Abstract booklet

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In an attempt to limit excessive use of paper, printouts will not be provided during the event.

Session 1 - Viral evolution and epidemiological modeling

Using mathematical models to analyze epidemic data and gain insights on epidemic dynamics and control

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Keywords. Epidemic dynamics, mathematical model, transmission

Abstract: Different types of epidemiological data can be collected during infectious disease epidemics. This includes surveillance data, serological surveys, cohorts, transmission studies and outbreak investigations. While these data can provide essential information on the epidemic and its drivers, interpretation can be difficult for a number of reasons such as missing data, under-reporting, selection bias, imperfect assays, complex dynamics... In this this talk, I will discuss how mathematical models have become an essential tool to strengthen the analysis and interpretation of these data, and gain key insights on epidemic dynamics and control strategies. The talk will be illustrated with examples considering various pathogens including respiratory and vector-borne pathogens.

Evolution of SARS-CoV-2: Now And Then

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To elucidate the virological characteristics of newly emerging SARS-CoV-2 variants in real-time, I launched a consortium, “The Genotype to Phenotype Japan (G2P-Japan)”. With the G2P-Japan consortium colleagues, we have revealed the virological characteristics of SARS-CoV-2 variants. In this talk, I briefly introduce the scientific activity of G2P-Japan consortium and would like to discuss the possibility for international collaboration to combat the outbreaks and pandemic that will happen in the future.

Comprehensive RNA virus detection from metatranscriptome data using NeoRdRp

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Keywords. Database, Bioinformatics, Viral evolution, Metatranscriptome, RNA virus diversity

Background. Metatranscriptome sequencing analyses have revealed diverse RNA viruses in various environments. However, comprehensive RNA virus detection methods have not been fully established due to a high diversity of RNA genome sequences. Therefore, we focused on RNA-dependent RNA polymerase (RdRp) that is essential for almost all RNA viruses and developed a hidden Markov model (HMM) dataset to enhance RNA virus detection.

Materials & Methods. We collected 557,197 RdRp-containing amino acid sequences from public databases as a seed RdRp dataset, including the RdRd dataset collection of four independent RNA virome studies. From these sequences, 19,394 RdRp HMM profiles were generated through 1) duplicate elimination and clustering using CD-HIT, 2) generation of multiple alignments for each cluster using MAFFT, and 3) segmentation of gap regions.

Results. We evaluated the performance of NeoRdRp2 using the UniProtKB database containing 565,254 amino acid sequences. As a result, hmmsearch with NeoRdRp2 detected 831 of 836 RdRp sequences; 188 sequences were incorrectly identified as RdRp (99.4% reproducibility and 81.6% specificity). A comparison with eight other RdRp (or RNA virus) search tools indicated that NeoRdRp2 provided comprehensive and balanced detection of RdRp sequences.

Conclusions. NeoRdRp could enhance the identification of unrevealed RNA viruses from various metatranscriptome sequencing data; the 19,394 HMM profiles and the 557,197 seed RdRp sequences and their annotation are freely available at https://github.com/shoichisakaguchi/NeoRdRp.

Species tropism of *Orthohepadnavirus*

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Keywords. HBV, Domestic cat hepadnavirus, entry receptor, NTCP

Background.
The *Orthohepadnavirus* genus includes the hepatitis B virus (HBV), which can cause chronic hepatitis and hepatocarcinoma in humans. Recently, a novel hepadnavirus in cats, domestic cat hepadnavirus (DCH), was identified as genetically close to HBV. DCH infection is associated with chronic hepatitis in cats, suggesting a similarity with HBV pathogenesis and the potential to use DCH as a novel animal model for HBV research. Although HBV uses the sodium/bile acid cotransporter (NTCP) as a major entry receptor, the equivalent receptor for DCH remains unknown. Here, we sought to identify the entry receptor for DCH.

Materials & Methods. We synthesized HBV and DCH-derived, FAM-labeled, myristoylated preS1 peptides. Human hepatocyte-derived cell line, Huh7 cells, transiently expressing human NTCP or cat NTCP were tested for binding to myristoylated preS1 peptides.

Results.
The DCH-derived preS1 peptide efficiently binds to cat NTCP and, importantly, human NTCP. Furthermore, we demonstrated that DCH utilizes human and cat NTCPs as a functional receptor. Consistent with findings with HBV, the G158 residue of NTCP determines the species specificity of DCH. Myrcludex B, a potent inhibitor, blocked the binding of the DCH-derived preS1 peptide to human and cat NTCPs.

Conclusions. The discovery that both HBV- and DCH-derived preS1 peptides efficiently bind to both human and cat NTCPs and that residue 158 of NTCP proteins determines the species-specific binding of the DCH preS1 peptide is a significant step in our understanding of the species tropism of *Orthohepadnavirus*. The fact that Myrcludex B blocked the binding of the DCH preS1 peptide further underscores the potential of our findings. This suggests that DCH and HBV may share cell entry molecules, opening up the possibility of inter-species transmission. Moreover, our study hints at the potential of DCH infection in cats as a novel model for HBV research, a prospect that could revolutionize the field.

Session 2 - Virus entry & restriction factors

Mode of hepatitis B and D virus interaction with its host entry receptor

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Keywords. HBV, HDV, entry, viral receptor, antiviral

Background. Hepatitis B and D virus (HBV, HDV) preS1 region in the large surface protein binds to its host receptor, sodium taurocholate cotransporting polypeptide/solute carrier family 10 member 1 (NTCP/SLC10A1), and determines virion infectivity, transmission efficiency, and host tropism. However, it has long been unclear how preS1 enables a high affinity and specific interaction with NTCP. So far, we have revealed the cryo-electron microscopy structure of the apo-state NTCP and preS1/NTCP complex (Park et al. Nature 2022; Asami et al. Nat Struct Mol Biol 2024). In this study, we analyze how preS1 establishes the stable binding to NTCP, based on the structural information.

Materials & Methods. HBV and HDV infection assays were performed using HepG2 cells stably expressing human NTCP gene. The preS1 binding activity to NTCP was visualized with a peptide consisting of the 2-48 aa region of preS1 conjugated with myristoyl group and TAMRA. Dynamics of preS1 in complex with NTCP was analyzed by molecular dynamics simulation using the model for the preS1/hNTCP complex as an initial structure.

Results. PreS1 binding and HBV infection assays using a series of preS1 mutants showed that the 2-19 aa, which formed a tandem loop in complex with the bile acid tunnel of NTCP, was essential for NTCP binding and the productive viral infection. We identified the counterpart amino acids in the transmembrane 1 and 8 in NTCP that played a key role in preS1 binding susceptibility. We also found that a more C-terminal preS1 region that contacted with the extracellular surface of NTCP augmented the NTCP binding affinity.

Conclusions. We propose a unique mode for preS1-NTCP binding whereby multiple functional sites coordinately binds to NTCP through both the bile acid tunnel and the extracellular surface in a stepwise manner. This study presents a significance of the induced-fit lasso structure of preS1 in receptor recognition to secure productive virus infection.

Entry and replication of human seasonal coronavirus HKU1.

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Abstract

Four endemic seasonal human coronaviruses causing common colds, HKU1, 229E, NL63 and OC43 circulate worldwide. After binding to cellular receptors, coronavirus spike proteins are primed for fusion by transmembrane-serine protease 2 (TMPRSS2) or endosomal cathepsins. HKU1 has been shown to bind 9-O-acelytated sialic acid but its protein receptor remained elusive. We recently identified TMPRSS2 as the functional high-affinity receptor for HKU1. We also elucidated the crystal structure of the HKU1 spike RBD in complex with TMPRSS2, showing that it recognizes residues lining the catalytic groove of the enzyme. Combined mutagenesis of interface residues and comparison across human and animal species highlighted important residues in TMPRSS2 as determinants of HKU1-CoV tropism. HKU1 has not yet been amplified in large amounts in cell culture systems. We have designed a panel of cell lines expressing different versions of TMPRSS2 and are currently testing their sensitivity to HKU1 infection. Our aim is to further characterize HKU1 entry, fusion, tropism, in comparison with other seasonal coronaviruses and SARS-CoV-2.

NEDD4-binding protein 1 suppresses HBV replication by degrading HBV mRNAs

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Keywords. HBV, host factor, N4BP1, anti-HBV effect, life cycle

Background. Chronic infection with hepatitis B virus (HBV) places patients at increased risk for liver cirrhosis and hepatocellular carcinoma. Although nucleos(t)ide analogs are mainly used for the treatment of HBV, they require long-term administration and may lead to the emergence of drug-resistant mutants. Therefore, to identify targets for developing novel anti-HBV drugs, we screened for HBV-suppressive host factors using a plasmid expression library of RNA-binding proteins (RBPs).

Materials & Methods. Our screen identified NEDD4-binding protein 1 (N4BP1) having an anti-HBV effects using hepatocellular carcinoma cell lines N4BP1 and HBV co-transfected, and N4BP1/NTCP expressing hepatocytes infected with HBV. Then, we generated KH-like and RNase domains of N4BP1 deficient mutants and assessed anti-HBV effects and assessed the interaction with HBV pgRNA by RIP-assay and Northen blotting. Primary human hepatocytes (PHHs) overexpressing N4BP1 also assessed anti-HBV effects with HBs/e Ag level.

Results. Overexpression of N4BP1 decreased relaxed circular DNA (rcDNA) levels, while knockout of N4BP1 expression rescued rcDNA levels. Both the KH-like and RNase domains of N4BP1 were required for the protein's anti-HBV effects. Overexpression of N4BP1 in NTCP-expressing hepatocytes and PHHs suppressed pregenomic RNA (pgRNA) and rcDNA production, but not covalently closed circular DNA (cccDNA). So we focused on HBV mRNAs, and N4BP1 overexpressing cells degraded pgRNA more effectively than KH-like and RNase domains deficient mutatnts. RIP-assay showed N4BP1 binds with HBV pgRNA suggesting that N4BP1 suppressed HBV replication by promoting HBV mRNAs degradation. PHHs overexpressing N4BP1 also showed suppressed HBs Ag and HBe Ag.

