Corneal Infection and Inflammation

Monday, May 05, 2014 11:00 AM–12:45 PM
S 330GH  Paper Session
Program #/Board # Range: 1688–1694
Organizing Section: Immunology/Microbiology

Program Number: 1688
Presentation Time: 11:00 AM–11:15 AM

Hmgb1: A target for treatment of Pseudomonas aeruginosa keratitis


Purpose: The purpose of this study is to examine high mobility group box 1 (Hmgb1), a prototypic alarmin and member of a family of danger associated molecular patterns (DAMPS), that mediates the systemic inflammatory response syndrome, and is elevated late in bacterial infection/sepsis, as an attractive target for disease treatment in P. aeruginosa infection.

Methods: C57BL/6 (B6) mice were treated with vasoactive intestinal peptide (VIP) or PBS, infected and Hmgb1 expression assessed by real-time RT-PCR, ELISA and immunostaining. Mice were then treated with Hmgb1 siRNA or scrambled control and effects also assessed by real-time RT-PCR, immunostaining and ELISA.

Results: VIP versus PBS treatment decreased Hmgb1 mRNA and protein expression levels. Immunohistochemistry showed less Hmgb1 positive staining in corneal epithelium and stroma after VIP treatment. To test the effects of Hmgb1 in disease, siRNA versus scrambled control treatment was used. siRNA treated B6 mice had improved disease outcome and also demonstrated downregulation of corneal mRNA and/or protein levels for Hmgb1. IL-1β, MIP-2, TNF-α, TLR4 and RAGE; antiinflammatory SIGIRR and ST2 were upregulated. Immunohistochemistry showed that silencing Hmgb1 decreased Hmgb1 and IL-1β positive staining in cornea. Hmgb1 siRNA treatment also reduced PMN levels and CXCL12 and CXCR4 expression.

Conclusions: These data provide evidence that silencing Hmgb1 leads to better resolution of P. aeruginosa keratitis, as its reduction results in decreased proinflammatory mediator levels, increased antiinflammatory TLR production, decreased, but sufficient, PMN infiltration and reduced Hmgb1/CXCL12 heterodimer formation required for signaling through CXCR4.

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IL-17A-Mediated Protection against Acanthamoeba Keratitis

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Purpose: Acanthamoeba Keratitis (AK) is a very painful and vision impairing condition of the cornea that is difficult to treat. Although past studies have indicated a critical role of neutrophils and macrophages in AK, the relative contribution of a proinflammatory cytokine, IL-17A that promotes migration, activation and function of neutrophils in the cornea is poorly defined. Moreover, the role of the adaptive immune response, particularly contribution of CD4+ T cell subsets such as Th17 and Tregs, in AK is yet to be understood.

Methods: C57BL/6 mice corneas were intrastromally injected with 2.5 × 10⁴ Acanthamoebae for corneal infection. Acanthamoeba infected mice corneas and local draining lymph nodes (dLN) were analyzed on day 5 and day 8 post infection (pi) for neutrophils, CD4+ T cells, Th1, Th2, Th17 and Treg cell composition by flow cytometry. In another set of experiments, corneal IL-17A expression was analyzed on day 1, 3, 5 and 8 pi. Furthermore, infected mice were treated with anti-IL-17A or isotype monoclonal antibody every alternate day starting from day 1 until day 7 pi. The severity of AK was scored on day 1, 3, 5 and 7 pi. On day 8 pi, corneas and dLN were collected to analyze the effect of anti-IL-17A treatment on various CD4+ T cell subsets by flow cytometry.

Results: Corneal Acanthamoeba infection induced both Teffector (Th1, Th2 and Th17) and regulatory T cell response in the cornea at all tested days pi. More importantly, our studies revealed that Acanthamoeba infection induces IL-17A expression and that IL-17A is essential for host protection against severe AK pathology. Accordingly, IL-17A neutralization in Acanthamoeba infected animals resulted in a significantly increased corneal AK pathology, increased migration of inflammatory cells at the site of inflammation and a significant increase in effector CD4+ T cell response in dLN. Further studies indicated that neutrophils and CD4+ T cells contribute to the source of IL-17A during early and late stages of AK respectively.

