Erasmus MC

Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT

Virus-host interactions in herpesvirus infections of human nervous tissues

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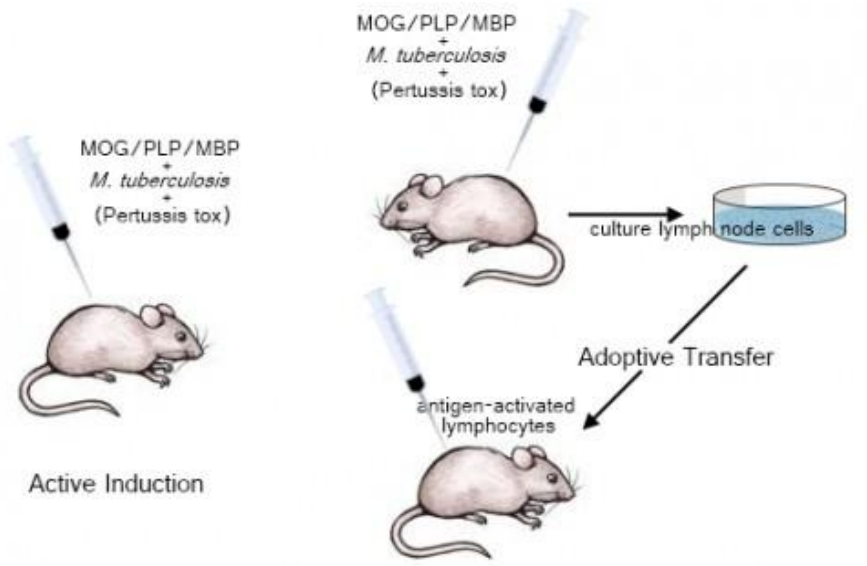
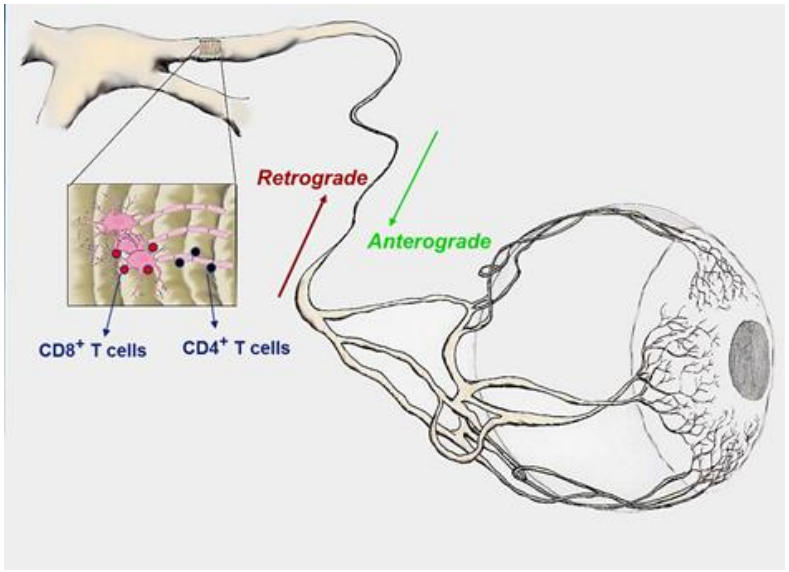
Annecy, November 30th 2018

Mouse models to study role T-cells in pathology multiple sclerosis and control herpesvirus latency

MS mouse model: EAE



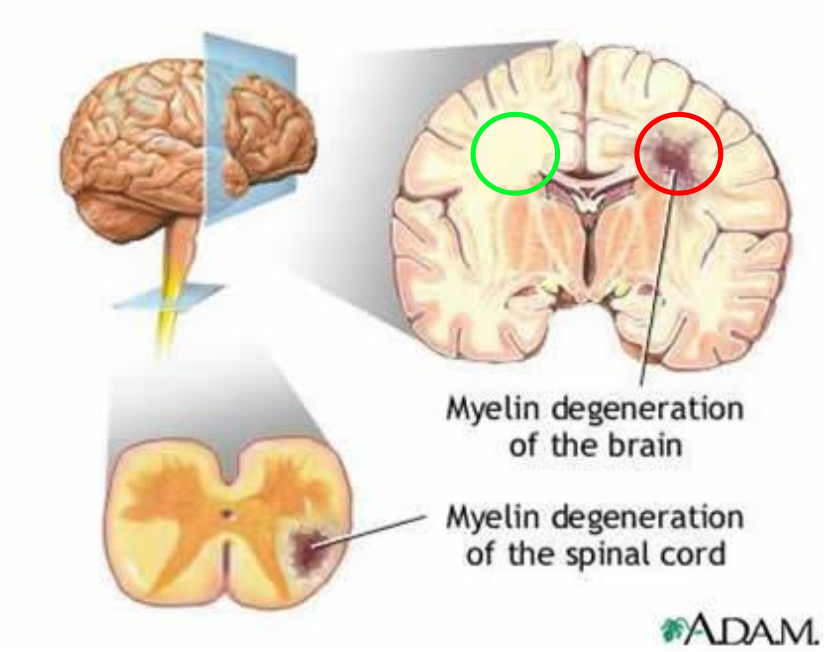
HSV-1 latency mouse model



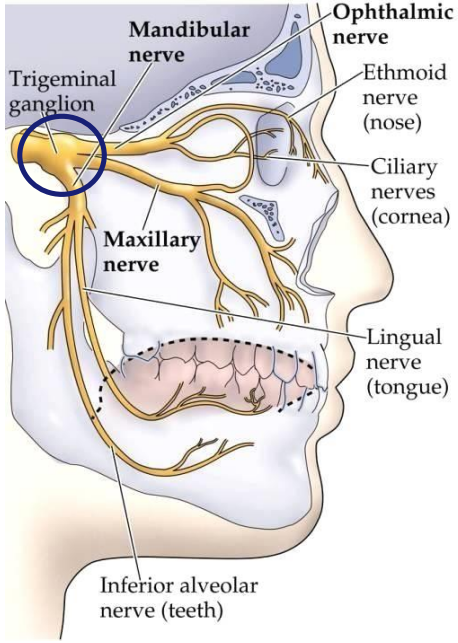
All (animal) models (of neurodegeneration) are wrong.
Are they also useful?
Richard M. Ransohoff (J Exp Med; 20NOV18)

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

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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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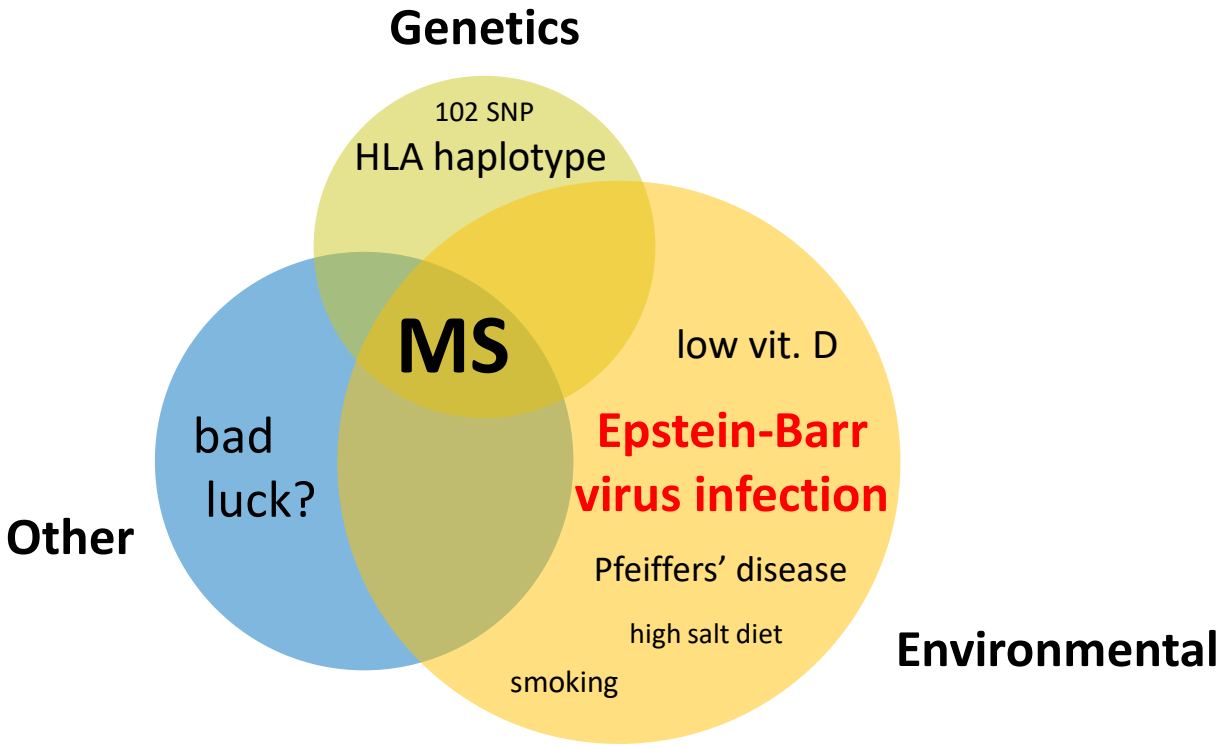
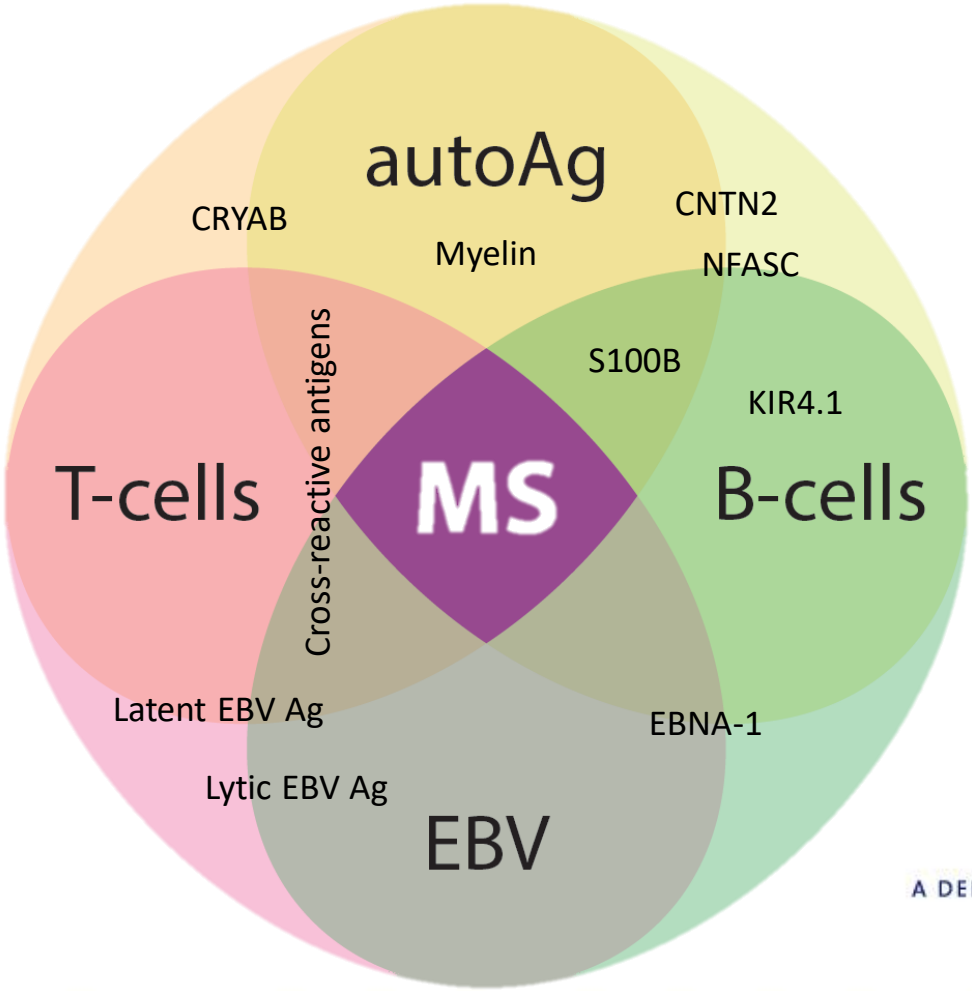
Multiple Sclerosis: risk factors



Central role T-cells in MS pathogenesis: which antigens are recognized?