Conclusions. In summary, we found that N4BP1 is a novel anti-HBV factor that inhibits HBV replication by promoting HBV mRNAs degradation.

Role of tetraspanins in hiv-1 and htlv-1 biofilms formation and transmission

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Keywords. HIV-1, HTLV-1, Viral biofilms, T cells, Tetraspanins

Background. The human retroviruses HIV-1 and Human T-Lymphotropic Virus type I (HTLV-1), mainly detected in CD4+T-lymphocytes, represent global health issues. HIV-1 can be transmitted by cell-cell contacts or by cell-free particles while HTLV-1 is almost exclusively transmitted during inter-cellular contacts, ie. by virological synapses and viral biofilms. Biofilms are adhesive structures polarized on the surface of infected T-cell that confine virions in a protective environment and allow their simultaneous delivery during infection. Our work aims to identify molecular determinants of these viral biofilms and their role in biofilm formation and function.

Materials & Methods. Our approach is based on a large-scale identification by mass spectrometry of proteins contained in viral biofilms isolated from chronically infected T-lymphocytes. To study the expression pattern of protein candidates and assess their presence in HTLV-1 biofilms, we used immunofluorescence coupled with confocal or stimulated-emission-depletion (STED) microscopy. shRNA silencing and drugs were used to conclude on candidate molecules' function.

Results.

We identify tetraspanins as appealing candidates, because these transmembrane proteins are involved in the organization of membrane protein microdomains and they regulate many cellular processes including cell adhesion and fusion. We previously showed that several tetraspanins were in the membrane of HIV-1 virions, but only CD81 was important for virus assembly and transmission in chronically infected T cells. We now show that several tetraspanins are also enriched in HTLV-1 biofilms and incorporated into the viral envelope of neosynthesized particles. We report that only the tetraspanin CD82 interacts with HTLV-1 Gag proteins which favors its polarization at the cell surface. We demonstrate that CD82 maintains HTLV-1 biofilm polarization and favors viral transmission, as its silencing induces a complete reorganization of viral clusters at the cell surface and reduces the ability of infected T-cells to transmit the virus. Finally, we show that CD82 cell-surface distribution was dependent on its glycosylation state, which also controlled the intercellular transmission of HTLV-1 biofilms.

Conclusion:

Altogether, these results suggest an enrichment of several tetraspanins into viral biofilms but distinct role for particular tetraspanins in viral clustering, biofilm adhesion, and polarization, which are essential for HIV-1 and HTLV-1 transmission.

Arone C, Martial S, Burlaud-Gaillard J, Thoulouze M-I, Roingeard P, Dutartre H, Muriaux D. HTLV-1 biofilm polarization maintained by tetraspanin CD82 is required for efficient viral transmission. mBio. 2023 Oct 27:e0132623. doi: 10.1128/mbio.01326-23

Abstract title: Interaction between APOBEC3 family proteins and HIV-1 in the myeloid cell line THP-1

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Keywords. APOBEC3 family proteins, HIV-1, G-to-A mutations, Vif, Deaminase-dependent mechanism, Deaminase-independent mechanism,

Abstract:

HIV-1 must overcome multiple innate antiviral mechanisms to replicate in CD4$^+$ T lymphocytes and macrophages. Previous studies have demonstrated that the APOBEC3 (A3) family of proteins (at least A3D, A3F, A3G, and stable A3H haplotypes) contribute to HIV-1 restriction in CD4$^+$ T lymphocytes. Virus-encoded virion infectivity factor (Vif) counteracts this antiviral activity by degrading A3 enzymes allowing HIV-1 replication in infected cells. In addition to A3 proteins, Vif also targets other cellular proteins in CD4$^+$ T lymphocytes, including PPP2R5 proteins. However, whether Vif primarily degrades only A3 proteins during viral replication is currently unknown. Herein, we describe the development and characterization of *A3F*-, *A3F/A3G*-, and *A3A*-to-*A3G*-null THP-1 cells. In comparison to Vif-proficient HIV-1, Vif-deficient viruses have substantially reduced infectivity in parental and *A3F*-null THP-1 cells, and a more modest decrease in infectivity in *A3F/A3G*-null cells. Remarkably, disruption of A3A–A3G protein expression completely restores the infectivity of Vif-deficient viruses in THP-1 cells both with and without interferon treatment. These results indicate that the primary function of Vif during infectious HIV-1 production from THP-1 cells is the targeting and degradation of A3 enzymes.

Session 3 - Anti-viral immune responses – Part I

Co-evolution of HIV-1 and HLA-C*14-restricted T cells

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Keywords.
Virus evolution, HIV-1, Escape mutation, HLA-C, CD8+ Cytotoxic T Cell

Background.
Co-evolution of HIV-1 with HLA-A/B-restricted CD8+ T cells were well known whereas that with HLA-C-restricted ones is not reported. Previous our study identified novel immunodominant HLA-C14-restricted epitopes by MS-based immunopeptidomics analysis. Several HLA-C14-associated mutations were found in NefYT9 (Nef120-128: YFPDWQNYT) epitope. We here investigated co-evolution of HIV-1 with NefYT9-specific T cells.

Materials & Methods.
Response to NefYT9 and its mutant peptides were measured by IFN-g Intracellular cytokine staining (ICS) assay of cultured epitope-specific T cells, and *ex vivo* ELISpots assay of PBMC from HIV-1-infected HLA-C*14^{+} Japanese individuals. PBMCs were stained with HLA-C14-tetramers to detect epitope-specific T cells. Virus sequences were analysed by PCR direct sequencing and NGS.

Results.
NefQ125H and NefQ125D mutations, which were significantly associated with the presence of HLA-C*14, were not recognized by NefYT9-specific T cells. NefYT9-specific T cells were predominantly found in individuals infected with wild-type virus whereas NefYT9-6H-specific and NefYT9-6D-speciifc T cells were in those infected with NefQ125H and with NefQ125D mutant viruses, respectively. Mixtures of wild-type and NefQ125H viruses and of NefQ125H and NefQ125D ones were detected in four and two individuals, respectively. A longitudinal analysis of an HLA-C*14+ individual demonstrated HIV-1 evolution in the order of wild-type, NefQ125H, and NefQ125D as well as T cell evolution from wild-type-specific T cells to NefYT9-6H-specific and then NefYT9-6D-speciifc T cells. These findings demonstrated 4-steps co-evolution between HIV-1 and T cells; 1. Selection of NefQ125H by NefYT9-specific T cells, 2. Induction of NefYT9-6H-specific T cells, 3. Selection of NefQ125D by NefYT9-6H-specific T cells, 4. Induction of NefYT9-6D-specific T cells.

Conclusions.
HLA-C-restricted T cells also contribute to the co-evolution of HIV-1 with T cells.

Immune landscape in functional cure of chronic HBV

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Keywords. Chronic Hepatitis B Virus ; Functional Cure ; virus-specific T cells

Abstract:

Over 250 million people worldwide are infected with hepatitis B virus (HBV), despite the availability of an effective prophylactic vaccine. While current treatments, such as nucleos(t)ide analogs (NA) and interferon-alfa are successful in controlling viral replication, they rarely lead to a complete cure. This is due to persistence of HBV covalently closed circular DNA (cccDNA) in infected hepatocytes. As a result, patients with chronic HBV remain at high risk of developing cirrhosis and liver cancer.

A functional cure, defined by the loss of viral surface antigen (HBsAg), has emerged as a more achievable treatment goal. Indeed, most adults with acute HBV infection can effectively control the virus, and a small fraction of patients with chronic HBV may also reach this functional cure after receiving antiviral therapy. Moreover, achieving functional cure associates with reduced risk of disease progression and liver cancer.

The antiviral immune response plays a crucial role in controlling HBV infection. In patients with chronic HBV, immune cells often become dysfunctional due to prolonged exposure to high levels of viral antigens. Recent studies have emphasized the important role of HBV-specific T cells as a key cellular component of functional cure. Harnessing these cells to fight back HBV has emerged as a promising strategy for developing new therapies aimed at achieving functional cure and reducing the burden of chronic HBV.

Abstract title: Distinct Localization of Cytotoxic and Cytokine-producing CD8⁺ T Cells in the Liver during OX40-mediated Virus Inactivation

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Keywords. Hepatitis B virus, CD8+ T cells, Tolerance, OX40, Spatial transcriptomics

Background. Hepatitis B virus (HBV)-specific CD8⁺ T cells terminate HBV infection by exerting cytotoxicity and producing antiviral cytokines. However, these effector functions are suppressed during persistent infections. This study aimed to identify molecular targets to reinvigorate HBV-specific CD8⁺ T cell responses and to decipher the spatial transcriptomic signatures of cytotoxicity and cytokine-mediated HBV suppression in the liver.
Materials and Methods. We used the HBV-specific T cell tolerance mouse model in which adoptively transferred HBV-transgenic naïve T cells are rendered dysfunctional after intrahepatic priming. Transcriptomic analysis was performed to identify candidate genes associated with intrahepatically induced functional defects. We also conducted spatial transcriptomic analysis to determine whether T cell effector functions are associated with particular microanatomical locations in the liver.
Results. The Intrahepatically induced functional defect of HBV-specific CD8⁺ T cells was associated with the upregulation of various co-inhibitory and co-stimulatory molecules, particularly OX40. Concomitant activation of OX40 signaling during intrahepatic priming transiently induced T cell functionality, resulting in the persistent inactivation of HBV transcription. Interestingly, spatial transcriptomic analysis revealed that dysfunctional CD8⁺ T cells were predominantly observed in the periportal area. In contrast, OX40-activated CD8⁺ T cells exhibited a more homogenous distribution throughout the liver, with virtually all of them upregulating genes associated with cytotoxicity. In contrast, CD8 T cells with high IFNγ expression were sparse and primarily localized in the centrilobular area.
Conclusions. These results indicate the therapeutic value of OX40 stimulation against chronic HBV infections and reveal the existence of distinct microanatomical niches for dysfunctional, cytotoxic, and cytokine-producing T cells in the liver.

