ABSTRACTS

GENES & PROTEINS

1-1 HHV-6B and its host receptor - What the discovery of viral receptor means for clinical practice

Oral

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HHV-6B primary infects to people during infancy and causes exanthema subitum. It can occasionally cause encephalopathy and leave residual damage. It is believed that more than 90% of adults are latently infected with the virus. Furthermore, HHV-6B frequently reactivates in hematopoietic stem cell transplant patients, occasionally causing encephalitis and being fatal, which is a problem. It also reactivates in drug-induced hypersensitivity syndrome, causing viremia, and it has been suggested that it may be involved in the exacerbation of the disease. Our group discovered that the host receptor for HHV-6B is human CD134 which is expressed in activated T cells. Furthermore, we found that the viral ligand is a glycoprotein tetramer (gH/gL/gQ1/gQ2 complex) present in the viral envelope. We also clarified the relationship between the discovery of the receptor and the onset of the disease. In this talk, I will outline the path to the discovery of the receptor and the subsequent elucidation of the disease's pathology.

1-2 Role of SUMOylation and PML nuclear bodies in HHV-6A genome integration

Oral

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Background: Human herpesvirus 6A (HHV-6A) is a member of the beta-Herpesvirinae subfamily, which also includes human herpesvirus 6B (HHV-6B), human herpesvirus 7 (HHV-7), and human cytomegalovirus (hCMV). A particularly fascinating feature of HHV-6A/B and HHV-7 is their ability to integrate their viral genomes into the telomeres of human chromosomes, a process not observed in other members of this subfamily. Telomeres, repetitive nucleotide sequences located at the ends of chromosomes, play a crucial role in protecting genetic material during cell division. The integration of viral genomes into these regions represents a sophisticated strategy for establishing a latent infection within the host. While telomeric repeats in the viral genome have been identified as critical for this integration process, the precise molecular mechanisms remain poorly understood, offering an exciting area for further research. In previous research, we demonstrated that PML nuclear bodies (PML-NBs) are important for the efficient integration of HHV-6B genomes into host telomeres. Building on this finding, the present study investigates the role of PML-NBs in the integration of HHV-6A genomes.

Results: Our results reveal that PML-NBs are key players for the efficient integration of HHV-6A. Additionally, we provide evidence that the immediate early protein 1 (IE1A) of HHV-6A localizes to PML-NBs. This localization influences the presence of IE1A at telomeric regions. Importantly, we explored the post-translational modification of IE1A through SUMOylation, a process in which small ubiquitin-like modifier (SUMO) proteins are covalently attached to specific lysine residues in target proteins. We identified the lysine 665 (K665) as a key SUMO acceptor site on IE1A. Additionally, the 638VIV640 sequence within IE1A functions as a potential SUMO-interacting motif (SIM). Next, we generated recombinant viruses and could demonstrate that replication of the IE1A SUMOylation-deficient mutant and SIM mutant was impaired and integrated less efficient into host chromosomes. To further elucidate these mechanisms, we studied different PML isoforms

and demonstrated that the C-terminal region of PML, containing a SIM site (amino acids 556–562), is important for IE1A SUMOylation.

Conclusion: In conclusion, we propose a model in which IE1A is recruited to PML-NBs via SUMOylation-dependent interactions, enhancing HHV-6A genome integration into host telomeres to achieve latency. This study provides critical insights into the interplay between viral proteins and host factors, offering new perspectives for targeting latency mechanisms.

1-3 X-ray crystallography of Human herpesvirus 6B gH/gL/gQ1/gQ2 complex which is a ligand of the cellular receptor

Oral

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Introduction: The tetrameric glycoprotein complex gH/gL/gQ1/gQ2 of HHV-6B serves as a key viral ligand on the entry process by recognizing the host receptor CD134 expressed on the activated T cells. The gH and gL are viral proteins shared among herpesviruses, while the gQ1 and gQ2 are specific for HHV-6A, HHV-6B, and HHV-7. It has been reported that the gQ1 and gQ2 are the determinants for the receptors; gQ1/gQ2s of HHV-6B and HHV-6A are required to recognize CD134 and CD46, respectively. The combination of gQ1 and gQ2 is also important for the function, suggesting that the gQ2 and gQ1 are tightly linked in the complex structure. In the previous study, we analyzed the structure of the HHV-6B gH/gL/gQ1/gQ2 complex by negative-stain electron micrography, and visualized the densities named Stalk and Palm corresponding to gH/gL and gQ1/gQ2, respectively. The Palm region was located at the tip of the Stalk resulting in an overall curved and elongated shape, however, the detailed structural features could not be revealed due to the limitation of the resolution.

Methods: In this study, X-ray crystallography is performed to reveal the molecular structure of gH/gL/gQ1/gQ2 complex at an atomic resolution. Purified HHV-6B gH/gL/gQ1/gQ2 ectodomain could be crystallized in a rectangular shape, but the X-ray diffractions by the crystals were poor (~10 Å) at the synchrotron radiation facility SPring-8. New crystals of semi-cylindrical shape were obtained by adding two Fab domains of antibodies, and diffraction spots greater than 4 Å resolution were observed by the crystals. The data was merged by an automated data processing system, and a diffraction dataset at a maximum 3.8 Å resolution was obtained.

Result: By the molecular replacement method using homology models of gH, gL, and the Fabs as search models, the electron density of the gH/gL/gQ1/gQ2 complex and two Fabs became visible. The observed structural features of the gH/gL/gQ1/gQ2 complex and Fabs will be discussed.

1-4 Study on interaction between HHV-6B gH/gL/gQ1/gQ2 complex and antibodies

Oral

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Background: Human herpesvirus 6B (HHV-6B) uses specific glycoprotein gH/gL/gQ1/gQ2 to enter the target cells. In terms of HHV-6B, the cell tropisms are mainly activated T cells, which express CD134 on its surface. The glycoprotein complex serves as a viral ligand and interacts with this receptor. Previous studies revealed that the tetrameric complex is formed as the gH/gL complex is associated with unique glycoproteins, gQ1 and gQ2. Antibodies against this viral surface antigens, i.e. glycoproteins gH, gL, gQ1, and gQ2, are important because they inhibit the interaction between this viral ligand and CD134, or restrict other functions of the ligand, hence in-depth analyses on these antibodies are needed to block this HHV-6B infection. In previous studies, we have isolated several murine monoclonal antibodies targeting the gH/gL/gQ1/gQ2 complex.

Methods: To characterize the binding mode of the antibodies, the interaction between the monoclonal antibodies and the gH/gL/gQ1/gQ2 complex were analyzed in detail. The HHV-6B gH/gL/gQ1/gQ2 ectodomain was prepared based on the protocols reported in our previous study. The interaction between the antibodies and the HHV-6B gH/gL/gQ1/gQ2 complex were analyzed through immunofluorescence assay (IFA), bio-layer

interferometry (BLI), and enzyme-linked immunosorbent assay (ELISA). First, we selected antibodies which show reactivity to the HHV-6B gH/gL/gQ1g/Q2 complex by IFA experiment. The reactive monoclonal antibodies were classified into three groups by the affinities determined by the BLI experiment. Competition assays by ELISA and BLI were conducted to test the overlap in the binding site among the antibodies.

Result: It was shown that the anti-gH monoclonal antibodies were not competing each other, suggesting that they recognize different epitopes in the antigen. The observed relationship between the binding sites of antibodies and the effect on the gH/gL/gQ1/gQ2 functions will be discussed.

HOST CELL INTERACTION - I

2-1 Oral Immunomodulatory Mechanisms of Human Herpesvirus 6B Infection: Potential Role for HHV-6B in Grade II-IV Acute Graft-vs-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation

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Objective: Human herpesvirus 6B (HHV-6B) establishes latency in CD4+ T cells in nearly all humans by adulthood and utilizes the CD134 (OX40) receptor on CD4+ T cells for cellular entry. HHV-6B commonly reactivates after allogeneic hematopoietic stem cell transplantation (alloHCT) and we previously showed that HHV-6B reactivation is associated with increased risk of grade II-IV aGVHD. Past studies have shown that high regulatory T cell (Treg) counts following alloHCT are protective against aGVHD, whereas CD134 has been shown to antagonize Treg function. We hypothesized that HHV-6B reactivation upregulates CD134 on CD4+ T cells as a possible mechanism explaining how HHV-6 contributes in aGVHD pathology.

Methods: We established an in vitro model of HHV-6B infection using the MOLT-3 T cell and THP-1 macrophage cell lines. Using qRT-PCR, Western Blot and flow cytometry we assessed CD134 expression and cell viability. We set up a MOLT3/THP-1 co-culture model with 2 distinct conditions: one in which cell-cell contact was allowed and another in which the MOLT3 and THP-1 cells were separated by a 0.4μm membrane (soluble factor setting).

Results: We show that HHV-6B infection of the MOLT-3 cell line resulted in a 1.76-fold increase in CD134 expression ($p<0.001$) compared to mock infection. We then show that CD134 upregulation is significantly augmented on MOLT-3 T cells on day 4 following HHV-6B infection when co-cultured in the presence of THP-1 macrophages (fold-increase: 1.76 [no co-culture] vs. 685.63 [soluble factor, $p<0.05$] vs. 113.75 [cell-cell contact, $p<0.05$]). These changes could also be observed via Western blot and flow cytometry. We also show that HHV -6B increases TLR9 mRNA transcription in THP-1 macrophages on day +1 post-infection.

Conclusions: Our data suggest that HHV-6B reactivation can greatly increase CD134, which also enhances the prospects for further HHV-6 replication. Our co-culture experiments suggest that antigen presenting cells are communicating a signal to T cells to drastically upregulate CD134 expression following HHV-6 infection, and that this effect is independent of cell-cell contact between macrophages and T cells. Initial data suggest that the TLR9 pathway may be involved in the process of augmented CD134 upregulation. Our proposed model lays out a positive feedback loop that would result in uncontrolled HHV-6 replication and increase the risk of aGVHD. Studies are underway to further characterize the relationship between HHV-6B infection with CD134 and TLR9 signaling cascades and to establish a new tissue culture model using PBMCs.

2-2 Oral Molecular mechanism responsible for the activation of RLR/MAVS-mediated signaling pathway in HHV-6 infection

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Background: Previous studies have shown that the activation of the RLR/MAVS signaling pathway, which could be inhibited by HHV-6 U26 gene product, plays an important role in antiviral responses.

Objective: To investigate the mechanisms of the activation of the RLR/MAVS pathway.

Methods: RNA immunoprecipitation and sequence assays were performed to identify the molecules for the activation of the RLR/MAVS signaling pathway during HHV-6 infection. Loss- and gain-function experiments were conducted to elucidate the identified molecules' function during HHV-6 infection.

Results: 1. We have confirmed the anti-HHV-6 role of RIG-1 and MDA5 through the knockdown of RIG-1 and MDA5 expression in HHV-6 susceptible cells and the assessment of the susceptibility change of knockdown cells to HHV-6 infection. 2. We constructed RIG-1 or MDA5 overexpression cells, and screened RNA molecules which named RNA5SP856 binding to them through RIP experiments. RNA5SP85 can activate the IFN-related signaling pathway which can be inhibited by the U26 gene product. 3. The infection of HHV-6 was enhanced by overexpressing RNA5SP85; conversely, the viral infection was decreased by knocking down its expression.

Conclusion: Mechanism for the activation of the RLR/MAVS signaling pathway during HHV-6 infection was detailed. Both RIG-1 and MDA5 play antiviral function during HHV-6 infection. A host small RNA molecule was identified as a key factor for the activation of the RLR/MAVS pathway during HHV-6 infection.

2-3 HHV-6A infection drives epigenetic alterations and tumor progression in papillary thyroid carcinoma cells

Oral

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Background: Thyrocytes are known to be permissive to HHV-6A infection. The virus contributes to the pathogenesis of autoimmune thyroiditis which may increase the risk of papillary cancer, considering that chronic inflammation is strongly linked to carcinogenesis. It has been reported that the well-differentiated papillary thyroid carcinoma (PTC), may progress to the more aggressive follicular thyroid carcinoma (FTC) and eventually to the anaplastic thyroid carcinoma (ATC). As for tumor initiation, the inflammatory/immune suppressive tumor microenvironment promotes tumor progression. Epigenetic changes, which include DNA methylation, histone modifications and microRNAs (miRNAs), may contribute to the expression of inflammation-associated genes and are known to be involved in all steps of carcinogenesis.

Objective: In this study, we asked if papillary tumor cells (BCPAP) infection by human herpes virus-6A (HHV -6A) could induce epigenetic changes and favor papillary cancer progression towards more aggressive forms of thyroid cancers.

Methods: FACS analysis, western blot analysis and qRT-PCR were used to evaluate HHV-6A infection and the effects induced in BCPAP cells.

Results: We found that HHV-6A infection of BCPAP cells induced several epigenetic changes, including miRNA dysregulation and histone modifications. The expression of miR-155, miR-9 and the miR-221/222 cluster was altered and the expression of methyltransferases such as EZH2 and G9a and acetyltransferases such as p300, PCAF and TIP60 was dysregulated as well. HHV-6A increased genomic instability and the secretion of pro-inflammatory cytokines, particularly IL-6. Moreover, the expression of PTEN, reported to act as an oncogene in mutp53-carrying cells such as BCPAP, was also upregulated following infection. These effects may be involved in cancer progression and the specific role of each epigenetic modification is under investigation in our laboratory.

Conclusions: These findings suggest that a ubiquitous herpesvirus such as HHV-6A, which displays a marked tropism for thyrocytes, could alter epigenetic landscape in BCPAP and promote the progression of PTC towards more aggressive forms of thyroid tumors.

2-4 Human herpesvirus 6B infection induces downregulation of a T cell surface antigen

Oral

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Objective: HHV-6B mainly infects T cells, which are pivotal cells in the generation of humoral and cell-mediated adaptive immune responses. T cell surface antigens are known to be involved in several immune activities such as immunological synapse formation that stimulates T cells. It is widely known that viruses sometimes modulate the expression of T cell surface antigens. Decreased expression of these antigens may lead to reduced host immune responses. In this study, we studied the possible association between T cell surface

antigens and HHV-6B infection.

Methods: First, we focused on one of the T cell surface antigens. Hybridoma cells producing monoclonal antibodies against this antigen were obtained by immunizing mice. We confirmed expression levels of this antigen in MT4, HSB-2, Sup-T1, JJhan and Molt-3 cells by Western blotting using this antibody. Protein expression levels of the surface antigen were higher in MT4 and Molt-3 cells, which are susceptible to HHV-6B, compared to those in HSB-2, Sup-T1 and JJhan cells. To investigate the association between HHV-6B infection and this antigen, we next examined the expression of this surface antigen in HHV-6B-infected MT4 cells by Western blotting. HHV-6B-infected MT4 cells decreased protein expression levels of this antigen compared to non-infected cells. To clarify the relationship between the infection stage and expression of this antigen, we treated HHV-6B-infected MT4 cells with the DNA polymerase inhibitor, foscarnet and examined expression of this antigen by flow cytometry.

Result: The antigen was down-regulated from the cell surface, even when DNA synthesis of HHV-6B was stopped by foscarnet. This suggested the immediate early- or early-expressing gene of the virus is related to down-regulation of this T cell surface antigen.

Conclusion: We indicated that one of the T cell surface antigens was down-regulated in HHV-6-infected T cells. This may affect viral induced-immune suppression.

2-5 The roles of the interaction of HHV-6A U14 and NF-kB p65

Oral

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Introduction: The transcription factor NF-κB plays a pivotal role in many cellular events such as apoptosis, cell proliferation, inflammation, and immunity. Numerous viruses modulate key components of the NF-κB pathway to evade antiviral responses or to promote viral infection. HHV-6A is a member of the Betaherpesvirinae subfamily and is frequently found in patients with neuroinflammatory diseases, although its role in disease pathogenesis has not been elucidated. HHV-6A U14 is a tegument protein that has physical and functional interactions with host cellular machineries and plays an important role during HHV-6A maturation. Recently, we reported that HHV-6A interacts with NF-κB p65 and activates the expression of the NF-κB-regulated genes. Activation of NF-κB is considered to promote viral gene expression, whereas it induces the genes involved in the antiviral mechanism. Thus, the balance of NF-κB signaling and regulation of its activation is critical for viral replication, although their details are not elucidated. Phosphorylation of NF-κB p65 at serine 536 (p.p65) is thought to be required for the activation; in contrast, this phosphorylation facilitates NF-κB p65 degradation, thereby terminating NF-κB transcriptional activity. Here, we focus on the interaction between HHV-6A U14 and NF-κB p65 to reveal the consequence of it.

Results: The results are as follows: (i) Co-expression of HHV-6A U14 and NF-κB p65 reduced the amount of U14 and NF-κB p.p65 through proteasome systems. (ii) These reductions depend on the activation of NF-κB p65. (iii) The amounts of NF-κB p.p65 and U14 were reduced in infected cells through the proteasome system.

Conclusion: It has been reported that NF-κB p.p65 is important for its activation and termination. HHV-6A U14-p65 interaction possibly promotes degradation of this complex to prevent deleterious inflammation caused by NF-κB activation. Thus, our results suggest the role of this tegument protein in evading and exploiting cellular NF-κB immune signaling cascades for their benefit through a tight temporal regulation.

HHV-6 & DRESS/DIHS, TRANSMISSION

3-1 Pathological implications of persistent HHV-6 infection in DIHS/DRESS

Oral

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Introduction: Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is characterized by fever, cutaneous eruptions, hematological abnormalities, multiple organ disorder, and reactivation of human herpesviruses such as HHV-6 and CMV. In addition, autoimmune inflammatory diseases such as type 1 diabetes and chronic thyroiditis may develop long-term after resolution of DIHS/DRESS. Reactivation of HHV-6 has been reported to be involved in clinical condition of DIHS/DRESS. We found that quite a few patients harbor high levels (> 1000 copies / 10^6 PBMC) of HHV-6 DNA in PBMC over 6 months after onset of DIHS. However, it was unclear what effects persistent HHV-6 infection has on DIHS patients.

Methods: We analyzed clinical symptoms, blood test findings, reactivation of herpes virus, expression of serum cytokines (IL-4, IL-5, IL-10, IFN-γ) and soluble IL-2 receptor for 11 DIHS patients with persistent HHV -6 infection and 30 patients with transient HHV-6 infection.

Results: Compared to the transient HHV-6 infection group, persistent HHV-6 infection group showed 1) more severe acute phase cutaneous and mucosal eruptions, 2) higher levels of HHV-6 and CMV DNA in acute phase, 3) higher levels of IL-4 and IL-5 in acute phase and higher levels of soluble IL-2 receptor in acute and late phase, 4) higher rate of chronic inflammatory complications such as type 1 diabetes, Hashimoto's disease, interstitial nephritis, arthritis, diffuse alopecia, vitiligo.

Conclusion: Persistent HHV-6 infection may have some influence on the pathological and immunological conditions of DIHS and may be involved in the development of post-DIHS chronic inflammatory complications.

3-2 HHV-6 & DRESS

Oral

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Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) is a severe drug reaction that has a mortality rate of 10%. DRESS usually presents as a diffuse infiltrated erythematous rash with facial oedema, lymphadenopathy, fever, hypereosinophilia and/or atypical circulating lymphocytes. Its severity is due to visceral involvement, including cytolytic hepatitis, renal failure, interstitial pneumonitis, meningoencephalitis, myocarditis or haemophagocytic syndrome. The time to onset after taking trigger drug typically ranges from 2 to 8 weeks. The number of drugs that may induce DRESS are limited and include allopurinol, anticonvulsants, minocycline, sulfasalazine, and cotriomaxole.

