Virus-host interactions in herpesvirus infections of human nervous tissues

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Annecy, November 30th 2018
Mouse models to study role T-cells in pathology multiple sclerosis and control herpesvirus latency

MS mouse model: EAE

HSV-1 latency mouse model

All (animal) models (of neurodegeneration) are wrong. Are they also useful?
Richard M. Ransohoff (J Exp Med; 20NOV18)
Dutch Brain Bank collects brain tissues from donors with a short post-mortem delay: < 6 hrs

CNS: Multiple sclerosis

PNS: HSV-1 and VZV latency

Unique specimens to study human neurotropic virus infections in humans
Virus-host interactions in herpesvirus infections of human nervous tissues

1. Determine the antigen specificity of T-cells in cerebrospinal fluid and brain tissue of multiple sclerosis patients.
2. Determine the viral transcriptome and its function in latently HSV-1- and VZV-infected human trigeminal ganglia.
3. Determine the antigen specificity of T-cells in latently HSV-1- and VZV-infected human trigeminal ganglia.
Virus-host interactions in herpesvirus infections of human nervous tissues (1/3)

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Multiple Sclerosis: risk factors

- Genetics
  - 102 SNP
  - HLA haplotype
- Environmental
  - low vit. D
  - Epstein-Barr virus infection
  - Pfeiffer's disease
  - high salt diet
  - smoking
- Other
  - bad luck?
Central role T-cells in MS pathogenesis: which antigens are recognized?
Intrathecal and cerebral T-cell responses in multiple sclerosis patients
No substantial T-cell reactivity towards MS-associated Ag in CSF-, NAWM- and WML-TCL
EBV-specific T-cells are enriched in CSF of patients with CIS and early MS

- Increased EBV-specific CD4 and CD8 T-cells in CSF of both CIS and early MS patients
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- Increased EBV-specific CD4 and CD8 T-cells in CSF of both CIS and early MS patients

- EBV-specific CD4 and CD8 T-cells frequencies correlate intra-individually
EBV-specific CD8 T-cells are enriched in chronic active demyelinating MS lesions.
Donor #6: in situ localization of EBV-specific CD8 T-cells in surplus MS lesion tissue.
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VZV infection: Varicella and Herpes Zoster
Old Dogma (e.g. Fields 2013):
Expression of ~10 VZV genes and ~6 VZV proteins in human neurons
HSV-1 and VZV transcriptome: RNAseq analysis lytic vs. latent infection

### HSV-1

**Lytic**

**Latent**

### VZV

**Lytic**

**Latent**
VZV Latency Transcript => VLT
Detection of neurons latently infected with HSV-1 and VZV in human TG by in-situ hybridization

~5% HSV-1 LAT<sup>POS</sup> TG neurons

~0.5% VZV VLT<sup>POS</sup> TG neurons
VLT inhibits VZV replication \textit{in vitro} by repressing viral ORF61 expression
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Herpetic Eye Diseases

1: Periocular dermatitis
2: Blepharitis
3: Conjunctivitis (with ulceration)

4: Infectious epithelial keratitis (dendritic keratitis)
5: Neurotrophic keratitis

6a: Stromal keratitis
   Immune stromal keratitis
6b: Stromal keratitis
   Necrotizing stromal keratitis
6c: Stromal keratitis
   Immune ring

7: Endotheliitis
8: Uveitis
9: (Epi-)scleritis
Asymptomatic shedding of HSV-1 and VZV at oral mucosa in latently infected individuals

VZV: incidental asymptomatic shedding at oral mucosa
HSV-1: ~every 13 days for 6 hrs high loads of infectious virus!
HSV-1 & VZV hide in human trigeminal ganglia: **Lifelong Latency**
T-cell responses in latently HSV-1 and VZV-infected human trigeminal ganglia

Infection with: mock, HSV-1 & VZV
Neuron-interacting T-cell clusters in ‘normal’ human TG
T-cell clusters in human TG interact with HSV-1 LAT^POS neurons
CD8 T-cells express CTL markers and CD137: antigen-driven T-cell retention?
Human TG-derived T-cells recognize HSV-1, but not VZV proteins
Complexity of CD4 and CD8 T-cell target antigen discovery for HSV-1

Genome: >80 genes

- Expressed in highly regulated fashion
- Not every gene is expressed during HSV life cycle

Where to start ....

Complete ORFeome
### HSV-1 antigens recognized by CD8 T-cells recovered from human TG

**Kinetic class of recognized HSV-1 proteins**

<table>
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<th>Patient ID</th>
<th>HLA allele</th>
<th>Immediate early</th>
<th>Early</th>
<th>Late</th>
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HSV-1 specific CD8 T-cells interact with neuron somata in human TG

**Symmetry: left = right TG**
- Left TG used for generation of T-cell lines and HSV-1 epitope definition
- Right TG used for *in situ* HLA-I/peptide tetramer stainings

**TG2 tissue sections**
- DAPI, nuclei *(blue)*
- Anti-CD8 *(green)*
- HLA-A*0201* tetramers *(red)*
  - ICP0\textsubscript{642-651}
  - ICP8\textsubscript{1096-1105}
Conclusions

- EBV-specific T-cells selectively infiltrate CSF and CNS tissue of MS patients: role EBV-specific T-cells in MS pathology?
- Novel VZV latency-associated transcript (VLT) is the key switch of lytic/latent VZV infection: potential gene to target in novel chickenpox vaccine.
- HSV-1-, but not VZV-specific T-cells are selectively retained in latently infected human TG: HSV-1-specific T-cells control HSV-1 latency!
- HSV-1 ICP6 & VP16 are immuneprevalent targets of intra-TG virus-specific T-cell response: potential HSV-1 subunit vaccine candidates.
Thank you for your attention

Questions?