Analysis of T-cell responses associated with age-dependent severity in a SARS-CoV-2 infected mouse model.

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Keywords. SARS-CoV-2, T cell, mouse model, pathogenesis, immune aging

Abstract:

COVID-19 pneumonia is prevalent in the elderly infected with SARS-CoV-2; however, its age-dependent pathogenesis is unclear. We established a SARS-CoV-2-infected Nr4a3-Tocky mouse model, which enables the analysis of the dynamics and responses of antigen-specific T-cell activation and identified different disease phenotypes depending on the mouse age. SARS-CoV-2-infected aged mice developed severe pneumonia, while adult mice exhibited mild pneumonia with transient weight loss, recovering subsequently. SARS-CoV-2-infected adult mice induced significantly higher frequencies of activated antigen-specific T cells in the lungs than SARS-CoV-2-infected aged mice, implying an inverse correlation between the induction of T-cell responses in the lung and pneumonia severity. Because the infiltration of inflammatory monocyte macrophages in the lungs in SARS-CoV-2-infected adult and aged mice was comparable, induced antigen-specific T-cell fractions in adult mice could contribute to the control of viral replication and suppression of lung inflammation. Our findings suggest that T-cell dysfunction due to immune aging is associated with the severity of SARS-CoV-2 infection.

Session 4 - Virus integration & tumorigenesis

Biology of unintegrated HIV-1 DNA: role in viral transcription and escape from innate immune sensing

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Keywords. HIV-1, Chromatin, Transcription, Innate immunity.

Background.
During retroviral infection, histone-free viral DNA copy is synthesized from viral genomic RNA. vDNA will ultimately integrate into the host genome to ensure its maintenance and expression. Understanding retrovirus-host interactions at the genomic level, and the peculiar mechanisms by which HIV-1 is imported into the nucleus, loaded with nucleosomes, integrate in, and interact with, the human genome will provide valuable information about lentiviral replication and HIV reservoir establishment and maintenance.
Materials & Methods.
To assess for histones loading and nucleosome positioning, we have used chromatin immunoprecipitation and micrococcal nuclease digestion of chromatin isolated from HIV-1 infected CD4 T cell. To assess for viral transcription we used HIV-1 carrying an invalidated mutation of the integrase gene expressing luciferase reporter.
Results.
We will present and discuss the nucleosomal architecture of HIV unintegrated DNA, its associated epigenetic marks and their impact on viral gene expression and escape from cGAS DNA sensor activation. We will also discuss the loading of non-canonical histone and histone repressive epigenetic marks and their impact on transcription from unintegrated viral DNA. Finally, we will address how adopting a nucleosomal dense and repressive chromatin structure, HIV-1 escapes from cGAS sensor and innate immune activation.
Conclusions.
Understanding the establishment of chromatin on newly synthesized retroviral DNA together with the identification of the host factors involved in their transcriptional silencing is important not only for better understanding of their biology but also for establishing retroviruses as a unique and reliable model to study the establishment of chromatin and its dynamics.

Exploring virus replication mechanisms with advanced technologies: cryo-EM and human respiratory organoids

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Keywords. Influenza virus, Filovirus, SARS-CoV-2, cryo-EM, human respiratory organoids

Background. We began our studies on influenza virus at Prof. Kida's Laboratory at Hokkaido University during our undergraduate years, and conducted studies on influenza and Ebola viruses at Prof. Kawaoka's Laboratory at the University of Tokyo during our graduate studies. Since moving to Kyoto University, we have continued our studies on virus replication mechanisms, including nucleocapsid formation of filoviruses and genome packaging mechanism of influenza virus using various electron microscopy techniques. Recently, we have also started studying virus replication mechanisms in the respiratory tract using human respiratory organoids.
Results. Thus far, we have revealed that influenza virus, which possesses eight-segmented single-stranded negative-sense RNAs as its genome, selectively packages eight distinct viral RNA segments into each progeny virion using revers genetics and conventional electron microscopy. In addition, we were the first to determine the high-resolution structure of the filovirus helical nucleoprotein-RNA complex, which serves as the core of the helical nucleocapsid, using cryo-EM. Furthermore, by using nasal organoids derived from human embryonic stem cells, we demonstrated that SARS-CoV-2 replicates in both nasal respiratory epithelium and olfactory neuroepithelium.
Conclusions. We aim to expand our current studies to gain a deeper understanding of the underlying mechanisms of selective genome packaging of influenza virus, transcription and replication of filoviruses, and pathogenesis of various respiratory viruses in human airways.

Retroviruses : integration and expansion in the genomics era

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Keywords. Clonal expansion, single cell, integration site, retroviruses

Background.
Retroviruses integrate a double-stranded DNA copy of their genome, the provirus, into the host cell genome. The provirus and the flanking host genome influence each other, with potential consequences for the long-term survival of the infected cell, and for both the viral and host transcription. Two different projects developed to address the challenge of integration site analysis and proviral sequencing of oncogenic retroviruses will be presented:

1. Simultaneous single-cell sequencing by Targeted G&T Seq (TGTSeq) of the provirus, integration site and transcriptome, in HTLV (Human T-cell leukemia virus type 1) infections.

2. Genetic diversity of JSRV (Jaagsiekte Sheep Retrovirus), responsible for respiratory cancers in sheep, by direct near full-length sequening of proviruses.

Materials & Methods.

1. TGTSeq - we adapted the G&T Seq protocol, combining the genome and transcriptome sequencing of single cells (Macaulay, 2015) to enrich the DNA fraction in proviral reads by probe capture (Miyazato, 2016).

2. Near full-length (nFL) amplification of the proviruses from twenty-one respiratory tumors by third sequencing technologies (Riocreux-Verney, 2024).

Results.
1. As a proof of concept, we validated the approach using HTLV-1 clones with a known unique integration site and proviral structure. The scDNA-Seq results showed that high sequencing depth and full proviral coverage was captured in 49% of the wells. For scRNA-Seq, 93% of single cell wells passed QC with little variation between clones. The TGTSeq is sensitive enough to capture the intra- and inter-clone diversity in viral transcript expression.

2. Phylogenetic analyses of JSRV nFL proviral sequences showed that circulating strains are distributed into two distinct clades, one of which is associated with a higher incidence of cancer in France.

Conclusions.
The results of these two studies are currently being used to analyse the effect of proviral structure and genomic integration site on viral and host cell transcription.

Structural study of Hepatitis B virus x protein (HBx)

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Keywords. HBV, HBx, cryo-EM, High-speed AFM

Background.
Current treatments for Hepatitis B virus (HBV) rely primarily on nucleotide analog antiviral therapies, which inhibit viral production but do not cure the infection due to the persistence of covalently closed circular DNA (cccDNA) in the nuclei of infected liver cells. Therefore, a definitive cure for chronic Hepatitis B should aim to eliminate or permanently silence cccDNA.
Transcription from cccDNA is regulated by the HBV x protein (HBx), which forms a complex with the host factor DDB1. This complex facilitates the proteasome-dependent degradation of the SMC5/6 complex, a repressor of cccDNA transcription. Notably, interventions targeting the HBx-DDB1 interaction have been shown to effectively restore Smc5/6 levels, leading to a decrease in viral protein and cccDNA levels. This mechanism not only promotes cccDNA transcription but also contributes to the development of hepatocellular carcinoma (HCC), partly through the inhibition of the DNA repair pathway. However, due to the complexity of HBx as a poorly soluble protein, its structural and functional analysis has been challenging. To date, research has primarily provided peptide-level structural information regarding its interaction with DDB1, leaving the comprehensive structure of HBx largely unexplored.

Materials & Methods.
In this study, to elucidate the structural basis of full-length HBx, we expressed and purified the full-length HBx protein complexed with DDB1 in human cells, forming the functional complex for HBV replication. To reveal the structure of the HBx-DDB1 complex, the purified complex was analyzed using Cryogenic Electron Microscopy (Cryo-EM) and High-Speed Atomic Force Microscopy (AFM).

Results and Conclusions.
In this study, we determine the cryo-EM structure of the HBx complexed with DDB1. Additionally, we present insights into the dynamics of HBx in the complex with DDB1, as observed through HS-AFM. Our biochemical analyses further reveal HBx in the the complex with DDB1 directly binds to a component of the SMC5/6 complex. These findings provide a detailed view of both the structural configuration and the dynamic behavior of HBx, which are critical for viral-host interaction.

Epigenetic viral imprinting and cancer risk during chronic viral hepatitis

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Abstract

Chronic diseases are often driven by epigenetic regulation including histone modifications. In a French-Japanese collaboration, we demonstrated that chronic hepatitis C infection perturbs the circadian oscillation of hepatic disease relevant gene expression and induces a pro-fibrotic transcriptional signature, which is partially persistent after anti-viral therapy (Lupberger et al. Gastroenterology 2019; Hamdane et al. Gastroenterology 2019; Jühling et al. Gut 2021; Mukherji et al. Nat. Comm. 2024). We hypothesize that other severe forms of viral hepatitis involve similar epigenetic dysregulations in the liver. Chronic infection with Hepatitis D virus (HDV) causes an accelerated progression to liver fibrosis, cirrhosis, and hepatocellular carcinoma. Antiviral therapy with bulevirtide reduces HDV load in patients with encouraging effects on liver disease parameters. However, effective chemo-preventive strategies to attenuate liver disease progression as well as reliable biomarkers are urgently needed to improve disease management.

Here we aim to identify the impact of chronic HDV infection on liver transcriptomics and epigenetics to identify molecular circuits of liver disease development, candidate biomarkers for liver disease progression and to characterize their response to antiviral treatment. Thereto, we infected human chimeric mice (FRG-NOD mice transplanted with human hepatocytes) with mock, HBV alone, or HDV/HBV. Snap-frozen liver tissues were analyzed 4 weeks post infection by bulk RNA-seq and ChIP-seq for histone marks associated with active transcriptional enhancers (H3K27ac and H3K4me1) and a mark for transcriptional repression (H3K27me3). Moreover, we validated predicted biomarker candidates in the blood of infected chimeric mice and patients by ELISA.

