HHS Public Access

Author manuscript

Microbes Infect. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Microbes Infect. 2018 ; 20(9-10): 526–530. doi:10.1016/j.micinf.2017.12.014.

Application of our understanding of pathogenesis of herpetic stromal keratitis for novel therapy

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Abstract

HSV-1 ocular infection can cause herpes stromal keratitis (SK), an immunopathological lesion. Frequent recurrences can lead to progressive corneal scaring which can result in vision impairment if left untreated. Currently, the acute and epithelial forms of SK are usually controlled using anti-viral drugs. However, chronic forms of SK which are inflammatory in nature, require the addition of a topical corticosteroid to the anti-viral treatment regimen. In this review, we highlight the essential events involved in SK pathogenesis which can be targeted for improved therapy. We also examine some approaches which can be combined with the current treatments to effectively control SK.

Keywords

Herpes simplex virus-1; Herpes stromal keratitis

1. Introduction

Herpes stromal keratitis (SK) in humans is caused by herpes simplex virus (HSV-1 and HSV-2) infection [1, 2]. Although, both sub-types of HSV can cause ocular disease, the most common cause of keratitis is HSV-1. Acute infection with HSV-1 is usually mild or subclinical and most individuals remain asymptomatic. When primary corneal lesions occur they affect the corneal epithelium, last up to 2 weeks and resolve with minimal damage. The course of events can be shortened by the use of antiviral therapy. However, an inevitable consequence of HSV-1 ocular infection is the establishment of latency in the trigeminal ganglia (TG) [3]. From this site, periodic reactivation can occur which results in replicating virus reentering the cornea along with clinical consequences in many cases. Such recurrent infections can result in stromal keratitis which is mainly caused by chronic inflammatory

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Conflict of Interest: The authors have no conflict of interest.

reactions in the corneal stroma. Frequent reoccurrences can impair vision and ultimately lead to blindness[4].

2. Animal models

Most of our current understanding of the pathogenesis of SK comes from studies done in mice and rabbit models[1]. The availability of inbred strains, transgenic mice and specific gene knockout mice, as well as a wealth of immunological reagents makes mice the most convenient model to use, particularly for studies to define mechanisms accounting for the inflammation. The virus mainly replicates in the epithelium which is followed by inflammatory lesions in the stroma that resemble SK lesions observed in humans. In mice latency is established in the TG, but spontaneous reactivation rarely occurs in mice and when it does, lesions are uncommon. However, some groups have used the mouse model to induce SK lesions following the application of a variety of reactivation stimuli. These include heat stress, epinephrine, sodium butyrate, immune-suppressive drugs such as dexamethasone, cyclophosphamide and UV-B radiation [5]. The reactivation model, however, is inconvenient to use, hard to establish and the percentage of animals exposed to the reactivation stimuli versus those that develop lesions is usually low, even in the hands of experts. Moreover when lesions are attained and mechanistic studies performed, the findings are quite similar to those described to occur in the primary infection model[1]. The rabbit model offers some advantages over the mouse. These include eye size and the fact that spontaneous HSV-1 reactivation from latency and shedding of virus in tears does occur, especially when infected with virulent strains such as McKrae and 17 Syn^+. However, it should be noted that while rabbits reactivate and shed the virus at the cornea they rarely develop SK. The other limitations of the rabbit model include costs, limited availability of inbred/transgenic strains and the few reagents available to study immune responses [5].,

3. SK Pathogenesis

SK is an immunopathological disease which involves components of both innate and adaptive immune responses[6]. HSV-1 ocular infection in mice results in a complex inflammatory response. During the initial acute phase, the virus is cleared from the cornea, but results in a robust inflammatory response with long-term consequences of chronic inflammation that persists after clearance of the virus. The infectious virus is usually cleared from the corneas by 6–7 days post infection, although the virus does invariably infect the TG where it sets up a latent infection[1]. The initial control of infection is thought to largely involve innate components of immunity[7]. The chief immune cell types include dendritic cells, natural killer cells and neutrophils, but their relative importance has not been carefully studied[8, 9]. The clearance of infection is not a silent event since several factors can be produced which likely contribute to tissue damage[7]. These include release of inflammatory mediators such as cytokines, chemokines, matrix metalloproteinases (MMP) and free radicals which enable the recruitment of neutrophils and other inflammatory cells into the infected corneas[7, 1]. Studies in murine models of SK have shown a pro-inflammatory role for cytokines such as IL-1, TNF-α, IL-6, IFN-γ and IL-17[10–15]. The cytokines, IL-1 and TNF-α which are likely synthesized by corneal cells, macrophages or monocytes during the early phase might be responsible for the production of mediators such as prostaglandins and

