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Infectious Diseases
Mouraya Hussein, PhD student

Novel CRISPR-Cas13d system to target highly conserved sequences of SARS-CoV-2 and other human coronaviruses

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The current SARS-CoV-2 pandemic is a major global health burden. Although protective vaccines are available, concerns remain as new viral variants continue to appear worldwide. Gene editing-based antiviral approaches, such as CRISPR-Cas, may offer an attractive alternative as it can be rapidly adjusted to new viral sequences. This study aimed at using CRISPR-Cas13 to target highly conserved viral RNA sequences, not only among different SARS-CoV-2 variants, but also among the other human coronaviruses, thus preparing for future zoonotic outbreaks of novel coronaviruses.
Complete genome sequences of SARS-CoV-2 variants were aligned in order to identify conserved sequences. Moreover, SARS-CoV-2 sequences were aligned with those of other human coronaviruses to identify broadly conserved sequences. Based on the selected target sequences, crRNAs were designed. The knock-down efficiency of the crRNAs was tested in vitro in transfected cells with a designed multi-target luciferase reporter construct. We identified a panel of crRNAs targeting highly conserved SARS-CoV-2 sequences with a high predicted activity. The majority of these crRNAs induced a strong reduction in luciferase signal. The antiviral activity was subsequently validated by scoring the crRNA knock-down efficiency in cells transfected with the SARS-CoV-GFP and SARS-CoV-2-mNeon replicons. Selected crRNAs demonstrated efficient targeting of the SARS-CoV and SARS-CoV-2 replicons, confirming that the designed crRNAs exhibit broader anti-coronavirus activity. Overall, this study demonstrates potent targeting of highly conserved SARS-CoV-2 sequences by the novel CRISPR-Cas13 system and emphasizes the importance of rationally designing gene-editing based antiviral strategies to prepare for future outbreaks of novel coronaviruses.

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Infectious Diseases
Moussa Djiimde, PhD student

Assessment of the impact of pregnancy and malaria infection on the variation of neutrophil levels in women from San, Mali

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Background: Severely decreased neutrophils levels can have life-threatening implications. Probably due immunological and hormonal changes, pregnant women are more likely to get malaria than non-pregnant women. It is essential to understand whether pregnancy induces changes in neutrophil levels and thereby poses a threat to the health of gravidae.
Methods: This is a cross-sectional analytical study assessing the impact of malaria in pregnancy on neutrophil level variation. The study was conducted in San Health District (Mali) and involved pregnant women infected or not by Plasmodium falciparum and non-pregnant healthy volunteers. Subjects were categorised as having neutropenia, normal neutrophil levels and neutrophilia. A linear regression model allowed to determine factors associated with neutrophil level variations in pregnant women.
Results: White blood cells mean count (4416.3, SD= 1313.8) while lower in healthy non-pregnant subjects, decreased from pregnant women without malaria infection (7673.8, SD= 10515.6) to pregnant women with malaria infection (5493.2, SD= 1528.6). Pregnant women in the malaria infected and non-infected groups had each 48.5% (98/ 202) cases of neutrophilia (48.5%). Surprisingly, 67 of the 71 cases of neutropenia (94.4%) observed in this study were in apparently healthy non-pregnant individuals. Categorising gestational age, the mean percentage of neutrophils level was significantly lower ($p<0.001$) in the first trimester (49.92%) compared to the second trimester of pregnancy (62.01%). A linear regression model showed that compared to early pregnancy, the second (OR= 3.301e+05, p= 0.001) and the third trimester (OR= 5.36e+05, $p< 0.001$) were strongly associated with an increase in neutrophil levels. The model also showed that each increase in malaria parasite density multiplies the neutrophil levels by 1.0001 times (OR= 1.0001, $p< 0.001$).
Conclusion: Data from Mali shows benign neutropenia in healthy non-pregnant people. This study suggests that, in absence of malaria infection, the second trimester of pregnancy is strongly associated with an increase in neutrophil levels.
Keys Words: Neutrophils, Pregnancy, Malaria, Mali

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Infectious Diseases
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Potent SARS-CoV-2 Omicron Neutralization by a Monoclonal Antibody Isolated from a Gamma-infected Individual

