ABSTRACT BOOK

2nd International Conference on INDIVIDUALIZED INFECTION MEDICINE

19-21 MARCH 2026

ORGANISERS

CiiM CENTRE FOR INDIVIDUALISED INFECTION MEDICINE

HZI HELMHOLTZ Centre for Infection Research

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CONTENT OVERVIEW

SELECTED ABSTRACTS

PRESENTED AS POSTER AND LIGHTNING TALK

1 Epigenetic regulation of cytokine responses in aging populations

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Inter-individual variability in cytokine responses critically influences susceptibility to infection, inflammation, and autoimmune disease. While genetic factors are known contributors, the role of epigenetic regulation, particularly DNA methylation, in shaping cytokine responses remains incompletely understood. We profiled ex vivo cytokine production under diverse bacterial, viral, and fungal stimulations in two independent cohorts of older adults: the SI cohort (n = 516) and the BCG Prime cohort (n = 384). Genome-wide DNA methylation and genetic data were integrated to quantify their relative contributions to cytokine-response variability. Epigenome-wide association studies (EWAS) were performed to identify cytokine-associated CpG sites (cCpGs), followed by replication in an independent cohort. Functional annotation, evolutionary enrichment analyses, and Mendelian randomization (MR) were used to assess biological relevance and causality. Cytokine-response variability was primarily driven by stimulus type, with genetic factors explaining a larger proportion of variance than DNA methylation (mean adjusted R^2: genetics 12.8% and DNA methylation 6.9% in the SI cohort; 14.2% and 11.5% in the BCG Prime cohort). Epigenome-wide association analyses identified 4,430 cytokine-associated CpGs (cCpGs; FDR < 0.05), annotated to immune regulators including IL1R1, CCR6, CCR9, STAT1, STAT6, IRF2, CD22, SMYD3, and KLF12. Enrichment analyses showed that cCpGs were preferentially located in enhancer regions and CpG shores and were enriched in immune-related pathways, including Toll-like receptor signaling, TNF signaling, and cytokine signaling. Cytokine-associated CpGs were significantly enriched in genomic regions under recent positive selection (Kolmogorov-Smirnov test, $P < 2.2 \times 10^{-16}$) and overlapped immune disease-associated GWAS loci, highlighting their evolutionary constraint and relevance to immune regulation and disease susceptibility. Mendelian randomization (MR) analyses identified 64 cCpGs with evidence of causal effects on cytokine production, highlighting loci in EHBP1L1, WDR37, BAIAP2L1, NIPAL2, and LSM5 as key regulatory hubs across multiple stimulation contexts. Together, these findings indicate that DNA methylation represents a functional layer of immune regulation that complements genetic variation and contributes to immune disease susceptibility.

2 Non-linear immune aging of human γδ T cells and age-specific responses in acute CMV infection

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Introduction: γδ T cells develop as the earliest T cells in early ontogeny and show diverse functions in homeostasis and diseases across the lifespan. However, their maturation from newborns to the elderly and how age-specific transcriptional states influence γδ T cell responses remain unclear.
Method: We performed single-cell RNA sequencing (scRNA-seq) of γδ T cells from infants, children, adolescents, and adults. These data were integrated with published datasets to build a lifespan reference atlas. Trajectory analysis and further in vitro validation were applied to study their developmental paths. Moreover, γδ T cell responses in neonates and adults with acute cytomegalovirus (CMV) infection were analyzed using scRNA-seq analysis.

Results: We generated a single-cell transcriptome atlas of 106,711 γδ T cells from 223 individuals spanning infancy to old age. Our results show that γδ T cell aging is non-linear, characterized by childhood transitions followed by relative stability throughout adulthood despite inter-individual variability. During childhood, transcripts shifted from developmental and mitochondrial features toward cytotoxicity and inflammaging, including maturation from GZMK^{+} intermediates to GZMB^{+}Perforin^{+} effectors at both RNA and protein levels. In acute CMV infection, both neonates and adults mounted robust Vδ1 T cell responses but via distinct strategies: neonatal Vδ1 T cells underwent de novo effector differentiation with balanced type 1- and type 2-associated programs, whereas adult cells were pre-armed with cytotoxic signatures and responded through proliferative expansion.
Discussion: Together, our study delineates human γδ T cell aging patterns, reveals age-specific antiviral responses, and provides a fully annotated resource for understanding γδ T cell biology in health and infection.
Outlook: We will employ in vitro co-culture of human γδ T cells with HCMV-infected precision-cut lung slices, combined with two-photon live imaging, to investigate age-specific γδ T cell responses.