leukotrienes[10]. This could result in the upregulation of chemokines such as MIP-1α and MIP-2 which serve as chemoattractants and further increase the influx of PMNs, macrophages and monocytes into the cornea[16]. Neutralization of IL-1 and TNF-α using antibodies diminished the severity of SK lesions in both the primary and recurrent infections in murine models[10, 11]. This indicates a key role for these two cytokines in the pathogenesis of the corneal disease. Another important cytokine produced during the early phase of infection is IL-6. Studies have demonstrated that IL-6 boosts the production of VEGF-A in the cornea and promotes corneal neovascularization and the ensuing tissue pathology[17]. In addition, IL-6 in combination with TGF-β can increase the differentiation of naive CD4 T cells into Th17 cells[18]. The role of IFN-γ and IL-17 which play a critical role in the initiation of chronic inflammation will be discussed latter in this review.

It is important to note that the cytokines produced during inflammation play variable roles with regard to tissue damage. For instance protective anti-inflammatory cytokines such as IL-10 play a critical role in regulating inflammation and help shield the host from pathologies and autoimmune diseases. Tumpey et al., and others have shown an important role for IL-10 in ameliorating SK lesions in the mouse model[19, 20]. These studies demonstrated that IL-10 mainly acted by reducing the synthesis of chemokines and the infiltration of neutrophils and T cells into the infected corneas.

A notable early event in the pathogenesis of SK is the generation of new blood vessels in the otherwise avascular cornea[21]. The vessels invade from the limbus and become clinically evident as early as 1 day post infection with the neovascularization process continuing to advance over the next 2–3 weeks period. These new blood vessels are often leaky and serve as a conduit for immune cells and their products to gain access to the cornea. The new blood vessels also contribute to vision impairment. The blood vessels develop in response to angiogenic factors that include, vascular endothelial factor (VEGF) and MMPs released from innate cell types[1, 22, 23]. Some studies suggest that in a normal cornea, corneal avascularity is maintained in part by the constitutive production of a soluble VEGF receptor 1 (sVEGFR-1) that binds to and inactivates VEGF[24]. A more recent study reported that ocular HSV-1 infection results in a rapid reduction in sVEGFR-1 in the cornea and this imbalance results in increased corneal VEGF levels[25]. The main sources of VEGF are corneal epithelial cells and infiltrating neutrophils. Thus in addition to VEGF inhibitors, blocking neutrophil infiltration into the corneas of HSV infected mice diminished both VEGF and MMP levels with a subsequent reduction in corneal angiogenesis[26].

In addition to corneal neovascularization, HSV-1 ocular infection can result in the destruction of corneal sensory nerves which innervate the cornea[27]. Two recent studies in mice showed that HSV-1 associated corneal nerve damage/retraction results in loss of corneal sensitivity and blink reflexes which results in desiccation that exacerbates SK[28, 29]. Importantly, Yun et al., reported that progression to severe SK associated with the loss of corneal nerve and blink reflexes was preventable by a technique called tarsorrhaphy (sewing together of eyelids), a clinical management procedure used to reduce corneal exposure and desiccation[28]. They also reported CD4 T cells involved in the inflammatory response negatively impacted on corneal nerve regeneration. In another study, Chucair-Elliot et al, showed that IL-6 was involved in corneal nerve degeneration after HSV-1

infection[12]. These are important findings as loss of corneal sensation and/or blink reflexes is one of the common symptoms of SK in human patients, and is associated with loss of sub-basal nerve plexus.

3.1. Role of Neutrophils

Neutrophils are the major component of the immune infiltrate in HSV-1 infected corneas in the mouse model[1]. Neutrophils are among the first cells to arrive after HSV-1 ocular infection[30]. Their numbers peak around 2 days post infection and decrease by day 5 post infection in the cornea[7]. As the influx of neutrophils into the HSV-1 infected corneas coincides with viral clearance, it is generally thought that they play an important role in viral clearance[30]. In fact, some studies demonstrated that depletion of neutrophils using the Gr-1 antibody delayed clearance of HSV-1 from the corneas of mice[9]. A major criticism of studies employing the Gr-1 antibody to deplete neutrophils is its lack of specificity as it depletes several other cell types including monocyte/macrophages and DC. A more recent study by the Hendrick's group demonstrated that the delayed clearance of HSV-1 from the cornea resulting from DC depletion was associated with decreased migration of NK cells and inflammatory monocytes, but normal migration of neutrophils occurred into the HSV-1 lesion into the cornea[8]. From these studies, it is possible that neutrophils may only be playing a partial at controlling primary virus infection. A second phase of neutrophil influx starts around day 7 post infection which coincides with the chronic/clinical phase of the disease. During the chronic phase, neutrophils are the dominant cell population comprising of 70–80% of the leukocyte infiltrate and likely contribute to the corneal opacity[1, 31]. Neutrophils cause tissue damage and inflammation by producing inflammatory mediators, degranulation and ROS production[31, 30]. Additionally, neutrophils are capable of releasing metalloproteinases such as MMP-9 which could degrade the extracellular matrix and they also facilitate corneal neovascularization by being a major source of VEGF[32].