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The worldwide pandemic caused by SARS-CoV-2 is a constant human medical threat due to the ongoing development of multiple vaccine and antibody-resistant variants. Since monoclonal antibodies (mAbs) can be used both therapeutically and prophylactically, it is our goal to discover novel broadly-reactive neutralizing mAbs. A stabilized autologous SARS-CoV-2 spike glycoprotein was used to enrich antigen-specific B cells from a Gamma-infected individual. Five novel mAbs retrieved from those B cells showed distinct functionalities and considerable neutralizing potency against multiple variants, with COVA309-2D11 being the most potent against the autologous strain, as well as against Omicron BA.1 and BA.2. When combined as cocktails or bispecific antibody formats, breadth and potency were significantly improved against all strains. In addition, structural analysis elucidated mechanism of cross-neutralization of the COVA309 mAbs. Altogether these data indicate that a Gamma-infected individual can elicit broadly neutralizing antibodies, which can neutralize the highly distinct Omicron variants.

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Infectious Diseases
Ana Chumbe Mendoza, PhD student

Antibody neutralization and potential effector functions directly after primary HCV is associated with the protection against reinfection

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Upon spontaneous clearance or after treatment of a primary hepatitis C virus (HCV) infection, reinfection is a serious risk, especially among HIV+ men who have sex with men (MSM) with high-risk behavior. HIV+ MSM at risk for acute HCV infection can be identified by the validated HCV-MOSAIC risk score. To study correlates of protective immunity, we analysed the antibody responses or lack thereof in MSM with continued risk behaviour who did or did not become reinfected following successful treatment of primary infection.
HCV non-reinfected (NR) participants of the MOSAIC cohort with at least 2 years of follow up following primary clearance and documented HCV risk score were selected. As a control group, reinfected (R) participants, matched for calendar time of primary HCV infection and follow-up duration were selected. Sera from two time points were used; (T1) 3-6 months after primary infection and (T2) 3-6 before reinfection or at a comparable follow-up moment since primary infection in the matched NR group. A panel of HCV pseudoparticles was used to assess neutralization breath and potency. A bead-based immunoassay (Luminex xMAP technology) with beads bearing membrane bound E1E2 envelopes was used to evaluate HCV-specific IgA, IgG (IgG1, IgG2, IgG3 and IgG4) and IgM binding and potential antibody-dependent effector functions (FcγRIIa, FcγRIIIa and C1q).
Age of infection, CD4 counts at T1, duration of infection or duration of treatment showed no significative differences between R and NR groups (Mann Whitney test, $p>0.05$ in all cases). Reported risk behaviour was higher among R than in NR gropus, median risk score at T1 of 3.4 versus 1.4, respectively (Mann Whitney test, $p=0.0003$). We found higher neutralization potency and breadth as well as higher IgG1 binding directly after primary infection in the NR group. We also observed an increased binding of FcγRIIa, FcγRIIIa and C1q in the NR group directly after primary infection, suggesting higher antibody dependent cellular phagocytosis (ADCP), antibody dependent cell mediated cytotoxicity (ADCC) and complement dependent cytotocicity (CDC) activities in this group. At the second time point, we only detected higher C1q binding in the NR group.
Several antibody-associated responses shortly after primary infection were associated with the protection against reinfection in MSM with continued risk behaviour. Memory B-cell rather than circulating antibodies may be playing a role in long-term immunity, as circulating antibodies were found shortly after primary infection but not later during follow-up. These results may guide future development of vaccines and new therapeutics to protect against HCV infection.

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Infectious Diseases
Norbert van Dijk, PhD student

Laboratory evaluation of the miniature direct-on-blood PCR nucleic acid lateral flow immunoassay (mini-dbPCR-NALFIA), a simplified molecular diagnostic test for malaria and visceral leishmaniasis