Pathophysiology of DRESS is complex and not fully understood. It involves genetic factors, drug exposure, herpesvirus reactivation (including HHV-6, CMV and EBV) and T cell immune response. HHV-6 reactivation is present in the majority of cases, particularly severe cases. We recently reported a multicentric retrospective series of severe cases, defined as death, transfer to the intensive care unit (ICU), or severe organ damage. HHV -6 reactivation was studied by PCR in blood and by detection of small non-coding transcripts (HHV-6 miRaU14) and a late viral protein (GP82/105) in skin biopsies (Bhupesh PRUSTY). Fifty two patients were included. Eight patients died, 13 were admitted to ICU. Liver was the most frequent organ involvement (88%), then renal failure (46%). Forty patients (77%) had at least one blood viral reactivation among HHV-6 (50%), EBV, and CMV. Median time from onset to detection of HHV-6 in blood was 24 days. Twenty skin biopsies of 19 patients were analyzed for the detection of MiR-aU14. MiR-aU14 was detected in 15/20 biopsies (with a median time of 11 days from onset) located in the inflammatory cells infiltrating the dermal region. There was combined GP82/105 positivity in 8/20 patients. Interestingly, MiR-aU14 was detected in 5 out of 8 patients with negative blood PCR.

The sequence of events linking DRESS manifestations and HHV-6 reactivation remains a topic of debate. Is HHV-6 reactivation a result of the hypersensitivity reaction, or is it an early event triggered by drug exposure? We demonstrated that reactivation of HHV-6 in skin lesions may precede the detection of HHV-6 in blood. Further prospective studies with fixed and repeated sampling times are required.

3-3 Salivary shedding of HHV-6B from nursery school children in Japan: An alternative route for the transmission of HHV-6B.

Oral

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Objective: In our previous study, maximum secretion was observed in 3-8 months after onset of exanthem subitem (ES), indicating the possibility that older children in these high secretion periods might be the source of HHV-6B transmission to younger infants. Thus, we hypothesized that not only older siblings, but also children in the same or older age group in nursery schools or kindergartens might be considered a potential source of infection.

Methods: A total of 88 children in nursery school A and 64 children in nursery school B located in the middle of Japan were enrolled. Their saliva samples were collected in the spring and autumn, and were examined for the presence of HHV-6B, HHV-7 and CMV DNA. Children who didn't go to nursery school were also examined as a control.

Results: In nursery school A, 19 children were positive for HHV-6B secretion in saliva in spring and most of these children were also positive in the autumn. The remaining 69 children were negative in the spring, but 37 of the 69 children were positive in the autumn. In nursery school B, the trends were the same. Although trends were similar for HHV-7, the results of CMV were quite different between nursery schools A and B. A drastic increase in detection rate of CMV was observed at nursery school A, while detection rate decreased at nursery school B.

Conclusions: Detection rates of HHV-6B drastically increased after 6 months at both nursery schools, suggesting that children in the same or older age group in nursery schools might be an alternative source of transmission and tends to become more prominent in the situation in which prevalence of ES is shifting to older ages. More precise comparison at a younger age (one-year-old group) supported the conclusion.

HHV-6 & CELL RESPONSE

4-1 The roles of nuclear egress complex are conserved in betaherpesviruses

Oral

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Background: Herpesviruses replicate their genomes and package them into capsids within the host cell nucleus. These capsids must translocate from the nucleus to the cytoplasm through a process designated as nuclear egress. The viral-encoded nuclear egress complex (NEC), which consists of the nuclear matrix protein and the nuclear membrane protein, plays a crucial role in this process. Although the role of the NEC for nucleo-cytoplasmic transport of capsids is conserved in Herpesviridae, some of the binding partners of the NEC components are specific to individual viruses. The NECs of alpha- and gammaherpesviruses recruit an endosomal sorting complex required for transport III (ESCRT-III) proteins to the INM for efficient nuclear egress of capsids. In contrast, the role of ESCRT-III on nuclear egress of betaherpesviruses including human cytomegalovirus (HCMV) and human herpesvirus 6A (HHV-6A) has not been elucidated.

Results: Here, we showed that ESCRT-III protein was recruited to the nuclear rim in cells expressing the NEC of either HCMV or HHV-6A. Abrogation of ESCRT-III machinery impaired HHV-6A replication and nuclear egress of capsid. We also demonstrated that inhibiting ALIX, the ESCRT-III adaptor protein that acts as a link between ESCRT-III and the NEC reduced HHV-6A replication.

Conclusion: From these observations, we concluded that HHV-6A NEC recruits ESCRT-III through ALIX to promote nuclear egress of its capsids. Thus, our results suggests that the recruitment of ESCRT-III to the nuclear membrane is a conserved function in Herpesviridae.

4-2 Activation of T lymphocytes by human herpesvirus 6B infected dendritic cells

Oral

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Background: HHV-6B is a member of the betaherpesvirus family that predominantly infects and replicates in CD4+ T lymphocytes and causes exanthem subitum in primary infection. The exact mechanism of HHV-6B infection into CD4+ T lymphocytes is still unknown in primary infection, but may involve dendritic cells (DCs) in its early infection.

Methods: Here, we infected dendritic cells with HHV-6B and analyzed the supernatants.

Results: There was a significant increase in several chemokine and cytokine levels in the supernatants from HHV-6B infected DCs compared to mock. Flow cytometry analysis showed stimulation of T lymphocytes cultured with the supernatants from HHV-6B infected DCs.

Conclusion: These results suggest that HHV-6B infected DCs may activate T lymphocytes to trigger the entry of HHV-6B into T lymphocytes in primary infection.

iciHHV-6/7 - I

5-1 The biology and consequences of HHV-6 integration

Oral

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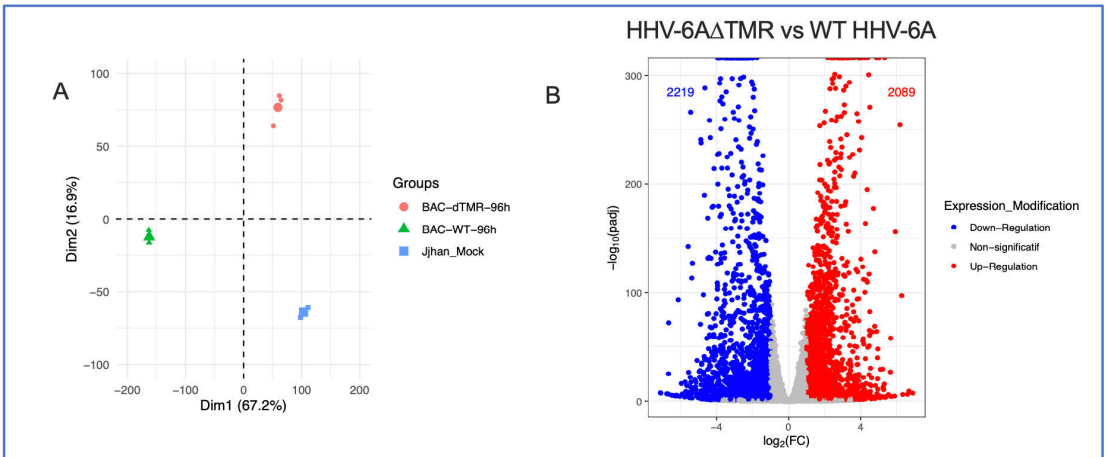
Human herpesvirus 6A and 6B (HHV-6A/B) can integrate their genomes within the telomere of chromosomes as a mean to achieve latency. The biological consequences of such integration of the biology of the cell remains poorly characterized. When integration takes place gametes prior to fertilization, individuals are born carrying an HHV-6A/B copy in every somatic cell. This condition, known as inherited chromosomally integrated HHV -6A/B (iciHHV-6A/B), varies in prevalence between 0.5-2% depending on the region of the world sampled. Large populational studies indicate that iciHHV-6 represents a risk factor for a number of diseases including angina, increased spontaneous abortion rate as well as systemic lupus erythematosus.

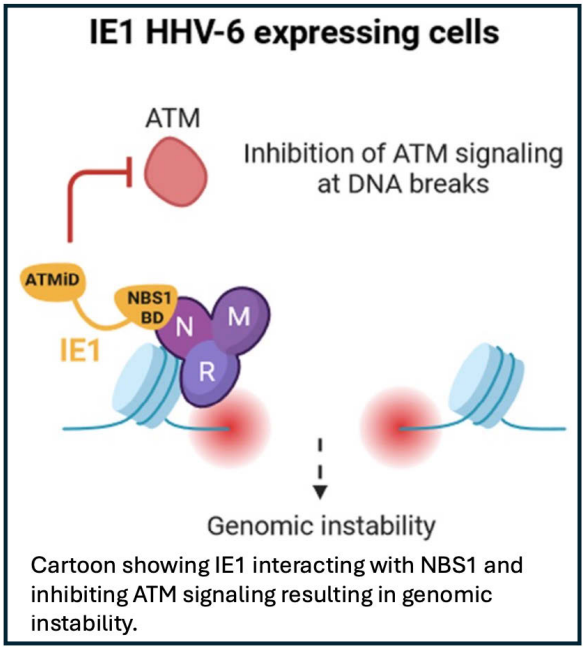
Our laboratory is very much interested in understanding the biology and consequences of HHV-6A/B chromosomal integration. During this presentation, I will report of the identification of several novel HHV-6A RNA transcripts mapping to the telomeric regions of the viral genome. An HHV-6A mutant (ΔTMR) lacking telomeric sequences fails to make such RNA, modulates cellular gene expression differently and is incompetent at chromosomal integration.



A) PCA plot showing divergent transciptomic proficles between mock, HHV-6A WT and HHV-6AΔTMR at 96h post infection.and B) Volcano plot comparing differentially expressed genes between HHV-6A WT and dHHV-6A ΔTMR.

In the integrated state, the HHV-6A/B genome is not entirely silent with spontaneous expression of a handful of genes, including U90 encoding for the immediate-early 1 (IE1) protein. Results will be presented indicating that IE1 interferes with the homologous recombination DNA damage repair pathway leading to genomic instabilities. Mechanistically, the amino terminus of IE1 interacts with the NBS1 protein causing a default in ATM kinase activation and absence of DNA break signaling.

Lastly, transcriptomic analysis of peripheral blood mononuclear cells isolated from iciHHV-6(n=29) and aged matched controls (n=53) will be presented. Viral and cellular differentially expressed genes will be discussed with potential consequences discussed.



Cartoon showing IE1 interacting with NBS1 and inhibiting ATM signaling resulting in genomic instability.

5-2 Human herpesvirus 7 genome integration and establishment of a genetic system

Oral

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Introduction: Human herpesvirus 7 (HHV-7) is a beta-herpesvirus and is the least understood among all human herpesviruses, despite its potential role in several diseases. Intriguingly, HHV-7 harbors telomeric repeat arrays (TMR) identical to the telomeres of the human genome. Similar repeats are present in the closely related human herpesvirus 6 (HHV-6) and facilitate the integration of its genome into the host telomeres; however, it remains elusive whether HHV-7 can integrate and what role its TMR may play. In addition, there is no reverse genetic system available, which tremendously hampered HHV-7 research until now.

Methods: Here, we first established an in vitro integration assay that allows the assessment of HHV-7 genome integration and maintenance in latently infected cells. Our data revealed that HHV-7 is stably maintained in latently infected U2OS cells. Integration was confirmed by fluorescence in situ hybridization (FISH) and nanopore sequencing, highlighting that HHV-7 can integrate its genome into the host telomeres. To address the role of the viral TMR in this process, we developed a bacterial/yeast artificial chromosome (BAC/YAC) system using transformation-associated recombination (TAR) cloning in yeast. The entire HHV-7 genome (156 kb) was captured in a BAC/YAC vector containing a GFP cassette. The resulting HHV-7 BAC/YACs clones were screened and confirmed by restriction fragment length polymorphism (RFLP) and next-generation sequencing (NGS).

Result: We successfully reconstituted the recombinant virus by nucleofection of SupT1 T cells and confirmed that it replicates comparable to wild-type virus. To assess TMR's role in virus integration, we generated TMR deletion viruses and could show that the sequences are dispensable for virus replication. In contrast, integration was severely impaired in the absence of the TMR.

Conclusion: Taken together, our study shows that HHV-7 efficiently integrates into human telomeres and relies on its TMR for this process, and we provide the first genetic system to manipulate this ubiquitous human herpesvirus.

5-3 Prevalence and effect of inherited chromosomally integrated human herpesvirus 6

Oral

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[¶]Drs. Petrovski, Dhindsa, and Lareau jointly supervised this work

Human herpesvirus 6 (HHV-6) is the only human infectious virus known to transmit through the germline. Despite the unique quality, the study of inherited chromosomally integrated HHV-6 (ici-HHV-6) and its impact on complex disease has been limited to anecdotal observations. Here, we utilized large-scale whole-genome sequencing data from the UK Biobank (UKB, n = 486,866) and the All of Us (AoU, n = 240,319) to assess the prevalence of ici-HHV-6 systematically and infer its associations with phenotypes in electronic health records (EHRs). Viral read counts, normalized to the human genome, were used to infer the prevalence of ici-HHV-6, which we estimate to be 1.35% in these cohorts. Additionally, we identified two individuals carrying up to six copies of the virus per cell, representing the highest germline viral load reported so far. Next, phenome-wide association studies (PheWAS) were conducted to explore potential associations with various phenotypic traits. PheWAS results showed a strong link between ici-HHV-6 and the most common type of skin cancer, basal cell carcinoma (BCC). Notably, compared to other heritable risk alleles for BCC in the UKB, ici-HHV-6B exhibited the second strongest effect size. Although previous studies have noted the presence of HHV-6 in BCC cases, our findings suggest that inherited viral integration, rather than infection acquired during life, increases susceptibility to BCC. We have established a screening platform to assess ici-HHV-6 functional genomics, and we will validate preliminary findings by overexpressing specific GWAS variants.

5-4 Establishment of tissue culture model with patient-derived iPS cells to study the pathogenic role of iciHHV-6

Oral

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Background: Inherited chromosomally integrated HHV-6 (iciHHV-6) is seen in 0.6% and 1.5% of populations in Japan and Western countries, respectively. The full length HHV-6 genome is integrated in the telomere in every nucleated cell. IciHHV-6 is proposed to associate with several diseases. However, due to the lack of suitable in vitro tissue culture model, it remains difficult to study molecular events of iciHHV-6 reactivation from the integrated genome and association with pathogenicity via viral reactivation.

Methods: Here we established a patient derived induced pluripotent stem cells (iPSCs) model to study iciHHV -6 reactivation. Because HHV-6A/ B are associated with neuropathogenicity, we focused on neural cell differentiation and examined effects on iciHHV-6 reactivation. First, lymphoblastoid B-cell lines (LCLs) were generated from PBMC from iciHHV-6 patients by EBV infection, and characterized location of HHV-6 integration by DNA-FISH. LCLs were then used to generate iPSCs by transduction of Yamanaka factors, and followed by differentiation to neural stem cells (NSCs).

Result: EBV episomes were lost during transition from LCL to iPSCs, while integrated HHV-6 genome copies were maintained in iPSCs and NSCs as expected. Stem cell markers and neural cell markers expression were confirmed by immunoblotting and RT-qPCR. HHV-6 immediate early and late gene expressions were detected in differentiated NSCs by RT-qPCR.

Conclusion: Our results demonstrated that HHV-6 gene expressions were induced spontaneously, when we triggered cell differentiation into NSCs. The transcription from integrated HHV-6 was induced by HDAC inhibitor and etoposide. Further transcription studies at single cell resolution may identify the key event for triggering iciHHV-6 reactivation.

iciHHV6/7 - II

6-1 Herpesvirus integration: Insights into the mechanism, latency and endogenization

Oral

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The establishment of latency allows herpesviruses to persist in the host for life. The dogma has been that all herpesviruses maintain their genome as a circular episome. However, several herpesviruses have been shown to integrate their genome into the telomeres of latently infected cells in recent years. Among these are human herpesvirus 6A (HHV-6A) and 6B (HHV-6B) that maintain their integrated virus genome in the absence of episomal DNA. Integration of HHV-6 also occurs in germ cells, resulting in individuals that harbor the integrated virus genome in every single cell of their body and transmit it to their offspring. This condition has been termed inherited chromosomally integrated HHV-6 (iciHHV-6). About 1% of the human population have this condition, while the biological and medical consequences for these individuals remain poorly understood. This presentation will highlight the recent advances in our understanding of the integration mechanism, epigenetic changes, and the evolutionary history of endogenous iciHHV-6.

6-2 Exploring iciHHV-6 disease associations in the Generation Scotland: Scottish Family Health Study using linked electronic health record data

Oral

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Objective: The clinical consequences of iciHHV-6 in the healthy population are poorly understood. The prevalence of iciHHV-6 in the Generation Scotland: Scottish Family Health Study(GS:SFHS) of 23,637 individuals was unexpectedly high at 2.74 % with an iciHHV-6B prevalence of 2.55 %. Detailed characterisation of iciHHV-6 and the ancestral lineages found in Generation Scotland has been carried out by Wood et al. (unpublished). We aimed to explore comprehensive linked electronic health record data to identify clinical associations with iciHHV-6 in this population. The primary objectives were to further analyse the previously reported association between iciHHV-6B and angina in this study and to further test a reported association with pre-eclampsia.

Methods: Linked electronic health records for hospital, maternity and psychiatry admissions as well as prescribing and death certificate data for individuals recruited to GS:SFHS were accessed dating from 1981 to 2024. A power simulation identified the prevalence above which a condition should occur in the population for the study to have the power to identify an OR of 1.3 or more. Subsequently, all conditions with a prevalence of greater than 0.2 % were assessed for association. Generalised linear mixed models were used to analyse associations between iciHHV-6 status and binary disease outcomes. Known risk factors were included as fixed effects and family structure as a random effect. A Holm Bonferroni correction was performed for multiple testing.

Results: Hospital admission data existed for 122,094 episodes from 18,202 participants between 1981 and 2021. Over 5 million prescriptions were recorded in this cohort between 1989 – 2020; 2,793,323 prescriptions were drugs prescribed more than 20 times. A significant association between iciHHV-6 status and angina (ORx [x-x])was confirmed. This was statistically significant only for iciHHV-6B. Analysis of other disease associations including the associations with pre-eclampsia is in progress and will be presented.

Conclusions: This is the largest study of electronic health record data in a population in which iciHHV-6 has been comprehensively characterised. We confirmed an association between iciHHV-6 and angina. Despite the higher prevalence of iciHHV-6 found in Scotland compared to other countries, larger studies are needed to analyse associations with rarer diseases or detect smaller effect sizes.

6-3 Epidemiology and clinical disease associations of iciHHV-6 in the UK

Oral

Michael Wood, Robin Young, Annette Lake, Matthew Kennedy, Charu Sharma, Kostas Papageorgiou, Natasha Jesudason, Sreenu Vattipally, John Cleland, Caroline Hayward, Ruth F Jarrett

University of Glasgow Centre for Virus Research, Glasgow, UK

Objective: Our aim is to understand the clinical impact of inherited chromosomally integrated human herpesvirus 6A and 6B (iciHHV-6A and 6B, collectively iciHHV-6) in the general population. A better understanding of iciHHV-6 is required to improve management of iciHHV-6-associated disease, to provide iciHHV-6-positive individuals with robust information about their condition, and to inform decisions about diagnostic testing for iciHHV6. To perform rigorous iciHHV-6 disease association analyses, a more detailed understanding of the epidemiology of iciHHV-6A and 6B, and their ancestral viral lineages, was required.

Methods: Our approach was to analyse several large cohort studies for iciHHV-6. Here, we focus on the UK Biobank (UKB), a cohort of almost half a million people resident in the UK. We screened nearly 490,000 UKB participants for iciHHV-6 using laboratory assays (417,000 participants), whole genome sequence data (70,000 participants), or a combination of both (30,000 participants), and determined viral species, genome composition and ancestral viral lineage. Statistical analysis was performed to identify associations with baseline questionnaire data and laboratory measurements; hospital in-patient records; and maternity, cancer, and death registry data. Based on data from the CARTaGENE study (Gravel et al., 2015) and our data from the Generation Scotland: Scottish Family Health Study, we tested the hypothesis that iciHHV-6 is associated with angina, but otherwise this was an exploratory study.

Results: The study prevalence of iciHHV-6A and iciHHV-6B were 0.3% and 1.1%, respectively, with significant regional variation within the UK. Consistent with our previous studies, iciHHV-6B prevalence was highest in Ireland and Scotland at around 3% whereas iciHHV-6A prevalence was highest in Wales and southern England. We also observed significant differences in the distribution of the common ancestral viral lineages. These results present significant challenges for disease association studies, which must take these differences into consideration.