3.2. Role of T cells

A major event that follows HSV infection in susceptible mouse strains is an inflammatory event orchestrated by T cells which are mainly of the CD4 T cell subset[1]. In some models, however, CD8 T cells do become more prominent although the lesions hardly become chronic since many animals die of herpes encephalitis. Studies using either CD4 knockout mice or mAb depletion of CD4 T cells have established the essential role of CD4 T cells in mediated SK immunopathology[33, 28]. CD4 T cells of the Th1 phenotype (IFN-γ, IL-2 producing cells) appear in the infected mouse corneas around day 6–7 post infection and their numbers in the cornea increase during the latter phase. The cytokines IFN-γ and IL-2, produced by Th1 cells play a key role as the initiators of chronic inflammation, probably by modulating the levels of chemokines/chemokine receptors[13]. More recent studies demonstrated a role for IL-17 and CD4 T cells producing IL-17 (Th-17 cells) in SK disease progression especially at a later stage of lesion development[14]. IL-17 increases the expression of the chemokine, CXCL1 which is crucial for the recruitment of neutrophils. These studies reported that IL-17R knockout mice or neutralization of IL-17 in mice delayed disease progression and reduced SK lesion severity. IL-17 expression was also reported in corneas of SK patients and may have a role in the recruitment of inflammatory cells into the corneal stroma during human HSK[34]. Both Th1 and Th17 CD4 T cells most probably

facilitate the massive infiltration of neutrophils into the infected cornea during the chronic phase of inflammation, as several studies have noted that the migration of neutrophils and the ensuing immunopathology are significantly reduced in the absence of CD4 T cells[1, 35].

Although, the ability of CD4 T cells to mediate SK is clear, the involvement of CD8 T cells in SK immunopathology and virus replication in the cornea is complicated. While studies done in most laboratories using the mouse model are in agreement that CD8 T cells are minimally involved in the development SK lesions, some groups implicate CD8 T cells in corneal disease. A study by Hendricks group showed that in the absence of CD4 T cells, a high infectious dose of HSV-1 can induce a transient SK mediated by CD8 T cells[36]. In addition, Allen et al., reported that CD8 T cells can mediate corneal scarring in mice infected with a mutant strain of HSV-1 (HSV-gK)[37]. Interestingly, Stuart et al., suggest that CD8 T cells might probably be playing more of a protective role by regulating the immunopathological response that causes SK lesions, as the disease was exacerbated in the absence of CD8 T cells[38]. Furthermore, recent studies by Conrady et al., demonstrated that CD8 T cells actively contributed to virus clearance from the cornea[39]. Thus the respective roles of different CD4 and CD8 subsets in SK remains an unresolved issue and depends to a large extent on the strains of both mice and viruses used for the studies.

Cells playing a protective role during SK pathogenesis are a subset of CD4 T cells which express the transcription factor FOXP3 and referred to as regulatory T cells (Treg). Studies from our lab and others have shown the importance of Treg in controlling aberrant inflammatory responses and preventing the associated tissue injury. Approaches manipulating Treg proliferation and function were found to be beneficial in suppressing SK lesions[40, 41]. Although the mechanism by which Treg function have not been fully deciphered, evidence suggest that Treg primarily cause suppression by producing anti-inflammatory cytokines such as IL-10, IL-35 and TGF-β[42]. Treg also use take up IL-2 thus depriving effector T cells from this crucial growth factor. They also induce a tolerogenic environment by directly interacting with dendritic cells (DC) and influencing the production of indoleamine 2, 3-dioxygenase (IDO), an enzyme which degrades the essential amino acid tryptophan and suppresses T cell responses[43]. Treg can also downregulate the capacity of DC to activate effector T cells. Another suppressive mechanism which Treg employ, is by expressing the inhibitory receptor CTLA-4 which blocks the effect of CD28 mediated co-stimulatory signals on effector T cells. However, some recent reports by the Bluestone group and others suggest that Treg could lose FoxP3 expression and suppressor function under major inflammatory conditions[44]. As Treg may also express the transcription factors TBET and RORγT in addition to FOXP3, they can become pro213 inflammatory in certain environments and start producing IFN-γ and IL-17. This condition is termed plasticity and it can have damaging consequences. It is generally thought that induced Treg populations are more unstable and susceptible to plasticity when compared to natural Treg populations. Notably, using fate mapping mice, Bhela et al., showed that Treg plasticity can occur in HSV-1-induced inflammatory environment and Treg may contribute to SK lesion severity by secreting the proinflammatory cytokine IFN-γ[45]. Plasticity in Treg is thought to occur as a consequence of epigenetic modifications in the Treg-specific demethylated region (TSDR). Drugs such azacytidine, retinoic acid and vitamin C which help maintain the demethylation

