332 Going viral!
Tuesday, May 09, 2017 11:00 AM–12:45 PM
Room 310 Paper Session
Program #/Board # Range: 2986–2992
Organizing Section: Immunology/Microbiology

Program Number: 2986
Presentation Time: 11:00 AM–11:15 AM
Role of $CD8^+$ T cells versus $CD8\alpha^+$ DCs in HSV-1 latency-reactivation
Homayon Ghiasi[2], Kevin R. Mott[2], Sariah J. Allen[2], Yasamin N. Ghiasi[1], Harry Matundan[2], Terrence Town[1]. [1]Zilkha Neurogenetic Institute, Department of Physiology & Biophysics, USC, Los Angeles, CA; [2]Ophthalmology Research/Surgery, Cedars-Sinai Medical Center, Los Angeles, CA.
Purpose: To evaluate whether $CD8^+$ T cells play a bystander or functional role during the course of latency-reactivation in trigeminal ganglia (TG) of latently infected mice.
Methods: Wild-type, $CD8\alpha^{-/-}$ (lack functional $CD8^+$ T cells and $CD8\alpha^+$ DCs), $CD8\beta^{-/-}$ (have functional $CD8\alpha^+$ T cells and $CD8\alpha^+$ DCs), $\beta 2m^{-/-}$ (lack functional $CD8^+$ T cells but have $CD8\alpha^+$ DCs), BXH2 (have functional $CD8^+$ T cells but lack $CD8\alpha^+$ DCs), and $CD4^{-/-}$ (have functional $CD8^+$ T cells and $CD8\alpha^+$ DCs) mice were ocularly infected with virulent (McKrae) or avirulent (KOS) strains of HSV-1. Level of viral gB DNA and LAT RNA in TG on day 28 PI and virus reactivation on day 28 PI were measured. Some of mice received adaptive transfers of BM-derived $CD8\alpha^+$ DCs or $CD8^+$ T cells before ocular infection.
Results: In these studies we have shown that TG from $CD8\alpha^{-/-}$ mice had a significantly lower level of latency and ex-plant reactivation than WT, $\beta_2m^{-/-}$, $CD8\beta^{-/-}$ or $CD4^{-/-}$ mice. Adoptive transfer of BM-derived $CD8\alpha^+$ DCs significantly increased the levels of latency in $CD8\alpha^{-/-}$ mice, while transfer of $CD8^+$ T cells had no effect. Similarly BXH2 mice had a lower level of latency-reactivation than their control mice. Finally, LAT expression and increased latency correlated with an increased level of PD-1.
Conclusions: Our results suggested that: (1) higher latency and subclinical reactivation correlated with the presence of more $CD8^+$ T cells expressing the PD-1 exhaustion marker; (2) $CD8^+$ T cells were not responsible for increase or maintenance of latency in ocularly infected mice and this was independent of their higher exhaustion phenotype or strain of virus; and (3) $CD8\alpha^+$ DCs directly or as a critical helper for other cell types, contributed to higher latency-reactivation and thus exhaustion in TG of latently infected mice independent of $CD8^+$ T cells.
Commercial Relationships: Homayon Ghiasi, None; **Kevin R. Mott**, None; **Sariah J. Allen**, None; **Yasamin N. Ghiasi**, None; **Harry Matundan**, None; **Terrence Town**, None
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Program Number: 2987
Presentation Time: 11:15 AM–11:30 AM
Routes of immunization differentially affect the dynamics of humoral immunity elicited by ocular HSV-1 vaccine
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Purpose: Routes of immunization differ among vaccines to maximize the efficacy of host immune protection in various pathogen-susceptible tissues. This study investigates how administration of a live-attenuated HSV-1 vaccine termed HSV-1 0ΔNLS in different tissues affects the quality of protection against an ocular HSV-1 challenge.
Methods: Eight-week old outbred CD-1 mice were immunized with a single administration of HSV-1 0ΔNLS intramuscularly in the flank (FL), subcutaneously in the footpad (FP), or via the intranasal (IN) mucosal route. Naive mice and mice immunized using a prime-boost FP+FL regimen previously shown to be highly efficacious were utilized as experimental controls. Humoral immune responses were assessed in serum, and animals were subsequently ocularly infected with the LD50 inoculum of HSV-1 McKrae. Animals were surveyed for viral burden in the cornea and nervous system and lymphocyte proliferation in regional lymph nodes during acute infection (day 7 post-infection) as well as corneal neovascularization and latent virus at day 30 post-infection. One-way ANOVAs were used for statistical analysis; data reflect 2-3 independent experiments.