We identified 748 unique transcripts in the livers of HDV/HBV-infected mice as compared with HBV mono-infected and mock-infected animals. Notably, most of these transcripts were highly upregulated by HDV infection and enriched by genes involved in HDV replication, inflammation, fibrosis, and cancer risk. Consistently, most of the genes coding for these transcripts are also associated with at least one histone mark linked to active enhancers and thus potentially representing a persistent viral footprint in the infected liver. Interestingly, 109 transcripts of this HDV signature potentially encoded secretory proteins based on signal peptide prediction. Top ranking candidate markers are positively correlated with the transcript levels and elevated in blood of HDV-infected mice and patients.

Overall, we revealed an HDV-specific transcriptional signature which is potentially part of a persistent epigenetic viral footprint. Signature components are differentially secreted to the blood of infected animals and therefore may serve as risk marker for disease progression and fibrosis. Further work is ongoing to address whether these proteins may serve as a prognostic biomarker for disease progression and/or therapeutic response such as bulevirtide and to predict and validate compounds able to reverse risk-associated transcriptional signatures.

HTLV-1-induced uncoupling of transcription and splicing in the context of 3D chromatin organization

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Keywords. HTLV-1, Alternative Splicing, 3D genome, NF-κB

Background
Human T-cell Leukemia Virus type 1 (HTLV-1) is the etiological agent of several inflammatory diseases and adult T-cell leukemia/lymphoma (ATLL). Unlike other retroviruses, HTLV-1 predominantly replicates through the clonal expansion of infected cells, driven by the viral proteins Tax and HBZ, which modulate host cellular gene expression. Specifically, we have demonstrated that both viral proteins disrupt the fine-tuned regulation of transcription and alternative splicing, processes essential for maintaining transcript diversity and appropriate expression levels required for cellular function.

Materials & Methods.
Transcriptional and alternative splicing alterations in HTLV-1-infected cells, as well as in cells ectopically expressing the viral proteins, were analyzed using exon array and RNA sequencing (RNA-seq) on the Illumina platform. Alterations in three-dimensional (3D) chromatin conformation were assessed via Hi-C, 4C, and 3C techniques. Causal relationships underlying molecular mechanisms were examined using defective Cas9 (dCas9) constructs fused to proteins of interest.

Results.
We observed that HTLV-1-infected cells *in vivo* display extensive alternative exon usage, which is particularly pronounced in tumor cells. These alterations include both linear RNA and circular RNA (circRNA) transcripts. At the molecular level, both Tax and HBZ contribute to these dysregulations by interacting with various helicases and disrupting the mechanisms coupling transcription and alternative splicing. Specifically, we identified an HBZ:DHX9 axis responsible for regulating circRNA expression profiles, which are altered in ATLL and contribute to the proliferation of HTLV-1 infected cells. For protein-coding linear RNAs, we demonstrated that Tax-induced NF-κB activation leads to the redistribution of the NF-κB subunit p65/RelA within gene bodies, where it modulates splicing by recruiting the RNA helicase DDX17 to its target exons. This function of NF-κB occurs within the 3D genomic context, where Tax-induced enrichment of p65/RelA at exon sites promotes chromatin contacts, leading to gene-gene coordinated regulation of transcription and alternative splicing for a subset of NF-κB-responsive genes.

Conclusions.
Our data provide new insights into the molecular mechanisms by which HTLV-1 alters gene regulation, underscoring the intricate relationship between transcription, alternative splicing, and 3D chromatin architecture. Future investigations should focus on the impact of these regulatory changes on the clonal expansion of infected cells and their contribution to disease development.

Session 5 - Viral pathogenesis & innate immunity

Why is Usutu virus the only mosquito-borne flavivirus to resist ISG20 exonuclease?

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Keywords. Mosquito-borne flaviviruses, Interferon-stimulated genes, ISG20, Innate immune response

Background.
Usutu virus (USUV) and West Nile virus (WNV) are two closely related emerging mosquito-borne flaviviruses. Their natural hosts are birds, but they can also cause severe neurological disorders in humans. Both viruses are effectively suppressed by type I interferons, cytokines produced by infected cells that block virus replication by inducing the expression of hundreds of antiviral genes called ISGs (Interferon-Stimulated Genes). Although ISGs interfering with the most widespread pathogenic flaviviruses, such as dengue virus, Zika virus or WNV, have been identified, no studies have yet been carried out on USUV. We therefore sought to determine whether USUV was sensitive to the same ISGs that interfere with WNV replication.

Materials & Methods.
To assess the sensitivity of USUV to key human ISGs, we performed a mini expression screen on 12 ISGs known to inhibit replication of most flaviviruses, including WNV. The mechanism of USUV resistance to ISG20 was unraveled by constructing chimeric viruses between WNV and USUV and by resolving the genomic RNA conformation of both viruses.

Results.
We show that the replication of USUV and WNV is inhibited through a common set of ISGs, with the notable exception of ISG20, which USUV is resistant to. Strikingly, USUV was the only virus among all the other tested mosquito-borne flaviviruses that demonstrated resistance to the 3′–5′ exonuclease activity of ISG20. Our findings reveal that the intrinsic resistance of the USUV genome, irrespective of the presence of cellular or viral proteins or protective post-transcriptional modifications, relies on a unique sequence present in its 3′–untranslated region. Importantly, this genomic region alone can confer ISG20 resistance to a susceptible flavivirus, without compromising its infectivity, suggesting that it could be acquired by other flaviviruses.

Conclusions.
This study provides new insights into the strategy employed by emerging flaviviruses to overcome host defense mechanisms.

Selective TLR ligands stimulation through mosquito saliva contributes *in vivo* pathogenicity of mosquito borne Flaviviruses.

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Keywords. mosquito, flavivirus, TLR, innate immunity, pathogenicity

Background. Innate immunity is the first line of defense against infection in a non-specific manner and is conserved in vertebrates. Recognition of viral components by various pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), induces the expression of numerous genes to eliminate pathogens. Mosquito-borne flaviviruses, such as Japanese encephalitis virus (JEV), dengue virus, and Zika virus, are transmitted by mosquitoes via injection of the virus along with saliva into the skin. While mosquito saliva is thought to contribute to efficient blood feeding, it is also involved in the efficient in vivo propagation of several mosquito-borne viruses. However, it remains unclear how mosquito saliva stimulates viral propagation in vivo.
Materials & Methods. JEV was subcutaneously inoculated into the footpads of mice along with mosquito salivary gland extracts (SGEs) or agonists of PRRs. The effects of SGEs or PRR agonists were determined by assessing viral pathogenicity and histopathology. Immune cell populations were identified using mass cytometry (the CyTOF system), and viral RNA was detected using the PrimeFlow assay.
Results. We demonstrated that SGEs enhance JEV pathogenicity in vivo by promoting the accumulation of immune cells and stimulating innate immune responses at the infection site. Among the innate immune signaling pathways, TLR2 stimulation specifically enhances flavivirus pathogenicity similarly to SGEs. Mechanistically, TLR2 ligands and SGEs activate neutrophils to secrete chemokines, which recruit viral-permissive monocytes to the infection sites. We also demonstrated that SGEs are capable of activating TLR2, and blocking TLR2 signaling mitigated the enhanced viral pathogenicity caused by mosquito saliva.
Conclusions. Our data suggest that the activation of TLR2 signaling due to mosquito bites might play a crucial role in the efficient viral propagation and pathogenicity of mosquito-borne flaviviruses.

Targeting the Integrated Stress Response to modulate viral replication

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Background: We are interested in the replication of RNA viruses pathogenic to humans, [retroviruses (HIV-1), arboviruses (Zika..), respiratory viruses (SARS-CoV-2)], the cell defense mechanisms to infection,as well as the viral response to infection, in order to propose new antiviral pathways, and evaluate the antiviral activity of compounds. Concerning HIV-1, we previously identified GCN2 as a cellular partner of HIV-1 integrase (IN)1. GCN2 is a cellular protein kinase involved in stress response. Upon autophosphorylation, GCN2 phosphorylates eIF2α, which results in the control of translation. GCN2 has been implicated in human burdens such as cancer and Alzheimer's disease.

Materials & Methods: Phosphorylation, integration assays in vitro. Integration sites sequencing. Mutagenesis. Cellular replication of native virus. Alpha-screen assay.
Results: We showed that GCN2 is activated during HIV-1 infection2 and phosphorylates HIV-1 IN. In cells, depletion of GCN2 increases infectivity, establishing a link between GCN2 and HIV-1 replication. Infectivity of HIV-1 was also increased in the context of viruses harboring IN mutations unable to sustain phosphorylation by GCN2. Although reverse transcription was not affected, integration was increased in these mutant viruses[3]. Further in vitro analysis demonstrated the formation of a complex between GCN2 and LEDGF/p75, a partner of HIV-1 IN. GCN2 does not phosphorylate LEDGF/p75 alone but the phosphorylation requires the presence of IN, suggesting a tripartite complex. We developed an AlphaScreen interaction assay to isolate modulators of GCN2/IN interaction4. We selected about twenty modulators of this interaction. A structure-dependent study of these molecules and derivatives identified[3] chemical series with the potential to inhibit the IN-GCN2 interaction.

Conclusion: Thus, targeting GCN2 itself or GCN2 interactions appears to be a viable therapeutic avenue for HIV and may pave the way for other diseases implicating GCN2.

