Streilein, 1996). In spite of the existence of mechanisms that minimize ocular inflammation, ocular inflammatory reactions nonetheless do occur. An example follows infection of the eye with herpes simplex virus (HSV). One form of herpetic keratitis results in a chronic inflammatory reaction in the stroma and this lesion represents the commonest infectious cause of blindness in the industrialized world (Pepose *et al*, 1996). It is becoming evident that several chemokines play

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key roles during the pathogenesis of Herpetic Stromal Keratitis (HSK) and it could be that future control measures may exploit our expanding knowledge of chemokine biology. This report focuses on the role of chemokines in experimental HSK that occurs in susceptible mouse strains infected via the cornea with HSV type 1.

Herpes stromal keratitis (HSK)

About 20% of individuals with herpes keratitis develop a chronic inflammatory reaction in the stroma referred to as herpes stromal keratitis (Liesegang, 1989). This lesion, which causes severe vision impairment, is considered as an immunopathological reaction to virus infection (Thomas and Rouse, 1997a). Solid support for this viewpoint comes from experimental studies in a mouse model. Thus, as initially shown by Metcalf *et al* (1979) and confirmed by many others, lesion expression requires the presence of immunocompetent T lymphocytes (Russel, 1984; Newell *et al*, 1989; Hendricks and Tumpey, 1990; Avery *et al*, 1995). In fact, $CD4^+$ T cells producing Type 1 (Th 1) cytokines are mainly responsible for orchestrating the inflammatory reactions (Niemialtowski and Rouse, 1992; Doymaz and Rouse, 1992; Hendricks *et al*, 1992a). Moreover, lesions may resolve if Th2 cytokines such as interleukin-10 (IL-10) are expressed in corneal tissues (Tumpey *et al*, 1994; Daheshia *et al*, 1997). Although there is general agreement that $CD4^+$ T cells are the principal cell types that organize the inflammatory lesions in HSK, usually viral antigens are not demonstrable during lesion progression (Babu and Rouse, 1996; Hendricks and Tumpey, 1990). Thus, it remains to be shown not only what factors cause the $CD4^+$ T cells' recruitment, but also the identity of antigens that the T cells recognize requires definition. Recently, at least three groups of investigators, including our own, have begun to evaluate the roles that various chemokines subserve during the pathogenesis of HSK (Chen *et al*, 1996; Yan *et al*, 1998; Thomas *et al*, 1998).

Following infection of the cornea, virus replication occurs primarily in epithelial cells and within 18 h, the underlying stroma becomes infiltrated by leukocytes that are mainly neutrophils (PMN). This response peaks in intensity at 48 h and then declines such that by 5 days after infection it may be barely apparent. This pattern of PMN invasion correlates with the time when virus can be detected (Tumpey *et al*, 1996; Thomas *et al*, 1997b). In fact, it is thought that the PMN are responsible for viral clearance although how they subserve this function remains undefined. Curiously, after the initial phase of corneal invasion by PMN, a second and more aggressive wave of cellular infiltration dominated by PMN ocurs starting around 8 days post-infection (Thomas *et al*, 1997b). This second wave corresponds with the initial ingress of $CD4^+$ T cells into the corneal stroma. Such cells are enriched in the activation phenotype and likely are only successful in invading the cornea once some angiogenesis has occurred in the normally avascular cornea. The second wave of inflammatory cell response is obvious clinically and this response may persist for weeks although it often progresses to a necrotizing form that necessitates euthanasia (in the mouse model). A summary of some critical events which occur in the pathogenesis is depicted in Figure 1.

The many issues during HSK pathogenesis that require explanation in terms of chemokine involvement include the initial wave of PMN invasion, angiogenesis, the preferential invasion of $CD4^+$ *versus* $CD8^+$ T cells starting around day 8 post-infection and the secondary clinically evident inflammatory reaction dominated also by PMN.

Inflammatory cell invasion

Following viral infection and replication within the corneal epithelium, several chemokine mRNAs are promptly expressed in corneal tissue and PMN invade the stroma (Su *et al*, 1996) (see Figure 2). The fact that virus needs to be present and capable of

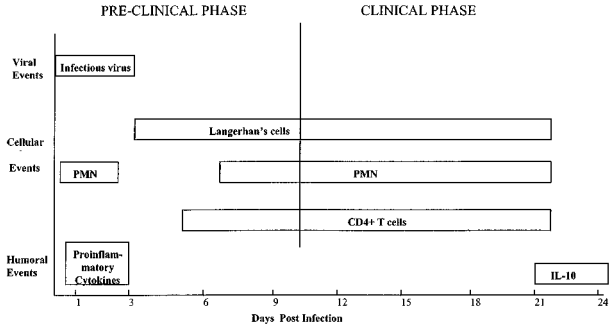
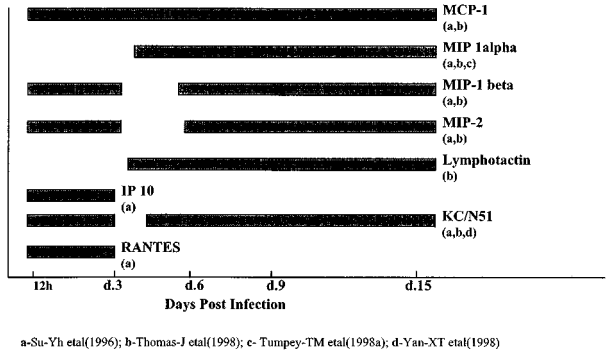