status of the TSDR region can be helpful in promoting the stability and improving the functionality of Treg especially under chronic inflammatory environments[46, 45].

4. Some novel treatment strategies for the management of SK

4.1. Anti-virals and corticosteroids

Anti-viral drugs are the first choice to treat herpetic ocular lesions in humans and these drugs effectively control acute HSV-1 infection as well as SK. In the United States, trifluridine solution and ganciclovir topical gel are the two drugs which are commonly chosen to treat dendritic epithelial ulcerations caused by acute HSV-1 infection[47]. Although acyclovir is used topically in Europe and other countries, it is only approved for oral therapy in USA[4]. A topical corticosteroid such as prednisolone is used along with an anti-viral for treatment of SK lesions[48]. Although this treatment modality has been effective, prolonged therapy with steroid drugs could cause problems such as cataracts. In addition corticosteroids may suppress immune responses which may result in virus reactivation. Furthermore long term therapy anti-viral usage can result in the development of resistant virus strains[49]. Moreover, the current anti-virals can only control active virus replication and cannot prevent virus reactivation[50, 48].

4.2. Specialized pro-resolving mediators

Recent advances in studies involving inflammation have uncovered a group of lipid mediators that include the lipoxins, resolvins, protectins and maresins which are collectively called specialized pro-resolving mediators (SPM)[51]. In animal experiments, synthetic versions of these lipid mediators exhibit potent anti-inflammatory and pro-resolving actions. Among the SPM, resolvins and protectins are mediators that are derived from the omega-3 poly unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Resolvins and protectins have proved to be efficacious in a range of experimental animal models of inflammatory diseases[52]. In mice infected ocularly with HSV, topical administration of resolvin E1 (RvE1) controlled SK and the extent of corneal neovascularization. RvE1 decreased the influx of neutrophils and effector $CD4^+$ T cells (both T_H1 cells and T_H17 cells)[26]. Furthermore, RvE1 dampened the production of pro-inflammatory cytokines and chemokines, including IFNγ, IL-6 as well as CXCL1 and decreased pro-angiogenic factors. Notably, RvE1 increased levels of the anti-inflammatory cytokine IL-10. Similar results were demonstrated for neuroprotection D1 (NPD1) and aspirin triggered resolvin D1 (AT-RvD1)[53, 35]. In addition, AT-RvD1 reduced the expression of pro-inflammatory miRNA such as miR-155, miR-132 and miR-223, which are involved in SK pathogenesis and corneal neovascularization[35]. AT-RvD1 causes attenuation of STAT1 which plays an important role in Th1 cell differentiation and IFN-γ expression. In rabbits infected with HSV-1, a combination treatment with pigment epithelial-derived factor (PEDF) plus docosahexaenoic acid (DHA) reduced dendritic corneal lesions, opacity, neovascularization and infiltration of CD4 T cells[54]. Importantly, these studies in HSV infected rabbits demonstrated that treatment with PEDF + DHA improved corneal nerve density, functional recovery of corneal sensation and induced the regeneration of damaged corneal nerves vital for maintaining ocular surface homeostasis. Overall, these studies illustrate the potential therapeutic benefits of SPM in the resolution of virus-

mediated inflammation. These lipid mediators may be highly effective and lack toxicity and in fact represent natural products shown in several systems to be upregulated and responsible for resolving inflammatory reactions