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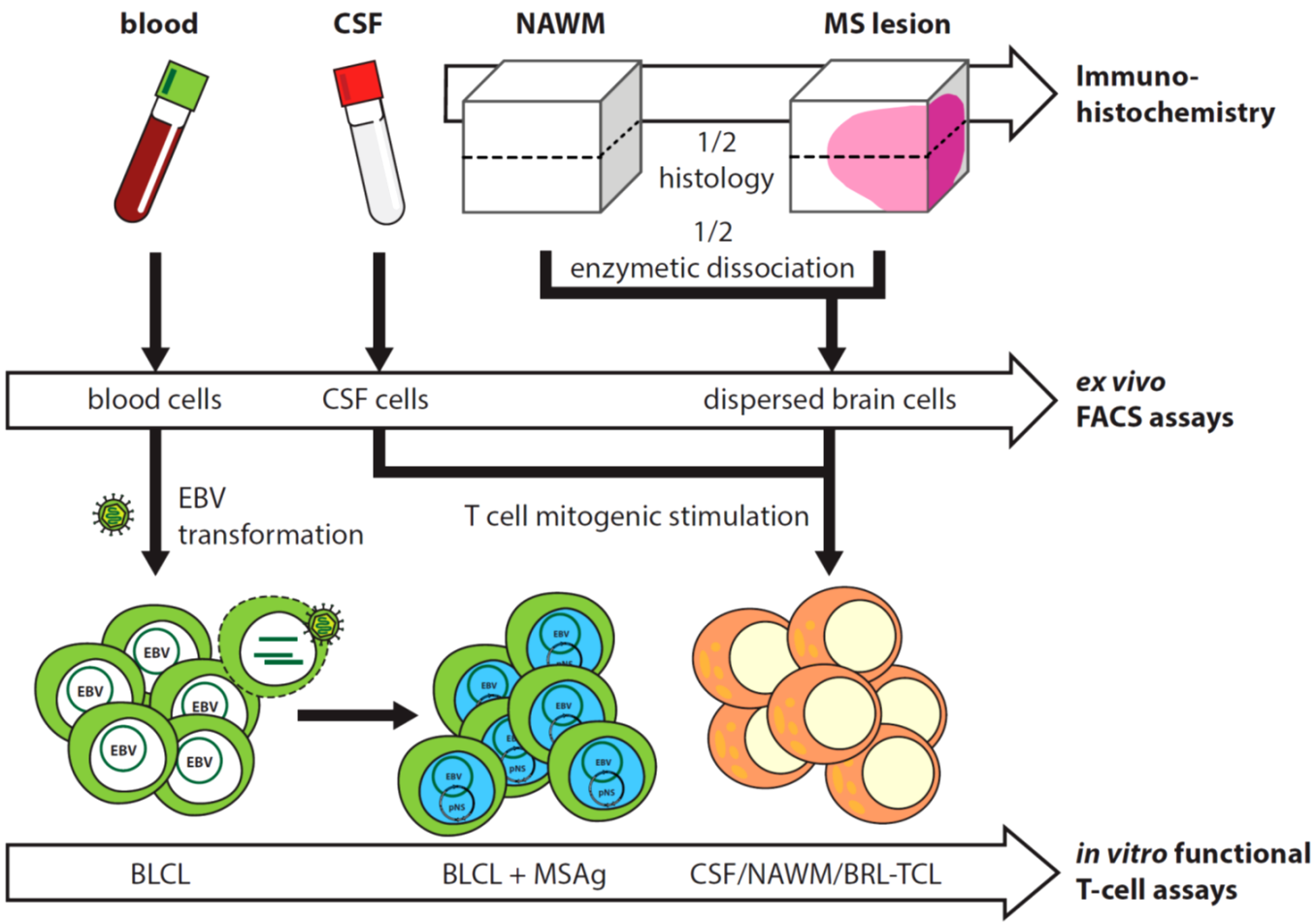
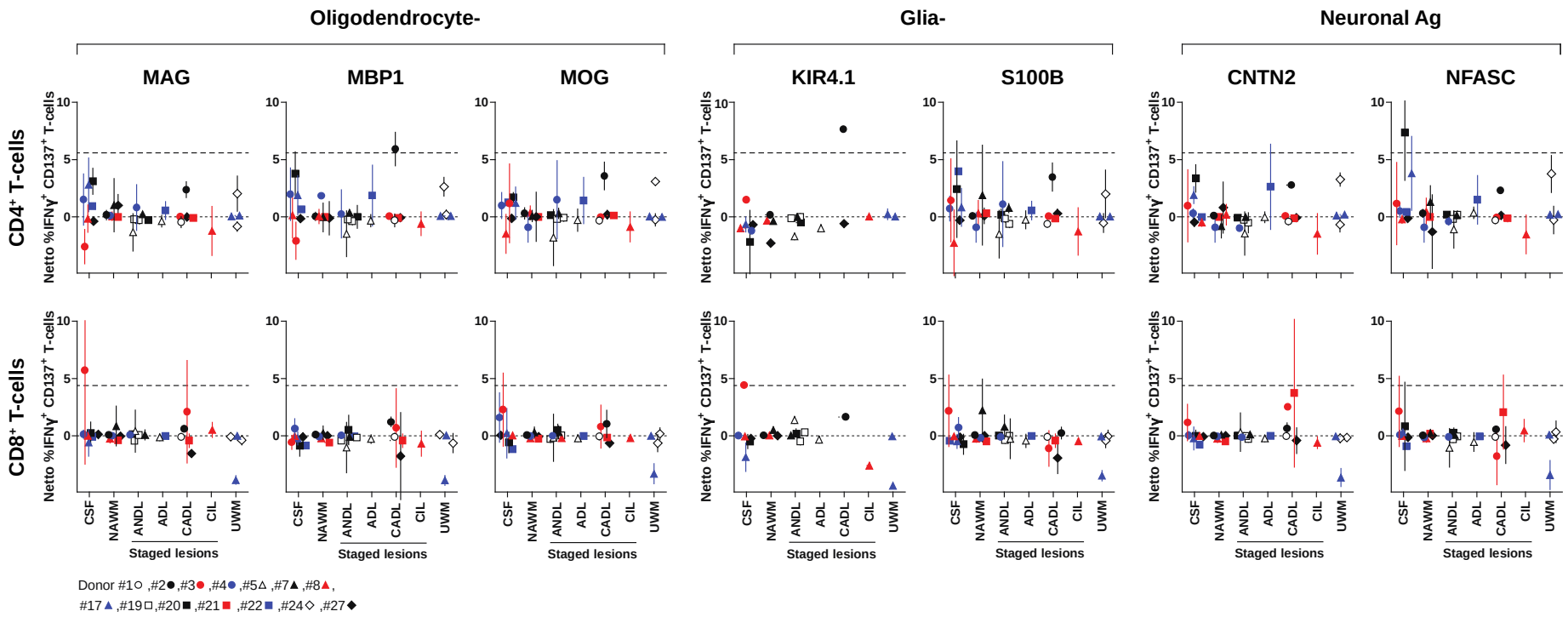
Intrathecal and -cerebral T-cell responses in multiple sclerosis patients



No substantial T-cell reactivity towards MS-associated Ag in CSF-, NAWM- and WML-TCL



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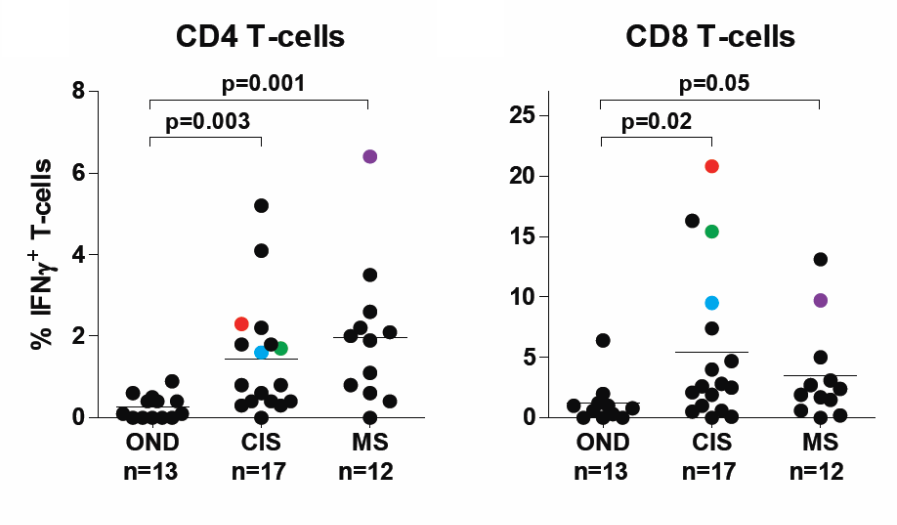
EBV-specific T-cells are enriched in CSF of patients with CIS and early MS