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Background
Currently used point-of-care (PoC) diagnostics for malaria suffer from poor sensitivity for low parasite densities. For visceral leishmaniasis (VL), PoC serological diagnosis is not suited for detection of relapses or as test of cure. Hence, more accurate parasitological diagnostic tests for routine clinical settings are warranted. The miniature direct-on-blood PCR nucleic acid lateral flow immunoassay (mini-dbPCR-NALFIA) is an innovative, easy-to-use molecular tool for the diagnosis of blood pathogens in resource-limited settings. This assay circumvents DNA extraction and makes use of a handheld, portable thermal cycler that can be powered with a solar-charged power pack. Result read-out is done using a rapid lateral flow strip. A laboratory validation was done to assess the performance of the mini-dbPCR-NALFIA for diagnosis of Plasmodium and VL infections in EDTA blood.
Methods
Diagnostic accuracy for malaria was assessed with a set of microscopy-confirmed Plasmodium-positive (n=29) and -negative blood samples (n=63). For VL, qPCR-confirmed L. infantum-positive (n=73) and -negative blood samples (n=73) were tested. P. falciparum limit of detection (LoD) was estimated with dilution series of three clinical P. falciparum blood samples. The LoD for L. donovani was estimated with a promastigote culture diluted in healthy donor blood.
Results
For Plasmodium, the assay had an overall sensitivity and specificity of 96.6% (95% CI, 82.2% - 99.9%) and 96.8% (95% CI, 89.0% - 99.6%), respectively. The P. falciparum LoD was 2 parasites per microlitre of blood. Sensitivity for VL was 94.5% (95% CI, 86.6% - 98.5%) and specificity was 97.3% (95% CI, 90.5% - 99.7%). VL LoD was 10 promastigotes per millilitre of blood.
Discussion
The mini-dbPCR-NALFIA is a sensitive, specific and relatively easy method for accurate detection of Plasmodium and VL infections in whole blood. Field trials are currently being conducted to evaluate the potential implementation of this assay in malaria and VL control programmes in different transmission settings.

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Infectious Diseases
Lisa van Pul, PhD student

Differences in the gene expression profile of HIV- and CMV-specific CD8 T cells in HIV infection

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Lifelong treatment of HIV infected individuals is essential since current therapies cannot eliminate the HIV reservoir. To gain immune control upon treatment interruption or to eradicate/reduce the viral reservoir during kick-and-kill strategies, a fully effective CD8 T cell response is thought to be essential. A highly efficient HIV-specific CD8 T cell response is found in rare long-term non-progressors (LTNP) that have natural immune control of HIV infection. Here we studied the transcriptional profile of virus-specific CD8 T cells to gain molecular insights into CD8 T cell functionality in HIV infection.
HIV- and CMV-specific CD8 T cells were isolated from PBMC of HIV-infected individuals from different groups: HIV progressors and LTNP groups (HLA-B57 and non-HLA-B57). Virus-specific CD8 T cells were sorted using MHC class-I dextramers presenting either HIV or CMV immunodominant peptides. In addition, CMV-specific CD8 T cells were isolated from blood donors (BD). The transcriptional profile of sorted cells was generated by RNA sequencing (QIAseq UPX 3' transcriptome kit).
Principal component analysis (PCA) of the transcriptional profiles of HIV-specific CD8 T cells of LTNP groups and progressors revealed distinct profiles between groups. However, differential gene expression analysis revealed only a limited number of differentially expressed genes (DEGs) when comparing progressors to the different LTNP groups. Pathway analysis of the identified DEGs revealed that most of the genes have functions in RNA and protein metabolism pathways, while some genes were involved in immunological pathways. These observations indicate that control of HIV infection in LTNP individuals is associated with increased functionality of their HIV-specific CD8 T cells. The transcriptional profile of CMV-specific CD8 T cells from the same participants showed almost no overlap in DEGs with HIV-specific CD8 T cells even though the DEGs were related to similar pathways. Compared to BD, the transcriptional profile of CMV-specific CD8 T cells of progressors showed increased expression of genes related to effector function and in a PCA both HIV-progressors and LTNP clustered separately from BD.
The findings in our study suggest that control of HIV infection in LTNP is associated with increased functionality of HIV-specific CD8 T cells. Results also indicate that the gene expression profiles are virus-specific and the CMV-specific CD8 T cell response is not hampered in these HIV infected individuals. Here we provide insights into the perturbed immune function of HIV-specific CD8 T cells during HIV infection.