4.3. Anti-angiogenic agents

The development of corneal neovascularization is mediated by several growth factors[55]. Among these, VEGF is considered to be the key regulatory factor. Recently, VEGF has been targeted for therapy with humanized mAb such as bevacizumab and ranibizumab which reduced angiogenesis in wet macular degeneration[56]. A few case reports in humans have reported that bevacizumab reduced the corneal neovascularization associated with herpetic SK, but more studies are needed to compare the effectiveness of these mAbs to other approaches at controlling HSV induced neovascularization[57]. Some reports showed that Src family of tyrosine kinases are responsible for VEGF-mediated vascular permeability and angiogenesis. A recent study in a mouse model using SRC inhibitors revealed significant effect on HSV-1 induced corneal angiogenesis[58]. Since blocking corneal neovascularization effectively reduces the development of SK, inhibiting angiogenic factors could represent a potential therapeutic approach to treat SK lesions.

4.4. MicroRNA

MicroRNA are small non-coding RNA which function in the regulation of genes involved in several processes which include inflammation[59]. Recent studies in the murine models of inflammation and autoimmune diseases have shown a crucial role for microRNAs[60]. Among these miR-132 was found to increase the levels of VEGF-A and pathological angiogenesis. A more recent study in a mouse model of SK demonstrated that the levels of miR-132 are upregulated in the infected cornea and silencing the expression of miR-132 using antogomir nanoparticles against miR-132 significantly reduced corneal neovascularization and SK lesions[61]. In addition, targeting miR-155, a pro-inflammatory microRNA dampened SK by specifically diminishing the influx of Th1 and Th17 cells into the infected cornea[62]. Besides these two microRNA, others might also be involved and further studies are required to decipher their function. Thus manipulating the expression of microRNA could be beneficial in controlling the severity of SK lesions.

4.5. Regulation of metabolism

One potential approach to therapy of SK which merits investigation is to exploit differences in metabolism expressed by the individual cell types present in herpetic SK lesions. T cells and other immune cells show differences in the utilization of major metabolic pathways to generate energy and synthesis of bio-products required for their proliferation, survival and function[63]. It has become apparent from recent studies that cells involved in driving inflammatory events such as CD4 Th1 and Th17 T cells, effector CD8 T cells and M1 macrophages may primarily derive their energy from glucose through the glycolytic pathway[63]. In contrast naive and memory T cells which have low energy requirements mainly use oxidative phosphorylation. In addition, cells involved in limiting inflammation such as Treg and M2 macrophages also use oxidative phosphorylation along with fatty acid oxidation[64]. Thus an approach to therapy could be to modulate metabolic pathways to favor the expansion of cell types involved in lesion resolution. Our group has achieved some

progress and we recently demonstrated that CD4 T cells from HSV-1 infected animals were metabolically active and displayed increased glucose uptake. Furthermore, inhibition of glucose uptake by treating HSV-1 infected mice with 2-deoxy-glucose (2DG), a glucose inhibitor limited the differentiation and functionality of CD4 T cells resulting in diminished SK lesions[65]. In addition, recent studies have demonstrated that blockade of Th1 and Th17 cell metabolism controlled inflammation in some other disease conditions[64]. Further studies are needed in the future to understand the role of different metabolic pathways involved in the generation and function of immune cells and this will provide valuable insights for the design of novel therapeutics to control inflammatory diseases such as SK.

5. Concluding remarks

HSV-1 is a ubiquitous and highly successful human pathogen and has evolved to hide in a latent stage in the sensory nerve ganglia. The virus upon reactivation can travel from the trigeminal ganglia to the cornea and initial infection can result in symptoms such as dendritic ulcerations. Frequent recurrences can lead to progressive corneal scaring and vision impairment. Unfortunately, a satisfactory prophylactic or therapeutic vaccine is currently not available to protect against HSV-1 infections. Controlling herpes virus infections remains a challenge and there is a need for the development of more effective therapies. Although, the current anti-virals and corticosteroids are effective in controlling SK in humans, long term usage can result in problems. Alternative approaches such as combination/supplemental therapy using anti-viral along with other reagents, for example, SPMs, anti-cytokine drugs, anti-angiogenic agents, metabolic drugs which target crucial steps in SK pathogenesis such as inflammatory mediators and corneal neovascularization may be more effective. Moreover, combination treatment may be a useful and successful therapeutic approach compared to individual drugs given alone, as combination therapy could have additive or even synergistic effects because drug combinations can target multiple dysregulated pathways involved in SK.

Acknowledgments

Research was supported by National Institutes of Health Grant EY005093 to BTR. We also thank the department of Biomedical & Diagnostic Sciences, University of Tennessee, Knoxville for supporting the research work through the COE funds to NKR.

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