Heparanse is a Host-Encoded Virulence Factor for Herpes Simplex Virus Ocular Infection

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**Purpose:** Ocular herpes simplex virus type-1 (HSV-1) infection is often accompanied by chronic inflammation and neovascularization. Very little understanding exists on intrinsic cellular factors that contribute directly to the development of corneal disease. Our study demonstrates a novel role for heparanse as a host encoded virulence factor that directly controls the emergence of corneal disease symptoms.

**Methods:** Multiple HSV-1 infection models including human corneal epithelial (HCE) cells, cultured human and porcine corneas, and murine corneal infection were used for the study. Flow cytometry, fluorescence microscopy, ELISA, and confocal fluorescence microscopy were used for monitoring and quantitative assessment of changes in the levels of heparanse, several cytokines and pro-angiogenic growth factors. Murine corneas (with or without fluorescein staining) were examined for tissue damage using slit lamp biomicroscope and scored using a four point scale. Quantitative RT PCR (qRT-PCR) was used for mRNA quantification and immunoblotting was used to assess viral protein levels.

**Results:** Here we demonstrate that upon HSV-1 infection of the murine corneas heparanse expression is upregulated and the enzyme can be found relocated to the nucleus of infected cells. As a direct result of heparanse upregulation, the levels of many different cytokines and growth factors including Interferon alpha and beta, TNF-alpha, IL-6, IL-8, RANTES, and VEGF go up. The upregulation of cytokine expression in corneal cells can be achieved without HSV-1 infection by overexpressing an active form of heparanse in cells but not by the full-length proenzyme. Transgenic overexpression of heparanse in murine corneas results in exacerbation of HSV disease and disease symptoms. Reverse is seen by downregulation of heparanse by shRNA transfection in the murine corneas.

**Conclusions:** Overall, our findings implicate heparanse as a novel cellular regulator and a virulence factor for corneal disease development upon HSV-1 infection. Our work establishes the existence of a unique intrinsic host factor that directly contributes to the inflammation and neovascularization seen during HSV-1 infection of the cornea. It also identifies a novel druggable pathway for ocular herpes management.

**Commercial Relationships:** Deepak Shukla, None; Alex Agelidis, None; Satvik Hadigal, None

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**Program Number:** 4324  
**Presentation Time:** 8:30 AM–8:45 AM  
**Program #/Board # Range:** 4323–4328  
**Organizing Section:** Immunology/Microbiology

**Purpose:** The correlate(s) of immunologic protection (CIP) requisite for an efficacious herpes simplex virus type 1 (HSV-1) vaccine remains unclear with respect to viral pathogenesis and clinical disease. This study investigated the hypothesis that neutralizing antibody is the primary CIP against HSV-1.

**Methods:** Eight-week old female CD-1 mice were vaccinated using a prime-boost regimen with a glycoprotein D subunit vaccine (gD-2) or a recombinant, live-attenuated virus (0ΔNLS). Vaccinated and naive mice were ocularly challenged with HSV-1 McKrae (LD90 inoculum) and surveyed for survival, corneal pathology, tissue viral burden, and neuronal latency as determinants of vaccine efficacy. CIP were assessed by serum virus neutralization assay, flow cytometry, and passive immunization. One-way ANOVAs were used for statistical analysis; data reflect 2-5 independent experiments.

**Results:** Directly vaccinated mice survived HSV-1 challenge with higher frequency than naive (p<0.01). Viral replication in the cornea and trigeminal ganglion (TG) were not detectable in 0ΔNLS-vaccinated mice during acute infection, yet viral replication was unabated in gD-2-vaccinated mice relative to naive controls. Latent virus was nearly undetectable by PCR in TG from 0ΔNLS-vaccinated mice, yet 10 and 104-fold higher in gD-2-vaccinated (p<0.01) and naive (p=0.001) mice, respectively. Directly vaccinated mice exhibited minimal T cell activation in draining lymph nodes and did not develop corneal neovascularization. Corneal sensation was reduced in naive and gD-2-vaccinated mice, yet preserved in 0ΔNLS-vaccinated mice (p=0.001). Protection correlated with serum neutralization antibody titers. Passive immunization with 0ΔNLS antiserum inhibited acute viral shedding, protected recipients from lethal encephalitis (90%), reduced viral latency in the TG (p<0.001 relative to naive), and largely prevented corneal neovascularization. However, 40% of mice immunized with gD-2 antiserum succumbed to HSV-1 challenge, while survivors exhibited severe corneal neovascularization and had no reduction in acute viral shedding or latent virus relative to naive mice (p<0.001 relative to 0ΔNLS antiserum). Mice receiving immunoglobulin-depleted antiserum showed no signs of protection.

**Conclusions:** Overall, the 0ΔNLS vaccine affords remarkable humoral immune protection against HSV-1 by impeding viral replication, dissemination, latency, and ocular pathology.