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Monocyte and macrophage subsets in HIV-1 infection

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Keywords. Monocyte, Macrophage, HIV-1, SIV

Although $CD4^+$ T cells are the well-known HIV-1 reservoir on ART, increasing evidence suggests that myeloid cells, including monocytes and macrophages, are also important viral reservoirs.
1. We initially found that peripheral $CD34^+CD14^+$ monocytes harbor HIV-1 proviral DNA even in the virologically suppressed individuals on ART. Therefore, we asked how $CD34^+$ monocytes are infected and persist. We identified $CD34^+CD14^+$ cells also in human lymph nodes expressing higher levels of HIV-1 receptors compared to the rest of monocytes, which suggested that they are counterpart of peripheral monocytes. Of note, these $CD34^+$ monocytes highly expressed CCR7 and S1PR1 regulating cell migration into and egress from lymph nodes, respectively. In addition, we detected SIV provirus in $CD34^+$ monocytes as well as T cells from SIV-infected rhesus macaques. Taken together, these results raise a new possibility that $CD34^+$ peripheral monocytes are infected with HIV-1 in lymph node owing to their high susceptibility and then return to circulation, which explains the reason for detection of proviral DNA in this subset even after long-term ART.
2. There have been several reports that embryo-derived macrophages continuously proliferate in tissues, which indicates a candidate of viral reservoir due to their longevity. However, it has not been analyzed much in human samples. We sorted peritoneal macrophages from ascites of cancer patients to collect two macrophage subsets which are supposed to be monocyte-derived ($CCR2^{hi}$) and embryo-derived ($CCR2^{low}$). RNA-Seq depicted higher expressions of fetus-derived markers such as TIMD4, LYVE-1 and FOLR2 in the latter subset. This subset also exhibited higher level of proliferation marker MKI67. Together with higher expression of HIV-1 receptors on this subset, these results indicate that this "TLF^+" macrophage subset can be preferentially infected with HIV-1 and serve as a tissue viral reservoir even on ART.

A multi-omics approach to highlight specific molecular properties of neglected endemic HTLV-1 subtypes

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Keywords. HTLV-1, molecular epidemiology, Tax, transcriptomics, interactomics

Background. While environmental and host factors have been thoroughly considered to explain HTLV-1 infection outcomes, viral genetic variability has been neglected in molecular studies, these being systematically made on the historical HTLV-1a cosmopolitan genotype. We thus aim to study the molecular properties of the neglected HTLV-1b and -1c endemic genotypes. Here, our working hypothesis is that Tax1a, one of the major oncogenic factors in HTLV-1a, engages in interactions with host cells in a different manner compared to Tax1b and Tax1c.
Materials & Methods. We use an unbiased multi-omics approach in Tax1a-, Tax1b- and Tax1c-expressing T cells, including quantitative proximity-biotinylation interactomics (BioID) to describe the protein-protein interaction network of specific Tax variants, kinomics (Pamgene technology) to identify signaling cascades modulated by Tax expression, and transcriptomics (RNA-sequencing) to describe the consequences on transcriptional reprogramming. We combine these analyses with ImageStream X cyto-microscopy to quantitatively compare the subcellular localization of specific Tax variants.
Results. While all Tax1 variants share common functions, several pathways, *e.g.* interaction with CREB, regulation of RNA metabolism, MAPK signaling and cell cycle, are differentially affected by Tax variants. Strikingly, our results suggest a distinct NF-kB activation mechanism by Tax1c, that may impact the quality of the Tax-induced signaling cascade and the identity of the NF-kB target genes modulated by Tax1c.
Conclusions. Our unbiased multi-omics molecular analysis of cells expressing Tax from endemic genotypes indicates that these variants differ in their ability to reprogram host cells. These results suggest functional variations between HTLV-1 subtypes, which could contribute to varying infection outcomes, and call for an extended consideration of genotype-associated specificities in the description of factors influencing HTLV-1-associated diseases.

Mucosal viruses: from pathophysiology of transmission to neuro-immune prevention

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Keywords. CGRP (calcitonin gene-related peptide); HIV-1 (human immunodeficiency virus type 1) / HSV-2 (herpes simplex virus type 2) co-infection; LCs (Langerhans cells); SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and COVID-19 (coronavirus disease 19); TRPV1 (transient receptor potential vanilloid 1)

Background: One important anti-viral defense mechanism involves interactions between sensory peripheral pain neurons termed nociceptors innervating all mucosal tissues, and immune/epithelial cells. We discovered that the nociceptor-derived neuropeptide CGRP acts on antigen-presenting LCs and inhibits LCs-mediated HIV-1 transfer to CD4+ T-cells and HSV-2 infection.
Recent results: 1) HIV-1 - CGRP secretion is induced upon activation of the Ca^{2+} ion channel TRPV1 in nociceptors. We found that natural TRPV1 agonists, namely capsaicin (CP) from chili peppers and cannabidiol (CBD) from *Marijuana*, activate TRPV1 also in LCs. In turn, CP/CBD induce secretion of CGRP from LCs that inhibits HIV-1 transfer. We further identified a novel inhibitory mechanism, involving CGRP-induced secretion from LCs of the matricellular protein thrombospondin 1 (TSP-1), which inhibits HIV-1 transfer via direct neutralization and/or ligation of its CD47 receptor; **2) HSV-2** - To better mimic the mucosal tissue architecture, we developed a microfluidic 'mucosa-on-chip' model, combining CGRP+ nociceptors with genital epithelial cells and LCs. In this model, HSV-2-infected LCs relay the virus to nociceptor axonal endings, leading to their degeneration. HSV-2 preferentially targets CGRP+ nociceptors, leading to reduction in their CGRP content; **3) SARS-CoV-2** - CGRP is abundant in the lungs, and due to its anti-hypertensive/inflammatory functions could control COVID-19 pulmonary symptoms. We found that CGRP acts on bronchial epithelial cells, decreasing their surface expression of SARS-CoV-2 entry receptors, subsequently inhibiting their infection. Moreover, we found elevated CGRP pulmonary levels in critical COVID-19 patients, which could mediate beneficial SARS-CoV-2 clearance.
Conclusions: Our studies identify mucosal neuroimmune and neuroepithelial interactions, mediated by CGRP with significant ant-viral roles. These studies support the development and re-positioning of CGRP/CP/CBD-based formulations that could be used clinically against a variety of mucosal viruses.

Session 6 - Anti-viral immune responses - Part II

Unraveling mucosal immune dynamics in chronic SIV infection: implications for HIV persistence and viral rebound

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Keywords. HIV-1/SIV, intestinal macrophages, CX3CR1, ATI, post-treatment control

Background:
Chronic HIV-1 and SIV infections are characterized by persistent immune activation and inflammation, even with antiretroviral therapy (ART). This study investigates the role of intestinal CX3CR1+ macrophages (MΦs) in immune dysregulation during chronic SIV infection and their impact on viral persistence after antiretroviral treatment interruption (ATI).

Materials & Methods:
We studied Cynomolgus macaques during chronic SIV infection and after ATI. We analyzed mucosal samples to assess the dynamics of CX3CR1+ MΦs and various T cell subsets.

Results:
In SIV-infected animals, we observed a significant accumulation of pro-inflammatory MΦs, which correlated with viral load. Post-treatment controllers (PTCs) showed restored MΦ homeostasis, suggesting their involvement in viral control. Chronic SIV infection led to alterations in mucosal T cell populations, with depletion of CD4+ T cells, particularly Tregs, in the colonic mucosa. While PTCs partially recovered Treg levels, non-controllers did not. Additionally, Th1 CD4+ cells increased, and Th17 cells showed a decreasing trend. A notable correlation between the accumulation of pro-inflammatory MΦs and Th17 cell depletion was identified. CD4+ T cells exhibited activated phenotypes, marked by upregulation of PD-1, HLA-DR, and Ki67 in infected and non-controller animals. Neutrophils were activated in infected animals, with an increase in CD66+CD32a+ cells, which were associated with pro-inflammatory MΦ accumulation and fecal calprotectin levels, indicating intestinal damage.

Conclusions:
This study reveals the significant role of CX3CR1+ MΦs and their interactions with mucosal immune cells in chronic SIV infection, contributing to immune dysfunction and gut damage. These findings highlight the need for strategies to preserve or restore CX3CR1+ macrophage function in future HIV cure research, especially to mitigate viral rebound after ART cessation.

Integrated single cell analysis of HTLV-1 specific CD8 T cells in peripheral blood (PB) and cerebrospinal fluid (CSF) from HAM/TSP patients.

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Keywords. HTLV-1, HAM/TSP, CD8 T cell, TCR affinity, Tax, Single cell analysis

Background.
Although several factors in CD4 T cells have been reported to be involved in development of HAM/TSP, the pathogenesis has not been fully understood yet. In this study, we analyzed role of HTLV-1 specific CD8 T cells in the pathogenesis by an integrated single cell analysis using PB and CSF cells from HAM/TSP patients.

Materials & Methods.
We obtained single cell data sets, gene transcriptome and TCR sequences, from PB and/or CSF cells from five ACs and thirteen HAM/TSP patients. Recognition of epitope by the HTLV-1 specific CD8 T cells/TCRs was analyzed by using HLA-I multimer. Single cell data was obtained by 10x Chromium system and the data analysis was performed by CellRanger and Seurat softwares. The integrated single cell analysis was performed for ACs (PB: 260-4,664 cells/case) and HAM patients (PB: 696-8,939 and CSF: 155-5,823 cells/case).

Results.
Cell-type clustering indicated that the number of CD8 T cells in HAM-CSF was significantly higher than that in PB and some clonotypes among the increased CD8 T cells are clonally expanded. Using HLA-I multimer, we found that dominant CD8 T cell clonotypes in HAM-CSF recognized HLA-A*24:02 restricted $Tax_{301-309}$ (twelve of thirteen HAM patients). Interestingly, the Tax specific CD8 T cells dramatically increased in the CSF than that in PB. Differential gene expression analysis between CSF and PB cells showed that T cell exhaustion, cell cycle and proapoptotic gene signatures were up-regulated in Tax-specific CD8 T cells of CSF. We also performed differential gene expression and ontology analysis by comparing AC- and HAM-PB cells and found that some gene clusters associated with T cell cytotoxicity and activation were up-regulated in the CD8 T cells in HAM-PB. Furthermore, Tax specific TCRs cloned from HAM-CSF showed higher TCR affinity against HLA-A24/Tax peptide complex than those of AC-PB.