We did not replicate the association with angina; ongoing analyses aim to understand the reasons for the difference between the UKB results and those from previous studies. In the exploratory analyses, some highly significant associations with iciHHV-6 were observed; however, the most significant were accounted for by place of birth or were not clinically relevant. Results will be described but no clear associations emerged.

Analysis of HHV-6 serology results from almost 9,000 UKB participants with available data showed a highly significant association between iciHHV-6B-positivity and both HHV-6 seropositivity and IE1B antibody levels, suggesting more viral antigen expression in iciHHV6B-positive individuals compared to those with exogenous infection.

Conclusions:

• Results from analysis of UKB to date do not show any clear iciHHV-6 disease associations suggesting that iciHHV-6-positivity is not catastrophic, at least in immunocompetent individuals. Most iciHHV-6 in the UK is accounted for by a relatively small number of ancient viral lineages and we cannot exclude the possibility that recently endogenised viruses are more likely to cause disease.

• In keeping with previous data, iciHHV-6B-positive individuals had higher antibody levels to HHV-6B antigens than controls, consistent with the idea that the integrated virus is not silent.

• Future disease association analyses must include stringent case–control matching or adjust for place of birth, or some equivalent measure, to avoid reporting of spurious disease associations.

6-4 Over 50 different lineages of iciHHV-6 are present in the UK Biobank and these lineages display significant variation in geographical distribution within and outside the UK

Oral

Michael Wood[1], Annette Lake[1], Charu Sharma[2], Kostas Papageorgiou[1], Natasha Jesudason[1],Jenna Nichols[1], Sreenu Vattipally[1], Matthew Kennedy[1], Caroline Hayward[2], Ruth F. Jarrett[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, [2]MRC Human Genetics Unit, IGC, University of Edinburgh, Edinburgh, UK

Objectives: The number of iciHHV-6 lineages, their relative abundance and origins across the globe are unknown. Here, we aimed to use the UK Biobank cohort to explore these aspects of the natural history of iciHHV-6 within and outside the UK.

Methods: In the largest study of its kind to date, we analysed nearly 490,000 individuals in the UK Biobank (UKB) using laboratory-based assays (417,000 individuals), whole genome sequencing (70,000 individuals), or a combination of both (30,000 individuals). The prevalence of iciHHV-6A and iciHHV-6B was 0.3% and 1.1%, respectively, with significant regional variation. Precise quantification with droplet digital PCR revealed that an unexpectedly high proportion (13%) of iciHHV-6 genomes had a non-canonical structure.

To address limitations in identifying ancestral lineages and integration sites, we characterised the telomere and telomere-like repeats at the junction between the human and HHV-6 genomes. Here, we have increased the number of lineages with a characterised junction from less than 10 to over 50, including more than 30 previously undescribed lineages. A combination of iciHHV-6 phylogenetics and a novel analysis of telomeric SNPs enabled us to predict the integration site of the viral genome in dozens of lineages, including lineages with as few as three iciHHV-6-positive individuals.

Results: The prevalence of each individual lineage of iciHHV-6 was independent and varied considerably. Many lineages contained only one or two individuals, while a small number comprised hundreds; the most common lineage was found in approximately 0.3% of UKB participants. Notably, some lineages exhibited pronounced geographical clustering, with specific lineages confined to small regions within the UK or linked to the countries of origin from the 40,000 UKB participants born outside the UK.

Conclusions: These data suggest that HHV-6 integrations are a global phenomenon and that the variation in prevalence is shaped by where the integration events occur geographically, as well as population dynamics such as migration and population bottlenecks. The prevalence and diversity of iciHHV-6 lineages indicate that heritable germline integrations are more common than previously appreciated and likely to be ongoing in the present day, albeit at an unknown frequency and with unknown consequences.

6-5 Investigation of iciHHV-6 integration sites and its integration mechanism at the DNA level

Oral

Joilson Xavier[1], Christiana Victoria Cismaru[1], Dilan Gün Serdar[1], Jana Reich[1], Yingnan Cheng[1], Benedikt B. Kaufer[1,2]

[1]Institute of Virology, Free University Berlin, Berlin, Germany, [2]Veterinary Centre for Resistance Research, Free University Berlin, Berlin, Germany

Introduction: About 1% of the human population harbors the inherited chromosomally integrated human herpesvirus 6 (iciHHV-6) in every cell of their body. However, the integration process of HHV-6 into the host telomeres remains poorly understood.

Methods: To assess differences in the integration sites, the HHV-6 integration mechanisms and reactivation at the DNA level, we developed a CRISPR/Cas9-targeted nanopore sequencing approach. We employed a set of four crRNAs designed to selectively cut both ends of the virus genome in proximity to its direct repeat (DR) sequences. Sequencing of a known iciHHV-6A isolate (KY315540) generated 140 reads mapping to viral DRs, with the longest read being 77.9kb long. DR-right-containing reads harbored ~2kb telomeric repeats adjacent to the virus genome, followed by human subtelomere sequences matching chromosome 18q. The presence of the virus genome in this chromosome arm was confirmed by FISH. Next, we employed the same targeted sequencing approach to investigate the role of the telomere arrays (TMR) at the ends of the HHV-6 genome in the integration process. We assessed U2OS cell clones infected with TMR mutant viruses that lack either the imperfect TMR (ΔimpTMR), the perfect TMR (ΔpTMR) or both TMR (ΔTMR).

Results: Sequencing of a ΔTMR U2OS clone generated up to 34kb long reads that mapped to the DRs, which were followed by sequences matching within chromosome 2p15, highlighting that the virus cannot integrate into host telomeres in the absence of its TMR. Both ΔpTMR and ΔimpTMR mutants generated DR-right reads containing chromosomal DNA, which mapped either outside or close to human telomeres for ΔpTMR and ΔimpTMR mutants, respectively.

Conclusion: Taken together, these results demonstrate on a sequence level that the HHV-6 TMR are essential

for proper virus integration into the human telomeres, highlight the utility of Cas9-targeted sequencing for generating long reads of virus-host junctions, and underscore the need for additional sequencing data to further characterize the HHV-6 integration process.

6-6 Oral Investigating the impact of superinfections on the reactivation of inherited chromosomally integrated HHV-6

Isabelle Dubuc[1], Annie Gravel[1], Michael Wood[2], Nicola Royle[2], Louis Flamand[1,3]

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Background: Approximately 96% of the world population have been exposed to HHV-6A/B during infancy. After primary infection, HHV-6A/B establishes latency and maintains its genome either as an episome (yet to be formally demonstrated) or as integrated virus. In addition, nearly 1% of the population possesses what is known as inherited chromosomally integrated human herpesvirus 6 (iciHHV-6), where a copy of the viral genome is integrated to a chromosome into every somatic cell. Recent studies have revealed associations between iciHHV-6 and various diseases, including angina pectoris, pre-eclampsia and increased spontaneous abortion rates. How iciHHV-6 contributes to these diseases remains elusive. Understanding the factors and mechanisms that trigger viral gene expression and/or reactivation may help explain how iciHHV-6 influences the development of certain diseases.

Methods: To study this, we screened by PCR more than 500 babies and have identified 6 iciHHV-6A+ babies. Endothelial (HUVEC) and smooth muscle cells (SMC) from umbilical cords were obtained. Considering that HHV-6 is found integrated at the telomeres, we first characterized the telomere lengths and the proliferative capacity of endothelial cells from iciHHV-6+ and control subjects. We then used HUVEC and SMC to investigate the effect of herpesvirus superinfection on the reactivation of HHV-6. Cells were infected with human cytomegalovirus and human herpes simplex virus type 1 for different time periods after which cells were processed for RNA extraction and RT-qPCR to assess the expression of viral genes, including U90, U54, and U100. Immunofluorescence was used to detect viral protein expression.

Results: Control and iciHHV-6 cells had similar overall telomere lengths. The telomere lengths of the chromosomes harboring integrated HHV-6 were also similar. In terms of population doublings, iciHHV-6+ demonstrated a significant reduction in proliferative capacity relative to control cells ($p=0.003$). Upon superinfection, most of the HHV-6 genes tested were upregulated following viral infection, and some of the corresponding proteins were detected by immunofluorescence.

Conclusion: These findings highlight major impact of HHV-6 integration on the long-term proliferative potential of endothelial cells, independent of telomere lengths. Furthermore, stimuli such as viral superinfections trigger the expression of HHV-6 genes from the integrated state but fail to cause HHV-6 reactivation. The quest of factors leading to HHV-6 reactivation from integration remains a subject of active studies.

iciHHV-6/7 - III

7-1 Pathogenicity of iciHHV-6: Does It Cause Disease in Humans?

Oral

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Human herpesvirus 6 (HHV-6) exhibits a rare phenomenon of viral genome integration into the host chromosome (inherited chromosomally integrated HHV-6, iciHHV-6), which is extremely uncommon outside of retroviruses. Since HHV-6B was established as the causative agent of exanthem subitum in 1988, the pathogenesis of primary infection in infants and reactivation in transplant patients has become increasingly understood. Meanwhile, researchers from Italy and Japan reported in the late 1990s that HHV-6 genomes were integrated into malignant lymphoma tissues, thereby revealing that this virus integrates into the host genome.

In the Japanese population, iciHHV-6 is observed at a frequency of approximately 0.6–1%, and cases with HHV -6A genome integration into chromosome 22 have been demonstrated to result from a founder effect. Large-scale genomic cohort studies abroad have reported an increased risk of angina pectoris and preeclampsia associated with iciHHV-6. Furthermore, our study has shown a significantly higher risk of miscarriage in pregnant women with iciHHV-6.

This presentation will review clinical virological analysis aimed at elucidating the pathogenic significance of iciHHV-6, with a particular focus on our recent work utilizing patient-derived iPS cells as a platform for investigating its pathogenic mechanisms.

7-2 Inherited chromosomally-integrated or endogenous human herpesvirus 6 as a disease risk factor

Oral

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Introduction: Unlike most herpesviruses which replicate without integrating into the host chromosome, human herpesvirus 6 (HHV-6) can integrate into telomeres. Remarkably, such integrations have occurred into human germline chromosomes at various times during our evolutionary history. As a result, about 1% of humans inherit a full copy of the HHV-6 genome and carry this sequence within every cell of their body. This phenomenon has been termed inherited chromosomally-integrated (iciHHV-6) or endogenous HHV-6 (eHHV -6). While infection by HHV-6 can manifest in various diseases, including encephalitis and skin eruptions, the impact of eHHV-6 on human health has not been fully clarified.

Methods: My colleagues and I have screened for eHHV-6 in various disease cohorts and biobanks, including the “All of Us” program and UK Biobank, and performed a phenome-wide association study.

Results: Through this work, we identified associations between eHHV-6B and diseases including systemic lupus erythematosus (SLE) and motor neuron disease. SLE disease activity in patients with eHHV-6B is substantially higher than in those without eHHV-6B. Moreover, we have observed immunological differences in subjects with eHHV-6B, including increased antibody titers against at least one exogenous HHV-6 protein, and have mapped a specific linear epitope to which subjects with SLE and eHHV-6B characteristically target. Work is ongoing to more specifically define the disease characteristics (e.g. organ involvement) of patients with eHHV -6 and lupus and pinpoint the neurological features of patients with eHHV-6B and motor neuron disease; updates in these areas are anticipated to be shared at the meeting.

Conclusion: These results reveal that a human endogenous virus with the potential for reactivation is associated with potentially self-reactive immune responses as well as autoimmune and neurological disease risk. Further work is needed to define the mechanistic basis of the risk mediated by eHHV-6 and translate these findings to personalized medicine.

7-3 Role of immune response against endogenous human herpesvirus 6 in autoimmunity

Oral

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Objective: Sequences of human herpesvirus 6 (HHV-6), known as inherited chromosomally-integrated HHV-6 (iciHHV-6) or endogenous HHV-6 (eHHV-6), are present in the genomes of approximately 0.5% of the population. While associations between eHHV-6 and some human diseases have been reported, its potential role in autoimmune diseases remains largely unexplored. In this study, we aimed to investigate the association between eHHV-6 and autoimmune diseases by analyzing biobank data and characterizing the immune response to eHHV-6 using epitope-specific antibody profiling.

Methods: We first analyzed biobank data from hundreds of thousands of individuals to explore associations between eHHV-6 and autoimmune diseases. Following the identification of a statistically significant association between eHHV-6 and systemic lupus erythematosus (SLE), we further investigated the potential contribution of eHHV-6 to immunity in SLE patients. Using phage-display immunoprecipitation sequencing (PhIP- seq), a method combining phage display and sequencing analysis, we profiled anti-HHV-6 antibodies in serum or plasma samples from SLE patients.

Results: Our biobank analysis revealed a statistically significant association between eHHV-6 and SLE, with a higher prevalence of eHHV-6 in SLE patients compared to healthy individuals. Furthermore, consistent with previous findings, eHHV-6 carriers exhibited tenfold higher anti-HHV-6B antibody titers than non-carriers. Critically, PhIP-seq analysis demonstrated that SLE patients with eHHV-6B exhibit a distinct immune response, characterized by antibodies targeting specific HHV-6B epitopes.

Conclusions: Our findings strongly suggest an association between eHHV-6B and SLE, with the immune response to eHHV-6B potentially playing a role in the disease's development or progression. This is the first study to identify a link between eHHV-6B and SLE, opening new paths for therapeutic strategies targeting eHHV-6. Further studies are necessary to dissect the molecular mechanisms underlying this association and to explore the therapeutic potential of targeting eHHV-6B in SLE.

7-4 Characterising inherited chromosomally integrated human herpesvirus 6 in four isolated European populations

Poster

Natasha Jesudason[1], Michael Wood, Lee Murphy, Caroline Hayward, Shona Kerr, Jim Wilson, Charu Sharma, Anne Richmond, Nicola Royle, Daniel Schwartz, Asa Johansson, Ruth Jarrett

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Objective: It has been estimated that inherited chromosomally integrated human herpesvirus 6 (iciHHV-6) affects around 1% of individuals worldwide; however, our recent data show that prevalence varies significantly between populations even over small distances. Furthermore, the ancestral lineages of iciHHV-6 contributing to the overall prevalence of iciHHV-6 differ regionally. The aim of the present study was to identify novel lineages of iciHHV-6, by analysing isolated populations, which offer a unique opportunity to identify rarer iciHHV-6 lineages due to founder effects. We determined the prevalence of iciHHV-6 in four isolated European populations and characterised the ancestral viral lineages using a panel of laboratory tests and data-mining methods.

Methods: We accessed DNA, host genome-wide genotyping and whole genome sequencing (WGS) data from participants in the VIKING study, Shetland (n=2053), ORCADES study, Orkney (n=2100) and the 10001 Dalmatians study, Croatia (n=4851). Whole genome sequencing data was used to screen the Northern Swedish Population Health Study (n=1047). PCR based assays were used to determine iciHHV-6 status, and viral species, composition and clade. Integration site PCR assays were used to amplify and size host-junction fragments and Sanger and Nanopore sequencing were carried out to confirm and characterise viral lineages. A genome-wide association of directly genotyped and imputed SNPs was performed for three populations to identify telomeric variants and haplotypes significantly associated with iciHHV-6. Whole genome sequence data were mined for iciHHV-6 with phylogenetic analysis of derived sequences.

Results: The prevalence of iciHHV-6B ranged from 0.09% in Croatia to 1.95% in Orkney, while iciHHV-6A was absent in Northern Europe, but found to be the prevalent species in Croatia (0.29%). GWAS analysis identified highly associated telomeric SNPs in the most frequently observed chromosomes of integration: 9q (Shetland), 17p (Croatia) and 1q (Orkney). 0.74 % of Orcadians have a 1q integrated lineage of iciHHV-6B, confirmed by the presence of a shared 1q haplotype, phylogenetic clade, and host-junction fragment. The viral sequences identified in Northern Sweden formed a new HHV-6B clade with a, so far unknown integration site.

Conclusions: The prevalence of iciHHV-6 and distribution of ancestral viral lineages varies significantly in different European populations. Data-mining accurately predicts prevalence and ancestral lineage and together with analysis of conserved host haplotypes could be used in the future to study other isolated populations to increase understanding of HHV-6 germline integration. In this study we identified two new iciHHV-6B lineages: B14_1q was unique to Orkney and Bx unique to Northern Sweden.

7-5 Deciphering early transcriptional landscape of HHV-7 infection and reactivation

Poster

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Human herpesvirus 7 (HHV-7) belongs to the betaherpesvirus family and is one of the least studied members of the Herpesviridae family. HHV-7 reactivation has been strongly associated with post-viral chronic illnesses like ME/CFS. In the recent past, we, for the first time, showed that HHV-7 integrated into human telomere like HHV-6. However, unlike iciHHV-6, the number of genetically inherited and chromosomally integrated HHV-7 cases is rare. We have created in vitro cell culture models carrying latent and chromosomally integrated HHV-7, which can be reactivated using chemical stimuli. Our abstract will utilize this model and lytic infection models of HHV-7 to decipher the transcriptional landscape of HHV-7 infection and reactivation. We hope to show key differences between HHV-6 and HHV-7 life cycles that can help us understand HHV-7 pathology.

7-6 A Case of Multiple Sclerosis in a Woman with Chromosomally Integrated Human Herpesvirus 6

Poster

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Introduction: ciHHV-6 occurs when the entire HHV-6 genome integrates into the host germline, passing to offspring in a Mendelian manner. Little is known whether individuals with ciHHV-6 develop immune tolerance to viral proteins or face risks of viral reactivation under immunosuppression, necessitating caution when using immunosuppressive therapies.

Case: A 48-year-old female presented with incomplete left-sided hemiplegia, paresthesia affecting the left upper extremity, and headache without fever or meningism. Contrast-enhanced magnetic resonance imaging revealed a periventricular oval lesion in the genu of the left internal capsule with a central venous sign, multiple T2-weighted hyperintense lesions in the deep white matter and subcortical regions, and spinal cord lesions at the C6-7 and Th10-12 levels. Cerebrospinal fluid（CSF） analysis showed oligoclonal bands, mononuclear pleocytosis, elevated protein, and HHV-6 DNA (2.3×10^4 copies/mL).

Autoimmune conditions such as multiple sclerosis (MS) and neuromyelitis optica spectrum disorder were initially suspected. However, serologic tests for anti-aquaporin 4 and anti-myelin oligodendrocyte glycoprotein antibodies were negative. The patient was treated with intravenous methylprednisolone and intravenous immunoglobulin was added after she developed retrobulbar optic neuritis in the right eye. Her clinical presentation remained consistent with MS, though her CSF findings were atypical. HHV-6 meningitis or meningoencephalitis was suspected as a potential trigger for her MS-like symptoms, and Foscarnet was administered for 19 days, normalizing CSF cell counts. The patient was discharged on day 23 with residual left leg weakness and reduced visual acuity in the right eye, both gradually improving. Repeated HHV-6 DNA

testing on day 18 showed a persistently elevated viral load (4.4×10^3 copies/mL), suggesting ciHHV-6. High HHV-6 DNA levels in blood cells, plasma, and hair bulbs confirmed the diagnosis via real-time PCR.

Conclusion: HHV-6 comprises two species: HHV-6A, tentatively linked to MS, and HHV-6B, the cause of exanthema subitum. Thus, patient's HHV-6B positivity leaves its association with MS unclear, however distinguishing active HHV-6 infection from ciHHV-6 is critical when considering immunosuppressive disease-modifying therapies for MS. Real-time PCR to quantify HHV-6 DNA in body cells is a key diagnostic method, particularly in asymptomatic patients with HHV-6 detected in their bodily fluid.

7-7 Avoiding unnecessary treatment in a case of chromosomally integrated HHV-6 initially suspected to be HHV-6 myelitis

Poster

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Objective: Human herpes virus 6 (HHV-6) causes exanthema subitum in infants, and its reactivation in adults is typically associated with encephalitis. With the development of the DNA examination, the number of positive HHV-6 DNA cases has been increasing. Here, we report a case initially suspected to be HHV-6 myelitis due to the detection of HHV-6 DNA in the spinal fluid, which was later confirmed to be chromosomally integrated HHV-6 (ciHHV-6).