Conclusions: Corneal Acanthamoeba infection induces both Teffector and Treg response in the cornea. Moreover, in sharp contrast to other corneal infections such as Herpes and Pseudomonas aeruginosa keratitis where IL-17A exacerbates corneal pathology and inflammation, findings reported in this study indicate that IL-17A response after Acanthamoeba infection plays an important role in host protection against invading parasites.

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Presentation Time: 11:30 AM–11:45 AM

IL-2/anti-IL-2 Complex Treatment Inhibits the Development but not the Progression of Herpetic Stromal Keratitis (HSK)

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Purpose: To determine if IL-2/anti-IL-2 complex (IL-2 complex) treatment given prior to or late after ocular herpes simplex virus-1 (HSV-1) infection affect the development of corneal opacity and angiogenesis.

Methods: C57BL/6 mice received intra-peritonal injection of freshly prepared IL-2 complex either prior to or late after ocular HSV-1 infection. IL-2 complex induced increased pool of naturally occurring CD4+Foxp3+ regulatory T cells (nTreg) were determined by flow cytometry. The development of the corneal opacity and angiogenesis was determined in different groups by slit lamp microscopy. Plaque assay was used to determine the viral load in infected corneas of PBS and IL-2 complex treated groups. Frequencies and absolute numbers of cytokine secreting CD4+ T cells in the corneas, lymph nodes and spleens of mice from different groups were determined by intracellular cytokine assay.

Results: In vivo administration of IL-2 complex is known to expand nTreg in uninfected mice. Our results showed that IL-2 complex treatment given three days prior to or four days after ocular HSV-1 infection optimally expand nTreg pool in secondary lymphoid
tissues on day 2 or day 9 post-infection, respectively. Interestingly, treatment-induced increased pool of nTreg at the time of T cell priming (Day 2 post-infection) significantly reduced the corneal opacity and angiogenesis. To determine the underlying mechanism, our results showed that increase in nTreg numbers at the time of priming lead to decrease in viral load in the corneas on day 2 but not day 4 post-infection when compared with control group. Lesser virus on day 2 post-infection was correlated with an increased influx of NK cells in infected corneas. Moreover, increased nTreg pool at the time of priming also resulted into decreased influx of CD4 T cells in infected corneas as determined on day 7 post-infection. There was also a significant reduction in the frequency of IFN-γ, IL-2 and TNF-α producing CD4 T cells in lymph nodes and spleen tissue of IL-2 complex treated than control groups of mice. On the other hand, increasing nTreg pool during contraction phase of T cell response or late after infection did not decrease the severity of HSK lesions.

Conclusions: We conclude that IL-2 complex induced increase in nTreg pool early but not late after HSV-1 infection is effective in reducing HSV-1 induced corneal opacity and angiogenesis.

Commercial Relationships: None; Susmit Suvas, None; Subhash Gaddipati, None; Kathleen Estrada, None; Pushpa Rao, None

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ATM Activation by HSV-1: a Critical and Targetable Step in Herpes Simplex Keratitis

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Purpose: Herpes keratitis (HK) remains the leading cause of corneal-derived blindness in the developed world, despite the availability of effective antiviral drugs. Treatment toxicity and the emergence of drug resistance highlight the need for additional therapeutic approaches. We examined ataxia telangiectasia mutated (ATM), an apical kinase in the host DNA damage response, as a potential new target for the treatment of HK.

Methods: Small molecule inhibitor of ATM (KU-55933) was used to treat HSV-1 infection in cultured human corneal epithelial cells, explanted human and rabbit corneas, and in mice. Infection productivity was assayed by plaque assay, real time PCR, Western blot, and disease scoring. Mechanistic studies relied on mutant viral strains, bacterial artificial chromosomes, and comet assays.