Conclusions.
These results indicated that HTLV-1 specific CD8 T cells may play a role in HTLV-1-associated inflammatory diseases and could be new target cells for the therapy and diagnosis.

Human broadly neutralizing antiviral antibodies

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Keywords. Humoral immunity, monoclonal antibodies, memory B cells, HIV-1, SARS-CoV-2, hepatitis viruses.

Abstract

Humoral immunity is fundamental to host defense against infections, driven by the remarkable diversity of antibody molecules that recognize and neutralize pathogens. Gaining a deeper understanding of humoral immunity through the molecular and functional characterization of human virus-specific monoclonal antibodies has been pivotal for advancing effective vaccine and antibody-based therapeutic strategies. This presentation will highlight our findings on virus-specific memory B-cell antibodies, with a special emphasis on broadly neutralizing antibodies identified against HIV-1, SARS-CoV-2, and hepatitis viruses.

SIV-neutralizing antibody induction: *extrinsic vs. intrinsic* phenotypes of immune perturbation

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Keywords
HIV, SIV, neutralizing antibody, immunosignaling, Nef, Inborn Errors of Immunity (IEIs)

Abstract:

Neutralizing antibody (NAb) responses are impaired throughout the course of HIV and simian immunodeficiency virus (SIV) infection. This occurs through multi-scale adverse virus-host interactions, including low antigenicity of Env, post-primed CD4+ T-cell targeting and histological dysregulation. While these logically explain for HIV/SIV neutralization resistance, how such resistance is overcome is somewhat “epistatic” and still remains ill-understood.

We have reported in the 2022 France-Japan meeting properties of NAb induction against a highly neutralization-resistant strain, SIVmac239, in rhesus macaques. The work highlighted an important conceptual link of such NAb induction with human inborn errors of immunity (IEIs), involving PI3 kinase signaling. The speaker will report updates, and this time will lay emphasis on introducing how antiviral immunity can sometimes be surprisingly well understood from findings in a completely different field of clinical immunology.

Overcoming the short-lived nature of tissue-resident memory CD8⁺ T cells in the lung

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Keywords. Tissue-resident memory, CD8 T cells, Lung, Respiratory virus infection, Protective immunity

Abstract:

Tissue-resident memory T (T_{RM}) cells are a unique subset of T cells that permanently reside in various peripheral tissues and play dominant roles in protective immunity at barrier surfaces. T_{RM} cells also contribute to cancer immunity, autoimmunity, allergy, and inflammatory disease, and are therefore major candidates for vaccine development and immunotherapy. However, how T_{RM} cells are maintained in different peripheral tissues and tumors remains poorly understood.

The use of mouse models of influenza virus infection with experimental approaches such as intravenous antibody staining and parabiosis have allowed us to precisely identify lung resident T_{RM} subsets. With this methodology, we have discovered that CD8⁺ T_{RM} cells in the lung are generated and maintained in transient niches created at sites of tissue damage and regeneration, and that these CD8⁺ T_{RM} cells are maintained independently of tissue-circulating effector memory T cells (T_{EM}). Interestingly, however, our study also demonstrated that lung resident CD8⁺ T_{RM} cells display a relatively shorter lifespan than T_{RM} cells in other tissues, which is due to the loss of these transient niches during tissue repair. Nevertheless, we have recently identified a specific CD8⁺ T_{RM} subpopulation that is selectively maintained in the lung following infection, raising the possibility that the preferential generation of this unique T_{RM} subset may prolong their longevity, as well as promoting T_{RM}-mediated protection against rechallenge. Thus, we are now investigating the mechanisms that drive the generation of this unique and long-lived CD8⁺ T_{RM} population upon influenza infection, which could greatly improve protocols for vaccine engineering.

Session 7 - Young investigators session

Ribosome profiling and immunopeptidomics reveal tens of novel conserved HIV-1 open reading frames encoding T cell antigens.

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Keywords. Translation, Alternative Open Reading Frame, Antigen, T Cell Response, Polyfunctionality

Background. Recent advances in omics have challenged the traditional understanding of Open Reading Frames (ORFs), showing that thousands of small ORFs (sORFs) encode polypeptides. Studies estimate that 85% of human translation products originate from non-annotated regions, particularly alternative ORFs (ARFs) in out-of-frame sequences. Notably, a significant fraction of peptides presented by MHC molecules is derived from sORFs. In the context of HIV-1, T cells are critical in controlling viral replication, sometimes down to undetectable levels in certain individuals. Our team and others have shown that HIV-specific T cells target peptides presented by MHC, which are derived from ARFs. However, the existence of ARFs within the HIV genome has only been inferred through indirect methods like T cell assays and HLA footprinting.

Material and Methods, & Results. In this study, we characterized the translatome of HIV-1 in infected CD4+ T cells. Through ribosome profiling, we provide an unbiased view of actively translated HIV mRNAs, revealing that the HIV-1 genome contains 98 ARFs corresponding to sORFs located in the 5' UTR or overlapping canonical HIV ORFs. Utilizing a database of HIV genomes, we found that most ARF-encoded amino acid sequences are highly conserved within clade B and C, with 8 ARFs more conserved than their overlapping canonical ORFs. Additionally, two complementary approaches—detection of ARF-specific T cells in PBMCs from people living with HIV (PLWH) and isolation of MHC-bound ARF-derived peptides via mass spectrometry—demonstrated that these ARFs encode viral polypeptides capable of inducing strong T cell responses. Notably, ARF-derived peptides are recognized by both CD8+ and CD4+ T cells in PLWH.

Conclusion. These findings expand the landscape of HIV-1 antigens, providing insights that could inform future vaccine design and suggesting the existence of HIV-1 microproteins or pseudogenes

MEX3B, an RNA-binding protein, strongly suppresses HIV-1 viral replication depending on its RNA-binding ability

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Keywords. HIV-1, RNA-binding protein, host defense against viral infection

Abstract: Antiretroviral therapy (ART) has greatly improved the prognosis of patients infected with human immunodeficiency virus (HIV-1), but it requires lifelong continuation of ART. In order to develop therapies to eradicate HIV-1 from the body, it remains important to understand the full context of the host's defensive immune response against HIV-1. To date, approximately 1700 RNA-binding proteins have been identified in humans, but the role of RNA-binding proteins in biological defense as an antiviral response is only partially understood. Based on expression screening, we found that MEX3B, an RNA-binding protein, strongly suppresses HIV-1 viral replication depending on its RNA-binding ability. As a mechanism, MEX3B binds directly to specific sequences of HIV-1 RNA and suppresses the release of infectious virions by repressing the translation of viral proteins. In addition, based on our analysis of CD4+ T cells in the peripheral blood of HIV-1-infected individuals, we found that people with high levels of MEX3B tended to be less likely to develop AIDs, suggesting that MEX3B may play a role in the development of AIDs.

Unraveling the relationship between NK cell polyfunctional capacity and HIV/SIV post-treatment control

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Keywords. Innate response, Post-treatment control, NK cells, Animal model

Background. We evaluated the relationship NK cell dynamics and SIV/HIV post-treatment control in both macaques and humans. The pVISCONTI study was designed to assess the underlying mechanisms of SIV post-treatment control in a Cynomolgus model of SIVmac251 infection, while human HIV post-treatment controllers (PTCs) belong to the VISCONTI cohort.

Materials & Methods. Two groups of Cynomolgus macaques were infected with SIVmac251 and received cART early (28 days post-infection (pi), n = 6) or late (6 months pi, n=6). Treatment was interrupted after 24 months and animals were monitored for further 12 months. NK cell phenotype was followed up by flow cytometry in blood and tissue. We also evaluated the evolution of human NK cell function by a 42-color spectral cytometry panel after stimulation with target cells or autologous infected CD4+ T-cells.

Results . In Cynomolgus, NKG2A is constitutively expressed at different levels in blood and tissue. NHP PTC showed increased levels of NK cells with an inhibitory phenotype ($NKp80^{+}NKG2A^{high}NKp30^{-}NKp46^{-}$) since the baseline that correlated positively with NK mediated viral inhibition during primo-infection and negatively with viral load after ATI. In humans, we observed non-traditional effector functions in a stimulation-dependent manner that were linked to a $NKp80^{-}NKp44^{+}NKp30^{-}NKp46^{-}$ phenotype.

Conclusions ; Our results indicate that NK cell phenotype and function have an impact on the achievement of SIV and HIV post-treatment control.

HIV-Tocky system to visualize proviral expression dynamics

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Keywords. HIV latency, Viral dynamics, Fluorescent reporters, Block and lock, HIV reservoir.

Background: Determinants of HIV-1 latency establishment are yet to be elucidated. HIV reservoir comprises a rare fraction of infected cells that can survive host and virus-mediated killing. *In vitro* reporter models so far offered a feasible means to inspect this population, but with limited capabilities to dissect provirus silencing dynamics. Here, we describe a new HIV reporter model, HIV-Timer of cell kinetics and activity (HIV-Tocky) with dual fluorescence spontaneous shifting to reveal provirus silencing and reactivation dynamics.

Materials & Methods: We established the HIV-Tocky system which uses a fluorescent Timer protein whose emission spectrum spontaneously shifts from blue to red to reveal HIV-1 provirus dynamics. This was done by infection of Jurkat, THP-1 cell lines, and primary CD4+T-lymphocytes with HIV Timer single-round virus to establish an in-vitro model. Timer fluorescence quantification was done by flow cytometry. Using Linker-mediated PCR, we identified integration sites of HIV-1 proviruses in bulk infected cells across FACs-sorted Timer populations. Limiting dilution was performed to obtain infected Jurkat/THP-1 single integration clones. Proviral load measurement was done by ddPCR. DNA-cap-seq was used to determine the provirus sequence and integration site for each Timer clone.

