Latent herpes simplex virus and varicella zoster virus infections of sensory neurons of the peripheral nervous system

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Research questions and background
Upon primary infection, herpes simplex virus type 1 (HSV-1) and varicella zoster virus (VZV) establish a lifelong latent infection of neurons located in sensory ganglia like the trigeminal ganglia (TG). Virus reactivation from the TG can result in symptoms ranging from mild disease to severe disorders or even death. The association of viral reactivation with immune suppression, and the observed retention of T-cells in HSV latently infected human TG, suggest a pivotal role of T-cells to control viral latency. In contrast to latent HSV infection, VZV latency is not associated with the retention of VZV-specific T-cells in the TG. Paradoxically, while HSV latency is restricted to the expression of 1 non-coding viral transcript, VZV latently infected neurons express several latency-associated genes and proteins. However, both the relationship between latent VZV transcripts and proteins and their function in VZV latency are unclear. We aim to elucidate the correlation between latent VZV transcripts and proteins and between the different latent VZV proteins.

Alphaherpesvirus infections are successfully treated with the antiviral drug acyclovir (ACV). Prolonged treatment of herpetic lesions may result in the development and subsequently enrichment of ACV-resistant (ACV:\textsuperscript{R}) viruses, commonly due to mutations in the viral thymidine kinase (TK) gene. It is generally assumed that ACV:\textsuperscript{R} alpha-herpesviruses do not reactivate or even establish latency in sensory ganglia. We have recently shown that ACV:\textsuperscript{R} HSV-1 does reappear in the same eye of patients with recurrent HSV eye disease. We determined the HSV-1 TK sequence variability and the prevalence of ACV:\textsuperscript{R} HSV-1 in the paired (left and right) TG samples in detail.

Methods and tissues used
The acquisition of paired TG biopsies with short to medium post-mortem intervals (PMI) offers the unique opportunity to characterize the VZV transcriptome and proteome expressed in latently infected human TG. In collaboration with colleagues (Drs RJ Cohrs and D Gilden) at the department of Neurology of the University of Denver (Denver, US), we analyzed the spectrum and quantity of all 77 unique VZV transcripts by multiplex VZV gene-specific PCR in VZV latently infected human TG (n= 43) with post-mortem intervals (PMIs) ranging from 3.7 to 24 hours.\textsuperscript{2}

The inconsistency of VZV protein expression (e.g., viral protein 62 and 63) in VZV latently infected human ganglia, without evidence for T-cell recognition of the respective VZV proteins locally has puzzled the VZV field for many years. Recently, it has been suggested that the VZV proteins detected in-situ may be staining artefacts due to cross-reactivity of specific blood group antigens expressed in the cytoplasm of sensory neurons. In collaboration with colleagues at the department of Neurology (Drs. SE Flowerdew and K HÜfner) of the Ludwig-Maximilians University (München, Germany) we performed in-depth immunohistochemical analyses on snap-frozen human TG (n=30) and dorsal route ganglia (DRG; n=9) specimens of blood group genotyped donors (n=30).\textsuperscript{2}

For the studies on HSV-1 TK gene variability and ACV resistance, the HSV-1 TK gene was amplified from whole TG-derived DNA, isolated from paired TG of 5 donors, and PCR products were ligated into a cloning vector and transformed into bacteria. Individual colonies (n >35 colonies/TG) were sequenced and the sequence variability analyzed. Genetic relatedness of the TK sequences was determined by phylogenetic tree analyses. Furthermore, the ability of a number of cloned TG-derived TK genotypes to convert ACV to its functional product (ACV mono-phosphate) was assayed using mass spectrometry\textsuperscript{3}

Results and conclusions
Multiplex reverse transcriptase PCR (RT-PCR) revealed no VZV transcripts in human TG with a PMI of<9 h. Real-time PCR indicated a significant increase (P= 0.02) in VZV gene 63 transcript levels, but not the virus DNA burden with longer PMI. Overall, both the breadth of the VZV transcriptome and the VZV gene 63 transcript levels in human cadaver TG increased with longer PMI. The data indicate that the long-held dogma that latent VZV infection is associated with the expression of
multiple transcripts is questionable and that this is most likely attributed to the long PMIs of human ganglia studied in earlier studies.\(^1\)

The number of immunohistochemically stained neurons was higher with mAb directed to immediate early protein 62 (IE62) compared with IE63. The VZV IE63-specific monoclonal antibody (mAb)-positive neurons always co-stained for IE62 but not vice versa. The VZV protein-specific mAb staining was confined to distinct large intra-neuronal vacuoles and restricted to bloodgroup A\(^{1\text{POS}}\) donors. Anti-VZV mAb staining in neurons, but not in VZV-infected cell monolayers, was obliterated after mAb adsorption against blood group A1 erythrocytes. The data presented demonstrate that neuronal VZV protein expression detected by ascites-derived mAb in snap-frozen TG and DRG of blood group A\(^{1\text{POS}}\) donors can be misinterpreted due to the presence of endogenous antibodies directed against blood group A1-associated antigens present in ascites-derived VZV-specific mAb preparations. Because these staining artefacts are not restricted to ganglia and virus-specific mAb preparations, in-situ studies on nervous tissue using ascites-derived mAb preparations should be carefully interpreted and data to be confirmed with other techniques like in-situ hybridization.\(^2\)

HSV-1 latently infected human TG contain several HSV-1 TK variants. One dominant ACV\(^{S}\) variant shared between the left and right TG, but different between TG donors. Notably, a small part of the TG-derived viral TK sequences (10-15\%) are derived, but no identical to the dominant ACV\(^{S}\) variant. They consist of ~4-10 different ACV variants, being either ACV-sensitive (ACV\(^{S}\)) or ACV\(^{R}\), and are not shared between the left and right TG of the same individual. The data suggest that ACV\(^{R}\) establish latent infection in human TG and poses a risk of recrudescent HSV-1 infections of the innervating tissue with ACV\(^{R}\) viruses.\(^3\)

**Publications**