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

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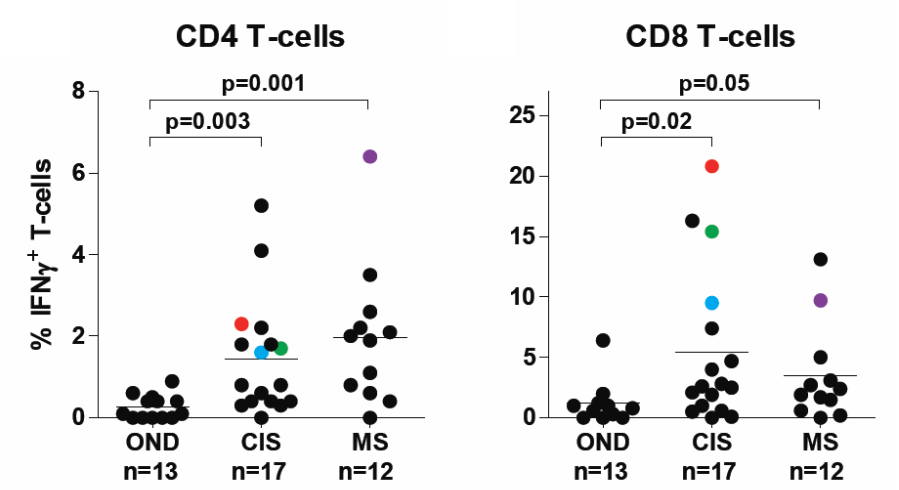
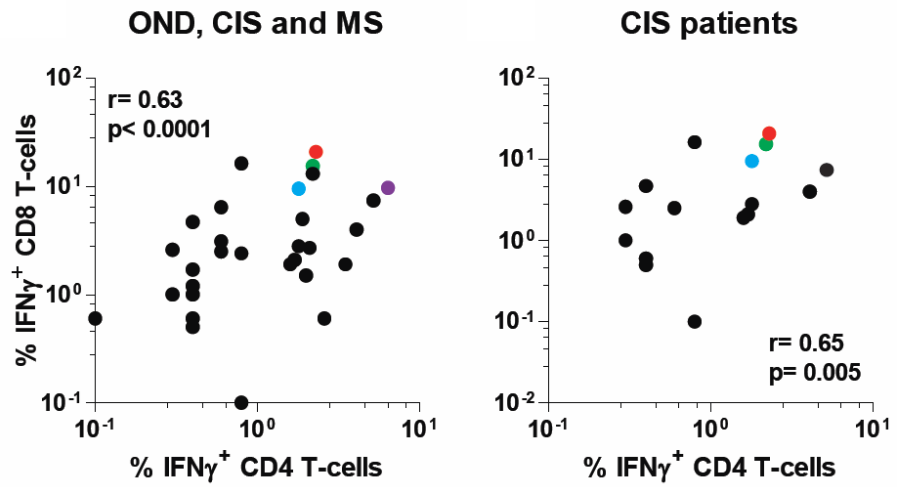
EBV-specific T-cells are enriched in CSF of patients with CIS and early MS





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

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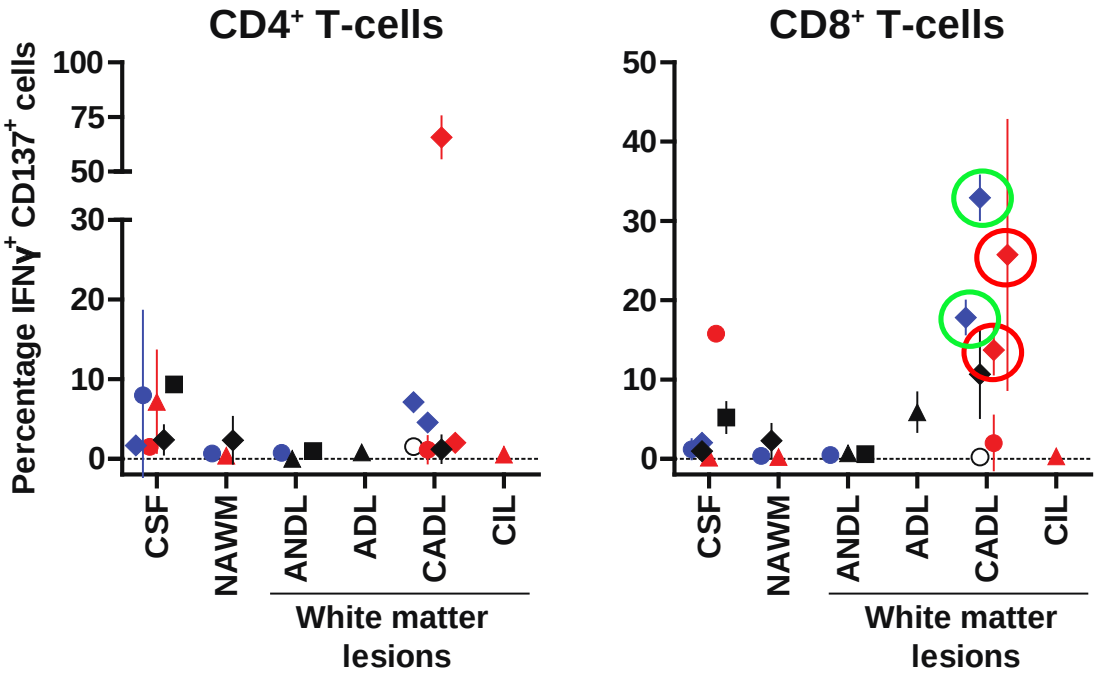
EBV-specific CD8 T-cells are enriched in chronic active demyelinating MS lesions



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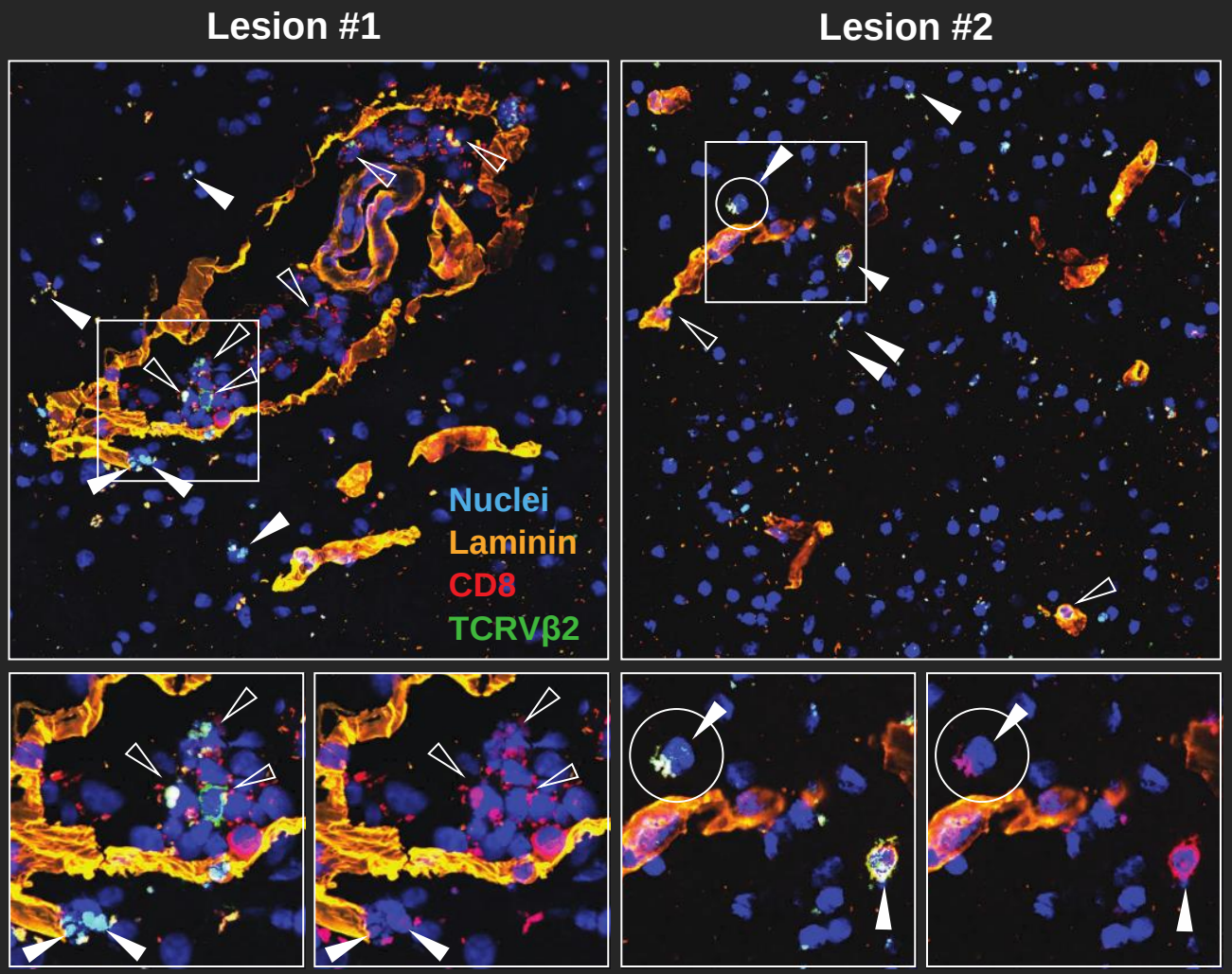
Donor #6: *in situ* localization of EBV-specific CD8 T-cells in surplus MS lesion tissue



Virus-host interactions in herpesvirus infections of human nervous tissues (2/3)