Results: Serum neutralizing antibody titers were not detected in naive animals but were present in immunized mice such that FP + FL > IN = FP > FL. The neutralizing antibody concentrations correlated with viral shedding and titers in the corneas and trigeminal ganglia following challenge. Viral replication was only blocked in the nervous system of the FP and FP+FL groups. Lymphocyte proliferation was significantly elevated in the mandibular lymph nodes of the naive group—as expected, was noted to be moderate in the single-vaccine FL, IN, and FP, groups, but was largely subdued in the FP+FL group. Corneal neovascularization was only prevented in the FP+FL and IN vaccine groups.
No correlation was discerned between the predominant HSV-specific antibody isotype classes and serum neutralization activity. All routes of immunization led to a significant reduction in the amount of virus that establishes a latent infection, while the highest efficacy was observed in the FP+FL group.
Conclusions: While a single vaccination mediates a degree of protective efficacy against HSV-1 ocular infection and resultant pathology, the prime-boost approach affords superior protection by eliciting higher concentrations of HSV-neutralizing antibodies.
Commercial Relationships: Daniel J. Carr, Rational Vaccines (S); **Meghan Carr**, None; **Hem R. Gurung**, None; **Derek J. Royer**, None; **William Halford**, Rational Vaccines (I), Rational Vaccines (F), Rational Vaccines (P)
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Program Number: 2988
Presentation Time: 11:30 AM–11:45 AM
Sensory nerve loss and sympathetic innervation characterize recurrent herpes stromal keratitis in mice
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Purpose: Herpes simplex virus type-1 (HSV-1) causes a potentially blinding corneal inflammatory disease called herpes stromal keratitis (HSK) that manifests as edema, opacity, neovascularization and hypoesthesia of the cornea. Mouse corneas are heavily innervated by sensory nerves, with few if any sympathetic nerves. Mice with primary HSK completely lose corneal sensory nerves leading to severe, diffuse inflammation that is maintained by subsequent hyperinnervation with sympathetic nerves derived from the superior cervical ganglion. In humans HSK is usually recurrent disease and ranges from focal areas of corneal opacity and vascularization to diffuse inflammation. Recurrent HSK in mice tends to be focal and

transient in C57BL/6(B6) mice, but severe and diffuse in NIH mice. Our goal was to characterize corneal nerve changes associated with recurrent HSK in these two mouse strains.
Methods: B6 and NIH mice were infected with HSV-1 McKrae and treated with anti-HSV-1 antibody to prevent primary HSK. Recurrent HSK was induced by UV-B corneal irradiation 30 days after primary infection. Whole mounts of irradiated and non-irradiated corneas of infected mice, or irradiated corneas of non-infected were stained with fluorescent antibodies to β III tubulin (all nerves), tyrosine hydroxylase (sympathetic nerves), and substance P (sensory nerves). Z-stacks spanning entire cornea thickness were acquired by confocal microscopy and nerve plexus density was quantified using FIJI software. ANOVA was used for statistical analysis.
Results: UV-B irradiation did not alter sensory innervation of non-infected corneas. Infected corneas of NIH mice showed mild loss of sensory nerve plexus prior to irradiation, but diffuse loss of sensory nerves and sympathetic hyperinnervation of the corneal stroma after irradiation. Infected corneas of B6 mice did not exhibit a significant reduction in the sensory nerve plexus prior to irradiation, but showed focal areas of sensory nerve loss with coincident sympathetic innervation after irradiation.
Conclusions: Loss of corneal sensory nerves and innervation by sympathetic nerves are closely associated with recurrent HSK in mice. Moreover, the degree of sensory nerve loss and sympathetic innervation appear to correlate with the severity of recurrent HSK. We are currently investigating the correlation between focal nerve changes and leukocytic infiltration in recurrent HSK in B6 mice.
Commercial Relationships: HONGMIN YUN, None; **Xiaotang Yin**, None; **Patrick M. Stuart**, None; **Robert L. Hendricks**, None
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Presentation Time: 11:45 AM–12:00 PM
Effectiveness of real-time PCR assessment for varicella-zoster virus-associated keratitis
Dai Miyazaki, Kodai Inata, RYU UOTANI, Daisuke Shimizu, Atsuko Miyake, Yumiko Shimizu, Yoshitsugu Inoue. Ophthalmology, Tottori University, Yonago, Japan.