Results: Robust and early ATM activation was detected in HSV-1-infected human corneal epithelial cells. Inhibition of ATM greatly suppressed viral replication in cultured cells and in explanted human and rabbit corneas, and reduced the severity of stromal keratitis in mice. The antiviral effect of KU-55933 in combination with acyclovir was additive, and KU-55933 suppressed replication of a drug-resistant HSV-1 strain. KU-55933 caused minimal toxicity, as monitored by clonogenic survival assay and fluorescein staining. Mechanistic studies show that ATM activation during HSV-1 infection occurs in the absence of DNA damage and is independent of viral genome replication, tegument proteins alone, or ICP0. Results of further investigations into the mechanisms behind ATM activation and its downstream significance will also be presented.

Conclusions: This study identifies ATM as a potential target for the treatment of HK and delves into the mechanisms underlying its involvement. ATM inhibition by KU-55933 reduces epithelial infection and stromal disease severity without producing appreciable toxicity. Additional experiments reveal a potential mechanistic model of ATM activation and subsequent significance in HSV-1 infection.

Commercial Relationships: Oleg Alekseev, None; Kelly Donovan, None; Jane Azizkhan-Clifford, None

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Presentation Time: 12:00 PM–12:15 PM
Mast Cells Contribute to the Innate Defense Against Corneal HSV-1 Infection

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Purpose: The contribution of mast cells (MC) to corneal defense against herpes simplex virus type 1 (HSV-1) infection is currently unknown. Due to their proximity to the cornea, we hypothesized MC influence the host immune response to acute HSV-1 infection.

Methods: Wild type (WT) and MC-deficient (KitW-sh) mice were infected with 1000 plaque forming units of HSV-1 per eye following corneal scarification and compared at 24-48 hours post infection (pi) for corneal edema by pachymetry and viral titer by plaque assay. The immune response to HSV-1 was analyzed by flow cytometry, ELISA, Luminex-based arrays and real time PCR to phenotypically characterize infiltrating immune cells and measure contributing soluble factors at the mRNA and protein level. Corneal limbal button whole mounts were imaged by epifluorescent and confocal microscopy. Neutrophils (PMN) were isolated from infected corneas by immunomagnetic separation for HSV-1 lytic gene detection and adoptive transfer.

Results: Tissue-resident MC circumscribe the cornea in close proximity to the limbal vasculature of WT but not KitW-sh mice and undergo degranulation following corneal infection. Corneas from KitW-sh mice were significantly more edematous throughout the first 48 hours pi commensurate with a significant increase in infiltrating PMN and viral titer at 24 and 48 hours pi, respectively. There were modest changes in some chemokines expressed in the cornea comparing WT to KitW-sh mice but no significant changes in other soluble factors screened at the RNA or protein levels. Furthermore, we observed PMN colocalize with HSV-1 antigen in lesions of infected corneas in both WT and KitW-sh mice. In addition, the HSV-1 lytic gene thymidine kinase was expressed in PMN isolated from corneas of infected mice. Viral sentinel CD118+ mice deficient in the type I interferon receptor succumbed to HSV-1 infection following intravenous adoptive transfer of PMN isolated from infected corneas.

Conclusions: Collectively, these results provide a striking novel insight to the viral surveillance and pathogenesis of corneal HSV-1 infection. We have demonstrated that MC are a contributory resident immune cell population that conditions the innate response to insults in the cornea. We have also shown that PMN, which have historically been considered beneficial phagocytes, contribute to the pathogenesis of HSV-1 infection by facilitating viral replication and dissemination.

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Presentation Time: 12:15 PM–12:30 PM
Trigeminal ganglion inflammation following corneal injury

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Purpose: The cornea receives sensory innervation from the trigeminal ganglion (TG). Trigeminal nerve
ablation is associated with neurotrophic keratitis in cornea. However, the effect of corneal nerve damage on the TG -and specifically on inflammation- has not been clarified yet.