1. Determine the antigen specificity of T-cells in cerebrospinal fluid and brain tissue of multiple sclerosis patients.
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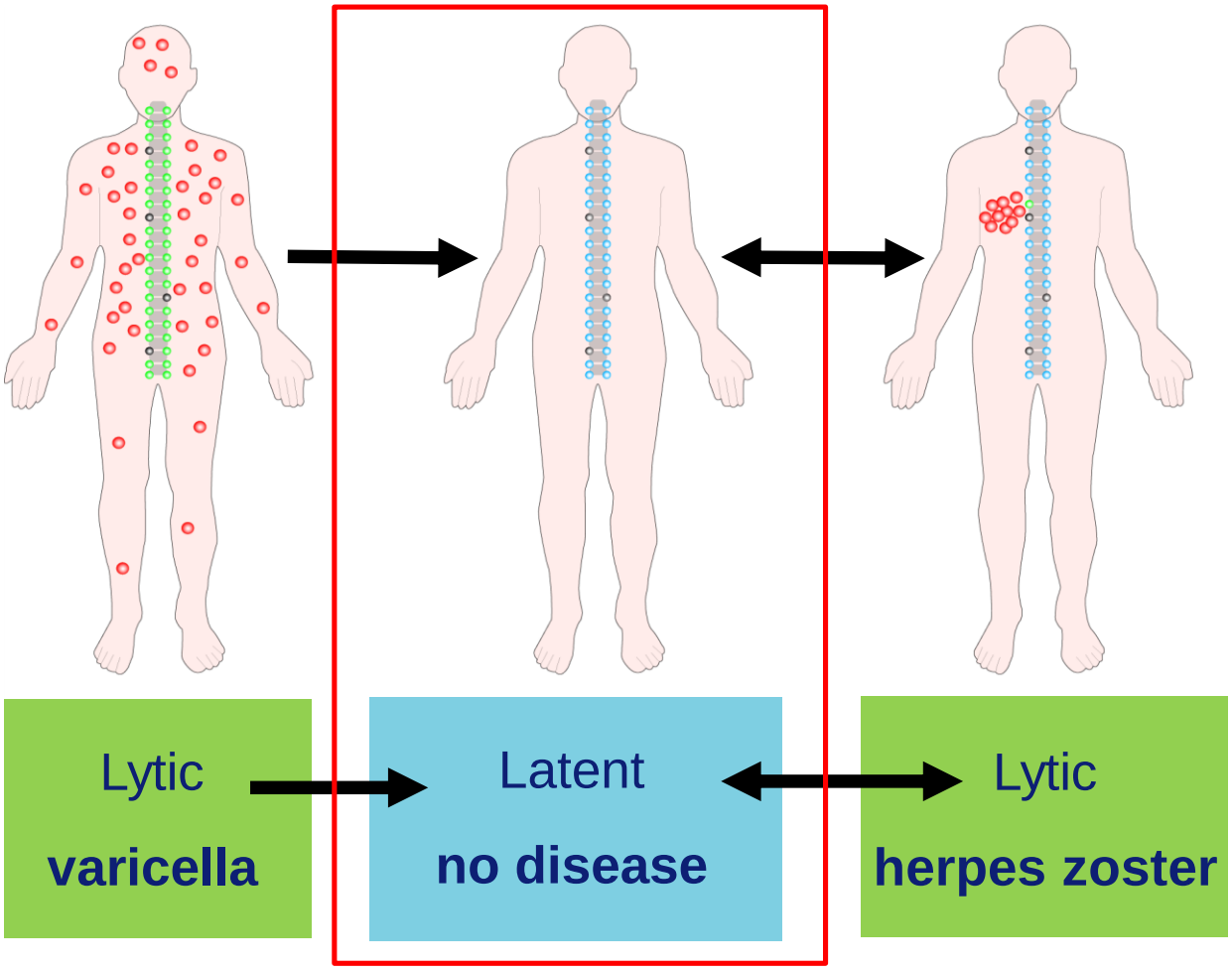
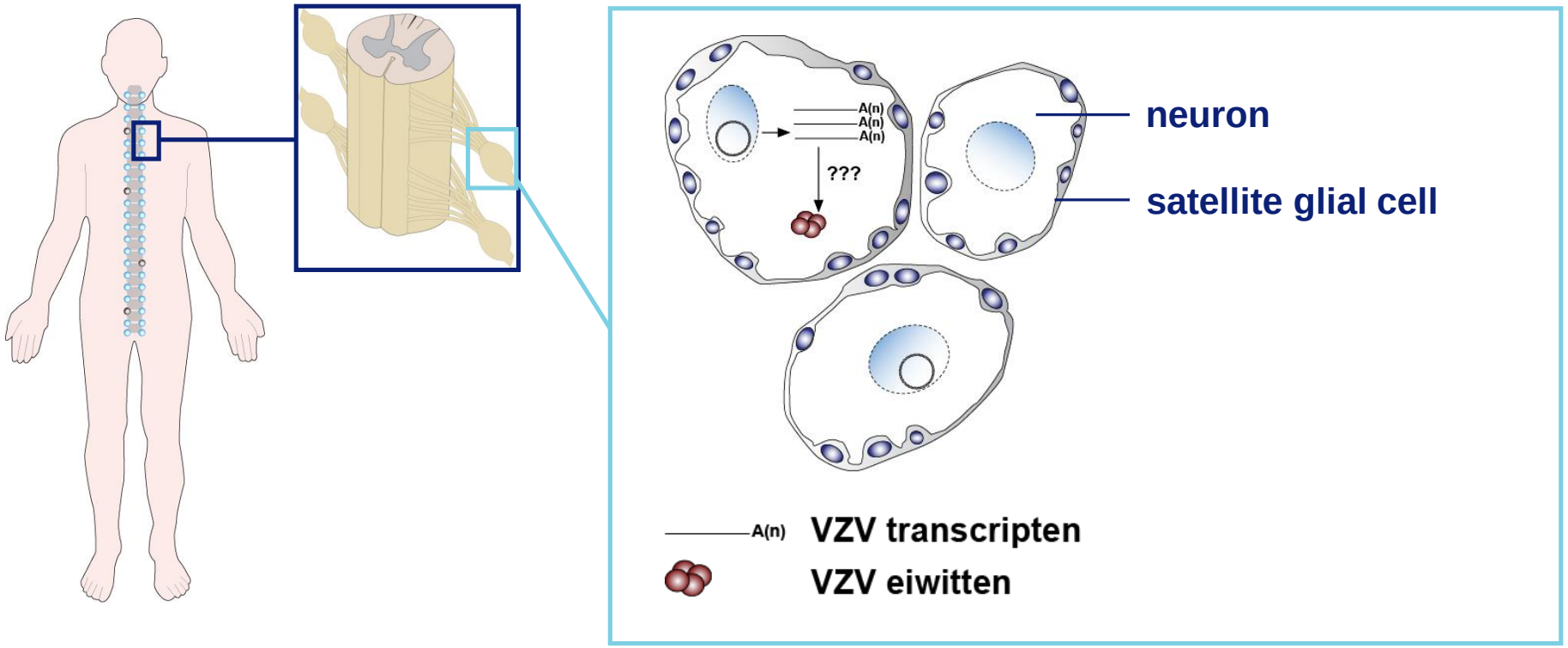
VZV infection:
Varicella and Herpes Zoster



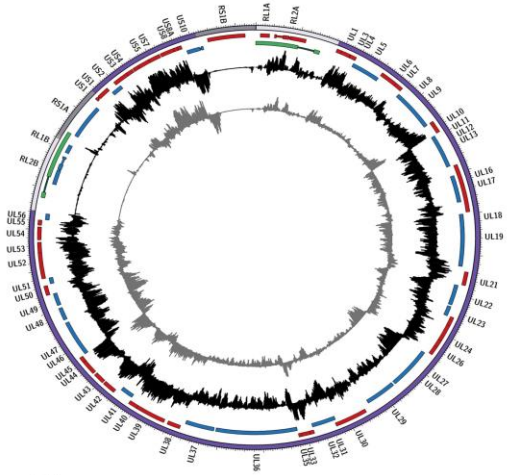
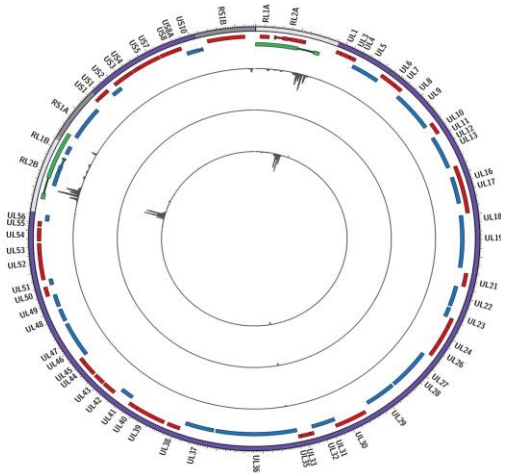
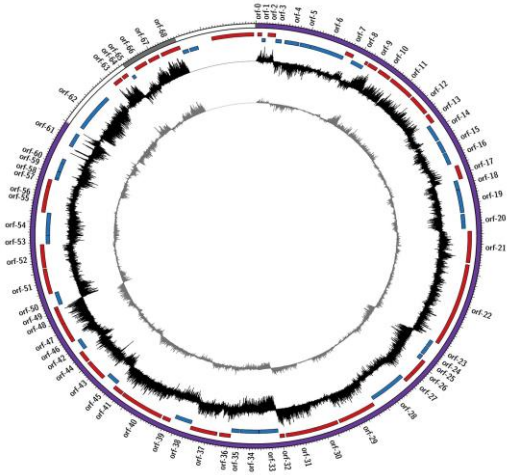
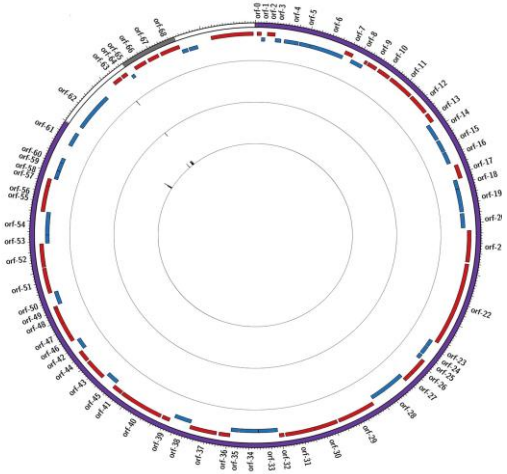
Long-held concept of VZV latency



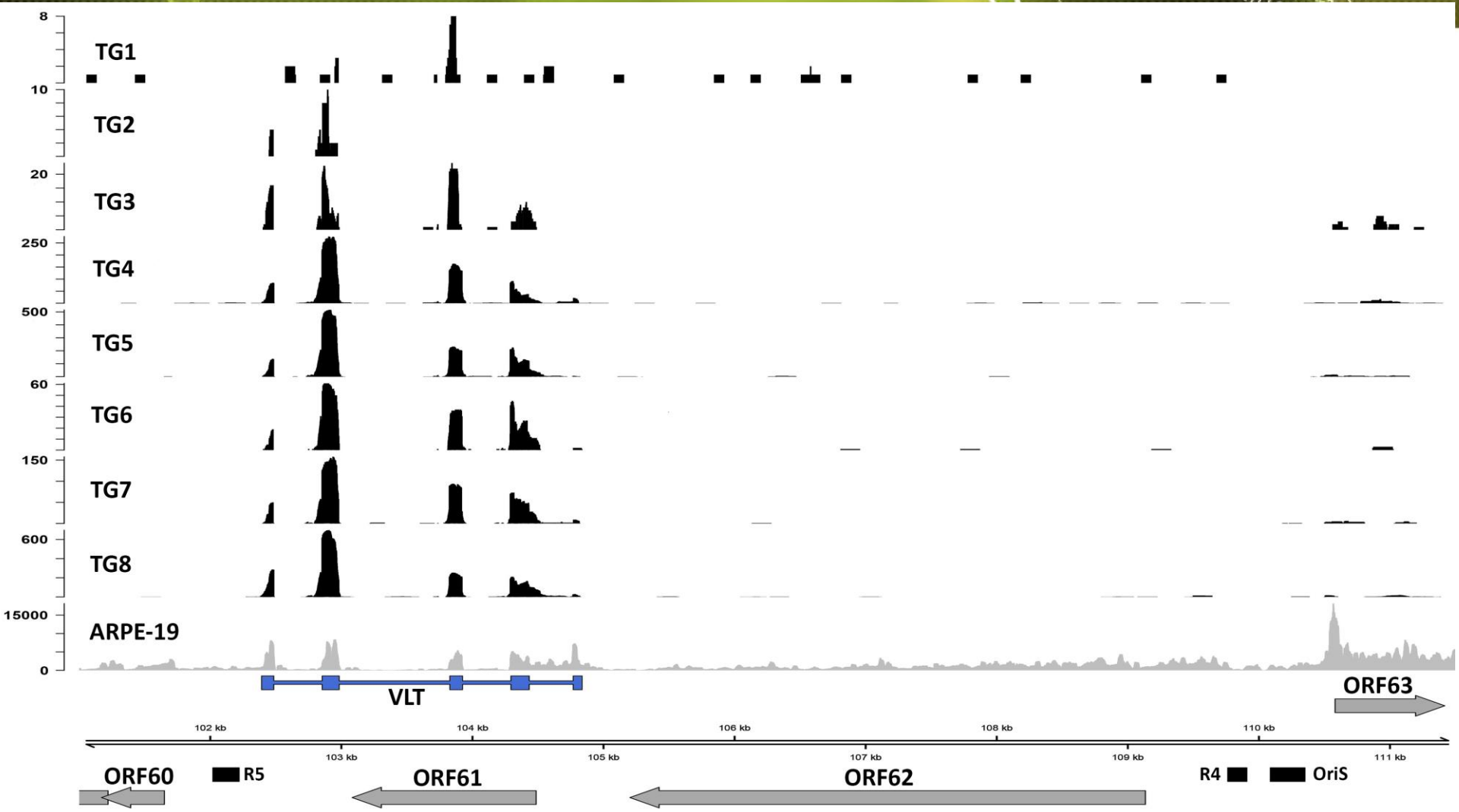
Old Dogma (e.g. Fields 2013):