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Infectious Diseases
Stefanie Kroeze, Postdoc

Specific plasma microRNAs are associated with CD4+ T-cell recovery during suppressive antiretroviral therapy for HIV

1, 2, 3, 4 Kroeze S, 1, 2, 3, 4 Kootstra NA, 3, 4 van Nuenen AC, 5 Rossouw TM, 6 Kityo CM, 7 Siwale M, 8 Akanmu S, 9 Mandaliya K, 10 de Jager M, 1, 2, 11 Ondoa P, 1, 2, 4, 12, 13 Wit FW, 1, 2, 4, 13 Reiss P, 1, 2, 4 Rinke de Wit TF, 1, 2, 4, 13, 14 Hamers

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Despite viral suppression mediated by antiretroviral therapy (ART) for HIV-1, it remains incompletely understood why in some people CD4+ T-cell recovery fails. Host microRNAs have been shown to play an important role in HIV-1 pathogenesis. This study investigated whether plasma microRNAs were associated with poor CD4+ T-cell recovery during viral suppression mediated by ART. In an African cohort of persons living with HIV (PLWH) who had plasma viral-load <50 cps/mL after 12 months of ART, we first screened the levels of 179 microRNAs in a random subset of participants from the lowest- and highest tertiles of CD4+ T-cell recovery after the first 12 months of ART (N=12 each). Eleven candidate microRNAs that differed between groups, were validated in an additional 113 participants from the same cohort. Using multivariable logistic regression, we found that higher levels of hsa-miR-199a-3p and hsa-miR-200c-3p before ART, and of hsa-miR-17-5p and hsa-miR-501-3p during ART, were associated with poor CD4+ T-cell recovery. Pathway analysis of hsa-miR-199a-3p, hsa-200c-3p, and hsa-miR-17-5p suggested a possible role for pathways involved in signaling by VEGF and MET, and RNA polymerase II transcription. Lastly, we compared plasma microRNA levels of PLWH with HIV-negative controls, and observed lower hsa-miR-326, hsa-miR-497-5p, and hsa-miR-501-3p levels before and during ART in all PLWH, and we observed higher hsa-miR-199a-3p and hsa-miR-200c-3p levels before ART in all PLWH, and during ART in participants with poor CD4+ T-cell recovery only. These findings add to the understanding of the role of microRNAs in persistent HIV-induced immune dysregulation during suppressive ART.

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Infectious Diseases
José Dekker, PhD student

How mutations in the hiv-1 3'-polypurine tract confer dolutegravir resistance

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The integrase strand-transfer inhibitor (INSTI) dolutegravir (DTG) is widely applied in combination antiretroviral therapy for HIV-infected individuals. Resistance to DTG is usually associated with mutations in the integrase gene, but a previous in vitro HIV selection study identified a mutation in the 3'-polypurine tract (3'PPT) that reduced DTG sensitivity (1), and mutation of this viral sequence was also observed in a patient with virologic failure on DTG monotherapy (2). We predicted that such PPT mutations may affect the reverse transcription process (3), in particular the start site of second-strand DNA synthesis and thereby the 5' end of the viral DNA that is the template for integration. We here set out to identify other PPT mutations that cause DTG resistance and to determine the molecular mechanism of PPT-mediated INSTI resistance.
We designed a library of HIV-1 genomes with a randomized PPT sequence. Culturing of this pool of PPT-variants on C8166 T cells in the presence of DTG resulted in the selection of viruses with different mutations in the 3'PPT. Single-cycle infection and multi-cycle replication experiments revealed that the selected 3'PPT mutations reduce viral fitness, yet improve virus replication with DTG. Intriguingly, replication of the 3'PPT-mutated viruses is activated by the HTLV-1 Tax protein that was recently shown to stimulate episomal (2-LTR circle) replication of an integrase-deficient HIV variant (4,5). Analysis of the integrated and non-integrated viral DNA products formed upon infection indicates that the 3'PPT mutations do not restore integration, but rather stimulate the production of such non-integrated HIV DNA products.
Our data indicate that several 3'PPT mutations cause DTG resistance. The mutations stimulate production of a non-integrating (episomal) DNA intermediate, which may allow a low level of integration-independent HIV replication. Further analysis of the mechanism of PPT-mediated DTG-resistance and the impact on viral fitness is important for a complete understanding of this potent drug class.
Acknowledgements
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Infectious Diseases
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Mapping the antibody repertoire to Staphylococcus aureus wall teichoic acid reveals a protective role for IgM during invasive infection