Case: A 33-year-old male patient presented to our hospital with a rapidly progressing gait disturbance over several days to weeks, muscle weakness, increased deep tendon reflexes, and decreased vibratory sensation in both lower extremities; therefore, we assumed that the culprit lesion was in the spinal cord. Magnetic resonance imaging of the spinal cord did not reveal any lesions, but cerebrospinal fluid examination revealed an elevated cell count and HHV-6 positivity on the BioFire FilmArray® meningitis/encephalitis panel (ME panel). Foscavir treatment was initiated for suspected HHV-6 myelitis, although it is rare in immunocompetent adults. Despite treatment, the symptoms worsened, and the number of HHV-6 DNA copies in the cerebrospinal fluid remained unchanged. Consequently, foscavir was replaced with ganciclovir, and methylprednisolone was added to address possible autoimmune myelitis. Concurrently, the possibility of ciHHV-6 was considered, because the HHV-6 DNA copy count in whole blood was almost identical to the white blood cell count. As a result, the antiviral drug was discontinued. Furthermore, an HHV-6 DNA assay system was established at our hospital, and HHV-6 DNA was detected in somatic cells obtained from pharyngeal swab specimens. In addition, HHV-6 DNA was detected in the pharyngeal swab specimens of the patient's father; therefore, a diagnosis of inherited ciHHV-6 was confirmed. Although the cause of myelitis remained unknown, the patient's symptoms did not deteriorate, and rehabilitation was planned for amelioration of symptoms.

Conclusions: In this case, the interpretation of quantitative whole blood HHV-6 DNA levels, combined with the detection of HHV-6 DNA in somatic cells other than the patient's blood cells, enabled a definitive diagnosis of ciHHV-6, thereby minimizing unnecessary antiviral treatment. With the widespread use of the ME panel and the increasing frequency of HHV-6 DNA detection in recent years, it is important to consider the possibility of ciHHV-6 early, especially in immunocompetent adults, to accurately assess the need for treatment.

HOST CELL INTERACTION - II

8-1 APOBEC3 generates a diversity of human T lymphotropic herpesviruses 6A and B

Oral

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Each of the nine human herpesviruses causes diverse pathologies, but the molecular mechanisms behind this diversity are poorly understood. Despite being T lymphotropic β-herpesviruses and sharing >90% genome similarity, Human herpesviruses 6A (HHV-6A) and 6B (HHV-6B) exhibit different pathogenetic effects. HHV -6B infection leads to exanthema subitum and encephalitis, while HHV-6A infection is considered asymptomatic. The underlying mechanisms driving these distinct outcomes in two closely related viruses are unclear.

Apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide-like (APOBEC) 3 enzymes cause mutations in the viral genome unless counteracted by viral defense proteins. We showed that APOBEC3s prevent the replication of HHV-6A, whereas HHV-6B can evade restriction. U28 was expressed more predominantly by HHV-6B than by HHV-6A and triggered the redistribution and degradation of APOBEC3 proteins. APOBEC3-mediated mutations were observed in HHV-6B DNA collected from patients, although the integrity of the viral genome was maintained in a proportion of the viruses for over 20 days. Intriguingly, the mutations were predominantly accumulated in the DNA collected from a patient with HHV-6A reactivation. The diversity observed in the HHV-6A genome in this patient was far greater than that of HHV-6B.

The interplay between APOBEC3 proteins and viruses in humans remains poorly understood, including whether this antiviral system restricts ubiquitous viruses. Our findings indicate that viral susceptibility to APOBEC3s could influence viral pathogenicity and the diversity of viruses.

8-2 Identification of stimuli that enhance human herpesvirus 6A (HHV -6A) replication and reconstitution

Oral

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Introduction: Bacterial artificial chromosome (BAC)-based systems have revolutionized herpesvirus research by facilitating precise genome manipulation and subsequent virus reconstitution. However, their application in HHV-6A biology has faced several technical challenges. These challenges include a low transfection efficiency in T cells, slow virus replication, and a strong preference of the virus for establishing latency over lytic replication. In our study, we developed approaches to improve the reconstitution process and enhance virus replication to overcome these technical challenges.

Methods: We systematically evaluated several strategies to enhance BAC transfections, including dimethyl sulfoxide (DMSO) and exonuclease V (ExoV) treatments. In addition, we tested a wide range of stimuli, including hydrocortisone (HC), mitogens (12-O-tetradecanoylphorbol-13-acetate [TPA] and phytohemagglutinin [PHA]), and inhibitors such as Janus kinase 1/2 (JAK1/2) inhibitor ruxolitinib (RUX) and hypoxia-inducible factor 1 alpha (HIF-1α) prolyl hydroxylase-2 inhibitor (IOX2), to further enhance viral reconstitution and replication.

Results: Our results revealed that treatment with DMSO and ExoV drastically improves BAC transfection efficiency, allowing a successful HHV-6A reconstitution in JJHan T cells. Furthermore, HC treatment significantly enhanced both reconstitution and replication of HHV-6A. Furthermore, we observed that host interferon (IFN) responses inhibit viral replication. Treatment with RUX effectively countered this antiviral response, resulting in higher viral yields. Finally, stabilization of HIF-1α using IOX2 significantly accelerated

virus reconstitution, emphasizing the important role of hypoxia signaling in HHV-6A replication.

Concusion: In conclusion, our study identified key stimuli and host pathways – including IFN-mediated and hypoxic responses – that influence virus replication. These findings drastically improved virus replication and reconstitution, and finally make the HHV-6A BAC-based system accessible to all researchers to study this ubiquitous human herpesvirus.

8-3 A host circular RNA circRELL1 is regulated by infection with human beta- and gamma-herpesviruses

Oral

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Objective: Circular RNAs (circRNAs) are a novel class of gene regulatory RNAs involved in virus-host interactions. Host circRNAs are predominantly non-protein-coding despite being produced by the splicing of premature messenger RNAs. circRNAs interact with other RNAs and proteins to regulate their functions. We have previously identified specific circular RNAs induced by all human gamma-herpesviruses, Epstein-Barr virus (EBV) and Kaposi sarcoma herpesvirus (KSHV). One of the infection-induced circRNAs, circRELL1, suppresses KSHV lytic infection and promotes the growth of latently infected cells. Although the mechanism by which both EBV and KSHV induce circRELL1 remains unclear, we found that circRELL1 expression can be induced by interferon responses, a universal host reaction to pathogens. Thus, we aimed to determine whether circRELL1 is also regulated by infection with evolutionarily close beta-herpesviruses, which may suggest that circRELL1 has pan-herpesviral functions.

Methods: Human cytomegalovirus (HCMV), herpesvirus 6A (HHV-6A), and herpesvirus 7 (HHV-7) were selected for this study. For HCMV, publicly available Ribo(-) RNA-sequencing datasets of the fibroblast cell line MRC5 were used to compare the circRELL1 transcript levels with and without infection. For HHV-6A and HHV -7, T cell lines HSB2 and SupT1, respectively, were used for primary infection by co-culturing infected and uninfected cells. circRELL1 transcript levels were determined using RT-qPCR. RNAi-mediated circRELL1 was performed in infected cells to measure its effect on viral transcript and episome levels.

Results: circRELL1 was upregulated by infection with HCMV at 3- and 5-days post infection (dpi), according to circRNA sequencing data analysis using the Circrnas in Host And viRuses anaLysis pIpEline (CHARLIE) pipeline. Similarly, co-cultures of HHV-7 infected and uninfected SupT1 cells led to the induction of circRELL1 in an increasing manner from 2 to 8 dpi. In contrast, HHV-6A infection of HSB2 cells suppressed circRELL1 expression. circRELL1 depletion in HHV6A-infected HSB2 caused no obvious changes in viral transcript levels, but viral genome copy showed a mild increase, suggesting an anti-lytic function of circRELL1.

Conclusions: In addition to EBV and KSHV, infection with beta-herpesviruses CMV, HHV-6A, and HHV-7 regulated the host circRNA circRELL1. Only HHV-6A infection reduced the circRNA levels, suggesting a regulatory mechanism different from that of other human herpesviruses. circRELL1 may be relevant to a wide range of herpesviruses.

8-4 Human herpesvirus 6B ribonucleotide reductase sequester NF-κB/p65 in the cytoplasm as an immune evasion mechanism

Oral

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Background: Human herpesvirus 6B (HHV-6B) belongs to the genus Roseolovirus of the betaherpesvirus subfamily, causing exanthema subitum and encephalitis Although viral ribonucleotide reductase (RNR) is conserved in betaherpesviruses, it has lost its enzymatic activity. Human cytomegalovirus (HCMV) belongs to the other betaherpesvirus genus, Cytomegalovirus; its RNR inhibits NF-κB signaling via interaction with the adaptor molecule RIP1. However, the significance of enzymatically inactive RNR in roseoviruses is unclear.

Results: Here we show that the RNRs from all three human roseoloviruses inhibit NF-κB activation. HHV-6B

RNR sequesters NF-κB subunit p65 in the cytoplasm and inhibits its translocation into the nucleus. Silencing HHV-6B RNR increased the expression of inflammatory molecules in infected cells.

Conclusion: This study reveals that inhibition of NF-κB is a conserved role of the RNR in betaherpesviruses, although this function has been acquired convergently.

8-5 *Oral* The HHV-6B U20 glycoprotein selectively binds to host ULBP family stress ligands, masking them from recognition by NKG2D and interfering with natural killer cell activation.

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Objective: HHV-6B impedes host responses by downregulating MHC-I molecules, hindering antigen presentation to CD8 T cells. Downregulation of MHC-I disengages inhibitory receptors on natural killer (NK) cells, resulting in activation and killing of the target cell if NK cell activating receptors such as NKG2D have engaged stress ligands upregulated by infection. Previous work has shown that HHV-6B downregulates three of the MHC-like stress ligands MICB, ULBP1, and ULBP3 recognized by NKG2D. The U20 glycoprotein of the related virus HHV-6A has been implicated in the downregulation of ULBP1, but the precise mechanism remains undetermined. We set out to investigate the role of HHV-6B U20 in modulating NK cell activity.

Methods: We used HHV-6B U20 expressed as a recombinant protein or transduced into target cells to investigate binding interactions with NK ligands and receptors and to assess effects on NK cell activation. We collected small-angle X-ray scattering data for the purified U20-ULBP1 complex, and used these to align molecular models derived from machine-learning approaches.

Results: We demonstrate that U20 binds directly to ULBP1 with sub-micromolar affinity. Transduction of U20 decreases NKG2D binding to ULBP1 at the cell surface but does not decrease ULBP1 protein levels, and blocks NK cell activation in response to cell-surface ULBP1. Soluble U20 has the same effect. U20 also binds tightly to ULBP5 and UBLP6 but none of the other five UBLP or MIC family NKG2D ligands.

Conclusions: These results suggest that U20 interferes with NK cell detection of HHV-6B-infected cells by a masking mechanism, binding to host ULBP-family proteins at the cell surface and preventing them from being recognized by NKG2D. Selective binding HHV-6 U20 to ULBP1, ULBP5 and UBLP6 matches the pattern of NK ligand expression on T cell subsets infected by HHV-6B, and evolutionary analysis indicated that host mutations blocking U20 binding to ULBP2, ULBP3 and UBLP4 have arisen twice in primate evolution, highlighting the complex evolutionary biology of the HHV-6 and NK cell arms race.

8-6 *Poster* HHV-6 dUTPase interacts with human cNOT1 to manipulate host transcription machinery and facilitate virus infection

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Herpesviruses are the only organisms whose genome encodes monomeric deoxyuridine triphosphatase (dUTPase) – an enzyme that catalyzes the hydrolysis of deoxyuridine triphosphate (dUTP), thus reducing the possibility of its integration into DNA and contributing to genome stability. Emerging studies show strong evidence of HHV-6 dUTPase's role in the physiology of herpesvirus latency, its involvement in latent virus reactivation, and its participation in various pathological conditions, such as decreased immune response, pain, fatigue, and others. The exact mechanism of this action remains unknown. Our findings show biochemical evidence for direct interactions between HHV-6 dUTPase (both HHV-6A and HHV-6B) and human CNOT1 protein – a subunit of the CCR4-NOT complex, which is responsible for the intracellular degradation of mRNA from the poly-A tail (3'-end), thus restricting the mRNA half-life. Our abstract will characterize this interaction and its potential role in the virus life cycle.

8-7 Single-Cell transcriptomic analysis of HHV-6 infection

Poster

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Objective: To elucidate the temporal regulation of viral and host gene expression during HHV-6A infection.

Methods: HHV-6A-infected cells were harvested at multiple time points post-infection and analyzed using single-cell transcriptomics by FastQC and STAR. Further mechanism analysis was performed for the new findings.

Results: 1. Distinct subpopulations of CD4+ T cells in cord blood mononuclear cells (CBMCs) with varying susceptibility to HHV-6A were identified, each exhibiting unique transcriptional signatures. 2. Different regions of HHV-6A genome exhibited significant transcriptional variability at various stages of infection, and novel HHV-6A transcripts were identified. 3. HHV-6A infection resulted in a notable downregulation of a set of immune-related molecules.

Conclusions: This study uncovers the regulatory dynamics of host and viral gene transcription following HHV -6A infection and identifies new viral transcripts, providing a foundation for further investigation into virus-host interaction mechanisms.

HHV-6 IN THE IMMUNOCOMPROMISED - I

9-1 American Society for Transplantation and Cellular Therapy Guideline: Management of HHV-6B After Hematopoietic Cell Transplantation and Chimeric Antigen Receptor (CAR)-T-Cell Therapy

Oral

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Background: The Practice Guidelines Committee and the Transplant Infectious Disease Special Interest Group of the American Society for Transplantation and Cellular Therapy have developed guidelines focusing on human herpes virus 6B (HHV-6B). A compendium-style approach was used to address a series of standalone frequently-asked questions (FAQs). Adult and pediatric infectious disease and HCT content experts developed these FAQs, and finalized recommendations after consensus was reached. The guideline focuses on the relevant risk factors, diagnostic considerations, prophylaxis, and treatment approaches relevant to HHV-6B infections after HCT.

Methods: The assembled expert opinion panel identified key challenges in the management of HHV-6B infection and best practice recommendations were formulated for relevant sections. Guidance is based on a literature review and discussion amongst the expert panel to reach consensus. Evidence-based grading was applied to guidelines regarding management.

Results/Discussion: Questions in the guideline include, but are not limited to:
- What is the incidence and clinical significance of HHV-6B reactivation after HCT?
- What is the incidence of HHV-6B reactivation after CAR-T-cell therapy?
- What diagnostic methods and specimens should be used to detect HHV-6B?
- When to suspect and how to diagnose chromosomally integrated HHV-6?
- What is the recommended approach to diagnose HHV-6B encephalitis?
- What is known about the role of HHV-6B in pneumonia after allogeneic HCT?
- When should therapy for HHV-6B be started and how long should it be administered?

9-2 Petabase-scale discovery of HHV-6 and HHV-7 genomes

Oral

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Humanity has collectively sequenced ~10 exabases of DNA sequencing data, including a vast repository of public sequencing data in the sequence read archieve (SRA). Here, we introduce methods for efficiency querying this resource at scale, which enables retrospective discovery of HHV-6 and HHV-7 (among other) viral sequences from every public dataset worldwide. Using this unbiased hypothesis generating resource, we provide evidence that HHV-6 reactivates in CD4+ T cells in vitro, which we extrapolate show HHV-6 reactivation in chimeric antigen receptor T cell therapy. Generalizing on this approach, we provide further (unpublished) evidence of HHV-6 reactivation occurring in human organoid models and clinical-grade tumor infiltrating leukocytes (TILs), broadening the importance of viral reactivation in ex vivo settings. Finally, using HHV-7 as an exemplar virus, we show how population-scale quantification of this virus can be correlated with genetic and phenotypic data to uncover novel associations with these endemic viruses. Our platform provides a general solution to study the human virome, a focus of our group for the decade to come.

9-3 Porcine Roseolovirus: Prevalence, Detection Methods, and Relevance for Xenotransplantation

Oral

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Porcine cytomegalovirus (PCMV), now reclassified as porcine roseolovirus (PRV), is a close homolog of human herpesvirus 6 (HHV-6) and HHV-7, sharing many biological properties. PCMV/PRV is widespread in pigs, with active replication detectable by PCR in nasal swabs, blood, and tissues of young piglets, while latency complicates detection in adults. Western blot assays using recombinant proteins containing highly conserved epitopes enable reliable diagnosis, showing 100% prevalence in tested German and Greek slaughterhouse pigs and up to 90% in wild boars. Antibodies in young piglets are often derived from the PCMV/PRV-positive mother sows through the colostrum, diminishing over time without infection. Evidence also indicates that PCMV/PRV can be transmitted via virus-positive oocytes. In preclinical xenotransplantation models, e.g., pig kidney or heart transplantations in non-human primates, PCMV/PRV transmission drastically reduced graft survival. Baboons receiving PCMV/PRV-positive pig hearts survived less than 30 days, whereas virus-free transplants extended survival to 195 days. The virus was found in all organs of the transplanted baboon by PCR and immunohistochemical methods. PCMV/PRV caused thrombotic microangiopathy, consumptive coagulopathy and multi organ failure accompanied by elevated levels of IL-6, TNFa and tPA-PAI-1 complexes. Since there is no evidence that PCMV/PRV infects primate including human cells, the virus likely interacts directly with the recipient´s immune and endothelial cells modulating immune functions and coagulation and exacerbating transplant complications. Notably, PCMV/PRV transmission occurred in the first pig-to-human heart transplant in Baltimore, and most likely contributed to the death of the patient. Treatment of the patient with antiviral drugs effective against human CMV failed. However, an intravenous immunoglobulin G (IVIgG) therapy may have temporarily reduced the virus load in the patient since we detected antibodies against HHV-6 which cross-react against PCMV/PRV in humans. Comprehensive screening using PCR and serological methods enables effective PCMV/PRV detection, and elimination strategies, including early weaning, have shown promise. Addressing PCMV/PRV is essential to improving xenotransplantation outcomes and mitigating associated risks.

LUNCH SEMINAR SYMBIO Room 407

10-1 New Insights into the pathogenicity of HHV-6 after HCT and CAR-T cell therapy

Oral

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HHV-6B is a DNA betaherpesvirus closely related to cytomegalovirus (CMV). HHV-6B infects most individuals during early childhood and establishes latency in a broad range of cells. HHV-6B frequently reactivates in immunocompromised patients and has been associated with a variety of complications. This talk will review new insights to implicate HHV-6B as a pulmonary pathogen after allogeneic hematopoietic cell transplant (HCT). The talk will also review recent studies that have improved our understanding of HHV-6 as a cause of encephalitis, and its association with mortality, after HCT. Finally, I will cover emerging data for HHV-6B reactivation and disease in patients treated with chimeric antigen receptor (CAR) T cell therapy.

HHV-6 IN THE IMMUNOCOMPROMISED - II

11-1 Oral HHV-6B encephalitis following allogeneic stem cell transplantation: Understanding and overcoming the challenge

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Around the year 2000, mysterious cases of encephalopathy following allogeneic hematopoietic stem cell transplantation began to be observed among transplant clinicians in Japan. These cases were characterized by memory impairment during the engraftment period, and MRI showed signs of limbic encephalitis. At that time in Japan, many were doubtful about the link between central nervous system (CNS) complications after transplantation and HHV-6. We started investigating whether HHV-6 could be the cause. In all six patients at our facility who developed limbic encephalitis, we consistently found high levels of HHV-6B reactivation at the time of onset. A nationwide multi-center study later confirmed that "HHV-6B reactivation can cause encephalitis" and that "HHV-6B encephalitis has become the leading cause of CNS complications after transplantation, with many cases remaining undiagnosed." Many data have established a causal relationship between HHV-6B and encephalitis. With support from the JSTCT, we conducted a nationwide observational study, which identified the risk factors for HHV-6B encephalitis and confirmed the effectiveness of foscarnet as a treatment. As a result, foscarnet became the first drug to be approved drug for HHV-6B encephalitis. Additionally, we developed clinical guidelines suggesting that if CNS symptoms occur after transplantation, HHV-6B encephalitis should be considered, and empirical antiviral therapy should be started when necessary. In Japan, these guidelines are now recommended for treating HHV-6B encephalitis. Recent nationwide surveys show that, compared to the period from 2007 to 2011, the prognosis of HHV-6B encephalitis cases in recent years (2019-2021) has improved (One-year survival rate: approximately 20% vs 50%). Over the past 20 years, research and efforts to understand HHV-6B encephalitis after transplantation have clarified its role, enabling us to treat cases that were previously poorly understood in 2000. This progress has led to better outcomes. We must focus on developing novel antiviral treatments or immunomodulatory therapies to reduce long-term sequelae and further improve survival rates. Additionally, creating preventive measures should be a priority for the future.