Purpose:
Varicella zoster virus (VZV) causes herpes zoster ophthalmicus by reactivation of latent virus, which leads to ocular complications, characterized by keratitis
or iridocyclitis. Although diagnosis is generally made by its acute skin episode, effectiveness of viral genome-based diagnosis for VZV-associated keratitis has remained obscure. We sought to evaluate how real-time PCR of VZV may improve its diagnostic efficacy as well as predict prognosis for better management of chronic and refractory cases.
Methods:
The medical records of 563 consecutive case series with suspected VZV-associated keratitis or infectious keratitis were retrospectively reviewed. All the patient underwent real-time PCR of VZV using corneal scraping samples at the Tottori University Medical Hospital between November 2005 and September 2016. Diagnostic accuracy of real-time PCR and clinical signs were determined by receiver operating characteristic analysis. Prognosis, described as the persistence of active disease and complications, were assessed for their associations using logistic regression and cox proportional hazard model.
Results:
For the VZV-associated keratitis (38 eyes), VZV copy number was $1.9\times10^7\pm 5.9\times10^7$ copy. Significant associations with the VZV amount was observed for facial skin eruption (OR; 4.1, 95% CI: 2.5- 6.5, P=0.000) and extraocular muscle paralysis (OR; 2.0, 95% CI: 1.2- 3.4, P=0.007), followed by IOP elevation, dendritic lesion, and mutton-fat keratic precipitate. VZV amount and facial skin eruption showed highest diagnostic accuracy calculated as area under the curve (AUC) of 0.92 and 0.96, respectively. The mean disease duration of VZV-associated keratitis was 108 days. Higher amount of VZV (per log) and the presence of iridocyclitis significantly prolonged the duration of active disease with hazard ratio of 0.75 (P=0.003) and 0.18 (P=0.002).
Conclusions:
Quantification of VZV genome is effective for predicting the persistence of active disease as well as diagnosis of VZV keratitis.
Commercial Relationships: Dai Miyazaki, None; **Kodai Inata**, None; **RYU UOTANI**, None; **Daisuke Shimizu**, None; **Atsuko Miyake**, None; **Yumiko Shimizu**, None; **Yoshitsugu Inoue**, None

Program Number: 2990
Presentation Time: 12:00 PM–12:15 PM
A rat model of facial Post Herpetic Neuralgia (PHN) induced by Varicella Zoster Virus (VZV)
Paul R. Kinchington[1], Benjamin Warner[1], William Goins[1], Mahesh Rao[2], Crystal Stinson[2], Phillip Kramer[2]. [1]Ophthalmology/ Mol Micro & Genetics, Univ of Pittsburgh Eye & Ear Inst, Pittsburgh, PA; [2]Biological Sciences, Texas A&M College of Dentistry, Dallas, TX.
Purpose: Facial zoster and Herpes Zoster Ophthalmicus (HZO) are caused by the reactivation of VZV from neuronal latency, and often result in problematic sequelae that impair vision and well-being. This includes debilitating prolonged pain in the facial/periorbital region (Post Herpetic Neuralgia, or PHN). The strictly limited human host specificity of VZV has made in vivo study of VZV pathogenesis, latency and reactivation rather difficult. However, live VZV innoculated into the footpads of rats induce chronic behaviors of pain. The goal of this work was to determine if facial, ocular and periorbital VZV PHN models could be developed.
Methods: Sprague Dawley Rats (n=4 to 6) were innoculated at the facial or periorbital region with live cell-associated VZV (1x10^5). Control rats recieved received uninfected cell equivalents or UV-irradiated VZV. Affective pain was asssessed weekly using the Fuchs Place-Escape-Avoidance-Paradigm (PEAP) involving periodic facial stimulation with 38g or 60g filaments in split light/dark chambers. Pain was assessed by avoidance of dark sides of testing chambers over 30 min periods. Both sexes were evaluated.
Results: PEAP testing relies on rat preference of the dark side of split dark/light testing chambers. Live cell-associated VZV inoculation, but not inoculation of UV-irradiated VZV or uninfected cells, induced signficant avoidance of the preferred dark side when facial sites of inoculation were stimulated. Behaviors suggesting pain initated rapidly by week 1, required VZV infectivity and lasted for 6-9 weeks p.i. A more prolonged and stronger pain response developed in female over male rats.