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Staphylococcus aureus is one of the leading causes of hospital-acquired infections with high overall mortality. Pre-existing immunity to S. aureus is common among healthy individuals due to natural exposure, although this is not always sufficient to protect from (re-)infection. Antibodies are believed to play a key role in cell-mediated bacterial killing through antibody opsonization and complement activation, which enhances bacterial uptake and immune cell recruitment. A large proportion of the anti-S. aureus antibody pool is directed against Wall Teichoic Acid (WTA), an abundant cell wall glycopolymer, which shows structural variation through glycosylation with N-acetylglucosamine (GlcNAc). Overall, three WTA glycotypes are currently distinguished, which elicit IgG antibody responses in healthy individuals. How these anti-WTA antibody profiles are affected during invasive S. aureus infection is not known but may provide insight in protective responses. We analyzed the antibody repertoire to S. aureus WTA in plasma from healthy individuals (n=31) and patients with culture-confirmed S. aureus bacteremia (n=38) on the intensive-care unit (ICU) using in vitro-glycosylated synthetic WTA fragments that resemble the three S. aureus WTA glycotypes. Robust IgM responses to all three WTA modifications were detected in >95% of healthy individuals, whereas on average WTA-specific IgM responses were significantly decreased (three-fold lower) in patients with S. aureus bacteremia and several patients lacked anti-WTA IgM. Moreover, decreased WTA-specific IgM responses was further associated with disease mortality within the ICU patients cohort. No differences in IgG2 responses were observed. Longitudinal analysis showed limited variation of WTA-specific antibody responses over the course of infection. In an additional cohort, we observed decreased IgM reactivity to intact S. aureus bacteria in serum of patients with invasive S. aureus infection compared to healthy individuals, suggesting that WTA presents a dominant surface target for IgM. This study supports the existence of a broad antibody repertoire to S. aureus WTA glycotypes, and hints towards a protective role of WTA-specific IgM antibodies against invasive S. aureus infections.

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Infectious Diseases
Marieke Kuijk, PhD student

Interference with lipoprotein maturation sensitizes methicillin-resistant Staphylococcus aureus to human
2 group IIA secreted phospholipase A2 and daptomycin.

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Methicillin-resistant Staphylococcus aureus (MRSA) has been classified as a high priority pathogen by the World Health Organization underlining the high demand for new therapeutics to treat infections. Human group IIA secreted phospholipase A2 (hGIIA) is among the most potent bactericidal proteins against Gram-positive bacteria, including S. aureus. To determine hGIIA-resistance mechanisms of MRSA we screened the Nebraska Transposon Mutant Library using a sublethal concentration of recombinant hGIIA. We identified and confirmed the role of lspA, encoding the lipoprotein signal peptidase LspA, as a new hGIIA resistance gene in both in vitro assays and an infection model in hGIIA-transgenic mice. Increased susceptibility of the lspA mutant was associated with faster and increased cell wall penetration of hGIIA. Moreover, lspA deletion also increased susceptibility to daptomycin, a last-resort antibiotic to treat MRSA infections. Exposure of MRSA wild-type to the LspA-specific inhibitors globomycin and myxovirescin A1 induced a lspA mutant phenotype with regard to hGIIA and daptomycin killing. Analysis of >26,000 S. aureus genomes showed that LspA is highly sequence 46 conserved, suggesting that LspA inhibition could be applied universally. The role of LspA in hGIIA resistance was not restricted to MRSA since Streptococcus mutans and Enterococcus faecalis were also more hGIIA-susceptible after lspA deletion or LspA inhibition, respectively. Overall, our data suggest that pharmacological blocking of LspA may disarm Gram-positive pathogens, including MRSA, to enhance clearance by innate host defense molecules and clinically-applied antibiotics.