11-2 Oral HHV-6 encephalitis following allogeneic hematopoietic cell transplantation: current understanding and challenges

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HHV-6 encephalitis, one of the major complications following allogeneic hematopoietic cel ltransplantation (allo-HCT), was first reported in 1994, and despite 30 years having passed since then, it remains an unresolved issue. This condition is particularly common after umbilical cord blood transplantation (UCBT), with reported incidences of 7.9–9.9%, compared to 0.5–1.2% following bone marrow or peripheral blood stem cell transplantation (Hill JA, Biol Blood Marrow Transplant 2012;18:1638) (Scheurer ME, Bone Marrow Transplant 2013;48:574) (Ogata M, Clin Infect Dis 2013;57:671). An analysis of 723 patients who underwent allo-HCT at our institution between 2006 and 2013 revealed that the cumulative incidence of early CNS complications (ECNSC) within 100 days was 19%. Among the 138 patients who developed ECNSC, HHV-6 encephalitis was the most frequent, occurring in 56 cases. UCBT was identified as an independent risk factor for ECNSC, with the high incidence of HHV-6 encephalitis following UCBT being the primary reason (Kageyama K, Bone Marrow Transplant. 2021;56:686).

Although the underlying mechanism explaining why HHV-6 encephalitis occurs more frequently after UCBT than the others remains unclear, it is hypothesized that immune responses mediated by immune cells present in umbilical cord blood play a significant role. This hypothesis is supported by findings that pre-engraftment immune reactions (PIR), immune-mediated complications unique to UCBT, is a risk factor and that the incidence of HHV-6 encephalitis varies greatly depending on the GVHD prophylaxis method used (Ogata M, Bone Marrow Transplant 2017;52:1563).

The prognosis after onset has been examined in multiple studies, showing survival in 20% of cases, death due to

encephalitis in 13%, to other complications in 44%, and to the primary disease in 23%. Even among survivors, more than half exhibited long-term neurological sequelae, such as memory impairment, apathy, disorientation, and epilepsy (Ogata M, Bone Marrow Transplant 2017;52:1563). An analysis of 30 long-term survivors at our institution found that 23 were able to live independently, 4 required minor assistance, and 3 needed intensive caregiving support (Kageyama K, et al. JSTCT 2022).

Despite significant advancements in understanding the pathophysiology, risk factors, clinical presentation, and treatment of HHV-6 encephalitis following allo-HCT, the overall incidence has not been effectively reduced. Further efforts are needed to establish appropriate preventive and therapeutic strategies.

11-3 Human herpesvirus 6–specific T cell immunity in allogeneic hematopoietic stem cell transplant recipients

Oral

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Objectives: Human herpesvirus-6 (HHV-6) reactivation is associated with severe clinical manifestations in allogeneic HSCT (allo-HSCT) recipients. The correlation of HHV6-specific immune responses after HSCT with clinical outcome is largely unexplored, especially with the use of newer transplant platforms as post-transplant cyclophosphamide (PT-Cy).

Methods: From February 2013 to October 2015 we conducted a prospective observational study to investigate HHV-6 reactivation in 208 adult patients who received allo-HSCT for hematological malignancies. All types of donors were included, and the stem cell source was mainly PBSCs. Viral load was monitored weekly by quantitative PCR in plasma. Numbers of IFN-γ-producing HHV-6 T cells were determined by enzyme-linked immunospot assay (ELISpot) by stimulation with the immunodominant viral protein U54.

Results: HHV-6 reactivation occurred in 63% of patients at 100 days. HHV-6 was detected in plasma for 85% of patients; 50% tested positive in other materials including bone marrow aspirates, gut biopsies, bronchoalveolar lavage, and cerebrospinal fluid. Only 40% of reactivating patients presented a clinically relevant infection (CRI), defined either as classical HHV-6 related EODs recognized by ECIL guidelines (proven encephalitis, delayed engraftment, or myelosuppression), or as other clinical entities possibly HHV-6–related with a weaker association (probable/possible encephalitis, pneumonitis, hepatitis, gastroenteritis). Main clinical manifestations were rash, fever, cytopenias, hepatitis, diarrhea, and encephalitis. Overall survival, relapse and TRM were not affected by HHV-6 reactivation. CRI was associated with increased occurrence of acute GvHD of all grades. Risk factors for HHV-6 reactivation included previous allogeneic HSCT, PT-Cy use, and therapy with corticosteroids. CRIs were associated with PT-Cy and steroids use, whereas higher polyclonal CD3+ cell counts appeared protective. Counts of circulating IFN-γ-producing HHV-6- specific T-cells were significantly higher in HHV-6 reactivating patients than in non-reactivating patients. We found that a threshold of 18 spot forming units (SFUs) at the IFN-γ ELISpot assay, performed 4 days after viral reactivation, is able to predict CRI, which manifests at least 15 days (median 30) from viremia.

Conclusions: In this study, we identified as risk factors for HHV-6 reactivation and CRI the use of PtCy and corticosteroids, and lower polyclonal CD3+ cell counts. CRI was associated with increased incidence of aGvHD. HHV6-specific T cells detectable by ELISpot are significantly associated with HHV-6 CRIs for both classical EODs and other clinical entities potentially HHV-6-related that we identified. These results represent a new promising tool to unravel the role of HHV6 positivity in allo-HSCT recipients, and may affect the future of HHV -6 monitoring and preemptive treatment.

11-4 Association between human herpesvirus-6 encephalitis and antiviral prophylaxis after allogeneic hematopoietic stem cell transplantation in the letermovir era

Oral

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Objective: The impact of letermovir (LTV)—an anti-cytomegalovirus (CMV) drug—on human herpesvirus-6 (HHV-6) encephalitis after allogeneic hematopoietic stem cell transplantation (HSCT) is unclear. We hypothesized that LTV prophylaxis may increase the incidence of HHV-6 encephalitis by reducing anti-CMV therapies after HSCT. To evaluate the association between HHV-6 encephalitis and antiviral prophylaxis, 7985 adult patients from a Japanese nationwide registry who underwent their first HSCT between January 2019 and December 2021 were analyzed.

Methods: The clinical data of HSCT recipients were obtained from the Transplant Registry Unified Management Program of the Japan Society for Transplantation and Cellular Therapy and the Japanese Data Center for Hematopoietic Cell Transplantation. The primary endpoint was the incidence of HHV-6 encephalitis on day 100 after HSCT. To evaluate the impact of LTV prophylaxis on HHV-6 encephalitis, we divided the entire cohort into three groups according to the antiviral drugs administered prophylactically immediately after transplantation: (1) broad-spectrum antiviral drugs including prophylactic foscarnet (FCN), ganciclovir (GCV), or valganciclovir (VGCV); (2) oral or intravenous LTV; and (3) other antiviral drugs that did not include FCN, GCV, VGCV, or LTV.

Results: Out of 7985 patients, 467 patients (5.8%) received broad-spectrum antiviral prophylaxis, 4911 patients (61.5%) received LTV, and 2607 patients (32.6%) received other antiviral drugs. The LTV group showed significantly lower incidence of CMV preemptive therapy or CMV disease at day 100 as compared to those with broad-spectrum antiviral prophylaxis or other antiviral drugs. Of note, 278 HHV-6 encephalitis cases occurred in 7985 patients, with an incidence of 3.6% (95% CI: 3.2–4.0%) on day 100 (median 24 days, range 10–196 days). The risk of developing HHV-6 encephalitis was significantly higher in patients who received broad-spectrum antiviral prophylaxis (11.5%, 95% CI 8.5–14.4%) compared to those who received LTV (2.8%, 95% CI 2.3–3.2%) or other antiviral drugs (3.8%, 95% CI 3.0–4.5%) ($p < 0.001$). These differences persisted when cord blood transplantation was analyzed separately (14.1%, 5.9%, and 7.4%, $p < 0.001$). In the multivariate analysis, CBT (HR: 2.90), broad-spectrum antiviral prophylaxis (HR: 1.91), and grade II-IV acute graft-versus-host disease requiring systemic corticosteroids (HR: 2.42) were independent risk factors for encephalitis (all $p < 0.001$).

Conclusions: The findings of this large modern database study indicate that broad-spectrum antiviral prophylaxis, rather than LTV prophylaxis, is paradoxically associated with HHV-6 encephalitis in the LTV era. This paradoxical finding needs to be further explored in future studies.

11-5 Clinical features and outcomes of Human Herpesvirus-6 DNAemia in critically ill patients: a retrospective multicenter analysis

Oral

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Objective: Human Herpesvirus-6 (HHV-6) DNAemia is not rare in intensive care unit (ICU) patients. However, evidence for a causal association of HHV-6 DNAemia with organ disease and with mortality is limited in this setting. In ICU patients with HHV-6 DNAemia, we sought to 1) assess the prevalence of HHV-6 disease, 2) identify risk factors for HHV-6 disease and 3) investigate its association with mortality.

Methods: Retrospective multicenter case-matched study in three ICUs from January 2011 to January 2022 of patients with HHV-6 viral load in the whole blood (genome equivalent copy/10 6 cells) detected during the ICU stay. Patients were classified as reactivation (i.e. DNAemia without imputable end-organ disease) or HHV-6 disease (i.e. DNAemia with at least one imputable end-organ disease). Classification was based on guidelines when available (i.e. encephalitis in hematopoietic stem cell transplant recipients) and a multidisciplinary expert adjudication committee. Patients with chromosomally integrated HHV-6 were excluded.

Results: One hundred and sixty-eight patients were included. Seventeen (10%) were classified as HHV-6 disease (i.e., HHV-6 DNAemia with attributable end-organ disease) and 151 (90%) as HHV-6 reactivation (i.e., HHV-6 DNAemia without any attributable end- organ disease). Immunodepression was significantly more frequent in HHV-6 disease patients (100% vs. 48%, p < 0.001). Eleven (65%) HHV-6 disease patients received hematopoietic stem cell transplantation (HSCT). End-organ diseases were encephalitis (n= 10) and pneumonia (n = 7). Best cut-off of whole blood viral load to predict HHV-6 disease was 500 gec/10 6 cells. A broncho-alveolar lavage cut-off of 3.35 log gec/10 6 cells predicted HHV-6 pneumonia with an area under the curve (AUC) of 0.81. The ICU mortality was 32% (n = 53). In multivariate analysis, HHV-6 disease remained independently associated with ICU (OR 4.90) and 90-day (HR 2.25) mortality after adjustment for comorbidities and baseline severity. Mortality remained significantly higher in the HHV-6 disease group (OR 4.30) when compared to a matched control population of ICU patients without HHV-6 DNAemia. Among the 17 patients with HHV-6 disease, only 9 (53%) received specific anti HHV-6 treatment during the ICU stay.

Conclusion: Our analysis suggests that HHV-6 disease develops in 10% of patients with HHV-6 detection in the ICU, mostly in the setting of allogeneic hematopoietic stem cell transplantation and is independently associated with ICU and 90-day mortality.

11-6 Association between HHV-6B reactivation, acute GVHD, and outcomes following allogeneic hematopoietic stem cell transplant recipients: a retrospective study.

Poster

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Objective: HHV-6B reactivation occurs in approximately half of allogeneic hematopoietic stem cell transplant recipients, typically between the 2-4 weeks post-transplant, and is occasionally associated with the development of HHV-6B encephalitis. Other potential HHV-6B-related complications post-transplant, such as myelitis, bone marrow suppression, and GVHD have been suggested, though causal relationships remain unclear. Notably, the recent studies suggested links between HHV-6B reactivation and aGVHD or non relapse mortality, but findings have been inconsistent. This study aimed to investigate the association between HHV-6B reactivation, acute graft-versus-host disease (aGVHD) and survival outcomes after allogeneic hematopoietic cell transplantation (allo-HCT).

Methods: This retrospective study analyzed 164 patients who underwent allo-HCT at Oita University Hospital, between May 2010 and April 2022. Monitoring of plasma HHV-6 reactivation was conducted weekly using RT-PCR from day 0 to day 70 post-transplant. Statistical analysis was done using EZR software.

Results: The median age of the patients was 56 years (range: 16–72 years), with a median observation period of 1,445 days (range: 29–5,307 days). Patients with HHV-6B reactivation were identified in 80 of 164 patients (49%), with a median onset at day 20 post-transplant. Patients with HHV-6B reactivation had significantly higher incidences of aGVHD (HR 2.6, P<0.01) and grade II-IV aGVHD (HR 2.75, P<0.01) compared to those without reactivation. No significant differences were found in non-relapse mortality, recurrence rates, or 1-year survival between the two groups.

Conclusion: This study suggests significant association between HHV-6B reactivation and an increased risk of

aGVHD, including grade II-IV aGVHD. These findings highlight the need for further investigation into the potential causal mechanisms linking HHV-6B reactivation and aGVHD.

11-7 Prevalence of Human Herpesvirus 6 infections in pediatric hematopoietic cell transplantation recipients from 2010 to 2019 in a single-center study

Poster

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Objective: It has been reported that human herpesvirus 6 (HHV-6) may cause serious diseases such as encephalitis, graft versus host disease, graft failure, and death in hematopoietic cell transplant (HCT) recipients, but data in Korea is limited. We report the prevalence and clinical manifestations of HHV-6 infection within one year after HCT in pediatric patients in Korea.

Methods: A retrospective study included autologous and allogeneic HCT recipients from January 2010 to December 2019 in Samsung Medical Center. Data on clinical manifestations and laboratory results of patients with HHV-6 infection were collected. Results for cytomegalovirus (CMV) and Epstein-Barr virus (EBV), human simplex virus (HSV), and varicella zoster virus (VZV) we also obtained.

Results: Among 969 pediatric HCT cases, HHV-6 viremia was observed in seven patients (0.7%). Two were male (28.6%), and the median age was 8 years (range, 2-18 years). Underlying diseases included four patients with acute lymphocytic leukemia (57.1%), one medulloblastoma (14.3%), one neuroblastoma (14.3%), and one adrenoleukodystrophy (14.3%). Transplantation types were autologous HCT (n=2, 28.6%), haploidentical transplant (n=2, 28.6%), and umbilical cord blood transplant (n=3, 42.9%). HHV-6 DNAemia was detected on median day 83 (range, 10-283 days) after HCT. Concurrent herpesvirus DNAemia was observed: CMV 28.6% (2/7), EBV 0% (0/7), HSV 0% (0/7). Patients with HHV6 DNAemia had fever (85.6%), rash (85.6%), pulmonary disease (28.6%), CNS complications (28.6%), and GVHD (42.9%). Among seven patients, overall mortality was 42.9% (n=3), and the median day from the first HHV-6 DNAemia to death was 29 (range, 18-32 days).

Conclusions: This is the first study to examine the data on HHV-6 DNAemia in pediatric HCT recipients in Korea. There is a need for increased awareness of HHV-6 monitoring with better test platforms for HCT recipients among pediatricians in Korea.

11-8 Clinical significance of HHV-6 DNA detection in different samples from patients before and after allo-HSCT

Poster

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Introduction: Infections caused by HHV-6 are one of the leading causes of complications and mortality in hematology patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Saliva and renal tubular endothelial cells are natural reservoirs for this virus.

Purpose: The aim is to study the frequency of HHV-6 detection in patients before/after allo-HSCT in natural reservoirs and the necessity of routine screening of patients for HHV-6A/B.

Materials and methods: The study group consisted of 92 patients (47 women and 45 men), aged 28 to 47 years. All patients underwent allo-HSCT at the National Medical Research Center for Hematology from October 2022 to January 2024. We studied blood, saliva and urine in all patients during the pre-transplant period, on day 0, and then once a week until +42 days. We performed PCR to determine the presence of HHV-6. In parallel all patients were examined for oral mucositis symptoms (NCI, fNCI, WHO) and acute cystitis symptom scores

(ACSS).

Results: The maximum frequency of detection of HHV-6 in blood was 16,1 %. HHV-6 was detected in blood at all terms after allo-HSCT, starting from day +14. The maximum frequency of detection of HHV-6 in urine was on day +35 (6,8%) It is important that HHV-6 was detected in urine before allo-HSCT in some recipients as well (2,2%). Maximal frequency of detection of HHV-6 in saliva was on day +7 (22%). In 19,1% HHV-6 was detected in saliva before. On day +7 after transplantation in patients with mucositis, only 15% of patients had HHV-6A/B detected in saliva. At the same time, in patients without mucositis, HHV-6 was detected in saliva in 25%. And at +14 days the situation remained the same. Thus, we found no association between detection of HHV-6 DNA in saliva and presence/absence of oral mucositis in the first 2 weeks after allo-HSCT and in the recovery period after allo-HSCT. Also we were able to perform genotyping of 18 HHV-6-positive samples for differentiation of HHV-6 variants. HHV-6B was detected in all of them.

Conclusions: Salivary glands are a natural reservoir for HHV-6, which implies that routine saliva screening for HHV-6 in patients after allo-HSCT is not indicated. In a subset of patients before allo-HSCT, HHV-6 was detected in the urine, and there were no symptoms of cystitis. Thus, detection of HHV-6 in natural virus reservoirs is not an indication for starting antiviral therapy.

NON-CNS DISEASE

12-1 Circulating tumor DNA sequencing for biologic classification and individualized risk stratification in patients with Hodgkin lymphoma – a deeper look at HHV-6-associated Patients

Oral

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Objective: We previously presented a biological classification of Hodgkin Lymphoma consisting of three subtypes based on plasma-derived circulating tumor (ct)DNA sequencing: Inflammatory immune escape Hodgkin Lymphoma is characterized by frequent copy number variations including immune escape variants such as high-level amplifications of the PD-L1 locus and an inflammatory tumor microenvironment. Virally-driven Hodgkin Lymphoma shows strong association with EBV and/or HHV-6 as well as a tumor microenvironment dominated by neutrophils and macrophages. Oncogene-driven Hodgkin Lymphoma is defined by a high tumor mutational burden including recurrent mutations in common oncogenic drivers (Heger et al. 2024, Journal of Clinical Oncology). Here, we look in-depth at the HHV-6-associated patients and will present this data at the meeting.

Methods: In this study, we applied circulating tumor DNA sequencing to 243 patients obtained from pivotal German Hodgkin Study Group trials to identify subtypes of Hodgkin Lymphoma. Independent validation of the subtypes was performed in 96 patients treated in the EuroNet-PHL-C2 study. Outcome differences of subtypes were assessed in an event-enriched clinical validation cohort comprising 72 patients from the HD21 trial, using a refined, validated, and clinically feasible assay. HHV-6-associated patients were identified by a cfDNA metagenomic approach, aligning reads not mapping to the human genome to bacterial, viral and fungal genomes followed by subsequent filtering steps. HHV-6-associated patients were compared to patients in the other clusters (i.e. not virally-driven Hodgkin Lymphoma) as well as to patients associated with EBV within the virally-driven Hodgkin Lymphoma cluster.

Results: Our genetic classification of Hodgkin Lymphoma (Heger et al. 2024, Journal of Clinical Oncology) identified a subgroup of Hodgkin Lymphoma patients with a distinct genotype, microenvironment and clinical phenotype that is associated with herpesviruses, namely EBV and HHV-6. While the association between Hodgkin Lymphoma and EBV is long known, the association between Hodgkin Lymphoma and HHV-6 is unclear and has been debated. In our study we identified HHV-6 in 25/243 (6.2%) patients by a cfDNA metagenomic approach. We are currently performing an in-depth study of these patients and will present details on their genotype, phenotype and microenvironment at the meeting.