3 Differentiation-induced reduction in functional diversity restricts the ability of cytomegalovirus-specific CD8 T cells to eliminate virus-infected cells

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Introduction: Human cytomegalovirus (HCMV) is one of the most influential pathogens that affects the functional diversity of CD8 T cell subsets and the expansion of virus-specific CD8 T cells. Nevertheless, it remains unclear why individuals with expanded CD8 T cells recognizing the pp65-HLA-A*02:01–restricted viral epitope NLVPMVATV (NLV-T cells) exhibit weakened immune control of HCMV reactivation.
Methods and Materials: To address individualized evolution of HCMV-specific CD8 T cell responses, we phenotypically and functionally characterized NLV-T cells in 116 healthy HCMV-positive donors, dividing them into two groups: those with low and those with high NLV-T cell frequencies (LF and HF, respectively). We phenotyped the cells using multi-color spectral flow cytometry and single-cell RNA sequencing coupled with TCR profiling and examined their killing properties against peptide-loaded and virus-infected target cells.
Results: Our comprehensive multimodal analysis revealed that NLV-T cells from HF donors exhibited a phenotype of advanced differentiation, marked by high levels of granzyme B and perforin expression, and efficiently eliminated peptide-loaded targets and HCMV-infected cells as long as cell surface HLA expression was unaffected. However, NLV-T cells from LF donors, possessing a less differentiated granzyme K-intermediate phenotype, demonstrated enhanced cytokine secretion and the ability to eliminate HCMV-infected cells, even in the presence of virus- induced HLA class-I downregulation.
Discussion and Outlook: Overall, these findings suggest that HCMV exploits CD8 T cell differentiation to evade immune protection. Hence, our data reveal individualized trajectories of HCMV-specific CD8 T cell differentiation that pave the way for personalized assessment of "immune aging" and the associated increased risk of coronary disease, frailty, and mortality in CMV-seropositive individuals. Furthermore, they underscore the need for personalized strategies in monitoring and managing HCMV reactivation, particularly in vulnerable populations such as transplant recipients.

4 Host control of persistent Epstein-Barr virus infection

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Introduction: Epstein-Barr virus (EBV) is a herpes virus that infects around 90-95% of the global population and subsequently establishes life-long persistence in B cells. EBV infection is associated with autoimmune and neoplastic diseases, such as Multiple Sclerosis (MS) or certain lymphomas. Despite its connection to disease development, the biological basis of host control during EBV persistent infection remains unknown.
Methods and Materials: Using genome sequencing (GS) data of blood samples from UK Biobank (UKB) and All of Us (AoU) participants we extracted short-reads mapping to the EBV genome (EBV-reads). In independent cohorts, we correlated the presence of EBV-reads (EBVread+) with qPCR and transcriptomics data. Finally, we analyzed how genetic and non-genetic factors are associated with EBVread+.
Results: In 16.2% of 486,315 UKB and 21.8% of 336,123 AoU participants, we detected EBV-reads and in independent cohorts, we demonstrated that EBVread+ is a surrogate for elevated EBV viral load. In UKB, EBVread+ associated with HIV infection, immunosuppression, and current smoking. Genome-wide association analyses uncovered strong associations at the Major Histocompatibility Complex (MHC). Outside the MHC, 27 genomic regions were associated, which implicated genes with known immunologic functions (e.g. ERAP2, CTLA4) and genes underlying monogenic susceptibility to EBV (e.g. CD70). Among participants with EBV-associated diseases, we observed a higher polygenic burden of EBVread+ for HLA-alleles at MHC class I in MS (driven by HLA-A*02:01), and at MHC class II in rheumatoid arthritis (RA). A phenome-wide association analysis uncovered a polygenic overlap of EBVread+ with inflammatory bowel disease, hypothyroidism, and type I diabetes.
Discussion and Outlook: Our study establishes by-products from human GS data as surrogate markers of EBV viral load and opens new avenues in studying persistent viral infections, which will inform future mechanistic studies and therapeutic approaches.