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Infectious Diseases
Lance Mulder, PhD student

Studying cytomegalovirus in human iPSC-derived neuronal cell types and human organoid models

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Background:
Human cytomegalovirus (CMV) is a DNA virus of the Betaherpesvirinae subfamily of the herpesviruses. During pregnancy, infected mothers can transmit the virus to their developing child resulting in congenital CMV (cCMV) infection potentially leading to long-term neurological sequalae such as hearing, visual and neurodevelopmental impairment. It remains unclear why certain cCMV infected children develop symptoms while others remain asymptomatic. Different CMV strains exhibit different infection behaviours in similar cell types and thus, evaluating the effects of symptomatic cCMV neonates-derived strains (cCMV strains) can help understand the underlying mechanisms of these differences in the brain. To do this we used human induced pluripotent stem cell (iPSC)-derived brain organoids as a model for the developing brain, and CNS relevant cell types to evaluate potential differences in neurotropism of two symptomatic cCMV strains.
Methods:
The cCMV strains were isolated by cell culture from urine of 2 symptomatic cCMV neonates with symptomatic cCMV infection and were used to infect brain organoids and 2D monocultures of CNS and BBB- relevant cell types. The BAC derived TB40/E-mCherry strain served as a control. Cerebral, forebrain and choroid plexus organoids were generated from iPSCs. The iPSCs were also used to generate neural progenitor cell, neuron and astrocyte 2D cultures.
Results:
Analysis of viral replication marker (UL86; late replication/ virus assembly marker) was performed by qRT-PCR on supernatant and lysed monolayer/ organoid cultures, collected at different time points. Viral replication is observed in two brain organoids models (cerebral and choroid plexus organoids) after 12 and 21 days post-infection (dpi). Neurotropism is confirmed by IF and shows localised virus in the organoids at 12 dpi.
Conclusion:
We successfully infected the different brain organoid models with cCMV strains and observed strain-dependent differences. The monolayer infections results should tell us more about the cell specific differences.

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Infectious Diseases
Olivier Papapietro, Postdoc

DOMINANT MUTATION IN TOPOISOMERASE 2-BETA CAUSES B CELL IMMUNODEFICIENCY

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Rare mutations that affect early stages of B cell development cause B cell immunodeficiencies and agammaglobulinemia. Here we studied two families with the autosomal dominant immunodeficiency with undetectable B cells and a rare combination of skeletal and urogenital defects, known as the BILU syndrome. Exome sequencing led us to identify a novel heterozygous missense mutation A485P in the TOP2B gene that segregated perfectly with the clinical phenotype in four patients from the two unrelated BILU families. Topoisomerase 2-beta (TOP2B) is an enzyme that regulates DNA topology through the introduction of transient double strand breaks. TOP2B has been implicated in regulation of transcription during cell differentiation. We demonstrated that the newly found mutation A485P affects stability and catalytic activity of TOP2B. Bone marrow analysis of the BILU patient showed a complete absence of CD19+ B cell progenitors, but normal T cell and myeloid progenitors, suggesting that TOP2B is critically important for the early stages of B cell differentiation. To investigate the emerging role of TOP2B in B cell development we used mass spectrometry and characterised the TOP2B interactome. We demonstrated that in pro-B cells TOP2B is a part of a multi-protein complex that regulates epigenetic control of gene expression and that its inhibition abrogates expression of the key transcription factors known to regulate B cell fate. Our work discovered a novel dominant mutation in TOP2B that causes a rare primary immunodeficency syndrome and revealed a previously unrecognised role of TOP2B in cellular commitment to the B cell lineage.

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Infectious Diseases
Pamela Capendale, PhD student

Cerebral organoids as a model to study genotype dependent potential of Parechovirus A to cause Central Nervous System related illnesses

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Parechovirus A (PeV-A) from the Picornaviridae family is among the most prevalent human viruses worldwide. The most prevalent genotype PeV-A1 usually causes respiratory and gastrointestinal infections in infants. The second most prevalent genotype PeV-A3 causes severe central nervous system (CNS) diseases such as encephalitis and meningitis. The cause for the differential outcomes for these genotypes is poorly understood. Here, we investigate the viral dynamics and tropism of genotype PeV-A1 and PeV-A3 in the CNS by using iPSC (induced pluripotent stem cell)- derived human cerebral organoids.