Conclusion: HHV-6-cfDNA can be detected in a subgroup of patients with Hodgkin Lymphoma. It is currently unclear, if HHV6-infection is the cause of lymphomagenesis in these patients. Details on HHV-6 associated patients in our cohort will be presented at the meeting aiming to understand these patients better.

12-2 Exploring the role of neonatal roseolovirus infection in autoimmunity

Oral

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Mounting evidence has demonstrated that autoimmune diseases are characterized by immune dysregulation that begins years before overt disease. Viral infections have been proposed to be a contributing factor to development of autoimmunity, but in the case of herpesviruses, the onset of autoimmune disease typically occurs years after the typical age of infection. Recent studies out of our lab demonstrated that neonatal infection with murine roseolovirus (MRV), which is genetically and morphologically highly related to HHV-6 and -7,

induces transient thymic atrophy and CD4+ T cell depletion. Interestingly, neonatal MRV infection resulted on durable immune dysregulation manifested as organ limited autoimmune disease. We identified the tropism of MRV in the thymus as well as specific pathways of immune and tolerance dysregulation. Interestingly, although type I IFN contributed to control of MRV replication, it also played an important role in MRV-mediated autoimmune disease. Additionally, we found that adult mice that were neonatally infected with MRV were predisposed to systemic autoimmunity but only after additional immune perturbation such as TLR7 stimulation. We showed that disease is associated with specific cytokine and chemokine alterations. Moreover, we demonstrated that neonatal MRV infection resulted in shifts in T cell activation and exhaustion in response to TLR7 stimulation, as well as gross alterations in immune cell localization. Our findings show that neonatal roseolovirus infection results in durable disruption of immunologic tolerance and predisposition to systemic autoimmunity after additional immune stimulation.

12-3 Human herpesvirus 6A/B and preeclampsia

Oral

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Introduction: Preeclampsia is a serious pregnancy complication associated with high morbidity and mortality rates worldwide. It affects millions of women annually, with an estimated 70,000 maternal and 500,000 infant deaths attributed to the condition each year. Preeclampsia is characterized by new-onset hypertension, accompanied by proteinuria or maternal organ dysfunction. This can lead to life- threatening complications such as kidney failure, pulmonary edema, seizures, or stroke. Though primarily considered a placental disorder, the exact causative factors of the condition remain unknown.

Hypothesis: In preeclampsia, placental stress alters the release of various factors into maternal circulation. Notably, this condition is marked by elevated levels of soluble fms-like tyrosine kinase-1 (sFLT-1) and reduced levels of placental growth factor (PlGF). Additionally, systemic inflammation characterizes preeclampsia. Previous studies have linked human herpesvirus (HHV)-6A/B to pregnancy complications. Our research hypothesizes that HHV-6A/B reactivation may be an underlying cause of preeclampsia.

Methods: To investigate this hypothesis, we analyzed data from Swedish population registers.

Results: We found that women prescribed antiviral medications during pregnancy had a significantly lower likelihood of developing preeclampsia ($p<0.01$; n=618,814). Furthermore, in early pregnancy, women who later developed preeclampsia exhibited higher plasma levels of the cytokine TNFSF14 before symptom onset ($p<0.001$; n=44). TNFSF14 plays a role in antiviral defense, particularly against herpesviruses. Our *in vitro* studies further support a link between HHV-6A/B and preeclampsia. Infection of trophoblast cells isolated from human placenta with HHV-6A or HHV-6B resulted in a significant increase in sFLT-1 release into the culture medium compared to uninfected controls ($p<0.05$; n=8). Additionally, PlGF release showed a trend toward reduction in HHV-6A/B-infected trophoblast cells ($p=0.09$; n=8), mimicking changes observed in preeclampsia.

Conclusion: These results support the hypothesis regarding an association between viral infections and preeclampsia.

12-4 The silent invader: investigating HHV-6 as a potential cause of infertility in the Indian population

Oral

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Introduction: HHV-6 is a ubiquitous virus and its role in infertility among women is upcoming. Infertility is associated with social stigma in India and in around 15% of cases, the cause remains undiagnosed. The present study has been designed to determine HHV-6 positivity among infertile women.

Methodology: A total of 406 (infertile) and 116 (fertile) endometrial biopsy samples were collected and

screened. 28 participants were lost to follow up and the remainder were divided into Group I (primary unexplained infertility), Group II (explained infertility), and Group III (fertile women). The endometrial biopsy samples were screened for human herpesvirus-6 (HHV-6) DNA using conventional nested PCR targeting the U67 and U94 gene. Additional blood samples were collected and tested for HHV-6 DNA from those study participants, positive for HHV-6 DNA in endometrial biopsy samples. Sanger sequencing among representative HHV-6 positive samples was carried out for specific variant analysis of HHV-6. HHV-6 specific mRNA analysis was conducted to check the active viral replication in the tissue samples.

Results: The results revealed that 21.30% (13/61) of women in Group I tested positive for HHV-6 in their biopsies, compared to 9.14% in Group II (29/317) and 6.89% in Group III (8/116) ($p<0.05$). Among the follow up blood samples of HHV-6 positive biopsy samples, 38.40 (5/13) of women in Group I were positive HHV-6, while only 17.24% (5/29) and 12.5% (1/8) were positive in Groups II and III respectively. Molecular analysis of viral variants showed that HHV-6B DNA was the major variant present in all three groups with some variations in Group I. For Group I, HHV-6 B was found in 83.3% (5/6) of endometrial biopsies and PBMCs, while HHV -6A was found in 16.6% (1/6) of endometrial biopsy and PBMCs. However in Group II and Group III all biopsies sequenced were HHV-6 B: (15/15) and (4/4) respectively. Interim results of m-RNAtranscript analysis showed that Groups I (55.50%, 5/9) and II (38.46%, 5/13) exhibited active viral replication (U67), as evidenced by lytic replication transcripts, while Group III showed latent transcripts (U94) (100%, 7/7), indicating a latent infection.

Conclusion: The findings reveal higher prevalence of HHV-6 among Indian infertile women both in the endometrial tissue and the systemic circulation, with active viral replication.

12-5 Impact of Human Herpesvirus 6A/B on Reproductive Health: Implications for Infertility, Placental Abnormalities, and Endometrial Receptivity

Poster

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Introduction: Human herpesviruses 6A and 6B (HHV-6A/B) are widespread pathogens linked to various health conditions, including neurological, pulmonary, and reproductive diseases. These viruses establish lifelong infections and can integrate into human chromosomes. While much remains unknown, HHV6A/B has been implicated in reproductive challenges such as unexplained infertility, and late-onset intrauterine growth restriction (IUGR). This abstract summarizes findings from studies exploring HHV6's involvement in placental abnormalities, infertility, and endometrial receptivity.

Materials and Methods: Histopathological and immunohistochemical analyses were conducted on 11 IUGR placentas and 11 control placentas to assess HLA-G expression and HHV-6 presence. In a cohort of women with unexplained infertility and controls, HHV-6 DNA detection, endometrial natural killer (NK) cell profiles, and cytokine levels in uterine flushing were evaluated. Further, human endometrial cells (HEC-1A) were infected with HHV-6A to analyze miRNA expression and trophoblast receptivity, employing RNA analysis and trophoblast cell adhesion assays.

Results: IUGR placentas exhibited elevated HHV-6 presence and increased HLA (Human leukocyte antigen)-G tolerogenic molecule, compared to controls, suggesting a link between HHV-6 infection, immune escape, and disrupted placental vessel remodeling. In unexplained infertility, HHV-6A DNA was detected in 43% of endometrial biopsies, with altered NK cell distribution and increased IL-10 and
reduced IFN-γ levels in uterine flushing. HHV-6A infection also induced significant changes in miRNA expression in endometrial cells, upregulating miRNAs linked to implantation failure and downregulating those associated with favorable pregnancy outcomes. HHV-6A-infected cells were less permissive to trophoblast adhesion, indicating impaired endometrial receptivity.

Discussion: These studies highlight the multifaceted impact of HHV-6A/B on reproductive health. Elevated HLA-G expression in IUGR placentas aligns with the virus's role in immune modulation, potentially compromising pregnancy outcomes. In infertility, HHV-6A alters NK cell profiles and cytokine balances, weakening the uterine immune environment necessary for successful implantation. Furthermore, HHV6A-induced miRNA alterations and reduced trophoblast adhesion underscore its role in disrupting implantation

processes. Together, these findings suggest that HHV-6A/B contributes to reproductive complications through immune, genetic, and cellular pathways. Further research is essential to confirm these findings and explore therapeutic interventions targeting HHV-6A/B to improve reproductive outcomes. These insights underline the significance of understanding HHV-6A/B in managing pregnancy-related disorders and infertility.

References:

[1]Bortolotti D, Gentili V, et al Rizzo R, Gafà R, Greco P. Late-onset intrauterine growth restriction and HHV-6 infection: A pilot study. J Med Virol. 2021; 93(11):6317-6322. doi: 10.1002/jmv.27138.

[2]Komaroff AL, Roberta Rizzo R, Ecker JL. Human Herpesviruses 6A and 6B in Reproductive Diseases. Front Immunol. 2021; 12:648945. doi: 10.3389/fimmu.2021.648945. Bortolotti D, et al. Rizzo R, Caselli E. HHV-6A Infection of Endometrial Epithelial Cells Affects miRNA Expression and Trophoblast Cell Attachment. Reprod Sci 2020;27(3):779-786. doi: 10.1007/s43032-019-00102-8.

[3]Pegoraro A, et al. Rizzo R, Adinolfi E. The P2X7 Receptor 489C>T Gain of Function Polymorphism Favors HHV-6A Infection and Associates With Female Idiopathic Infertility. Front Pharmacol 2020 11:96. doi: 10.3389/fphar.2020.00096.

[4]Caselli E, et al. Rizzo R. HHV-6A Infection of Endometrial Epithelial Cells Induces Increased Endometrial NK Cell-Mediated Cytotoxicity Front Microbiol 2017; 8:2525. doi: 10.3389/fmicb.2017.02525.

[5]Roberto Marci M et al. Rizzo R. Presence of HHV-6A in Endometrial Epithelial Cells from Women with Primary Unexplained Infertility PLoS One. 2016; 11(7):e0158304. doi: 10.1371/journal.pone.0158304.

12-6 Poster Pityriasis rubra pilaris with simultaneous emergence of human herpesvirus-6 reactivation in a patient with drug-induced hypersensitivity syndrome

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Background: Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disorder of unclear etiology. While viral infections such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV) have been implicated in some cases, this is the first reported instance of PRP occurring concurrently with human herpesvirus-6 (HHV-6) reactivation in a patient with drug-induced hypersensitivity syndrome (DIHS).

Case Presentation: An 85-year-old male presented with high fever, disseminated erythema, facial edematous erythema, and lymphadenopathy after 32 days of allopurinol use. Laboratory findings included elevated liver enzymes, atypical lymphocytes, and increased C-reactive protein. Skin biopsy confirmed DIHS with a severity score of 9. Following cessation of allopurinol and initiation of high-dose prednisolone (50 mg daily), erythema resolved within ten days. Four weeks later, new papulosquamous eruptions appeared, characterized by orange-red keratotic plaques on the palms, soles, and follicular papules on the dorsal feet. Histopathological examination revealed features consistent with PRP. Concurrently, real-time PCR showed transient elevation of HHV-6 DNA (4.2×10^4 copies/mL), which subsequently normalized. No serological evidence of CMV, EBV, or HHV-7 was found. The PRP lesions resolved with topical corticosteroids, coinciding with the decline of HHV-6 DNA.

Discussion: This case highlights a potential link between HHV-6 reactivation and the development of PRP in DIHS. Although PRP has previously been reported after infections with EBV, CMV, HSV, VZV, and SARS-CoV -2, this is the first report associating HHV-6 with PRP. Similar PRP-like eruptions have been observed in graft-versus-host disease, further suggesting immune dysregulation as a shared mechanism. The role of HHV-6 in these eruptions remains speculative, requiring further investigation.

Conclusion: The simultaneous emergence of PRP and HHV-6 reactivation in this case suggests a potential role for HHV-6 in the pathogenesis of PRP in DIHS. Future studies are warranted to elucidate the mechanisms underlying this association.

12-7 Poster Herpesvirus-6 infection of primary placental cells elevates sFLT-1 levels: a potential contributor to pre-eclampsia progression

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Overview: Pre-eclampsia, one of the leading causes of maternal and fetal mortality worldwide, is a pregnancy complication closely associated with placental dysfunction and characterized by new-onset hypertension and multi-organ dysfunction during pregnancy. The biomarkers soluble fms-like tyrosine kinase-1 (sFLT-1) and placental growth factor (PlGF) are used clinically for pre-eclampsia screening and diagnostics with elevated levels of sFLT-1 and reduced levels of PlGF being correlated with disease severity. sFLT-1 is an antiangiogenic protein, inhibiting angiogenesis and vascular permeability that are processes essential for both a successful immune response and for a healthy pregnancy. In women with pre-eclampsia, the placenta becomes the primary source of sFLT-1, which enters maternal circulation and contributes to systemic endothelial dysfunction leading to high blood pressure, swelling and organ damage. Despite its significant impact, the etiology of pre-eclampsia remains poorly understood. Several contributing factors have been identified, including viral infections. It has been shown that women carrying a child with inherited chromosomally integrated human herpesvirus 6 (iciHHV-6) have an increased likelihood of developing pre-eclampsia, highlighting a link between herpesvirus-6 (HHV-6) and this condition.

Objective: This study aimed to investigate the in vitro effects of HHV-6A and 6B infection on primary placental cells to explore its potential link to the development of pre-eclampsia.

Methods: Placental cells were isolated from term placentas (n=8) donated after elective Csection at Uppsala University Hospital, Uppsala, Sweden. Cells from each woman were infected with HHV-6A (U1102) and HHV -6B (Z29). Infection was confirmed using qPCR for all samples. Cell media was collected every 24 h for four days. PlGF and sFLT-1 levels were measured by use of ELISA on day 4 and normalized to total protein content. Human chorionic gonadotropin (hCG) levels were measured daily to confirm correct cell differentiation. Data was analyzed using repeated measure ANOVA to compare the infected samples to the uninfected control sample of each woman.

Results: Successful infection of the primary placental cells was confirmed for all samples. PlGF levels were not significantly different between infected and uninfected controls ($p=0.088$) and hCG levels were significantly lower ($p=0.01$) in cells infected with HHV-6B. sFLT-1 levels were significantly higher in both HHV-6A and 6B infected cells compared to healthy controls ($p<0.001$).

Conclusion: Our study shows that both HHV-6A and 6B infection of placental cells increase sFLT-1 levels compared to controls, suggesting that HHV-6 may contribute to progression of pre-eclampsia and exacerbate disease severity.

12-8 Human Herpesvirus 6 Infection as a Potential Driver of Idiopathic Pulmonary Fibrosis (IPF)

Poster

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Objective: To investigate the potential role of viral infection in the pathogenesis of IPF, a progressive and fatal lung disease of unknown origin.

Methods: A comprehensive virome analysis was performed using next-generation sequencing (NGS) on explanted lung tissue from IPF patients and control subjects. To validate the presence of HHV-6, quantitative PCR (qPCR) was employed. Furthermore, flow cytometry-sorted lung cells were subjected to qPCR to determine the cellular tropism of HHV-6.

Results: NGS analysis revealed the presence of HHV-6 in the lung tissue of 40% of IPF patients compared to only 8% of control samples. qPCR analysis confirmed these findings, demonstrating a higher sensitivity with 64% and 24% positivity rates in IPF and control groups, respectively. We sorted lung tissue by FACS and found HHV-6 viral DNA in epithelial cells, but not in CD4 T cells or endothelial cells confirming viral tropism for

epithelial cells. Species-specific qPCR confirmed all detected cases were HHV-6B, except for one iciHHV-6A case.

Conclusion: Our findings suggest a strong association between HHV-6 infection and IPF. The preferential localization of HHV-6 to lung epithelial cells implicates a potential role for this virus in the initiation and progression of lung fibrosis. These results warrant further investigation into the mechanisms by which HHV-6 contributes to IPF pathogenesis and the potential development of antiviral therapies to target this virus. Our future studies will explore whether the anti-HHV-6 humoral immune response is defective in IPF patients with the use of a custom designed protein micro array; and the possibility that HHV-6 integrates into specific chromosome/telomeres in IPF patients and drives a TPE-OLD (telomere position effect over long distances) mechanism.

12-9 Unveiling the role of HHV-7 in human angiosarcoma

Poster

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Background: Human herpesvirus-7 (HHV-7) is a ubiquitous, CD4+ T cell lymphotropic virus. It establishes lifelong latency and may reactivate, particularly in immunocompromised individuals. We had previously detected the presence of HHV-7 in two-thirds of angiosarcoma clinical samples. HHV-7-positive (HHV-7+) tumors were shown to be relatively enriched for B- and mast cells and had high tumour inflammation signature scores compared to HHV-7-negative tumours.

Objective: To investigate HHV-7 pathogenesis in host cells and its role in angiosarcoma.

Methods: A SupT1 infection model was established. Images and cell pellets were collected at Days 0 and 4 (early infection phase) and Days 8 and 14 (late infection phase) for bulk RNA-seq analysis. Gene expression data was validated with quantitative PCR (qPCR). RNA-seq and Nanostring gene expression profiling with the Pancancer IO360 panel were previously performed on HHV7+ fresh frozen and FFPE angiosarcoma samples respectively.

Results: Infected SupT1 cells exhibited cytopathic effects (CPEs) such as membrane blebbing, syncytia formation and growth arrest during early infection phase. Cell lysis, followed by recovery of cell growth, were observed during late infection phase. RNA-seq revealed significant pathway alterations across the infection course, supporting these observations. Early infection showed notable upregulation in arachidonic acid metabolism and antigen processing and presentation pathways, indicating an anti-viral response in host cells. The downregulation in actin cytoskeleton and cell cycle pathways were seen, corresponding to the observed CPEs. During late infection, there was a significant downregulation in antigen processing and presentation pathways (padj. <0.05), suggesting immune evasion the virus, along with a significant downregulation of tumor suppressor genes such as RNF43 and CBFA2T3. Through the course of infection, a general upward trend of oncogenic and inflammatory pathways was seen, with the latter being observed in HHV-7+ angiosarcomas as well. Narrowing down on specific genes, we observed the significant downregulation of HLA-F and upregulation of HK2 in both late-stage SupT1 infection and HHV-7+ angiosarcomas, implicating these genes in immune evasion and tumor progression.

Conclusions: Our study provides the first RNA-seq profiling of HHV-7 infection in host cells. The study reveals alterations in key cellular pathways that promote an immunosuppressive and pro-inflammatory environment. These changes likely facilitate tumor progression and immune evasion in angiosarcoma.

12-10 Analysis of clinical and pathogenic differences between apparent and inapparent infection of HHV-6B

Poster

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Objective: During primary HHV-6B infection, most patients develop typical exanthem subitum(ES); however, some do not present with the typical clinical course of ES. The reason for the discrepancy remains unclear. The purpose of this study was to elucidate the pathophysiological differences between patients with primary HHV -6B infection with and without the typical clinical course of ES.

Methods: Between June 2015 and September 2020, febrile children aged 5 and younger who visited the pediatric outpatient department at Fujita Health University Hospital and underwent blood tests were enrolled in this study. Detection of serum HHV-6B DNA and measurement of antibody titers in acute phase serum were carried out to determine primary HHV-6B infection. Among these patient samples, 131 serum samples were used for analysis of cytokine/chemokine levels by using the Milliplex® Map 38-plex human cytokine/chemokine magnetic bead-based panel.

Results: There were 145 (84%) typical clinical course of ES cases and 28 (16%) cases without the typical clinical course, respectively. The median age of patients without ES (1.8) was significantly older than patients with ES (1.2) ($P<0.001$). Frequency of hospital admission was significantly higher in patients without ES (86%) than those with ES (66%) ($P=0.034$). Median white blood cell (WBC) counts (6,100 vs 7,750 /μL; $P=0.005$), neutrophil counts (2,992 vs 5,500 /μL; $P<0.001$), and C-reactive protein (CRP) levels (0.60 vs 0.94 mg/dL; $P=0.024$) were significantly higher in patients without ES than those with ES. Among the 37 biomarkers, IL-6 ($P=0.001$), IL-1Ra ($P=0.002$), TNF-β ($P=0.005$), IL-15 ($P=0.013$), IL-17A ($P=0.026$), IL-17E/I -25 ($P=0.001$), and IL-4 ($P=0.005$) were significantly higher in patients without ES than those with ES. PDGF-AB/BB ($P=0.001$) and IL-17F ($P=0.003$) were significantly lower in the patients without ES than those with ES.