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Arvo 2016 Annual Meeting Abstracts

Program Number: 4325
Presentation Time: 9:00 AM–9:15 AM

Transparent and Self-defense Polyvinyl Alcohol Films

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Purpose: Microbial keratitis (MK), the microbial infection of the cornea, is a leading cause of ocular morbidity and blindness globally. Contact lens wear, ocular inserts, ocular bandages etc. constitutes the major risk factor for MK. To overcome these microbial-associated complications, one approach is to develop biocompatible, optically transparent and inherently antimicrobial soft materials that can be used to design safe ocular devices.

Methods: In the present work, we are reporting the laccase-catalysed oxidative polymerization of 14 catecholamines/catechols for the preparation of optically transparent, mechanically robust, thermally stable, broad spectrum antimicrobial polyvinyl alcohol films. UV-visible spectroscopy, Fourier-transform infrared spectroscopy, atomic force microscopy, thermogravimetric analysis, stress-strain studies are carried out to understand the optical, surface and bulk properties of the films. Antimicrobial evaluation has been carried out for the films using both Kirby-Bauer disc diffusion method as well as using microbroth dilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) protocol.

Results: Among all the investigated compounds, adrenalin (AD), pyrogallol (PG) and hydroquinone (HQ) are identified as the important phenolic compounds to generate efficient antimicrobial PVA films with optimized optical, surface and bulk properties. Interestingly, PVA films reinforced with oxidized/polymerized products of pyrogallol and adrenalin display potent antimicrobial activity against pathogenic Gram-positive, Gram-negative and yeast strains. Whereas hydroquinone reinforced PVA films display excellent antimicrobial properties against Gram-positive bacteria only.

Conclusions: Owing to the their optical transparency, oxygen permeability and high thermal stability combined with antimicrobial properties, the reinforced films could play a promising role in designing antimicrobial ophthalmic devices such as contact lens wares, ocular inserts, ocular bandages and intraocular lenses.

Commercial Relationships: Chetna Dhand

Program Number: 4326
Presentation Time: 9:15 AM–9:30 AM

Disruption of outer blood retinal barrier by Toxoplasma gondii-infected monocytes is mediated via FAK signaling pathway

Hyun Beom Song1, 2, Hyoung Oh Jun1, Jin Hyoung Kim1, Sang-Mok Lee1, Jiwon Shin1, Min-Ho Choi1, Jeong Hun Kim1, 2

Purpose: In patients with ocular toxoplasmosis, disruption of retinal pigment epithelium is frequently observed. The retinal pigment epithelial layer constitutes outer blood retinal barrier (BRB) that lies between retina and leaky choroidal vasculature. In this study, we investigated the effect of monocytes infected with Toxoplasma gondii on in vitro model of outer BRB.

Methods: Retinal pigment epithelial cells, ARPE-19, were cultivated on transwell to form a confluent monolayer. Then, human mononuclear cells, THP-1, infected with Toxoplasma gondii or their conditioned medium were treated and the barrier function was evaluated by measurement of transepithelial electrical resistance (TEER) and immunocytochemistry of tight junction proteins. Additional treatment with FAK inhibitor (PF-573228) or neutralizing antibody against IL-8 was performed to investigate the associated signaling pathway.

Results: Twenty-four hours after the treatment with infected monocytes or their conditioned medium, TEER was decreased and tight junction protein was disrupted. Interestingly, additional treatment with FAK inhibitor attenuated the decreased TEER and disrupted tight junction protein induced by the conditioned medium. After demonstrating increased concentration of IL-8 in conditioned medium from infected monocytes, we found additional treatment with neutralizing antibody against IL-8 could decrease phosphorylation of FAK and attenuate the decreased TEER and the disrupted tight junction protein.

Conclusions: Monocytes infected with Toxoplasma gondii can impair outer BRB. The paracrine effects of infected monocytes on outer BRB are mediated via FAK signaling that is activated by IL-8 from monocytes.

Commercial Relationships: Hyun Beom Song

Program Number: 4327
Presentation Time: 9:30 AM–9:45 AM

Continuous Topical Antibiotics Infusion through a Morgan Lens in Sight-threatening Pseudomonas Keratitis

Whitney Smith, Joshua Duncan, Joseph M. Miller, Mingyu Wang, Department of Ophthalmology, University of Arizona College of Medicine, Tucson, AZ.

Purpose: Despite standard treatment, Pseudomonas keratitis can continue to progress, resulting in loss of vision or eye. This study is to demonstrate that a Morgan lens can be highly effective in delivering topical antibiotics in cases of refractory keratitis.