Cerebral organoids were cultured for 67 days before inoculation with PeV-A1 and PeV-A3. Echovirus 11 (Echo11) was included as a positive control. Production of viral particles and their infectivity were quantified using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and median tissue culture infectious dose (TCID50) assay respectively. Changes in cytokine expression upon infection were quantified by RT-qPCR.

RT-qPCR and TCID50 data shows replication of PeV-A1, PeV-A3, and Echo11 and the production of infectious particles in the cerebral organoids over 10 days. Upregulation of cytokine expression was observed upon infection with PeV-A3 and Echo11 for pro-inflammatory cytokines TNF- α, IFN-γ and IFN- α2. Notably, even though PeV-A1 genotype also productively infected cerebral organoids, no significant cytokine upregulation was observed upon PeV-A1 infection.

For the first time, we demonstrate in vitro infection of PeV-A using iPSC-derived human brain organoids. Difference in CNS related pathology between the two genotypes of PeV-A is not due to inability of PeV-A1 to infect the CNS, as both PeV-A1 and PeV-A3 were shown to productively generate (infectious) particles. Upregulation of pro-inflammatory cytokines upon PeV-A3 infection indicates that PeV-A3 related CNS illness could be related to an increased pro-inflammatory response in the host.

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Infectious Diseases
Kwinten Sliepen, Postdoc

Structure of the hepatitis C virus E1E2 glycoprotein complex

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Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma in humans, and afflicts more than 58 million people worldwide. There is no effective vaccine against HCV. The HCV E1 and E2 glycoproteins on the virion are essential for viral entry and comprise the only antigenic target for neutralizing antibody responses. The molecular structure of E1E2, as well as how the E1E2 heterodimer binds broadly neutralizing antibodies, remains elusive. We present the cryo-electron microscopy (cryoEM) structure of the membrane-extracted full-length E1E2 heterodimer in complex with broadly neutralizing antibodies (bNAbs) AR4A, AT1209 and IGH505 at ~3.5 Å resolution. We resolve the long sought-after interface between the E1 and E2 ectodomains and reveal how it is stabilized by hydrophobic interactions and glycans. This structure deepens our understanding of the HCV fusion glycoprotein and delivers a blueprint for the rational design of novel vaccine immunogens and anti-viral drugs.

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Infectious Diseases
Nikitha Vavilthota, PhD student

Antimicrobial peptides: Potent alternative to antibiotics for treating hard-to-heal-wounds.

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Hospital acquired infections (HAIs) are infections acquired in health-care settings, they are a severe world-wide problem and constitute a vast societal challenge as they pose a serious threat to health, long term well-being and life. Current antibiotics resistance in bacteria poses an additional burden of HAIs. The most common HAIs are urinary tract, wound or surgical site infections. Exposure of subcutaneous tissue following a loss of skin integrity (i.e., a wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Group of multi-drug resistant bacterias belonging to the ESKAPE panel are the common colonizers of wounds and are capable of forming biofilms. Biofilms have been recognized as the major contributor of hard-to-heal status of chronic wounds. Biofilm encased bacterias are 10-1000 fold more resistant to conventional antibiotics. Presently there is an unmet clinical need in wound care for theranostics tools that allow early detection and treatment of infected wounds.
In this light, the European Marie Skłodowska training network STIMULUS "Stimuli Responsive Materials for the Rapid Detection and Treatment of Healthcare Associated Infections" aims at creating medical devices that signal when an infection is present and thereby reduces the unnecessary prescription of antibiotics and hence the spread of antibiotic resistance. An important feature is the release of potent antimicrobials by an externally triggered stimulus to treat the infection.
Antimicrobial peptides (AMPs) present an attractive and potent option as an antimicrobial agent. They are mostly cationic, and amphiphilic broad-spectrum host defense antimicrobials that are produced by all organisms ranging from prokaryotes to humans. We aim to encapsulate these AMPs in stimuli responsive nanocarriers such as liposomes for triggered release of agents to treat chronic infections without resistance development. Presently I am developing in-vitro and in-vivo skin infection models such as human skin equivalents and mice skin stirp models to test the antimicrobial activity of these AMPs.