Conclusions: This new VZV facial chronic pain model expands upon the footpad VZV chronic pain model, and permits modeling of facial and trigeminal pain seen in VZV HZO patients and viral infection of trigeminal ganglia. While pain develops following a VZV primary infection (unlike human PHN, which follows VZV reactivation and zoster), it may open avenues to test novel anti-VZV and PHN therapeutics and permit study of VZV -ganglia interactions leading to pain. The data indicate viral gene expression is required

for pain development, even though our work indicates rats are not fully permissive for VZV replication. Sex based differences mirror an increased female to male propensity for PHN in humans.
Commercial Relationships: Paul R. Kinchington, None; **Benjamin Warner**, None; **William Goins**, None; **Mahesh Rao**, None; **Crystal Stinson**, None; **Phillip Kramer**, None
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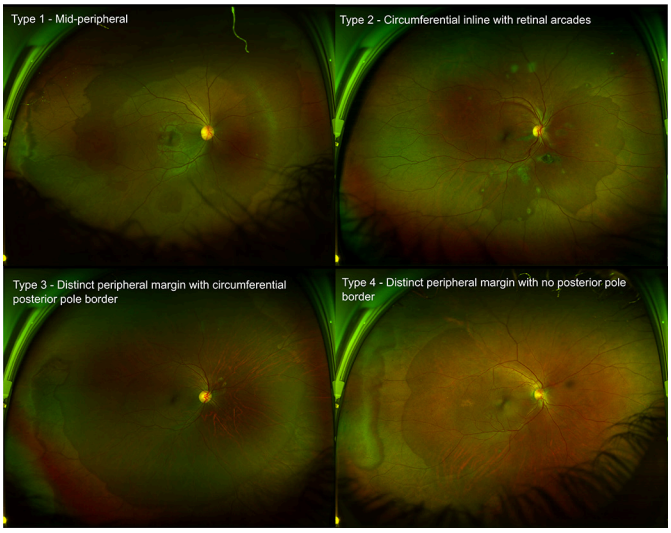
Presentation Time: 12:15 PM–12:30 PM
Longitudinal Changes of Geographic Retinal Darkening in a Cohort of Ebola Survivors
Paul J. Steptoe[1], Nicholas V. Beare[4, 2], Janet T. Scott[1], Matthew J. Vandy[3], Lloyd Harrison-Williams[3], Fayiah Momorie[5], Alimamy D. Fornah[5], Julia M. Baxter[2], Craig K. Parkes[2], Rahul Dwivedi[2], Foday Sahr[5], Simon P. Harding[4], Malcolm G. Semple[1]. [1]Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom; [2]St.Paul's Eye Department, Royal Liverpool Hospital, Liverpool, United Kingdom; [3]Ophthalmology Department, Connaught Hospital, Freetown, Sierra Leone; [4]Department of Eye and Vision Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, United Kingdom; [5]Department of Ophthalmology, 34th Regiment Military Hospital, Republic of Sierra Leone Armed Forces Joint Medical Unit, Freetown, Sierra Leone.
Purpose: Little is known regarding the longitudinal retinal sequelae in Ebola virus disease (EVD) survivors. An Ebola-specific retinal lesion and associated areas of geographic retinal darkening (GRD) were observed amongst EVD survivors in a previous case-control study. GRD, also known as dark-without-pressure are little understood but lesional OCT correlates to retinal ellipsoid layer hyporeflectivity. We performed an observational study in a cohort of EVD survivors to observe for longitudinal changes.
Methods: 137 Ebola survivors were invited for ophthalmic examination including widefield retinal imaging (Optos® Daytona, Dunfermline, UK) in Freetown, Sierra Leone. Median age was 31 (IQR 23.5-39). M:F ratio::1:0.78. Median time from Ebola treatment unit discharge to ophthalmic review was 479 days (range 97-977 days). Patients with identified GRD and/or Ebola retinal lesions were invited for examinations between January and November 2016.
Results: 95 presumed Ebola-specific retinal lesions were identified in 12.4% (17/137) of survivors. Mean number of lesions per eye was 3.9 (range 1-24, SD 5.19). All were systemically well and visually asymptomatic. 84.2% of these had a circumferential dark retinal halo. 18.8% (12/137) of survivors had areas of GRD (33.3% bilateral). Four patterns were identified (Figure 1): a) mid-peripheral (MP) (N=5), b) circumferential inline with the retinal arcade (CA) (N=3), c) distinct peripheral margin with circumferential posterior pole border (N=4), d) distinct peripheral margin with no posterior pole border (N=5). Mean period of review was 210 days (range 62-290, SD 77.3). All MP GRD areas showed recession over time, one of which developed perivascular infiltrates which resolved without treatment. All CA areas showed evidence of expansion. 66.7% had co-existent white-without-pressure.