Results: The HIV-Tocky system was successfully established in T-cell lines, primary T-cells, and the THP-1 cell line. Timer fluorescence transitions were validated to correlate with Provirus expression dynamics. Integration site analysis of various Timer fractions identified two latent populations: a directly latent, and a recently silenced subset, with the latter having integration features suggestive of stable latency. HIV-1 provirus silencing dynamics were more accurately captured through HIV-Tocky fluorescence, this was reflected in better identification of latency-promoting agents' effect in comparison to the conventional GFP model.

Conclusions: Our HIV-Tocky proposed model can help address the heterogeneous nature of HIV reservoirs and offers new possibilities for evaluating eradication strategies.

Biological effect of cytokine specific auto-antibodies on immune responses in a healthy population

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Abstract

The presence and pathological effect of cytokine specific auto-antibodies (C-aAb) has been observed in autoimmune diseases and more recently in COVID-19 cases. However, their presence in the plasma of healthy individuals raises questions about their biological effect and impact during different immune responses.

To address this question, we measured levels of c-aAb against IFNα, IFN□, CSF2, IL-1α, IL-6, and IL-10 in the plasma of 1,000 healthy donors of the Milieu Interieur (MI) cohort. Using TruCulture® whole blood stimulations, we observed a significant and strong association of IL-1α c-aAb with LPS induced IL-1α protein levels. LPS and E. Coli stimulation conditions showed differential gene expression associated with IL-6 c-aAb, while gene expression after Poly:IC stimulation was associated with CSF2, IFN□ and IFN□□c-aAb plasma titers. Furthermore, whole blood stimulation supplemented with plasma containing high titer c-aAb showed widespread association of anti-IFNα and anti-IL-6 c-aAb with both baseline and induced gene expression. This experimental effect on c-aAb gene expressions was cross validated for certain genes in the MI cohort.

Our results demonstrate a biological effect of C-aAb on immune responses of healthy donors, at low concentrations previously thought to be non-neutralizing. Interestingly, their presence strongly impacted the regulation of immune gene expression even in the Null condition. Further investigation of the prevalence of C-aAb and their neutralizing activity in population-based cohorts will provide a better understanding of their role in shaping immune response variability between individuals.

Structural regulation of NTCP receptor function on hepatitis B virus entry

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Keywords. Hepatitis B virus, entry, viral receptor, species specificity, host tropism

Background. Hepatitis B virus (HBV) infects limited animal species such as human and chimpanzee, but not most old-world monkeys including cynomolgus macaque. Non-susceptibility to HBV in cynomolgus macaque is attributed to the lack of binding of macaque-derived receptor homologue, NTCP (mNTCP), to the preS1 region in the HBV surface antigen. Despite of a high sequence identity (96%) to human-derived NTCP (hNTCP), how mNTCP obstructs viral binding is unknown. In this study, we aimed to clarify why mNTCP cannot function as an HBV receptor.

Materials & Methods. The structure of the mNTCP-bile acid complex was solved by cryo-electron microscopy. PreS1-NTCP binding activity was evaluated to treat HepG2 cells transfected with wild type or mutant NTCPs with TAMRA-conjugated 2-48 aa region of preS1 peptide and observed cell-bound fluorescence. HBV infection assay was performed using the NTCP-transfected HepG2 cells. To examine bile acid transport activity, NTCP-transfected HepG2 cells were treated with [^{3}H]-taurocholic acid and measured the intracellular radioactivity.

Results. Superposing the mNTCP-bile acid complex structure on that of the hNTCP-preS1 complex showed that Arg158 of mNTCP located at the entrance of the bile acid tunnel induced a steric clash with the main-chain of the preS1 N-terminal region. Cell-based assays confirmed that substitution at position 158 in mNTCP to any 19 non-glycine amino acids did not allow preS1 binding and HBV infection, but showed tolerable transporter activity. Moreover, molecular dynamics simulations showed that macaque-type residue on the extracellular surface of NTCP induced larger fluctuation of the preS1 C-terminal region that caused destabilization of NTCP-preS1 binding.

Conclusions. This study presents structural insights in which multiple sites in NTCP are coordinately involved in preS1 binding, and mNTCP loses the activity for preS1 binding at both sites, resulting in securing the strict restriction of HBV in macaque. Our data provide a model to determine the functionality of NTCP as a viral receptor.

GAS7 F-BAR Protein Regulates Macrophage Constitutive Antiviral Defense through Macropinocytosis

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Abstract

Macrophages play critical roles in innate immune surveillance. They are essential for the degradation of pathogens, through both inducible and constitutive intrinsic mechanisms. Inducible responses are triggered by pathogen-associated molecular patterns (PAMPs), activating pathways such as type-I IFN responses, oxidative burst, and chemokine secretion. However, pathogen degradation also heavily depends on constitutive mechanisms including – but not limited to – phagocytosis, autophagy, and proteasome-mediated viral component degradation. This study identifies the GAS7 (Growth Arrest Specific-7) F-BAR protein (a cytoskeleton-associated factor already known to be involved in neuronal dendrites formation) as a crucial constitutive barrier against pathogen infection in macrophages. By silencing GAS7 in macrophages, we found that it restricts viral replication across major viral groups (e.g., HIV-2, HSV-1, Zika, Sindbis, Sendai, measles virus) independently of type I IFN responses, and inhibit intracellular bacteria growth in a Salmonella Enterica model. Through yeast two-hybrid and mass spectrometry analyses, we determined GAS7 interactome. We found GAS7 to interact with various component of the actin cytoskeleton such as the WAVE2 complex, along with various proteins involved in macropinocytosis processes. Microscopic examination showed that GAS7 silencing in macrophages impairs macropinosome formation and maturation. Thus, although such defect in macropinocytosis was associated with less viral capture by the myeloid cells, it led to higher productive infection. Altogether, our data suggest that GAS7 block productive viral entry by promoting viral degradation in a process involving macropinocytosis and the macrophage actin network. Understanding such constitutive antiviral defense mechanisms is of interest for developing broad antiviral therapies.

Characterization of a novel broadly neutralizing antibody against flaviviruses

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Keywords. Flavivirus, Dengue virus, Human antibody, Broadly neutralizing antibody, Antibody dependent enhancement

Abstract: The genus flavivirus includes many arthropod-borne pathogens that cause severe infectious diseases in humans, such as Dengue (DENV), Zika (ZIKV), and Japanese encephalitis (JEV). Flaviviruses pose a threat to global public health, necessitating the development of countermeasures. The envelope (E) protein is the main target of neutralizing antibodies against flaviviruses. Flavivirus infection tends to elicit cross-reactive antibodies due to the conserved structures among divergent flavivirus E proteins. However, majority of cross-reactive flavivirus antibodies are poorly neutralizing and are often exhibiting antibody-dependent enhancement (ADE) activities, which poses a great barrier to develop flavivirus vaccines. In this study, we identified two novel cross-reactive monoclonal antibodies, NFV-1 and NFV-2, against DENV1, ZIKV, and JEV E proteins, whose epitopes do not overlap with any cross-reactive antibodies previously characterized. Especially, NFV-1 broadly neutralizes most of the Flaviviridae, including DENV 1-4, ZIKV, JEV, West Nile virus, Saint Louis encephalitis virus and yellow fever virus. Interestingly, while many of the known flavivirus antibodies exhibit ADE activity at sub-optimal concentrations for neutralizing activities, NFV-1 does not. Moreover, NFV-1 shows the prophylactic and therapeutic activities in mice infected with flaviviruses. These findings suggest the existence of a novel, highly conserved neutralizing epitope among flavivirus E proteins and provide new insights into the mechanism of ADE.

T follicular helper cells in SIV-infected rhesus macaques

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Keywords. Max. 5, Tfh, sequencing, multiplex analysis

Background. Progressive loss of memory CD4 T cells is a characteristic of human immunodeficiency virus (HIV) infection, in which mitochondria alterations contribute to the death of T cells. The laboratory has previously demonstrated in SIV-infected rhesus macaques a profound remodeling of the germinal center (GC) architecture associated with the depletion of T follicular helper (Tfh) cells, a subset of memory CD4 T cells, which are essential for B cell differentiation and GC development. Despite early antiretroviral therapy (ART), Tfh represent viral reservoirs in visceral tissues. Therefore, we hypothesized that viral persistence in this population may not only contribute in the dysregulation of B cells but also in altering microenvironment within GCs.
Our objectives were to determine (i) the dynamic and profile of B and (ii) of Tfh cells in relationship with TCR repertoire, and (ii) metabolites in the microenvironment of infected lymphoid tissues as it may contribute in the shaping of immune cells.

Materials & Methods. The profile and dynamic of B and Tfh cells were assessed by flow cytometry. Tissues analyzed are the spleen and mesenteric lymph nodes from both SIVmac251-infected and ART-treated RMs. We performed bulk and single-cell RNA transcriptomic analyses. For monitoring *in situ* microenvironment within B follicles, we developed new tools including multiplex confocal microscopy combined with mass spectrometry analysis.

Results. Interestingly despite early ART, we found that the profiles and dynamics of B and Tfh cells are partially restored. Transcriptomic analyses revealed altered profiles of both B and Tfh cells that neither back to that observed from non-infected RMs. Using nonsupervised analysis, Tfh can be splited into different sub-populations showing distinct signatures. In addition, to their depletion, alterations in Tfh may impact on GC microenvironment. Preliminary data indicated specific metabolomic signature associated with microanatomical structures of lymphoid tissues.

Conclusions. SIV infection induces alterations in Tfh and B cell function in lymphoid organs that persist despite treatment.