Expression of ~10 VZV genes and ~6 VZV proteins in human neurons

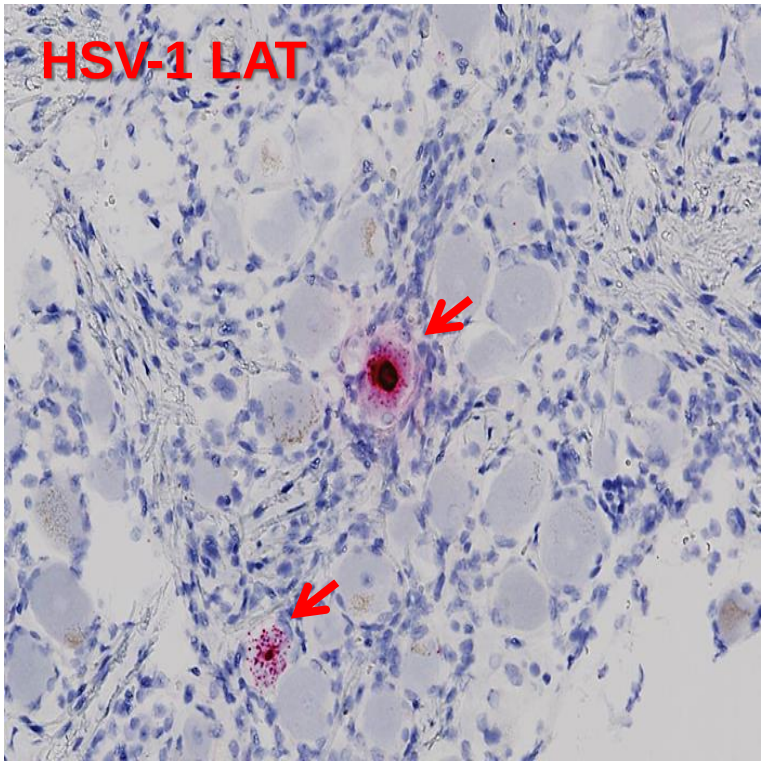
HSV-1 and VZV transcriptome: RNAseq analysis lytic vs. latent infection

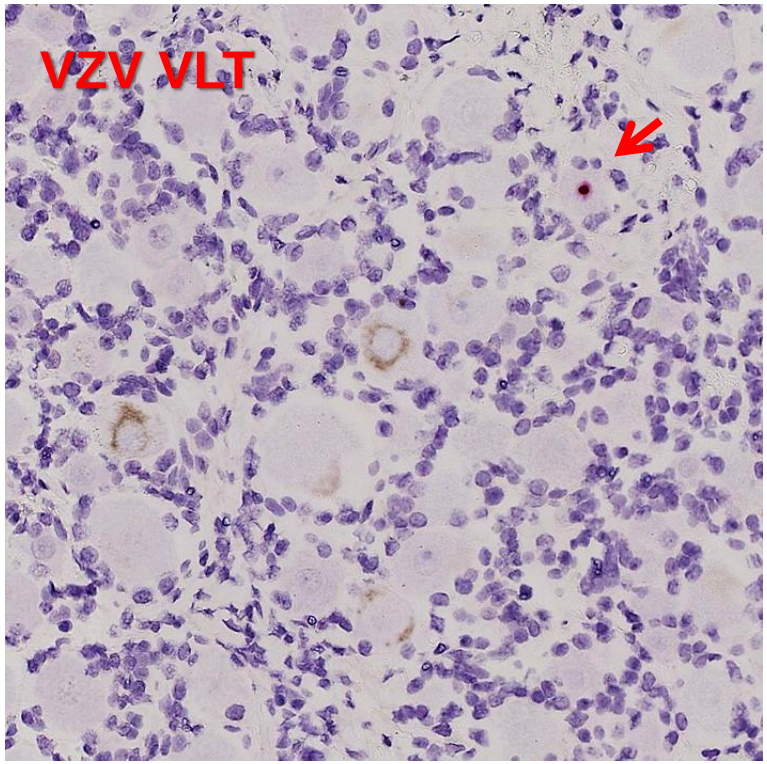
	Lytic	Latent
HSV-1		
VZV		

VZV Latency Transcript => VLT



Detection of neurons latently infected with HSV-1 and VZV in human TG by in-situ hybridization

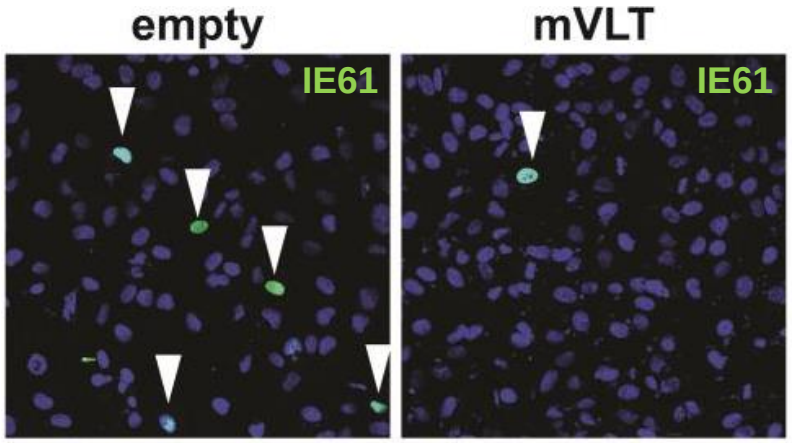


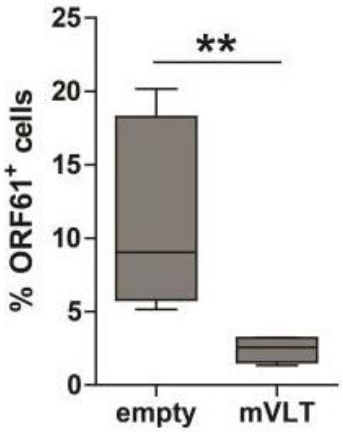


~5% HSV-1 LAT^{POS} TG neurons

~0.5% VZV VLT^{POS} TG neurons

VLT inhibits VZV replication *in vitro* by repressing viral ORF61 expression





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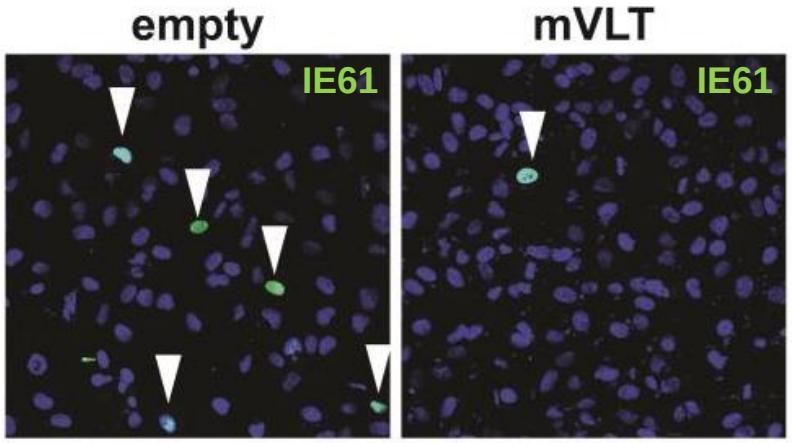

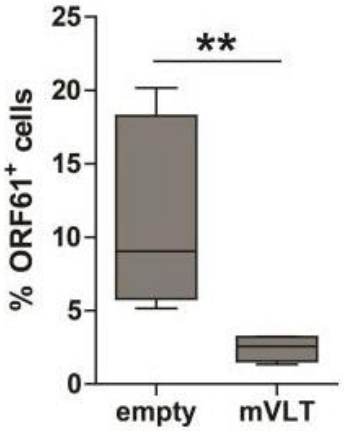

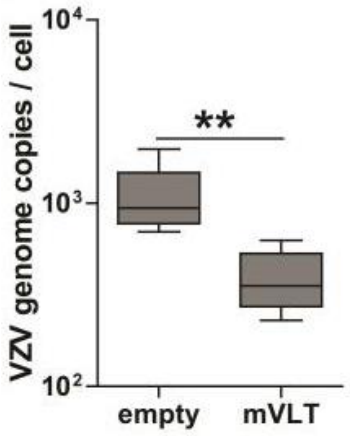

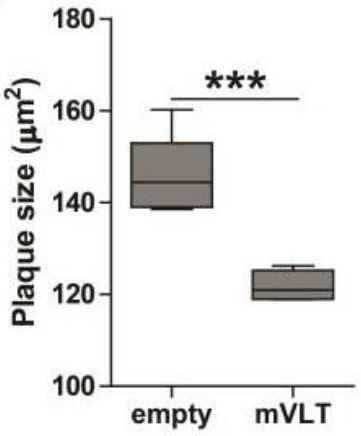

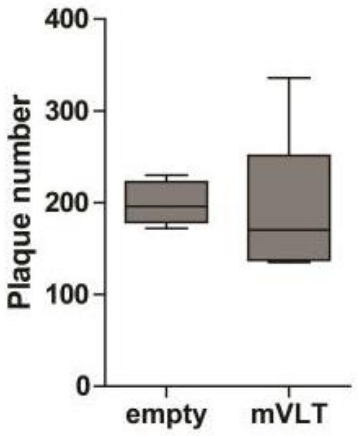

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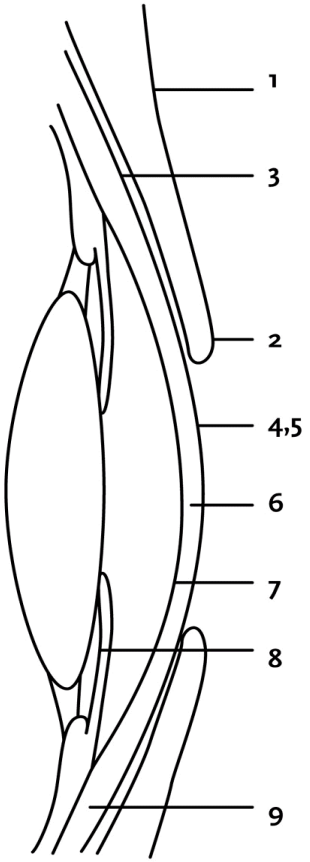
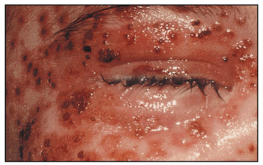
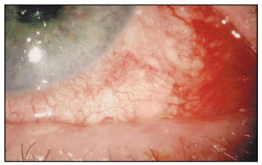
Herpetic Eye Diseases



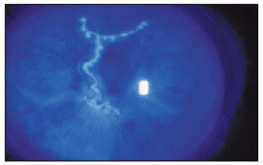


1: Periocular dermatitis

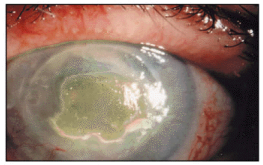
2: Blepharitis



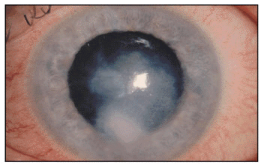
3: Conjunctivitis (with ulceration)



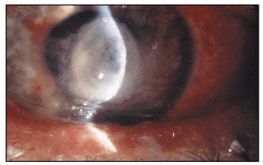
4: Infectious epithelial keratitis (dendritic keratitis)