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Infectious Diseases
Deeksha Rajkumar, PhD student

Novel Antimicrobial Release Coatings and Medical Device Technologies

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Medical device (Biomaterials)- associated infection is a major risk in the use of biomaterials. These infections are most frequently caused by staphylococci. Generally, these infections are difficult to treat due to tolerance or resistance to antibiotics. Biofilm formation on the implant surface contributes to phenotypic tolerance, and possibly to antimicrobial resistance (AMR) and persistent infection. The bacteria can also survive intracellularly in peri-implant tissue, increasing their resistance to antibiotic treatment. As many antibiotics are incapable of eliminating bacteria in biofilms on the implant surface and in peri-implant tissue, it is imperative to develop and validate alternative antimicrobial technologies. If these technologies are successful, they may also improve the efficacy of existing antibiotics, allowing for combination therapy. The objective of this project, within the Dutch Antimicrobial Resistance Technology development and Biofilm Assessment Consortium (DARTBAC), is to foster the development of novel antimicrobial technologies to combat antibiotic resistance. These technologies include Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs), SAAP encapsulated polymers, Bioactive glass S53P4 and Photosensitizers. This study will thus offer novel antimicrobial strategies as well as sustainable methods for evaluating antimicrobial activity and cell cytotoxicity. This way, the project aims to contribute to safer medical devices and lower risks of antibiotic resistance.

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Infectious Diseases
Laure van Hofwegen, PhD student

Investigating the gene expression profiles underlying biomaterial-associated infection

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The most common complication related to implantation of a biomaterial is biomaterial-associated infection (BAI). BAI is predominantly caused by S. epidermidis, a commensal bacterium that causes pathology in the presence of a biomaterial, which leads to chronic infection, revision surgeries and in severe cases, loss of function. In this project, the molecular details of the host response in BAI, as well as the foreign body response (FBR) to biomaterial, will be studied by gene expression analysis. Based on these data we will further study the host response using in vivo mouse models for bone or subcutaneous implant infection. Ultimately, we aim to identify biomarkers that can predict infection susceptibility to biomaterial implantation.

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Infectious Diseases
Jade Jansen, PhD student

Elimination of the HIV-1 viral reservoir using DDX3 inhibitors

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The persistence of the HIV-1 reservoir containing transcriptional latent HIV-1 in effectively treated people living with HIV-1, remains the biggest obstacle to a cure. A proposed cure strategy, 'shock-and-kill', is aimed at reactivation of latent HIV-1 (shock) and subsequent elimination via immune mediated pathways or HIV-1 related cytotoxicity (kill). One of the major hurdles of this strategy is induction of death of reactivated HIV-1 infected cells.
Interestingly, HIV-1 hijacks the function of cellular host factors such as nuclear export and translation by DDX3 for efficient replication. Therefore, inhibition of host factor DDX3 has the potential to limit HIV-1 replication as well as triggering apoptotic processes via accumulation of viral RNA. Here we investigated the functional impact of DDX3 blockage using a small molecule DDX3 inhibitor (DDX3i) on latency reversal and elimination of the viral reservoir.
The effects of DDX3 inhibition was analyzed in the latency cell line J-lat (expression of GFP upon reactivation) by flow cytometry. Increasing concentrations of DDX3i treatment did not lead to enhanced latency reversal in the J-lat cells. Treatment with the DDX3i however resulted in a large proportion of the reactivated cells undergoing apoptosis. Notably, DDX3i treatment in combination with a latency reversal agent (LRA) resulted in a larger proportion of reactivated cells (21%) as compared to the LRA treated cells only (12%). In addition, DDX3i treatment resulted in a 2-fold increase in reactivated cells undergoing apoptosis.
To further investigate the effect of DDX3 inhibition, a dual reporter HIV-1 was used to infect dendritic cells (DCs) to discriminate between latent and transcriptionally active HIV-1 infected cells by flow cytometry. One clear latent and one transcriptionally active HIV infected DC population could be distinguished by flow cytometry: 18% latent and 48% active infection. Notably, treatment with DDX3i together with a LRA strongly decreased the proportion of latent and transcriptionally active infected DCs (13% and 17%, respectively).
To conclude, here we show that inhibition of DDX3 leads to selective cell death of both active and latent infected HIV-1 cells in the different models used. Our data suggest that the inhibition of DDX3 leads to cytotoxicity of HIV-1 infected cells