**Methods:** A corneal alkali burn was created in the right eye of CD1 mice. After 4 and 8 days, mice were analyzed by in vivo MRI before and post iv. administration of USPIOs contrast (marker of macrophages) using a 7T scanner. To assess USPIOs uptake, T2 relaxation time distribution was analyzed in both left and right TG. After MRI analysis, longitudinal sections of the TG were stained for Prussian blue to detect USPIOs+cells and for inflammatory CD markers to characterize these infiltrating cells. On day 1, 4 and 8, Real-Time PCR analysis was performed to measure the expression of pro-inflammatory cytokines in the TG, also after 4 days of topical anti-inflammatory treatment with 0.2% dexamethasone, only in the alkali-burned eye.

**Results:** The corneal alkali burn induced a significant CD45+leukocyte infiltration in the right TG after 4 (1.7 fold) and 8 (2.2 fold) days. This infiltration was prevalent in the anterior part of the TG on day 4. In vivo MRI follow-up showed an increase of USPIOs+macrophages in the right TG at both time points, specially on day 8 (2.2 fold). Specifically, USPIOs uptake was predominantly found in the anterior part of the right TG on day 4. The Prussian Blue+USPIOs+macrophages were observed only in the right TG, and showed a M2-phenotype (CD45+F4/80+CD206+). Alkali burn induced a significant time-dependent increase of pro-inflammatory cytokines (IL-1β, TNFα and VEGF-A) in both the TGs, but prevalent in the right TG. The expression of IL-1β and VEGF-A were significantly reduced in the right TG following corneal treatment with dexamethasone.

**Conclusions:** Our findings support the involvement of brain structures (i.e. TG) after ocular surface damage: a corneal injury is able (i) to induce infiltration of leukocytes, in particular of M2-macrophages, in the TG and (ii) to increase pro-inflammatory cytokines in the TG; (iii) this inflammation was attenuated following anti-inflammatory therapy in the cornea. These results have relevant implication in understanding the pathophysiology and, potentially, in the treatment of many ocular ailments.

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**Presentation Time:** 12:30 PM–12:45 PM

**Role of plasmacytoid dendritic cell in the immune regulation in sutured inflamed cornea**

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**Purpose:** Plasmacytoid dendritic cells (pDC) represent a highly functional subset of bone marrow (BM)-derived cells and play a key role in linking the innate and adaptive immune responses, but little is known about the nature and the role of pDCs in the cornea. The aim of this study is to investigate the role of corneal pDC in the immune responses in corneal inflammation

**Methods:** Corneal inflammation was induced by placement of 3 intrastromal 10-0 nylon sutures. Corneal pDCs were depleted by subconjunctival (s.c.) injection of diphtheria toxin (DT) or by PBS (sham controls) into BDC-A2-DTR mice. Cornea opacity was scored in a 0-5 scale. Corneas and draining lymph nodes (dLNs) were excised and single cell suspensions obtained for flow cytometry assay by staining with antibodies to CD45 (BM-derived cells), Gr-1 (neutrophils), NK 1.1 (NK cells), F4/80 (macrophages), CD11c (dendritic cells), CD3, CD4, CD8 (T cells), and CD19 (B cells)

**Results:** We observed a significant and surprising increase in the opacity in pDC-depleted corneas at day 7 (3.5) and 14 (3.5) vs. (0.8 and 0.6 respectively, P<0.001) as compared to sham controls. Similarly, a significantly increased influx of CD45+ (3-fold) was noted in pDC-depleted corneas as compared to sham controls was seen. Also, there was an increased density of neutrophils (3.1-fold), macrophages (2.8-fold) and dendritic cells (3-fold) as compared to sham controls; while T cells, NK cells and B cells didn’t show significant changes. Depletion of corneal pDC did not have an effect on CD4+ T cells and NK cells in dLNs on days 7 and 14. However, there was a modest increase in total T cells, CD8+ T cells (1.35-fold), as well as B cells (1.3-fold)

**Conclusions:** This data demonstrate that corneal pDC may mediate innate and adaptive immune responses in the cornea. Depletion of pDC results in increased influx and shift in balance of infiltrating cells in the cornea during inflammation. Furthermore, pDC may play a role in regulating the adaptive immune responses in dLN.

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