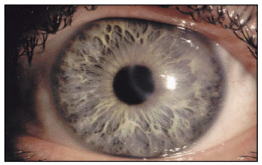
5: Neurotrophic keratitis



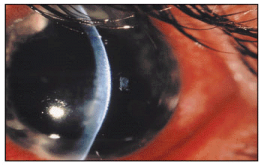
6a: stromal keratitis
Immune stromal keratitis



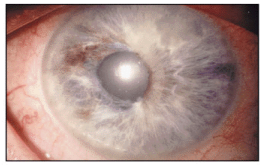
6b: stromal keratitis
Necrotizing stromal keratitis



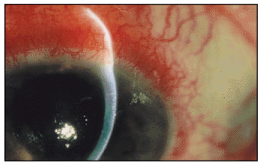
6c: stromal keratitis
Immune ring



7: Endotheliitis



8: Uveitis



9: (Epi-)scleritis

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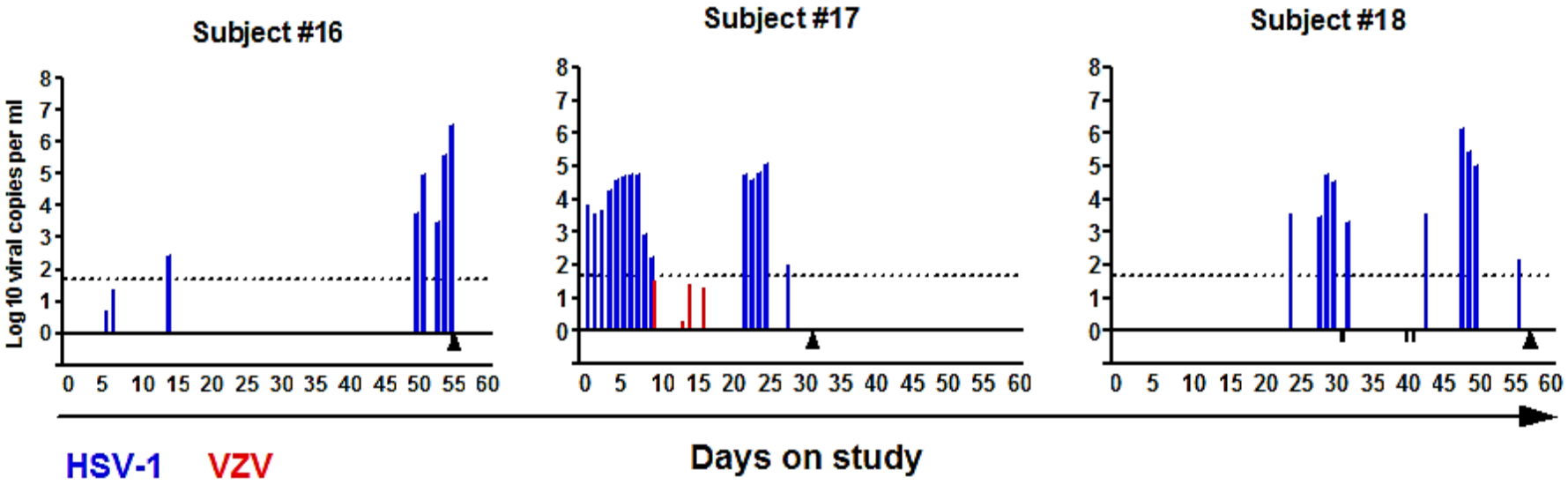
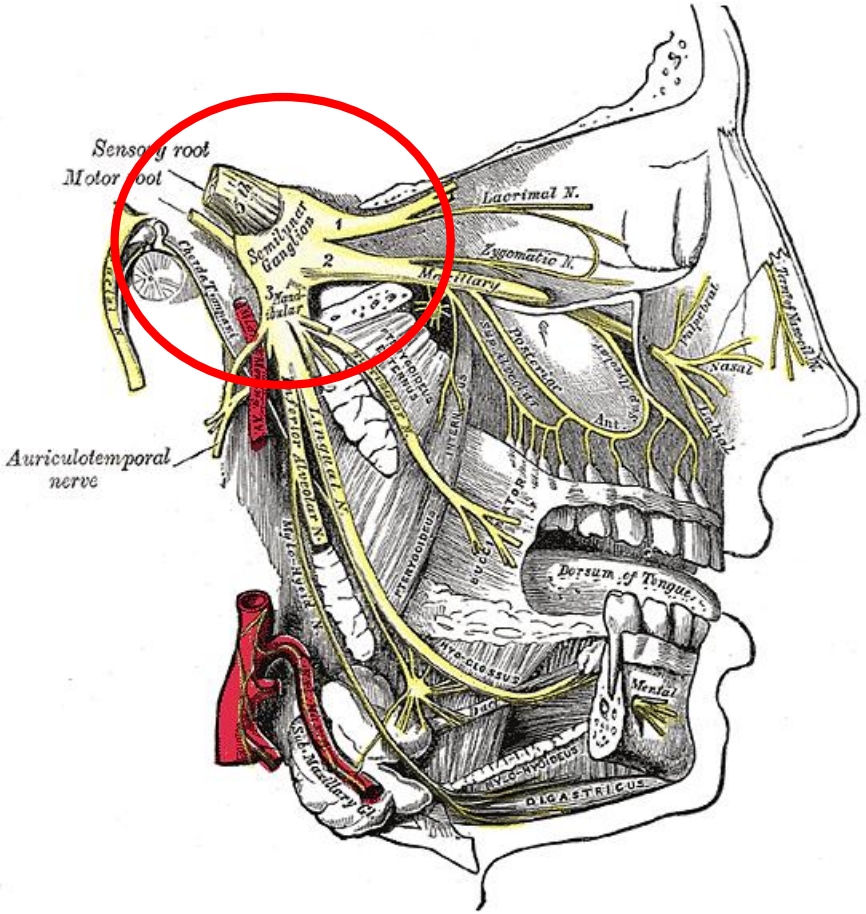
Asymptomatic shedding of HSV-1 and VZV at oral mucosa in latently infected individuals

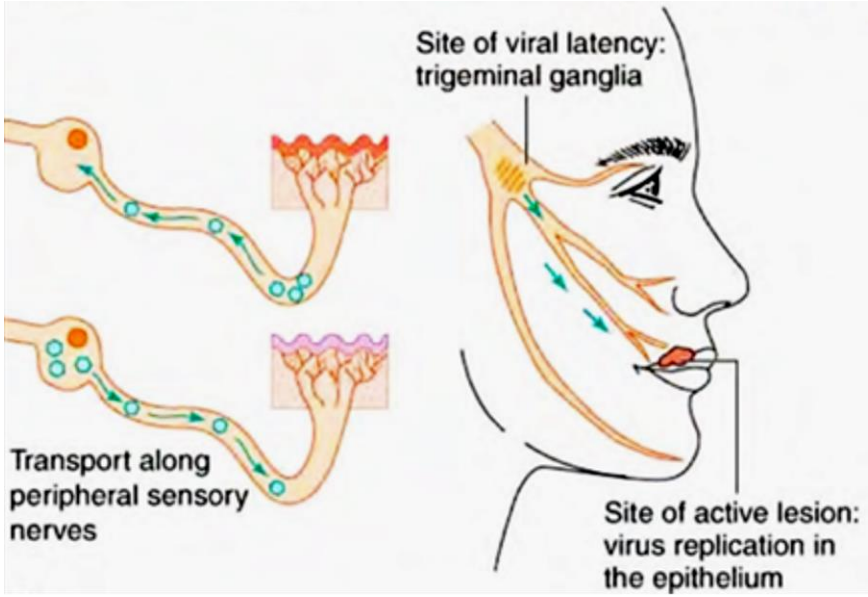


VZV: incidental asymptomatic shedding at oral mucosa

HSV-1: ~every 13 days for 6 hrs high loads of infectious virus!

HSV-1 & VZV hide in human trigeminal ganglia: Lifelong Latency





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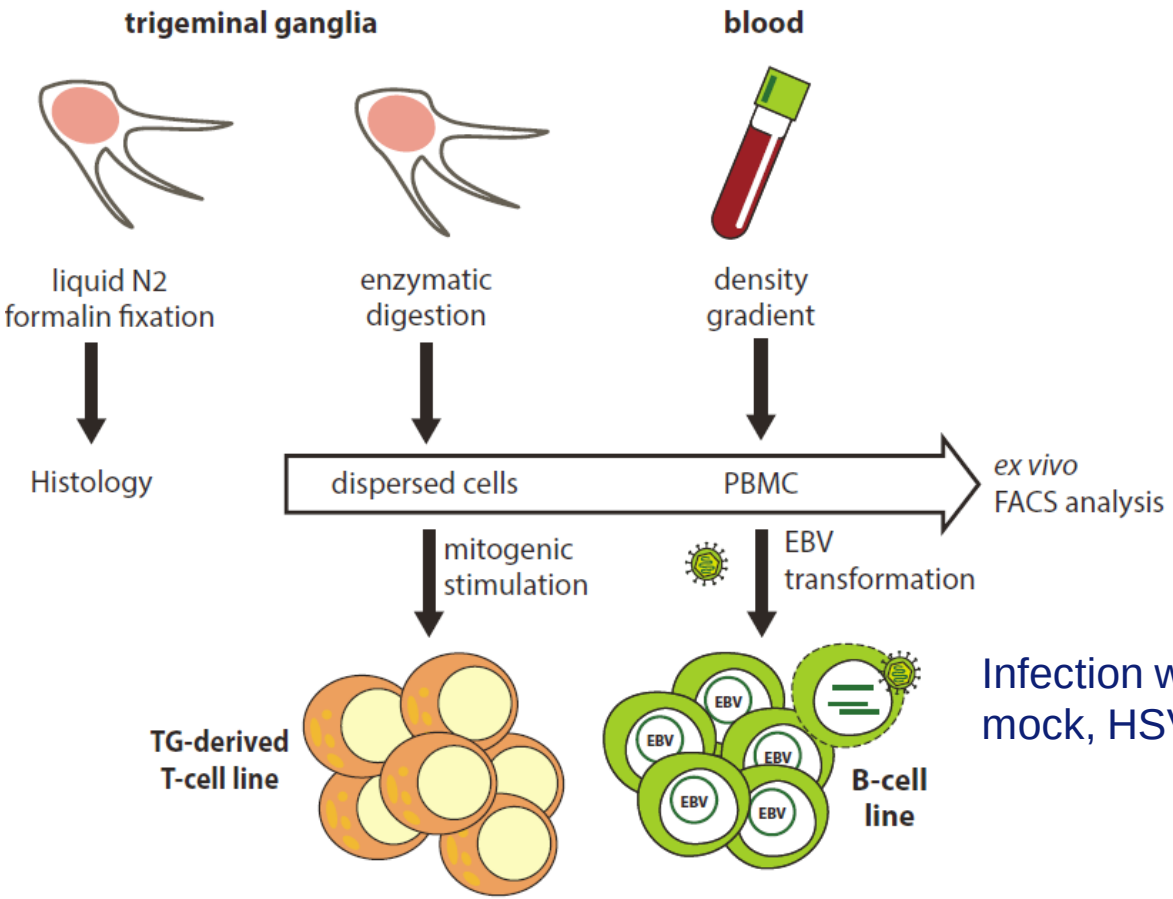
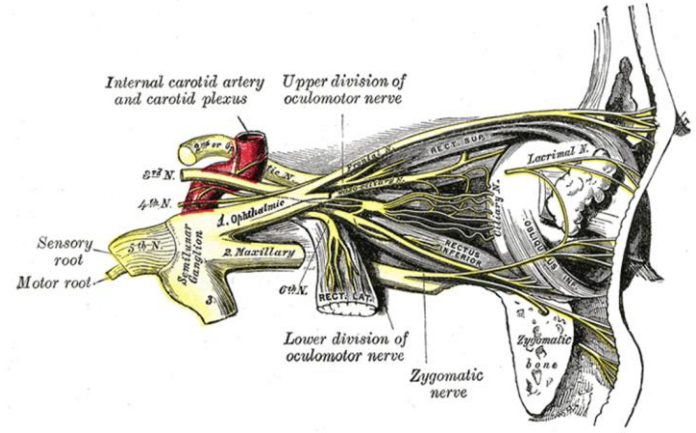
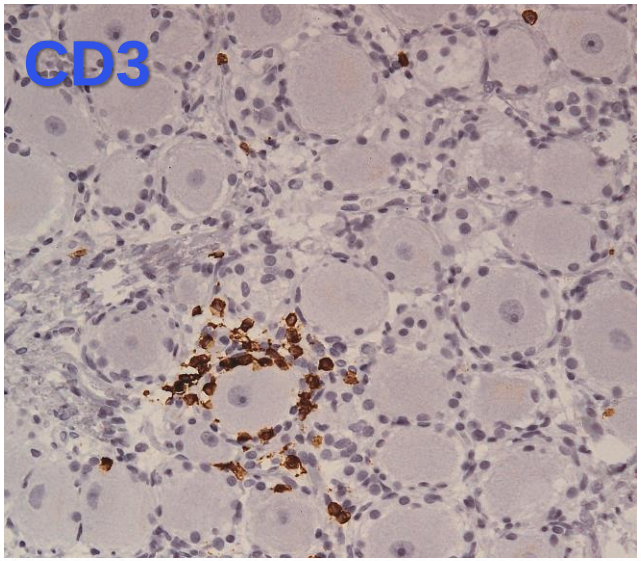
T-cell responses in latently HSV-1 and VZV-infected human trigeminal ganglia