5 IFNAR1 deficiency impedes the regulation of immediate early viral genes and impairs viral immunity in human macrophage models

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Introduction: Type I interferons (IFN-I) are critical for antiviral defense, yet the role of IFN-I signaling in human macrophages during viral infection remains incompletely understood.
Methods and Materials: We generated induced pluripotent stem cells (iPSCs) from a patient with IFNAR1 deficiency and pronounced viral susceptibility and differentiated them into macrophages (iMacrophages). Phenotypic and functional characterization was performed, including flow cytometry, Western blotting, and qPCR. Susceptibility to Influenza A virus (IAV), herpes simplex virus-1 (HSV-1), and human cytomegalovirus (HCMV) was assessed in IFNAR1-deficient (IFNAR1def) and wild-type (WT)

iMacrophages, with or without IFNα pre-stimulation. A reporter-HCMV strain was used to monitor viral gene expression dynamics.
Results: IFNAR1def iMacrophages exhibited normal morphology and macrophage marker expression but lacked IFN-I signaling: no phosphorylation of TYK2 or STAT2, and no induction of the IFN-stimulated genes (ISGs) IFIT1, IFIT2, and MX1 upon IFNα stimulation. While both WT and IFNAR1def iMacrophages showed similar baseline susceptibility to IAV, IFNα pre-treatment suppressed IAV replication only in WT cells. IFNAR1def iMacrophages were highly susceptible to HSV-1 (~85% infected cells) compared to WT iMacrophages (~7% infected cells). HCMV infection rates were comparable between genotypes (~62% for WT vs 89% for IFNAR1def iMacrophages), but IFNα significantly reduced infection only in WT iMacrophages (~29%). Reporter assays revealed that IFN-I signaling is essential to block the immediate-early HCMV gene expression.
Discussion: Our findings demonstrate that IFN-I signaling in human macrophages is indispensable for controlling HCMV and HSV-1 infection, particularly in regulating immediate-early viral gene expression. The differential impact on distinct viruses highlights the context-specific role of IFN-I in antiviral immunity.
Outlook: This study highlights the value of this iPSC-based in vitro model to provide a human-relevant platform to dissect IFN-I mechanisms and evaluate targeted therapies for viral infections in immunodeficient patients.

6 Systems Immunology Reveals Distinct Immune Signatures of Inhaled and Intramuscular SARS-CoV-2 Vaccination in Humans

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Background: Most licensed vaccines against respiratory pathogens are delivered intramuscularly (IM), inducing strong systemic but limited mucosal immunity. Inhaled vaccines may overcome this limitation by eliciting local immune responses. Yet, the molecular pathways engaged by mucosal versus IM vaccination in humans remain poorly defined. We applied a systems immunology approach to comprehensively profile responses to a Modified Vaccinia virus Ankara vaccine candidate encoding a prefusion-stabilized SARS-CoV-2 Spike protein (MVA-ST) administered as a booster, leveraging two parallel phase 1 trials of inhaled and IM delivery.
Methods: Healthy adults from two phase 1 trials received a single booster dose of MVA-ST: inhaled (10^7 IU; Hannover cohort, n=23) or intramuscular at three escalating doses (Hamburg cohort, n=30; lowest dose matched to inhaled). Longitudinal multi-omics profiling – including transcriptomic, proteomic, and cellular analyses – was performed on blood and bronchoalveolar lavage samples in the inhaled vaccine group. Transcriptional responses were compared between delivery routes and benchmarked against public mRNA vaccine datasets.
Results: Inhaled MVA-ST induced limited systemic antibody responses but robust antigen-specific T cell immunity, evidenced by IFN-γ production after ex vivo Spike peptide stimulation. Systems-level analyses demonstrated enrichment of effector CD8+ T cells in the respiratory tract with minimal systemic perturbation. Transcriptional profiling revealed shared innate and proliferative programs across delivery routes, but with temporal uncoupling of gene programs linked to humoral versus cellular immunity. Machine learning applied across