Figure 1 Critical events during HSK pathogenesis.



a-Su-Yh etal(1996); b-Thomas-J etal(1998); c- Tumpey-TM etal(1998a); d-Yan-XT etal(1998)

Figure 2 Some chemokines that may be relevant during the HSK pathogenesis.

replicating has been established by showing feeble chemokine responses if virus was neutralized with antibody or corneas are infected with replication defective mutants or UV inactivted viruses (Su *et al*, 1996; Thomas *et al*, 1998). In both instances, PMN invasion was also reduced as was the development of clinical HSK (Su *et al*, 1996). The cellular source of chemokines and the nature of the induction stimulus remains ill-defined. Cells infected by the virus is the most logical candidate source, but once virus replication is well underway in a cell, host cell mRNA is curtailed (Fenwick and Clark, 1982). Some evidence does exist, however, that viral immediate early protein expression is all that is necessary to induce chemokine expression in cell *in vitro* (Thomas *et al*, 1998). An alternate cellular source is nearby cells that are exposed to products released from HSV infected dying cells. The important topic of the cellular source of various chemokines in the HSV infected eye must await the refinement of approaches such as *in situ* hybridization combined with immunocytochemistry. Since PMN are by far the most prominent cell immigrants to the cornea following infection, one would presume that CXC chemokines would predominate in the early phase. Indeed CXC chemokines such as MIP-2 and KC, have been shown but in addition C-C chemokines such as MCP-1 is also present (Thomas *et al*, 1998). In fact of six chemokines measured in our studies only two tested ones, MIP-1α and lymphotactin, were lacking during the first few days after infection. Despite the representation of multiple chemokine species following HSV infection, some studies do indicate that single chemokines may be crucial. Thus the Lausch group have shown that neutralization of the CXC chemokine MIP-2 may markedly suppress the initial PMN invasion (Yan *et al*, 1998). Even more significant suppression was obtained; however, when both KC and MIP-2 were inhibited by specific antibodies. It is curious to know what function chemokines such as these are playing during the early phase. One possibility is that they serve to attract other cells to the cornea that participate in lesion pathogenesis and likely also act as an additional source of chemokines, these include Langerhans cells (Hendricks *et al*, 1992b) and natural killer cells (Bouley *et al*, 1995).

In spite of the continued presence of several chemokines mRNAs in the eye, after 2–3 days of infection PMN invasion slows and is usually no longer evident by day 5. The absence of PMN also coincides with the disappearance of infectious virus, an observation taken to indicate that PMN likely functions in some way to counteract virus replication (Thomas *et al*, 1998). Why PMN invasion ceases in the apparent continual presence of PMN attracting chemokines has no satisfactory explanation. However, it could be that an appropriate gradient of chemokine protein from the central cornea to the limbus (site of blood vessels) is no longer present. Alternatively some chemokines inhibitors could be produced either from infected cells or perhaps from dying PMNs themselves that inhibit chemokine protein recognition. An additional event which occurs around the time that the initial wave of PMN invasion ends, is the appearance of two additional chemokines, MIP1α and lymphotactin (Thomas *et al*, 1998). What causes their upregulation is not known but these chemokines are likely responsible mechanistically for the most crucial phase of HSK pathogenesis. This is cornea invasion by $CD4^+$ T cells that is followed by a secondary and more massive ingress by several inflammatory cell types but in particular PMN (Chen *et al*, 1996; Thomas *et al*, 1998). As discussed in the subsequent section, CD4 invasion may require neovascularization of the normally avascular cornea. Such angiogenesis might also be an essential function of certain chemokines (Strieter *et al*, 1995a).

Recent studies from the Lausch laboratory have provided provocative evidence that the secondary wave of inflammatory cell invasion during HSK pathogenesis is primarily dependent on the chemokine MIP-1α (Tumpey *et al*, 1998b). Thus, perhaps surprisingly given the large number of chemokines seemingly detectable, knock out mice unable to produce MIP-1α fail to show the secondary inflammatory cell invasion and do not develop clinical HSK (Tumpey *et al*, 1998b). In addition procedures which appears to selectively inhibit MIP-1α, such as the use of specific neutralizing antibody or the application of the cytokine IL-10 which abrogates MIP-1α production (Tumpey *et al*, 1998a), both suppress HSK. More information is required regarding the role of MIP-1α and other chemokines during $CD4^+$ T cell and the secondary PMN wave before the process can be fully understood. Other unresolved issues in HSK pathogenesis include an explanation for the markedly preferential invasion by $CD4^+$ *versus* $CD8^+$ T cells to the infectd cornea. Interestingly the $CD4^+$ T cells appearing in the cornea during HSK appear to represent mainly Th1 type $CD4^+$ T cells (Niemialtowski and Rouse, 1992; Hendricks *et al*, 1992).