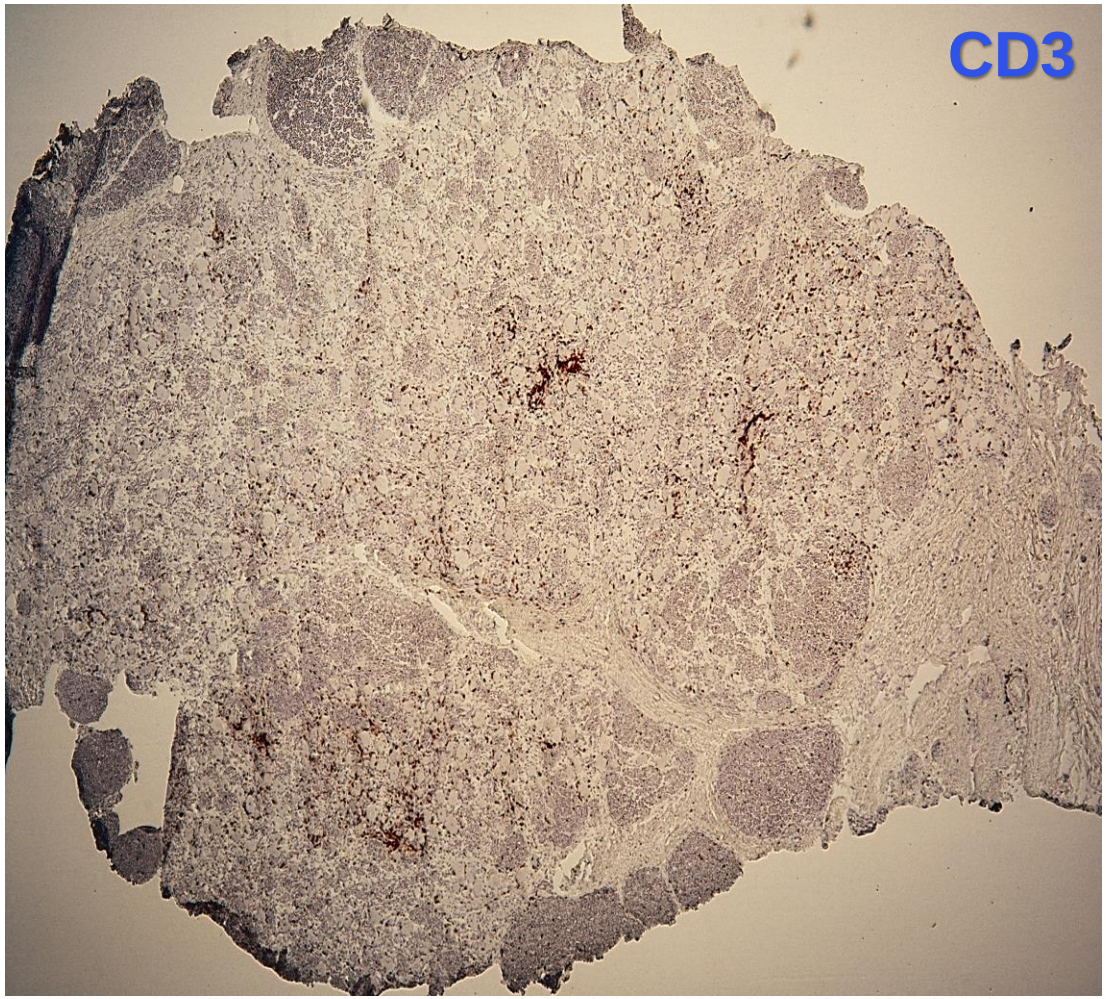


Infection with:
mock, HSV-1 & VZV

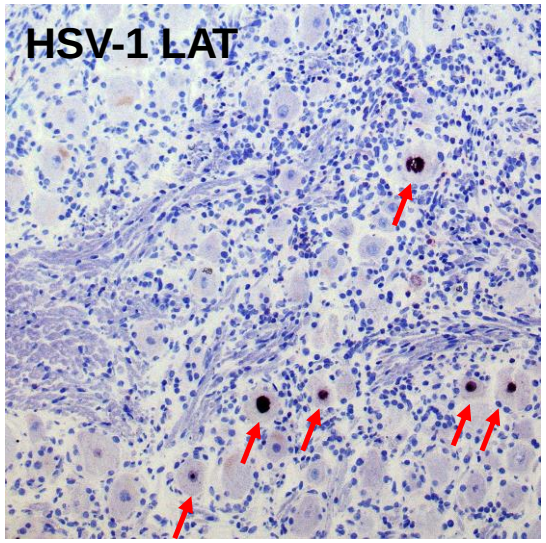
Neuron-interacting T-cell clusters in ‘normal’ human TG

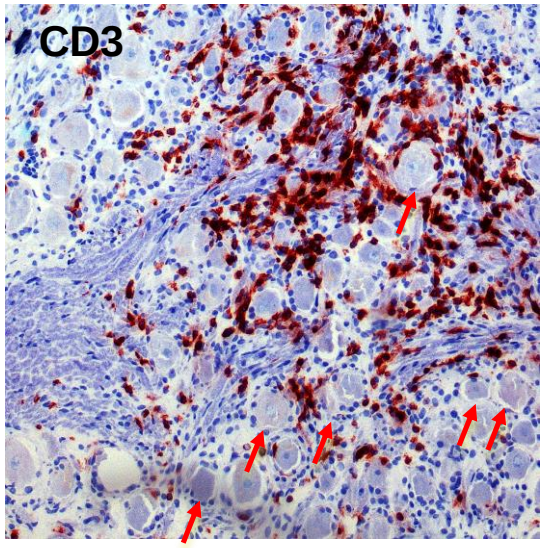


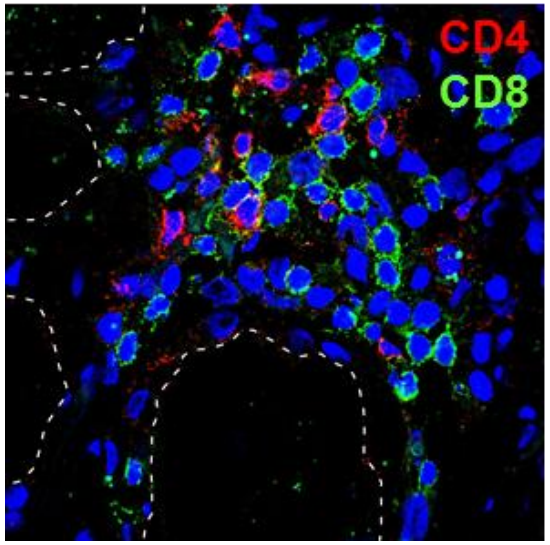




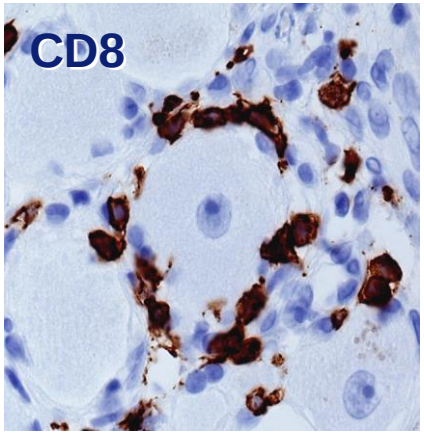
T-cell clusters in human TG interact with HSV-1 LAT^{POS} neurons

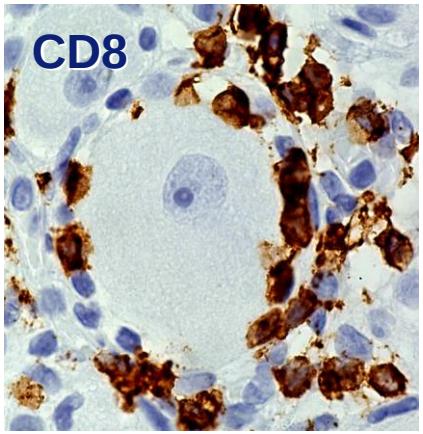


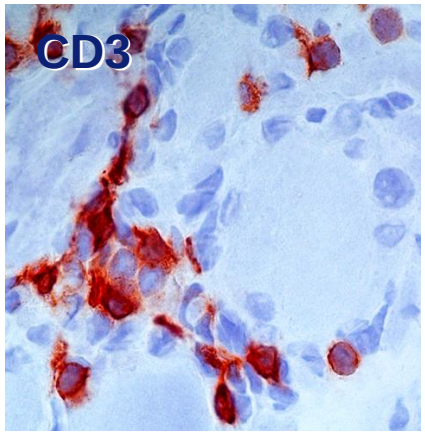


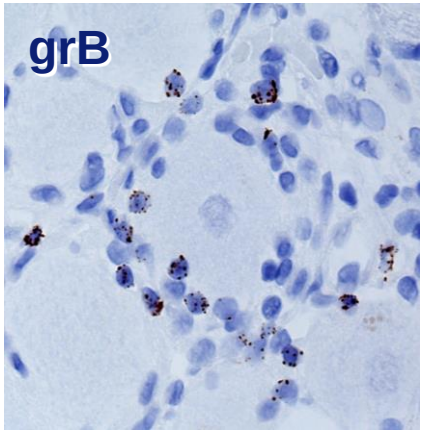


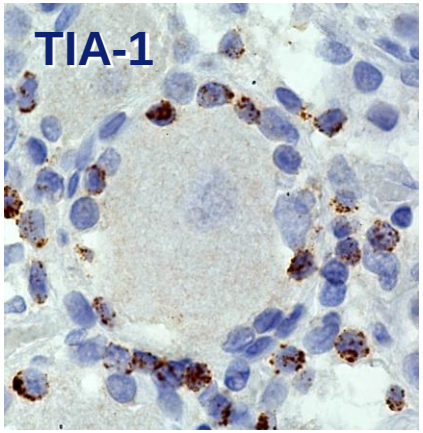
CD8 T-cells express CTL markers and CD137: antigen-driven T-cell retention?