Conclusions: The patients without ES were older and more likely to be hospitalized than ES patients. The inflammatory responses were stronger in patients without ES than ES patients,which was also supported by biomarker analysis.

KEYNOTE

13-1 Discovery of HHV-6B

Oral

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Human herpesvirus 6 (HHV-6) was first isolated from patients with lymphoproliferative disorders in 1986 and it mainly replicates in lymphocytes of T-cell lineage. This virus is classified into two variants such as HHV-6A and HHV-B. It is now known that HHV-6B is the causative agent of exanthem subitum (ES). ES is a common disease of infancy characterized by high fever and rash for a few days. Symptoms are usually mild, but febrile convulsion often occurs, suggesting the invasion to central nervous system. HHV-6 seroprevalence decreases from 0 to 5 months of age, as maternal antibody wanes. Beginning at about 6 months, seroprevalence increases rapidly, with almost all children becoming positive by 2 years of age. Infants appear to be protected against HHV-6 infection by maternal antibody. HHV-6 infects latently in monocyte/macrophage after the primary infection, and reactivated by some factors. Interestingly, HHV-6 genome found to be integrated in chromosomes of some individuals. Diseases possible associated with HHV-6 infections are reported such as chronic fatigue syndrome (CFS), Multiple sclerosis (MS), Progressive multifocal leukoencephalopathy (PML) and Drug Hypersensitivity.

HHV-6 & CNS DISEASE - I

14-1 HHV6-A in MS etiology

Oral

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Background: Both Epstein-Barr virus (EBV) and HHV-6A have been suggested as risk factors for the development of multiple sclerosis (MS). In later years it has been shown that MS is preceded by a prodromal phase with elevated levels of serum neurofilament light chain (sNfL), a marker of axonal injury.

Objective: Our aims were 1) to study if HHV-6A serostatus was associated with the level of sNfL in the multiple sclerosis prodrome, which would support a causative role of HHV-6A, and 2) to assess if HHV-6A and EBV seroreactivities interact regarding the risk of developing MS.[1,2]

Method: We used our Swedish biobank material of serum samples collected several years before the clinical onset from 670 MS cases, and 1:1 matched controls.

Results: In cases, seropositivity of HHV-6A was significantly associated with the level of sNfL (+11%, 95% CI 0.2–24%, P = 0.045), and most pronounced in the younger half of the cases (+24%, 95% CI 6–45%, P = 0.007). We also found a significant interaction between EBV and HHV-6A seroreactivities in those above the median age of 24.9 years (attributable proportion due to interaction = 0.44).

Conclusion: HHV-6A antibodies both precede and are associated with a higher degree of axonal injury, supporting that HHV-6A infection may contribute to the development of MS in a proportion of cases. Our findings also support that HHV-6A and EBV infections may interact in MS development.

[1]Grut V, Biström M, Salzer J, Stridh P, Jons D, Gustafsson R, Fogdell-Hahn A, Huang J, Butt J, Lindam A, Alonso-Magdalena L, Bergström T, Kockum I, Waterboer T, Olsson T, Zetterberg H, Blennow K, Andersen O, Nilsson S, Sundström P. Human herpesvirus 6A and axonal injury before the clinical onset of multiple sclerosis. Brain 2024 Jan 4;147(1):177-185. doi: 10.1093/brain/awad374.

[2]Grut V, Biström M, Salzer J, Stridh P, Jons D, Gustafsson R, Huang J, Bergström T, Kockum I, Waterboer T, Olsson T, Sundström P. Interactions between high seroreactivity to human herpes virus 6A and Epstein-Barr virus in MS development – a presymptomatic case-control study. Ann Neurol 2024 Aug;96(2):302-305. doi: 10.1002/ana.27009

14-2 Whole genome sequences reveal a higher prevalence of HHV-6 integration in synucleinopathies

Oral

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Objective: The infectious hypothesis of Alzheimer's disease (AD) theorizes that pathogens like herpesviruses may play a role in dementia-related neurodegenerative diseases through multiple mechanisms, including viral genome integration. While some herpesviruses, such as human herpesvirus 6 (HHV-6), are known to integrate into human host genomes, the frequency of viral genome integration in dementia patients is generally unknown. The goal of this study was to characterize the prevalence of herpesvirus genome integration in whole genome sequences (WGS) from dementia and control cohorts.

Methods: The publicly available PathSeq tool was used to analyze over 7,500 total WGS from control, frontotemporal dementia/amyotrophic lateral sclerosis spectrum (FTD/ALS), Lewy body dementia (LBD), multiple system atrophy (MSA), and AD cohorts for the integration of pathogen genomes, with a focus on

neurologically relevant herpesviruses including HHV-6.

Results: Low positive PathSeq scores less than 8,000 for HHV-6 were consistent with the integration of variably sized HHV-6 genome segments. The LBD and MSA cohorts had a significantly higher prevalence of individuals with this partial HHV-6 genome integration when compared to the control cohort. This higher prevalence in both synucleinopathy cohorts was not found in the other herpesviruses analyzed in this study.

Conclusions: These findings suggest that herpesvirus genome integration, specifically the integration of HHV -6, may play a role in a subset of LBD and MSA patients. Further investigation of the possible role of herpesvirus genome integration in the pathogenesis of neurodegenerative disease and dementia is warranted.

14-3 Engineering all-human models of the impact of HHV6 on the brain, using human iPSC-derived 2D and 3D cultures.

Oral

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HHV6 infection is associated with rare but severe neurological outcomes, and has been implicated in chronic diseases of the brain. While the neuronal and glial tropism of HHV6 has been investigated in various models, our understanding of the events occurring after a viral incursion into the central nervous system continue to elude us. This is in part due to a historical lack of access to primary samples representing early stages of infection, and lack of tractable experimental models that best represent human brain biology. Following the advent of reprogramming, we developed novel tissue culture models of interaction between neurons and glia. In particular, we pioneered methods to generate microglia, the brain's resident sentinels, from human pluripotent stem cells. We also engineered conditions to study their biology in co-cultures that better replicate their physiological properties. We applied these technologies to the study of inflammatory etiologies in neurodevelopmental such as autism, and neurodegenerative disorders such as multiple sclerosis or Alzheimer's disease. In our systems, we can recapitulate mature neuronal activity as well as myelination, uniquely in the presence of microglia. We have made strides towards the development of inflammatory demyelination models. All our efforts benefit from the reductionist scale of our models, which contain only the four primary cell types of interest to us, namely excitatory neurons, astrocytes, oligodendrocytes and microglia. Importantly, these four cell types are always isogenic to each other, and can be matched to individual patients. We can control the composition of the cultures, and build organoids that contain controllable numbers of the different cell types, to maximize reproducibility. To study these cells individually and together, we developed a single serum-free medium, ensuring that our results are devoid of culture artefacts coming from blood product exposure, a common confounding problem. Monotypic cultures allow us to study the cell-autonomous impact of disease factors, including viruses like HHV6A/B, while organoids are instrumental in understanding the complex tissue-level cellular interaction between virus exposed cells and their uninfected neighbors. We identified that microglia are likely a key underrated targets for HHV6, and are affected very differently by viral subtypes: HHV6A triggers their acute cell death, while HHV6B does not. However, both viruses affect the cells and elicit major antiviral and inflammatory responses. Astrocytes displayed a major differential response to the viruses, with productive infection by HHV6A complete with fusion events and evidence of viral replication and persistence, while HHV6B elicited minimal host transcription. In organoids, we observed that all cell types, including oligodendrocytes, mounted an antiviral response. Remarkably, the extent of this response was similar for HHV6A and HHV6B, hinting at tissue level vulnerabilities that are absent in 2D cultures. In particular, a cluster of reactive astrocytes emerged in infected organoids, and this impact was similar for HHV6A and HHV6B. We have evidence of acute alterations of neuronal electrophysiology following exposure to HHV6A. We are actively investigating the consequences of microglial death on surrounding cells. Microglial have emerged as key determinants of brain health, and their reactions can have indirect effects on the electrical units of the brain, the neurons and the oligodendrocytes, via astrocytic intermediates. Elucidating these events will pave the way for targeted interventions in acute infections, and help us establish causal relationships between HHV6 exposure and long-term neuronal and glial health, with implications for chronic diseases across the lifespan.

14-4 Investigating the differential vulnerability of microglia and astrocytes to HHV-6A exposure, and the impact of selective infection on neuronal function

Oral

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Background & Objective: Nearly everyone on earth is infected for life by a human herpesvirus. Approximately 90% of infants have already been infected by HHV-6 by the age of two[1,2]. HHV-6 is closely associated with neurological disorders, including epilepsy, encephalitis, and chronic diseases such as multiple sclerosis[3,4,5]. Understanding its effects on the central nervous system (CNS) is challenging due to the limited availability of primary human samples[6,7]. Stem cell engineering offers a novel platform to study HHV-6 in CNS cells derived from pluripotent stem cells (PSCs). We aim to investigate the differential vulnerability of microglia to HHV-6 exposure, viral persistence in astrocytes, and the electrical changes in neurons caused by HHV-6 infection.

Method: We differentiated induced pluripotent stem cells (iPSCs) into neurons and glial cells using NGN2-induction or small molecule supplementation. We infected 2D neuronal and glial monocultures and 3D immune-competent cortical spheroids with HHV-6 at a multiplicity of infection (MOI) of 3. To identify differentially regulated pathways, we performed RNA-sequencing and single-cell RNA-sequencing. Using live imaging at three frames per hour over 72 hours, we monitored microglial monocultures to assess cell death and phenotypic changes after HHV-6A exposure. We measured lytic cell death in microglia through LDH assays. To assess viral neurotropism, we conducted immunofluorescence staining of infected monocultures for immediate early (IE1) and late viral proteins (GP102, GP116). To investigate acute and chronic effects on astrocytes, we infected astrocytes with HHV-6A, examined their proliferation over 72 hours, and evaluated persistent infection 30 days post-exposure. We used multi-electrode array (MEA) recordings to analyze neuronal electrical activity in 2D monocultures for 24 hours and 7 days post-exposure to HHV-6A.

Results: HHV-6A efficiently infected iPSC-derived microglia, astrocytes, and neurons. Infection activated interferon response pathways, notably IFI6 and IFIMT3, in both 3D spheroids and 2D monocultures. Microglial monocultures exhibited a 95% decrease in viability, along with reduced cell speed and displacement. Infected astrocytes displayed a decline in proliferation during acute infection over 72-hours of initial infection and persistent viral presence 30 days post-exposure with the formation of syncytia. Neuronal infection at an MOI of 10 reduced firing synchronicity between neurons, while enhancing rhythmicity and bursting of individual neurons.

Conclusion: HHV-6A elicits a pronounced pro-inflammatory response in CNS cells. Microglia and neurons are acutely affected, whereas astrocytes serve as viral reservoirs, enabling potential reactivation. These findings advance our understanding of HHV-6A's impact on the CNS and may inform therapeutic strategies to protect against infection or suppress reactivation.

[1]Okuno, T., *et al.* (1989). Seroepidemiology of human herpesvirus 6 infection in normal children and adults. *Journal of clinical microbiology*, *27*(4), 651–653.

[2]Scheurer, M. E., *et al.* (2013). HHV-6 encephalitis in umbilical cord blood transplantation: a systematic review and meta-analysis. *Bone marrow transplantation*, *48*(4), 574–580.

[3]Bertolani, M. F., *et al.* (1996). A study of childhood febrile convulsions with particular reference to HHV-6 infection: pathogenic considerations. *Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery*, *12*(9), 534–539.

[4]Kawamura, Y., *et al.* (2015). Pathogenic Role of Human Herpesvirus 6B Infection in Mesial Temporal Lobe Epilepsy. *The Journal of infectious diseases*, *212*(7), 1014–1021.

[5]Challoner, P. B., *et al.* (1995). Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(16), 7440–7444.

[6]Reynaud, J. M., & Horvat, B. (2013). Animal models for human herpesvirus 6 infection. *Frontiers in*

microbiology, *4*, 174.
[7]Ptaszyńska-Sarosiek, I., *et al.* (2019). Post-mortem detection of six human herpesviruses (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6) in trigeminal and facial nerve ganglia by PCR. *PeerJ*, *6*, e6095.

14-5 Pathogenic role of herpesvirus dUTPases in chronic diseases

Oral

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HHV-6 and other herpesviruses including Epstein-Barr Virus (EBV) are often associated with chronic neurological diseases. However, there is insufficient mechanistic data to link herpesviruses to any disease pathology directly. All the human herpesviruses including HHV-6 and EBV acquire lifelong latency in humans. Reactivated HHV-6 and EBV infection is associated with multiple human diseases. A long-appreciated yet elusive pathogenic role exists for herpesvirus early protein, deoxyuridine 5'-triphosphate pyrophosphatases (dUTPase). We followed an unbiased approach to characterize the potential molecular function of all the nine herpesvirus dUTPase proteins. Here, we show that even though all nine human herpesvirus dUTPases share a unique monomeric structure and overlapping sequence homology, the cellular interactome of each protein is unique. We observed that HHV-6B and EBV dUTPases are the only herpesvirus dUTPases that are predominantly nuclear located. We conducted a massive parallel interactome analysis of all the nine herpesvirus dUTPases. EBV dUTPase, being nuclear located, interacts with major spliceosome-associated proteins U2AF1 and U2AF2 and induces widespread alternate spliced non-coding transcripts. EBV dUTPase interacts with many mitochondrial small ribosome proteins, fragments mitochondria and decreases OxPhos, potentially by interfering with their mitochondrial transport. Our results provide unparalleled information on the function of herpesvirus dUTPases, particularly that of EBV. We anticipate targeting individual herpesvirus dUTPases, providing exciting therapeutic options for treating herpesvirus-associated diseases.

HHV-6 & CNS DISEASE - II

15-1 Cutting-edge strategies to decipher the clinical impact of HHV-6

Oral

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Our focus on the tissue-resident virome has driven the development of innovative methods to study persistent viruses. The high seroprevalence and genoprevalence of HHV-6 present a challenge to the differentiate between health and disease, highlighting the cirtical need to demonstrate viral reactivation in order to decode its intricate interactions with the host and uncover disease associations.

Through the optimization of cutting-edge molecular detection tools, we have uncovered the reactivation of iciHHV-6 in two transplant recipients and, remarkably, identified HHV-6 sequences integrated into mitochondrial DNA. These exciting findings open the door to a deeper understanding of the virus' potential impact on health and disease.

Building on our recent research on EBV in Multiple Sclerosis, I will present a novel approach to uncover the significance of HHV-6 in the CNS. These advanced methodologies are essential for revolutionizing the detection and monitoring of viral reactivation, pushing the boundaries of our understanding of persistent viruses and their role in disease.

15-2 HHV-6B SITH-1-Induced Brain Acetylcholine Deficiency is a Key Driver of Post-acute Sequelae of SARS-CoV-2 Infection and Improved by Donepezil

Oral

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Post-acute sequelae of SARS-CoV-2 infection (PASC) occurs in 5-10% of COVID-19 patients but no treatment has been established. The PASC symptoms of fatigue and malaise, and its depressive symptoms are a major social issue because they reduce patients' QOL and ability to work. While the cause of PASC is undetermined, reactivation of Herpesviridae viruses is a likely candidate. We discovered that antibody titers of SITH-1, a latent protein of human herpesvirus-6B (HHV-6B) and a risk factor for depression, were higher than in normal controls in 62.8-71.2% of PASC patients. In addition, in a mouse model, SITH-1 expression was involved in malaise and depressive symptoms via reduced intracerebral acetylcholine production. Furthermore, in a double-blind study on 73 patients administered the acetylcholine-esterase inhibitor donepezil, in those positive for anti-SITH- 1 antibodies, there was a significant reduction in Chalder Fatigue Scale scores, an indicator of fatigue and malaise. There was also a significant improvement in depressive symptoms. These results suggest that HHV-6B SITH-1 is involved in the majority of PASC patients and that donepezil may be effective in these patients.

15-3 Proteomic analysis of serum and cerebrospinal fluid in children with HHV-6B associated encephalopathy and complex febrile seizures

Oral

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Objective: Exanthema subitum (ES) is a generally febrile exanthematous disease of infancy by primary HHV -6B infection. However, primary infection can cause complications such as complex febrile seizures (cFS) and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD). While cFS spontaneously resolves, AESD can cause serious sequelae. Since these complications have convulsions, it is difficult to distinguish between them at the time of onset. In this study, proteomic analysis was performed in order to find biomarkers to distinguish between them.

Methods: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed in patients with AESD and cFS virologically diagnosed with primary HHV-6B infection and healthy controls. Specimens were serum and cerebrospinal fluid (CSF). Proteins were screened with a fold change greater than 1.5 or less than 2/3 and a P-value of less than 0.05. Enrichment analysis was conducted by Metascape. ELISA was performed using a large number of samples for the screened proteins for validation.

Results: Nineteen proteins in serum were screened to distinguish between AESD early seizure and cFS. MARCKS had the lowest P-value, and GOLM1 was differentially expressed between AESD early seizure and other patient groups. These proteins were validated by ELISA. The pathway of glycolysis was upregulated in patients with AESD. Although serum GOLM1 and MARCKS were not significantly different between patients with AESD early seizure and cFS, those were significantly higher at early seizure phase than the convalescent phase in patients with AESD (MARCKS; P=0.016, GOLM1; P<0.001). Moreover, MARCKS was higher in AESD than ES (P=0.015). There were no significant results in the enrichment analysis of CSF. Although CETP was screened by LC-MS/MS, it was not validated by ELISA.

Conclusions: In this study, the pathway of glycolysis was upregulated in patients with AESD early seizure. Glycolysis is an anaerobic reaction, and metabolizes glucose to pyruvate or lactate to produce ATP. It has been reported that glycolysis was upregulated in patients with herpes simplex encephalitis and West Nile encephalitis. Moreover, MARCKS in serum was elevated in patients with AESD Early seizure. MARCKS is a substrate for protein kinase C, and expressed on macrophages and promotes inflammatory responses. It has been associated with schizophrenia, bipolar disorder and Alzheimer's disease. Taken together these results in this study and previous reports, it was suggested that the pathway of glycolysis and MARCKS might be involved in the pathogenesis of AESD. In the future, further research of this pathway or protein may lead to diagnosis, prevention, and treatment of AESD.

15-4 *Oral* Association between adeno-associated virus 2 and neurological complications of pediatric human herpesvirus 6B infection.

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Objective: The adeno-associated virus (AAV), belonging to the Parvoviridae family, has emerged as a pivotal tool in clinical gene therapy due to its broad tissue tropism. Although AAV is generally considered non-pathogenic and requires co-infection with "helper viruses" for replication, concerns remain about the safety of high-dose viral therapies, including immune responses and adverse effects such as hepatotoxicity. Notably, several studies reported that co-infection with AAV2 and helper viruses such as adenovirus and herpesviruses were related to worldwide outbreaks of pediatric unexplained acute hepatitis in 2022. Since children are frequently exposed to primary viral infections, co-infections with AAV2 and herpesviruses may contribute to conditions other than hepatitis. This study retrospectively analyzed the association between AAV2 co-infection and neurological complications of primary human herpesvirus-6B (HHV-6B) infection in children.

Methods: A total of 158 pediatric patients with primary HHV-6B infections, with or without neurological complications, diagnosed between 2005 and 2022, were retrospectively analyzed. Patients were categorized into three groups: (1) encephalitis/encephalopathy (n = 36, mean age: 1.0 year), (2) febrile seizure (n = 39, mean age: 1.4 years), and (3) no neurological complications (n = 83, mean age: 1.6 years). DNA was extracted from

sera obtained at diagnosis. Primary HHV-6B infection was defined as the detection of viral DNA in serum and the absence of HHV-6B immunoglobulin G antibodies. Real-time PCR assays were performed to detect AAV2 and HHV-6B DNA, targeting the ITR and U31 regions, respectively.