Comparative efficacy of mRNA and self-amplifying RNA vaccine platforms in immune response induction

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Keywords. self-amplifying RNA vaccine, mRNA vaccine, $CD8^+$ T-cell responses

Abstract:

In recent years, the integration of messenger RNA (mRNA) technology with nanotechnology has made significant strides in developing innovative vaccine approaches. The accumulation of preclinical and clinical evidences suggests that mRNA vaccines are effective in combating infectious diseases and cancers, showing safety and good tolerance in both animals and humans. These vaccines induce strong antibody and $CD4^+$ T-cell responses, providing protection against various health threats. However, our research has revealed that mRNA vaccines are less effective at inducing $CD8^+$ T-cell responses and the $CD8^+$ T-cell responses are not long-lasting. Therefore, further improvements are necessary to address these limitations.

Self-amplifying RNA (saRNA) platform has the potential to overcome this challenge. saRNA can self-amplify *in vivo*, leading to prolonged antigen expression and sustained immune responses. We investigated the efficacy of saRNA vaccines and found that in experiments with the non-human primates, antibody titers remained one year after saRNA vaccine administration. Furthermore, our research using mouse models demonstrated that saRNA vaccines induced polyfunctional $CD8^+$ T cells capable of producing multiple cytokines simultaneously. These findings suggest that the saRNA vaccine platform not only offers sustained immunity but also enhances the quality of $CD8^+$ T-cell responses.

Currently, we are focusing on $CD8^+$ T-cell responses by utilizing constructs that tandemly incorporate $CD8^+$ T-cell epitopes restricted by HLA-A*02:01 for various infectious diseases and cancers. Administration of these constructs to HLA-A*02:01 transgenic mice confirmed the induction of epitope-specific $CD8^+$ T cells. Based on these results, we are conducting in vivo assays using the transgenic mice and in vitro assays using human peripheral blood mononuclear cells (PBMCs) to discuss the differences between mRNA and saRNA platforms.

Session 8 - New hopes: Vaccines & therapies

Dengue T cell vaccine development

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Keywords. Dengue virus, asymptomatic infection, T cell vaccine, mRNA/LNP vaccine, NS epitopes

Background
Dengue, the most common mosquito-borne disease caused by 4 serotypes of dengue virus (DENV), is increasing in prevalence due to climate change and travel to endemic regions. Current dengue vaccines aim to induce balanced neutralizing antibodies against the 4 DENV serotypes using tetravalent live attenuated chimeric viruses. However, phase 3 trials revealed imbalanced protection, raising concerns about antibody-dependent enhancement (ADE), a phenomenon where pre-existing antibodies may exacerbate subsequent infections with different DENV serotypes. We used an integrated approach of transcriptional profiling and immunological analysis to compare a Cambodian population of strictly asymptomatic viremic individuals with clinical dengue patients. Whereas inflammatory pathways and innate immune response pathways were similar, T cell immunity with feedback mechanisms controlled the immune response were highly activated in asymptomatic viremic individuals. We, therefore, designed a DENV-NS poly-epitope to activate dengue specific T cells for the next generation dengue vaccine.
Materials & Methods
To evaluate the ability of the DENV-NS poly-epitope to express the antigenic peptides in the context of different HLA class I molecules, we established its in vivo immunogenicity by measuring, after DNA immunization and electroporation, the activation of DENV-specific CD8 T cells in transgenic mice expressing the human HLA class I alleles. We then engineered a lipid nanoparticle encapsulated modified mRNA vaccine encoding DENV-NS and tested immunogenicity and protection in these human HLA class I transgenic mice, after transient blockade of the interferon type I receptor.
Results
Significant protection was observed, after two immunizations, without induction of DENV neutralizing antibodies.
Conclusions.
Collectively, these data strongly support the development of T cell-based vaccines targeting immunodominant T cell epitopes that generate potent virus-specific T cell responses conferring immunity against DENV infection.

Mucosal vaccination and delivery systems

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Keywords. Mucosal vaccines, nanoparticules, mRNA vaccines, infectious diseases.

Background. Current vaccines are mainly administered via intramuscular injection, which induces good systemic immunity but limited mucosal immunity. However, mucosal immunity represents a powerful tool to avoid infection or reduce human-to-human transmission, in particular due to the secretion of mucosal antibodies. Therefore, vaccination through mucosa can outperform parenteral vaccination strategies by inducing local protective immunity at mucosal sites, acting as the first line of defence against mucosal infections, as well as a broad and effective systemic immunity. Nasal and sublingual administration of vaccines were shown to induce mucosal immune responses (antibody-mediated and cytotoxic T-cell responses) in the upper and lower respiratory tract, stomach, small intestine, reproductive tract as well as a robust systemic response. These routes are thus promising for the development of vaccines against both respiratory viruses or sexually transmitted viruses.
Materials & Methods. The development of efficient vaccine delivery systems has to overcome technological as well as biological barriers such as the presence of mucus and the immune mucosal tolerance. In our group, we designed nanovectors to protect the antigen and deliver it to the mucosal immune system.
Results. Polymeric, lipidic as well as hybrid nanovectors carrying either viral proteins or mRNA were produced and characterized for sublingual and nasal delivery. The encapsulation of adjuvants (required for mucosal administration) and the subsequent analysis of the immune response in mice to model antigens showed that the highest protection is reached by a combination of parenteral and mucosal route of administration.
Conclusions. The development of mucosal vaccines, though challenging, is promising and exciting in the field of vaccination and will probably tackle some of current issues in vaccinology when combined with mRNA technology.

Development of an antibody-drug conjugate（ADC）targeting CADM1 in adult T-cell leukemia/lymphoma cells.

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Keywords. Antibody-drug conjugate, Adult T-cell leukemia/lymphoma (ATL), HTLV-1, Malignant lymphoma.

Background. Effective therapeutic approaches to improve the prognosis of Adult T-cell leukemia/lymphoma (ATL) has been long explored. CADM1 is a cell adhesion molecule, which is overexpressed in ATL cells and HTLV-1 infected T cells. Recently, a remarkable efficacy of ADC such as brentuximab vedotin (anti-CD30 antibody-VcMMAE) has been reported in hematological malignancies. Therefore, the aim of this study is to develop an ADC targeting CADM1 to improve the prognosis of HTLV-1-related diseases including ATL.
Materials & Methods. An anti-CADM1 monoclonal antibody was developed by immunizing rat with a mixture of CADM1 isoforms expressed in ATL cells. Developed rat IgG was engineered to rat-human chimeric antibody (chimeric IgG). Physicochemical and biological properties of chimeric IgG were analyzed, followed by biokinetic analysis in immunodeficient mice (NOJ). Finally, the efficacy of the ADC (chimeric IgG-VcMMAE) was examined.
Results. The chimeric IgG showed specific binding to CADM1 on the cell surface of ATL and HTLV-1 infected T-cells with a high affinity, and internalization followed by localization in lysosomes. The ADC specifically and significantly suppressed cell growth of CADM1+ T-cell lines. Adittionally, the ADC effectively reduced ATL and HTLV-1 infected T-cell populations in ex vivo cultured indolent ATL-patient PBMC. Biokinetic analysis chimeric IgG showed a specific targeting against CADM1+ human T-cell lines grafted in NOJ mice. Finally, the ADC treatments in NOJ mice grafted ATL-derived cell line showed significant reduction of the tumor volume compared with PBS treated control.
Conclusions. Anti-CADM1 ADC showed specific and effective elimination of CADM1+ cells in vitro and in vivo, providing evidence that the CADM1-targeting ADC is an effective therapeutic candidate to improve the prognosis of ATL.

Distinct adjuvanticity for de novo or recall CD8⁺ T cell responses

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Keywords: CD8⁺ T cells; viral infections; vaccines; therapies; T cell exhaustion

Background. CD8⁺ T cells are critical for controlling viruses like HIV, SARS-CoV-2 or even influenza and preventing associated diseases. However, inducing potent virus-specific CD8⁺ T cells using therapeutic strategies faces several hurdles, in particular associated with contexts of waning immune competence, such as hyper activation and chronic inflammation. Our aim is to develop optimal approaches to either prime or boost virus-specific CD8⁺ T cell responses in humans.
Materials & Methods. We study the effects of different molecules, including ligands for pattern recognition receptors and cytokines on the priming and boosting of antigen-specific CD8⁺ T cells using an *in vitro* human system in different settings.
Results. We show here that naive and memory T cells have distinct requirements in terms of adjuvanticity to mount effector responses. The STING agonist cGAMP and type I IFNs are optimal for priming of naive T cells, but not for boosting of memory T cells. The TLR8 ligand ssRNA40, which combines the effects of inflammatory cytokines and IL-12, emerges as a particularly potent molecule to promote the expansion of memory T cells and their acquisition of effector molecules, even in conditions of T cell exhaustion associated with HIV-1 or SARS-CoV-2 infection.
Conclusions. These considerations to induce effective T cell responses are important for the design and efficacy of vaccine and immunotherapy approaches.

The challenge establishing an HIV cure strategy by using the NHP model.

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Keywords. Functional cure, T-cell responses, innate immune responses

Abstract:

With the spread of the combination of antiretroviral therapy (cART), it is becoming common to control the onset of AIDS even if a person is infected with HIV. However, cART treatment does not fully restore the immune system, and in HIV-infected patients under cART (known as people living with HIV: PLWH), there are cases of chronic inflammation even if the viral load in the blood can be controlled below the detection limit due to the side effects of cART over the years. As a result, PLWH has a higher risk of AIDS-related illness and death than people of the same age without HIV.
In PLWH under cART, latent HIV infected cells tend to gradually decrease over time because viral replication is suppressed. However, its half-life is extremely long, there is always a certain amount of latent infected cells in the body. Thus, by interrupting cART, latently infected cells are reactivated and viral replication is activated. Therefore, in order to create a cART-free state, it is essential to eliminate these latent infected cells.
Among the various approaches to achieve this goal, we have studied the possibility/reality of "Kick (Shock) & Kill" strategy, which could apply to all patients and has advanced to clinical trials. For the functional cure study, we established the SIV chronic infection cART model of cynomolgus monkeys and conducted mutual analyses between virus-infected cells and host immune cells. In this talk, I would like to summarize our data from the NHP model and discuss how we can apply these data to a future clinical trial.

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