Conclusions: We provide the first evidence of GRD in EVD survivors and propose a classification scheme based on imaging appearance. We found examples of GRD expanding and receding over time. The ellipsoid layer anatomically correlates to a high density of retinal mitochondria. Changes in mitochondrial refractivity have been shown to equate to enzymatic activity. Hypo-reflectivity of this layer, seen in GRD may therefore indicate visualisation of a viral effect upon retinal mitochondrial metabolic state. These findings may have systemic parallels in relation to Post-Ebola syndrome.



Commercial Relationships: Paul J. Steptoe, The Dowager Countess Eleanor Peel Trust (F), Bayer (F); **Nicholas V. Beare**, None; **Janet T. Scott**, Wellcome Trust (F); **Matthew J. Vandy**, None; **Lloyd Harrison-Williams**, None; **Fayiah Momorie**, None; **Alimamy D. Fornah**, None; **Julia M. Baxter**, None; **Craig K. Parkes**, None; **Rahul Dwivedi**, None; **Foday Sahr**, None; **Simon P. Harding**, None; **Malcolm G. Semple**, Wellcome Trust (F)
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Presentation Time: 12:30 PM–12:45 PM
Zika Virus Causes Chorioretinal Atrophy in Mouse Eyes and Infects Primary Human Cells Lining the Blood-Retinal Barrier
Pawan Kumar Singh[1, 2], John-Michael Guest[1], Mamta Kanwar[1], Joseph Boss[1], Nan Gao[1], Gary W. Abrams[1], Fushin X. Yu[1, 2], Ashok Kumar[1, 2]. [1]Kresge Eye Institute, Wayne State University School of Medicine, Detroit, MI; [2]Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI.
Purpose: Zika virus (ZIKV) infection has been liked to cause several ocular abnormalities, including chorioretinal atrophy, maculopathy, and uveitis in both newborn and adults. Thus, it is imperative to study ZIKV pathogenesis in the eye and develop animal models to identify potential targets for interventions. We hypothesized that ZIKV gain entry into the eye by directly infecting the cells lining the blood-retinal barrier (BRB).
Methods: ZIKV (strain PRVABC59) was administered intravitreally in adult C57BL/6 and ISG15 KO mice of either sex. The development of retinal abnormalities/lesions was monitored by fundus examination up to 7 days. Viral burden in the infected eyes and/or retina were estimated using qRT PCR and immunostaining of viral envelope proteins. For *in vitro* studies, human primary RPE cells, ARPE19 cell line, human retinal vascular endothelial cells (HRvEC), choroidal endothelial cells (CEC), human Müller glia (MIO-M1), and mouse photoreceptor cells (661W) were challenged with ZIKV and their permissiveness was assessed by immunostaining. ZIKV-induced retinal cell death was determined by TUNEL staining and assessing cleaved caspase 3. Induction of antiviral response was measured by qRT PCR analysis of various inflammatory and type-I interferons (IFNs).
Results: ZIKV challenge caused retinal lesions in both WT and ISG15 KO mouse eyes. However, the disease severity was more in ISG15 KO mice coinciding with increased viral replication and retinal cell death. WT retina exhibited strong inflammatory and

antiviral immune response as compared to the ISG15 KO mouse retina. Among the various retinal cell types, Pr. RPE and HRvEC were more permissive to ZIKV replication whereas photoreceptors were least permissive. ZIKV challenge caused retinal cell death as evidenced by increased TUNEL and Cl.casp-3 positive cells. ZIKV incited *in vitro* inflammatory and antiviral immune response resulting in increased expression of ZIKV entry receptors AXL, MERTK, and TYRO3. Blockade of AXL inhibited viral replication in retinal cells. **Conclusions:** Together, our mouse model of direct ZIKV inoculation into the eye mimics features of ZIKV-associated retinal lesions in humans. Our data on permissive nature of BRB provides the cellular basis of potential ZIKA entry into the eye. Furthermore, our study provides the first evidence that ISG15 plays a role in retinal innate defense to ZIKV infection.

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