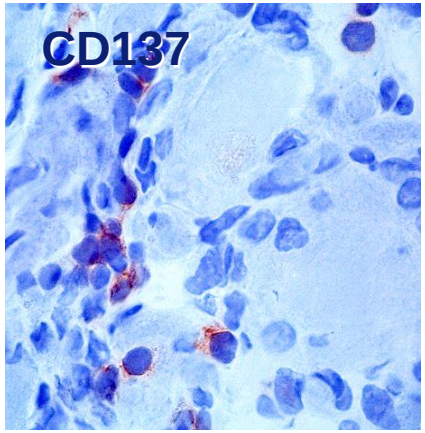






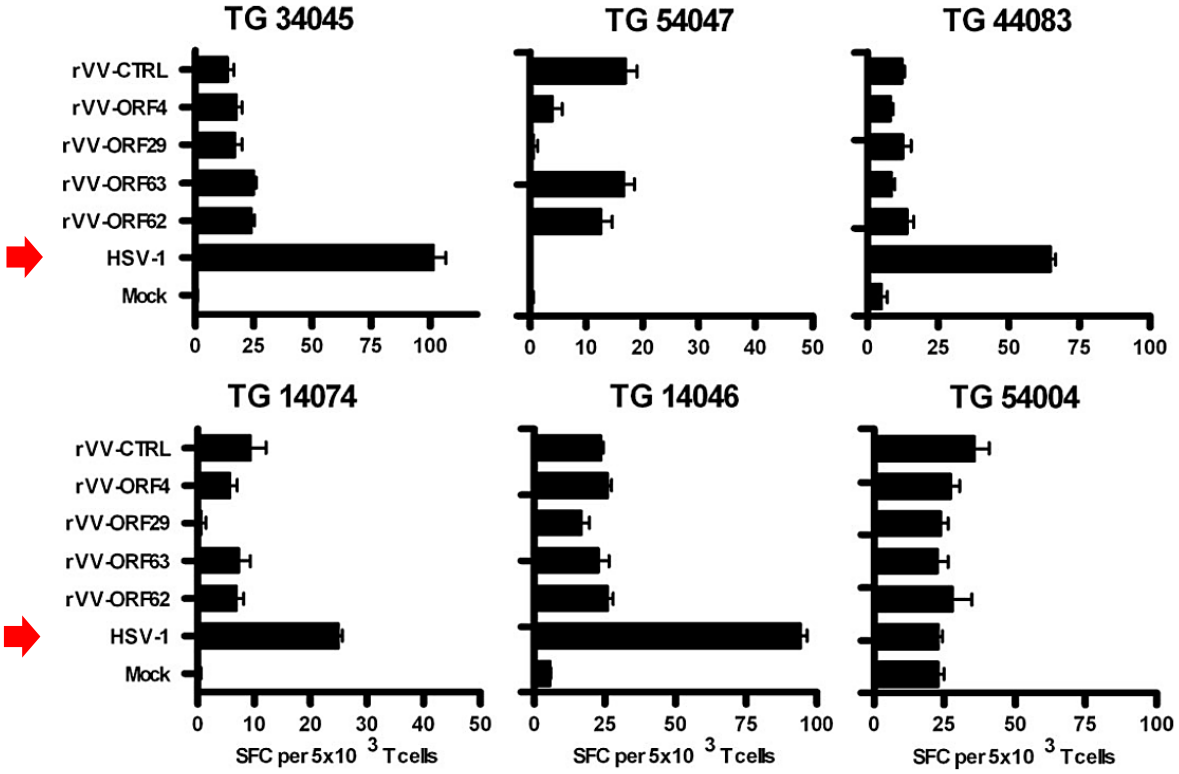






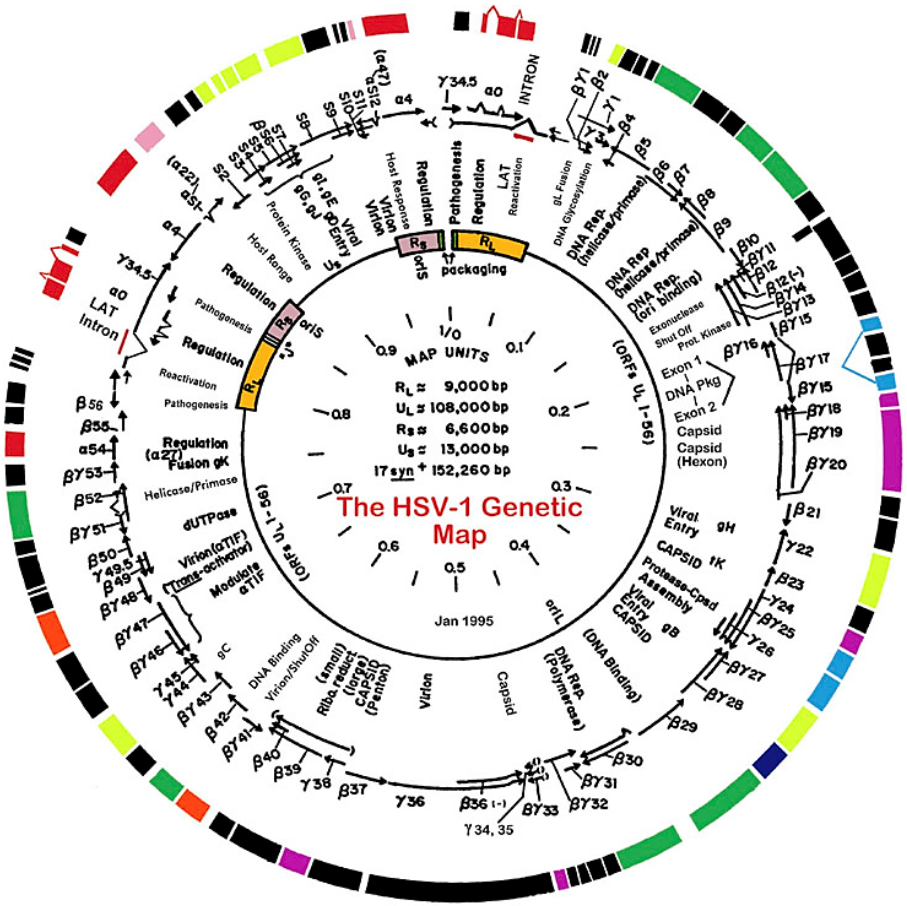
A DEPARTMENT OF Erasmus MC

Human TG-derived T-cells recognize HSV-1, but not VZV proteins



A DEPARTMENT OF Erasmus MC

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

A DEPARTMENT OF Erasmus MC

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

		Kinetic class of recognized HSV-1 proteins		
Patient ID	**HLA allele**	**Immediate early**	**Early**	**Late**
TG1	A*0201	-	-	UL6
	A*0201			gB
TG2	A*0201	ICP0 aa642-651	ICP8 aa1096-1105	-
	B*1501	-	ICP6	-
TG3	A*0101	VP16 aa090-099	-	gL aa066-074
	A*0101	VP16 aa479-488		gK aa201-209
TG4	A*0201	-	-	UL25
TG5	B*4001	-	ICP6	-
TG6	A*2902	-	-	VP13/14 aa508-516
	B*0702	-	TK	VP11/12 aa386-394
TG7	A*0301	ICP4 aa1096-1105	-	-
	A*3101	-	ICP6	-
	B*4001	VP16 aa163-175	ICP6	-
TG12	B*4001	-	ICP6	-

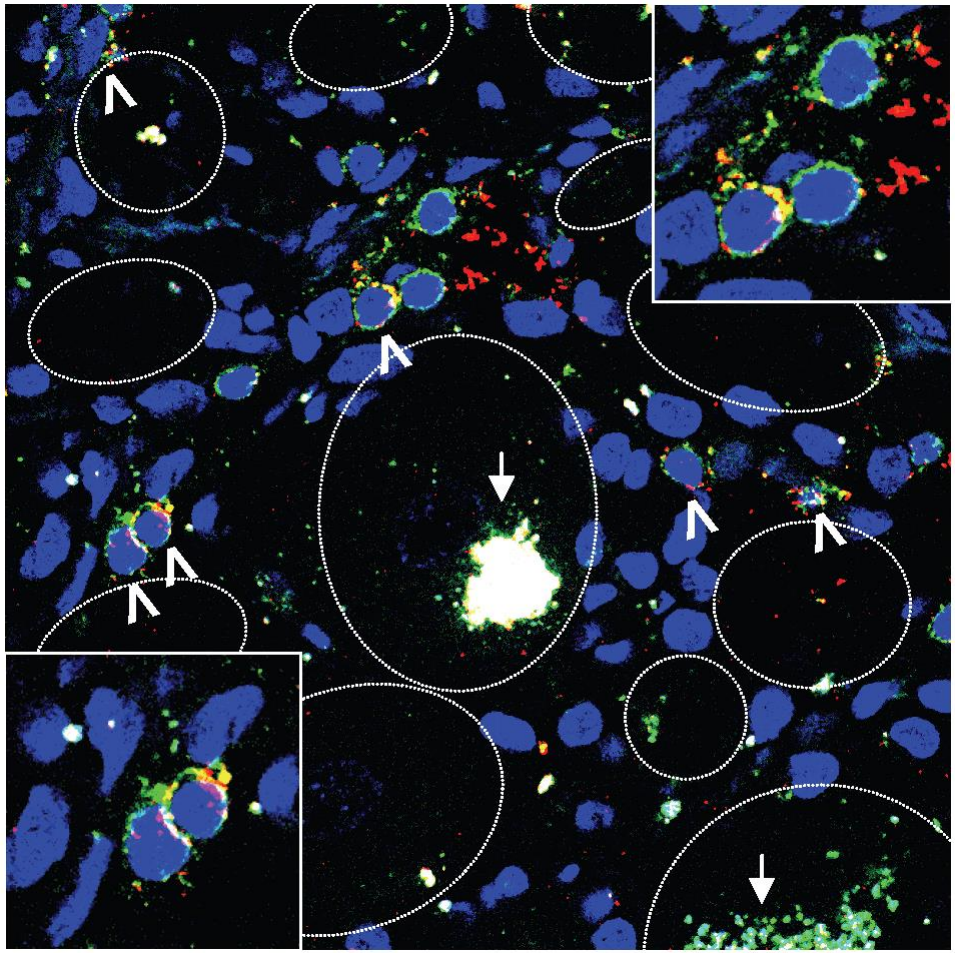
HSV-1 specific CD8 T-cells interact with neuron somata in human TG

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**)
  - $ICP0_{642\text{-}651}$
  - $ICP8_{1096\text{-}1105}$



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?