Methods: Two patients (three eyes) were treated in this report. Patient 1 was an 11-year-old female with a diagnosis of contact lens-related Pseudomonas keratitis in the right eye. Despite a 2-week treatment including topical 15 mg/ml tobramycin q1h and ciprofloxacin QID, corneal perforation appeared imminent (Figure 1A). Patient 2 was an 11-month-old female inpatient with Apert syndrome on ventilator support for complicated pneumonia. Lagophthalmos led to exposure keratitis and bilateral Pseudomonas keratitis. Fortified antibiotics q1h over two days did not contain the infection (Figure 1B). In all eyes, a Morgan lens (MorTan, Inc., Missoula, MT) was inserted under the eyelids and connected to standard IV tubing (Figure 2A). In patient 2, the Morgan lens was further secured by bilateral temporary tarsorraphy (Figure 2B). Cefazidime 50 mg/ml was the key topical antibiotic infused at 20 ml/hour over the ocular surface.

Results: Three days after initiation of the infusion, corneal culture became negative in all eyes. The infusion was continued for at least a week to ensure eradication of the infection before switching to standard topical antibiotic regimens. Amniotic membrane graft and/or topical steroid were used as necessary in the acute recovery phase.
to control inflammation. A combined cataract and corneal transplant 9 months later in patient 1 resulted in a best spectacle corrected vision of 20/60. In patient 2, the corneas remain epithelialized with stable scars and await future transplant.

**Conclusions:** This application of a Morgan lens is non-invasive, requires minimal training and monitoring by caregivers. It can deliver high concentrations of antibiotics to the entire ocular surface and possibly the intraocular tissues as well. Continuous lavage is performed in standard concentrations at a rate sufficient to keep pathogens from accumulating. IV connectors allow for an easy switch between medications or simultaneous administration of multiple medications, and titration of dosing. Additionally, monitoring of therapeutic effects is not hindered.

Commercial Relationships: Whitney Smith, None; Joshua Duncan, None; Joseph M. Miller, None; Mingwu Wang, None

**Program Number:** 4328  
**Presentation Time:** 9:45 AM–10:00 AM  
**Establishment of a new comprehensive polymerase chain reaction (PCR) strip kit to diagnose infectious eye diseases**

Satoko Nakano1, Sunao Sugita2, Yasuhiro Tomaru3, Takako Nakamuro1, Hiroshi Takase4, Norio Shimizu3, Manabu Mochizuki4, Toshiaki Kubota1. 1Ophthalmology, Oita University, Oita, Japan; 2Laboratory for Retinal Regeneration, Center for Developmental Biology, RIKEN, Kobe, Japan; 3 Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan; 4 Ophthalmology, Tokyo Medical and Dental University, Tokyo, Japan.

**Purpose:** To improve previously reported comprehensive PCR system (Ophthalmol., 2013;120:1761-8) and establish a new PCR kit, and to report a pilot clinical study to test its usefulness in the diagnosis of infectious eye diseases.

**Methods:** A multiplex solid-phase PCR strip kit was established. In each well, specific probes and primers of genomic DNA of 2-3 microorganisms were fixed, dried and stored at room temperature until use. A total of 24 common pathogens of infectious eye diseases including all 8 types of human herpes virus (HHV), HTLV1, Adenovirus, Mycobacterium Tuberculosis, Treponema pallidium, P. acnes, bacterial 16S ribosomal DNA (rDNA), Candida spp, C. glabrata, C. krusei, Aspergillus, Fusarium, fungal 28S rDNA, Toxoplasma gondii, Toxocara canis, Chlamydia and Acanthamoeba were selected for the PCR strip kit. After IRB approval was obtained, a pilot clinical study to test the usefulness of the PCR strip kit was performed using ocular samples from 22 patients with infectious eye diseases and 8 controls with non-infectious eye diseases at Oita University Hospital.

**Results:** The PCR strip kit detected all genomic DNA of tested positive control microorganisms, but not negative control. In the clinical study, among 22 patients with various infectious eye diseases, HSV1 (n = 1), VZV (n = 3), CMV (n = 2), EBV (n = 3), HHV6 (n = 2), HTLV-1 (n = 3), Treponema pallidum (n = 1), bacterial 16S (n = 5), Candida spp (n = 4), Aspergillus (n = 2), Fungal 28S (n = 5) were detected, but none in control patients. The clinical presentations of PCR positive patients were in accord with those of respective infectious diseases and the results were further conformed by our previous comprehensive PCR. On the other hand, P. acnes was detected in 15/22 (68.2%) of patients and 8/8 (100.0%) in controls. Bacterial 16S was detected in 5/22 (22.7%) of patients and 6/8 (75.0%) of controls. It is of note that P. acnes and bacterial 16S were detected in the end of PCR cycles and found to be low concentration by quantitative real-time PCR. No pathogens other than P. acnes and bacterial 16S were detected in the control samples.

**Conclusions:** The new multiplex PCR strip can be used for rapid comprehensive diagnosis of the infectious eye diseases. A prospective multicenter clinical study is necessary to determine its usefulness in infectious eye diseases.

Commercial Relationships: Satoko Nakano; Sunao Sugita, None; Yasuhiro Tomaru, None; Takako Nakamuro, None; Hiroshi Takase, None; Norio Shimizu, None; Manabu Mochizuki, None; Toshiaki Kubota, None

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