Results: AAV2 DNA was detected in 11% (4/36) and 8% (3/39) of patients with encephalitis/encephalopathy and febrile seizures, respectively. In contrast, AAV2 was not detected in patients without neurological complications (0/83). The frequency of AAV2 detection was significantly higher in patients with encephalitis/encephalopathy and febrile seizure compared to those without neurological complications ($p = 0.01$ and $p = 0.03$, respectively). The median AAV2 DNA loads in encephalitis/encephalopathy and febrile seizure case were 2.2×10^6 and 2.5×10^4 copies/mL, respectively, with no significant difference between the two groups. In four encephalitis/encephalopathy cases suspected of viral co-infection, serum AAV2 and HHV-6B DNA loads decreased over time. AAV2 DNA was detected in the cerebrospinal fluid of two of four cases.

Conclusions: These findings suggest that co-infection of AAV2 and HHV-6B is associated with neurological complications such as encephalitis/encephalopathy and febrile seizure in pediatric patients. An abnormal immune response to AAV2 or its central nervous system tropism may contribute to these complications.

15-5 Plasma proteomic profiles of pediatric patients with human herpesvirus 6B encephalitis following umbilical cord blood transplantation

Oral

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Objective: Human herpesvirus 6B (HHV-6B) reactivation is commonly observed following allogeneic hematopoietic stem cell transplantation. Although typically asymptomatic, HHV-6B reactivation can lead to HHV-6B encephalitis, a condition associated with high mortality and long-term sequelae. Umbilical cord blood transplantation (UCBT) is considered a risk factor for HHV-6B encephalitis. This study aimed to investigate the pathogenesis of HHV-6B encephalitis by comparing plasma proteomic profiles between pediatric UCBT recipients with HHV-6B encephalitis and those with asymptomatic HHV-6B reactivation.

Methods: Preserved plasma samples from four pediatric patients with HHV-6B encephalitis (median age: 5.1 years) and three with asymptomatic HHV-6B reactivation (median age: 8.7 years) were retrospectively analyzed. HHV-6B encephalitis was diagnosed based on neurological symptoms and HHV-6B DNA detection in cerebrospinal fluid, while asymptomatic HHV-6B reactivation was defined by increased HHV-6B DNA loads in whole blood without clinical symptoms. Plasma proteomic profiling was performed using liquid chromatography–mass spectrometry (LC–MS). Samples collected prior to the onset of HHV-6B encephalitis or asymptomatic reactivation were used to compare protein expression. Pathway enrichment analysis was conducted to identify biological pathways associated with the differentially expressed proteins.

Results: A total of 260 proteins were identified and quantified in plasma samples using LC–MS. At the onset of HHV-6B encephalitis or asymptomatic reactivation, 20 and 24 proteins were significantly upregulated compared to pre-onset levels, respectively. Among these, 11 proteins were exclusively upregulated in patients with HHV-6B encephalitis. S100-A9 and -A8, primarily expressed by neutrophils and monocytes, were the most and the second most upregulated protein in patients with HHV-6B encephalitis. Elevated plasma S100A8/A9 heterodimer levels were further confirmed using ELISA in three of four patients with HHV-6B encephalitis. In contrast, significant downregulation of 29 and 40 proteins was observed in HHV-6B encephalitis and asymptomatic reactivation, respectively, with eight overlapping between the groups. Pathway analysis revealed neutrophil degranulation as the most enriched category among upregulated proteins in HHV-6B encephalitis. Additionally, proteins related to the protein-lipid complex remodeling pathway were upregulated in HHV-6B encephalitis compared to asymptomatic reactivation.

Conclusions: Proteomic analysis revealed distinct plasma protein profiles between HHV-6B encephalitis and asymptomatic HHV-6B reactivation in pediatric UCBT recipients. The inflammatory response mediated by S100A8/A9 proteins may play a critical role in the pathogenesis of HHV-6B encephalitis. Proteomic analysis

may provide new insights into the host response to HHV-6B reactivation and development of HHV-6B encephalitis following hematopoietic stem cell transplantation.

15- 6 Using single-cell SLAMseq to understand static vs dynamic behavior of PBMCs in Chronic Fatigue Syndrome

Poster

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Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS) is a complex disease with many potential unknown triggers. Immune and metabolic alterations are the most common features in ME/CFS patients. The development of innovative systems biology approaches over the last decade has helped us understand many unknown aspects of the disease at the single-cell level. However, the common belief that permanent characteristic alterations in ME patients at genetic and epigenetic levels can be quantified at the molecular level has not led to any findings that can be translated to clinics. In 2018, for the first time, single-cell SLAMseq (scSLAM-seq) was used to show that genes are not only up or down-regulated. They are also switched on and off under specific physiological requirements. We hypothesize that this dynamic nature of genes and pathways defines ME/CFS. Cell-specific and stimulus-specific variations in pathways also add more complexities to the patient's response to external stimuli like infection, exercise, food, temperature etc. Single-cell SLAM- seq was initially pioneered for studying rapid transcriptional responses at the single-cell level. This approach initially relied on flow cytometry-mediated sorting individual cells into 96-well plates. While the published approach was restricted to analyzing dozens to a few hundred cells, we developed scSLAM-seq for state-of-the-art droplet-based single-cell RNA-seq platforms, i.e., 10x Chromium sequencing. As a proof-of-concept study done in PBMCs collected from ME/CFS patients and healthy individuals, we have gathered strong evidence suggesting a potential role of monocytes in modulating immune response in ME patients under various stimuli like extracellular ATP and viral infection. We have identified key alterations in pathways that explain the altered behavior of platelets and mast cells in a significant subset of ME patients, paving the way to a potentially successful treatment strategy. Our study deciphers the dynamic differences in behavior of various subsets of blood cells in ME patients.

15-7 Two cases of acute cerebellitis encephalopathy associated with primary HHV-6B infection

Poster

Yoshiyuki Asai, Ayami Yoshikane, Gen Furukawa, Naoko Ishihara, Hiroki Miura, Junichi Kawada, Tetsushi Yoshikawa

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Introduction. Although acute encephalopathy is a frequent neurological complication of primary HHV-6B infection, cerebellar manifestations are rarely seen, and there have been no reports of dysarthria as a sequelae. We report two cases of older children with exanthem subitum who presented with mutism during the acute phase of HHV-6B encephalopathy and subsequently developed dysarthria as a sequela. In this study, we report a case of HHV-6B encephalopathy in the acute phase.

Case 1: A 4-year-old boy with normal development. On day 3 of fever, he was admitted to the hospital with a tendency toward somnolence. An electroencephalogram (EEG) showed high amplitude slow waves, and a head MRI showed high-signal areas in the corpus callosum and deep white matter of the cerebrum on diffusion-weighted images, leading to the diagnosis of acute encephalopathy, and steroid pulse therapy was started. On the 5th day of illness, the patient developed fever and skin rash, and serum real-time PCR was positive for HHV -6B. He had been mute and unable to maintain a sitting position since admission but started to speak approximately on the 35th day and was able to walk unassisted. On the 49th day, cerebral blood flow scintigraphy showed decreased blood flow in the cerebellum. Six months after the onset, she was able to form three-word sentences but still had ataxic dysarthria.

Case 2: 3-year-old girl with normal development. She was admitted to the hospital because of decreased responsiveness on the third day of fever. On the 5th day of illness, her fever resolved and a skin rash appeared. Primary HHV-6B infection was confirmed by seroconversion of HHV-6B IgG antibody. An electroencephalogram (EEG) showed high amplitude slow wave contamination, and the patient was diagnosed with acute encephalopathy and given steroid pulse therapy. On the 15th day, the patient response increased, but she remained mute and still had difficulty sitting without assistance. On the 23rd day, cerebral blood flow scintigraphy showed decreased cerebellar blood flow. Even after she was able to walk unassisted, her speech was

limited to babbling.

Conclusion: We have experienced two cases of cerebellitis associated with primary HHV-6B infection in older children, both of whom developed long-term dysarthria as a sequela. Cerebellar symptoms can be difficult to identify in infants; however, recent increases in cases of primary HHV-6B infection in older children, such as the two cases presented here, may have made these symptoms easier to recognize. It is important to remain vigilant for similar cases, as cerebellar involvement may represent one form of the central nervous system complications associated with primary HHV-6B infection.

LUNCH & SWEETS SEMINAR (BIKEN) Room 407

16-1 Vaccine developments

Oral

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About four years ago, the new coronavirus pandemic spread worldwide, frightening us. There were also dangerous infectious diseases that affected the population and claimed many lives. For example, about 100 years ago, there was a new type of influenza called the Spanish flu, 200 years ago, cholera, and 300 years ago, the plague, and during that time, smallpox infections continued around the world. Vaccination is a way to artificially build up resistance (immunity) in us, but it was not used until the smallpox vaccine (vaccination) about 200 years ago. In recent years, many infectious diseases can be prevented by vaccination. There are two types of vaccines: one that gives immunity by putting in the body a large amount of inactivated pathogen, and one that gives immunity by putting a live (attenuated) pathogen (live vaccine) into the body and multiplying it throughout the body. Mesenger RNA vaccine is the former. However, when a new infectious disease suddenly appears, like the current COVID-19, it is difficult to take measures. At that time, the mRNA vaccine is useful quickly. Historically, many live vaccines such as smallpox, polio, and measles have been used to control infectious diseases. In my talk, I am going to talk on the development of live varicella vaccine.

DIAGNOSTICS & TREATMENT

17-1 Prospects for an HHV-6 cure

Oral

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The human herpesviruses share the common characteristic of establishing lifelong infections in their hosts, which can have substantial and detrimental health effects. Despite this, research into curative therapies for herpesviruses lags significantly behind that of other chronic viruses like HIV and hepatitis B. In this talk, I will argue that at least some human herpesviruses, specifically the alphaherpesviruses, are among those most amenable to cure. Importantly, the aspirational goal of cure can galvanize and energize the field of herpesvirus research. Drawing on our work with alphaherpesviruses and the broader field of viral cure research, I will discuss the arguments for pursuing an HHV-6 cure, potential approaches that might be applied, and the unique aspects of HHV-6 biology that must be addressed before a cure can be realized.

17-2 Epigenomic orofiling of HHV-6 infections using rapid readout of viral DNA methylation

Oral

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Objective: Human herpesvirus 6A/B (HHV-6) is responsible for infectious complications such as encephalitis and febrile seizures. HHV-6 is diagnosed late and often imprecisely since qPCR diagnostics cannot differentiate lytic (infectious) from latent (silent/dormant) HHV-6 states. Inherited chromosomally integrated HHV-6 (iciHHV-6) is characterized by the integration of the HHV-6 genome into every nucleated cell within the human host, resulting in persistently high viral DNA loads that further confound the interpretation of qPCR assays. Herpesvirus latency is maintained through epigenetic repression via DNA methylation of viral genes. Lytic reactivation requires DNA methylation removal. We hypothesized that HHV-6 DNA methylation is a surrogate for virus activity that holds potential for future biomarker applications.

Methods: We established a hybrid PCR-mass spectrometry assay that enables us to profile HHV6A/B DNA methylation. The assay delivers quantitative data on dozens of methylation sites across the HHV-6 genome, covering important regulatory sites of lytic and latent virus activity. It can be performed in 12 hours and is highly robust in cell-free plasma/serum DNA with as low as 400 HHV-6 total DNA copies. We utilized this assay on freshly/latently HHV-6-infected cell lines (MOLT3, SupT1, THP-1), two iciHHV-6 lymphoblastoid cell lines and patient-derived tissue/liquid biopsies (n=11). We further used Nanopore DNA sequencing to comprehensively read out native DNA methylation in an iciHHV-6B line.

Results: We report distinct low (MOLT3), intermediate (SupT1, THP1) and high (iciHHV-6) DNA methylation patterns for HHV-6. HHV-6 gained DNA methylation over time in cell culture, and this increase was exacerbated when cells were challenged with the antiviral ganciclovir (GCV). As a proof-of-concept we analyzed HHV-6 DNA methylation in a patient who presented with seizures and persistently high HHV-6 DNA levels despite high-dose GCV treatment. Analysis of patient matched plasma, blood mononuclear cells and bone marrow revealed consistently high levels of HHV-6 DNA methylation. Coupled with our in vitro data, we highlight DNA methylation patterns linked specifically to iciHHV-6 ($p < 0.001$). Nanopore sequencing revealed a full viral iciHHV-6B methylome with high global DNA methylation but also localized DNA methylation absence.

Conclusions: Antivirals like GCV require phosphorylation by lytic viral kinases and are thus active against lytic HHV-6 only. There is an unmet clinical need for better diagnostics that can guide informed screening, diagnosis and finally treatment of HHV-6 associated conditions. We propose that DNA methylation readout can identify patients with HHV-6 infection/reactivation who will respond favorably to antiviral therapy and

distinguish them from iciHHV-6.

17-3 Oral HIV Nanoheroes: novel CRISPR-Cas9-based CD4 + cell-specific HHV-7 vectors to cure HIV

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Objective: HHV-7 is a generally asymptomatic virus that infects a majority of the global population, often during early childhood. It primarily targets CD4 + cells, an interesting commonality with human immunodeficiency virus (HIV). So far, HIV remains incurable due to the integrated provirus, for which excision by CRISPR-Cas9 offers a potential cure strategy. However, conventional delivery methods face challenges such as sequestration by off-target cells and failure to cross tissue barriers, preventing them from effectively reaching HIV-infected cells in vivo. We propose a novel vector, HIV Nanoheroes, based on replication-incompetent HHV -7. HIV Nanoheroes will deliver CRISPR-Cas9 ribonucleoproteins targeting the viral genome and the CCR5 coreceptor, resulting in the removal of the HIV provirus and ablation of CCR5 surface expression preventing re-infection.

Methods: Several structural HHV-7 proteins were selected for conjugation to Cas9. Prior to HIV Nanohero production, these structural proteins were linked to monomer red fluorescent protein 1 (mRFP1). We used a CRISPR-Cas9-based method of homology directed repair (HDR), where HEK293T and SupT1 cells were lentivirally transduced to express Cas9, as verified by ELISA (507-2588 ng/mL Cas9), and sgRNAs targeting the selected HHV-7 capsid and tegument proteins. mRFP1 donor plasmids were simultaneously transfected during HHV-7 infection to yield fluorophore-tagged HHV-7 mutants. Isolation of these mutants was mediated by plaque purification or single cell sorting. In the next step, HDR will be used to exchange mRFP1 for sgRNA-Cas9 ribonucleoproteins and non-fluorescent viruses will be selected.

Results: Using our CRISPR-Cas9-based method, we produced five different mRFP1-tagged HHV-7 mutants. Propagation and characterization of these mutants is ongoing. We have identified sgRNAs that efficiently excise an essential large genome fragment from HIV proviruses in 50.89 ± 10.34 % of an HIV-infected cell line and additionally disrupt essential open reading frames (ORFs) in 21 ± 6 %. Further, we selected a sgRNA that disrupts CCR5 with 76 ± 8 % efficacy. To validate HIV Nanoheroes for their efficacy to cure HIV in vivo, we have established the NSG-SGM3 humanized mouse model for HIV infection, exhibiting 62.9 ± 18.9 % huCD45 cells/total PBMCs, with 33.27 ± 16.50% hCD4/hCD45 cells, and allowing HIV propagation (viremia levels of 10 5.8 -10 6.2 vRNA copies/mL plasma) making it a valuable model for evaluation of our novel cure strategy.

Conclusions: Together, the specificity of HHV-7 in targeting cells within the tropism of HIV, combined with the accuracy and efficiency of selected gRNAs, make the HIV Nanoheroes a promising tool for a definitive HIV cure.

17-4 Poster Protective effects of a novel ketone-based compound on corneal cells Infected with human herpesvirus 6A: an in vitro study

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Introduction: Human Herpesvirus 6A (HHV-6A) is a neurotropic virus implicated in various diseases, including encephalitis and potential links to neurodegeneration. While much of the existing research focuses on systemic viral behavior, its effects on corneal cells—a critical interface for viral entry—remain underexplored. It has been shown that HHV-6 may be another causative agent for corneal ulcers and conjunctivitis1,2. This study evaluates the protective effects of a novel ketone-based compound known for its anti-inflammatory and neuroprotective properties, on corneal cells infected with HHV-6A.

Materials and Methods: An in vitro infection model was established using corneal epithelial cells exposed to HHV-6A (0.1 PFU). Three experimental conditions were tested: pre-treatment with 10mM the ketone-based compound 1 hour before infection, co-treatment during infection, and post-treatment 1 hour after infection.

Infection efficiency was assessed via fluorescence microscopy using HHV-6A-expressing green fluorescent protein (GFP) (courtesy of Prof. Benedikt Kaufer). Additionally, the effects of the ketone-based compound on gene expression, inflammatory cytokine secretion, mitochondrial activity, and apoptosis markers were quantified using ELISA, and mitochondrial activity assay, respectively.

Results: HHV-6A successfully infected corneal cells, with GFP expression peaking at 3 days post-infection (DPI). All the three conditions of the ketone-based compound treatment significantly reduced infection efficiency in all conditions, with the most pronounced effects observed in post-treatment ($p < 0.01$). Inflammatory cytokine levels, including IL-6 and TNF-α, were elevated in infected cells but were normalized following the ketone-based compound treatments ($p < 0.05$). Mitochondrial activity, assessed via Mitotracker staining, was restored to baseline in the ketone-based compound-treated groups, suggesting a protective effect against viral-induced mitochondrial dysfunction. Apoptotic markers, such as caspase-3, were significantly reduced in the ketone-based compound-treated cells compared to untreated infected controls ($p < 0.001$). Gene expression analysis (RNA seq) resulted in pathway analysis with 314 genes. The ketone-based compound treatments enhanced Interferon alpha and beta signaling pathway (R-HAS-909733), antiviral innate immune response (GO: 0140374), pathways of nucleic acid metabolism and innate immune sensing (WP4705). Real-time RT-PCR analysis of selected genes from these pathways revealed a significant upregulation of IFI16, IFIT1, IFIT2, IFIT3, STAT1, CCL2, RIG-I, and CREB5 in HHV-6A-infected cells treated with the ketone-based compound, in all the three conditions.

Discussion: These findings indicate that the ketone-based compound mitigates HHV-6A-induced cytotoxicity in corneal cells. By reducing viral infection efficiency, normalizing inflammatory responses, inducing immune response and protecting mitochondrial function, the compound demonstrates potential as a therapeutic intervention for viral infections affecting ocular tissues. The similar impact of pre-, co-, and post-treatment conditions underscores the versatility of the ketone-based compound in various stages of infection. Future research should focus on in vivo validation and exploration of the ketone-based compound's mechanisms at the molecular level to confirm its applicability in clinical settings.

References:

1. Ana Boto-de-los-Bueis, Maria P Romero Gómez, Almudena del Hierro Zarzuelo, Eugenia G Sanchez, Soraya Mediero, Susana Noval. Recurrent ocular surface inflammation associated with human herpesvirus 6 infection. Eye Contact Lens. 2015; 41(3):e11-3. doi: 10.1097/ICL.0b013e3182a70a1b.

2. Toshiomi Okuno, Laura C Hooper, Roxana Ursea, Janine Smith, Robert Nussenblatt, John J Hooks, Kozaburo Hayashi. Role of human herpes virus 6 in corneal inflammation alone or with human herpesviruses. Cornea 2011; 30(2):204-7. doi: 10.1097/ICO.0b013e3181e2e9be.

KEYNOTE

18-1 The path forward for HHV-6 research

Oral

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There has been significant progress in demonstrating the clinical significance of HHV-6 recent years. However, there is to date no therapeutic or preventative agent for HHV-6 that is approved by regulatory agencies with a specific indication for HHV-6. There are several important steps that must be undertaken to make HHV-6 a therapeutic target that will be pursued by pharmaceutical companies. This will take a coordinated approach of stakeholders, including the HHV-6 research community, academic clinicians, pharmaceutical industry, and regulators. This presentation will outline the path forward to accelerate the development of agents that treat or prevent HHV-6.