The situation with HSK pathogenesis stands in contrast to another chronic inflammatory disease of the mouse cornea, namely that caused by Oncocerca volvulus (Pearlman *et al*, 1995). Here inflammation responses are dominated by $CD4^+$ T cells, but such cells in this instance are principally Th2 type T cells (Pearlman *et al*, 1995). A comparison of the chemokine microenvironment during HSK and Oncocerca keratitis is likely to be most revealing.

Chemokines and angiogenesis

The pathogenesis of HSK involves the development of new blood vessels in the normally avascular

cornea. Indeed, it is likely that angiogenesis is a necessary step to facilitate $CD4^+$ T cell and other inflammatory cell invasion to the central cornea during the clinically evident phase of HSK. Although the cornea has been a favorite tissue to study angiogenesis, the identity of specific angiogenic factors involved in neovascularization associated with HSK remains to be elucidated. Since certain chemokines are well known to have angiogenic activity, these represent likely candidates in the HSK system. For example, CXC chemokines with the ELR motif act as potent angiogenesis factors at least in the rat cornea (Streiter *et al*, 1995b). Curiously, other chemokines such as some CXC chemokines lacking the ELR motif, may inhibit angiogenesis (Cao *et al*, 1995). Some of the angiostatic chemokines exert their function by binding to heparan sulfate on target cells (Luster *et al*, 1995). Interestingly, heparan sulfate is also an important molecule involved in HSV entry into target cells (WuDunn and Spear, 1989). Thus it is conceivable that HSV may bind heparan sulfate and block any angiostatic factors thus driving the process towards angiogenesis. This speculative possibility requires evaluation as does the provision of direct evidence that any particular chemokine is involved in HSV induced angiogenesis. Both issues are under investigation in our laboratory.

Cellular interactions in HSK

Because the role of chemokines appears to firstly involve the establishment of gradients that recruit leukocytes to areas of inflammation, the identification of receptive cells is crucial for the understanding of chemokine molecular function. As with the chemokines themselves, multiple molecules may act as chemokine receptors (Rollins *et al*, 1997). Indeed, the outcome of actual events during HSK can as much depend on the expression of appropriate chemokine receptors on target cells and tissues as the production of the signaling chemokine molecules themselves. For instance, the differential expression of chemokine receptors on $CD4^+$ and $CD8^+$ T cells may account for the preponderance of $CD4^+$ T cells in HSK lesions. Additionally, leukocytes recruited by specific chemokine gradients presumably undergo phenotypic changes these may include upregulation of additional cell surface adhesion molecules thus permitting additional cell-cell or cell-matrix interactions to occur (Smith *et al*, 1997). Furthermore, most of these leukocytes, following activation, could become an additional source of cytokines and chemokines thus introducing increased flexibility to the local interactions involved in any inflammatory event. Thus, the events associated with clinical HSK expression, although initiated by viral replication and release of mediators from infected and uninfected corneal epithelial cells, may critically depend on the type, amount, and temporal release of chemokines and their subsequent interactions with receptors expressed on leukocyte and matrix cell surfaces. Although in HSK we are beginning to understand the role of certain chemokines, as yet nothing is known about the role of chemokine receptors nor the secondary interactions that could occur.

Conclusion

Clearly, chemokines form essential participants during ocular inflammation. They likely participate in selective cell recruitment as well as in a process of vital importance during ocular disease, namely angiogenesis. We are in the early phases of identifying some particular players during the pathogenesis of HSV induced corneal disease. Some chemokines, such as MIP-2 and KC, appear to be vital during the induction phase of HSK. However, we do not know the nature of agonists for chemokine induction nor the cellular site of their synthesis. HSV viral replication appears to act as a major stimulus for certain chemokine production but whether the major source is the virus infected cells themselves or products released from dying cells that stimulate other cells remain to be clarified. Most interestingly, the clinical phase of HSK involves a prolonged inflammatory event that progresses in the absence of demonstrable virus or viral antigens. Apparently, key chemokines such as MIP-1α are essential for the orchestration of the clinical phase. Thus it is important to identify what acts as the antagonist for MIP-1α induction as well as its cellular source. Moreover, it will be of interest to establish if chemokine manipulation during clinical HSK proves to be a useful way of managing the human disease. For example, if IL-10 application could be used to shut off MIP-1α induction or activity, this would be a useful form of therapy and well worth clinical evaluation.

The topic of chemokines and their receptors during ocular immunopathogenesis is very much in the discovery phase. We expect the next few years to add greatly to our knowledge and expect that some of this information may actually have practical significance for lesion control.

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