inhaled and IM MVA as well as mRNA vaccine datasets identified an early blood transcriptional signature predictive of subsequent antibody production that was confirmed in an independent validation cohort.
Outlook: Inhaled MVA-ST elicits robust mucosal T cell immunity and distinct transcriptional programs, advancing systems-level understanding of vaccine delivery routes. These findings may inform rational design of next-generation mucosal vaccines against respiratory pathogens.

7 A distinct monocyte transcriptional state links systemic immune dysregulation to pulmonary impairment in long COVID

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The mechanisms driving immune dysregulation in long COVID disease remain elusive. Here we integrated single-cell multiome data, immunological profiling and functional assays to investigate immune alterations across multiple cohorts. A transcriptional state in circulating monocytes (LC-Mo) was enriched in individuals with mild–moderate acute infection and accompanied by persistent elevations of plasma CCL2, CXCL11 and TNF. LC-Mo showed TGFβ and WNT–β-catenin signaling and correlated with fatigue severity. Protein markers of LC-Mo were increased in individuals with pronounced fatigue or dyspnea, and those with severe respiratory symptoms showed higher LC-Mo expression. Epigenetically, LC-Mo exhibited AP-1- and NF-κB1-driven profibrotic programs. LC-Mo-like macrophages in bronchoalveolar lavage samples from individuals with severe respiratory symptoms displayed a profibrotic profile, and individuals with a high LC-Mo transcriptional state showed impaired interferon responses after stimulation. Collectively, our findings define a pathogenic monocyte transcriptional state linking systemic immune dysfunction to persistent long COVID disease, providing mechanistic insights and potential therapeutic targets.

8 Long COVID Imprints a Persistent IL-6 Driven Trained Immunity Program That Bridges Infection to Autoimmunity

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Trained immunity (TI) reprograms innate cells to acquire long-lasting functional memory, but its role in post-acute COVID-19 syndromes remains unclear. Here, we combine single-cell multiomic profiling of peripheral blood mononuclear cells from 25 individuals, with functional mouse studies to define the molecular architecture of COVID-19- and long COVID–induced TI. Joint single-nucleus RNA- and ATAC-seq identified persistent reprogramming of monocytes characterized by constitutive interferon activation and stable IRF -driven regulatory circuits. Integrative analysis revealed three distinct TI phenotypes: interferon-primed (IP_IFN), interferon-antigen-presenting (IP_Antigen), and trained-inflammatory (TM_Inflam), extending the two-program TI model previously described for influenza vaccination. Long COVID monocytes exhibited a dominant IL-6–mediated communication axis with B cells. Functional validation in in vivo NBA2 mice demonstrated that IL-6 blockade disrupted monocyte–B-cell crosstalk, reduced autoantibody production, and prevented frailty phenotypes, whereas infection reproduced the IL-6–dependent autoimmune cascade. These data identify long COVID as a condition of persistent IL-6-driven trained immunity, linking monocyte reprogramming to maladaptive B-cell activation and systemic dysfunction. Our findings establish IL-6-regulated TI as a mechanistic bridge between infection and autoimmunity and highlight it as a potential therapeutic target for long COVID.

9 Decade-long persistence of adaptive γδ T effectors in recurrent malaria

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Human adaptive γδ T (Vδ1$^+$) cells participate in immune responses to infections and cancer, showing durable clonal expansion and sustained functional competence. However, the lack of longitudinal studies with integrated clonal-phenotypic resolution limits our understanding of their functional plasticity, and longevity mechanisms. Here, we employed single-cell RNA and γδ TCR sequencing (scRNA-seq and sc-γδTCR-seq) to profile peripheral γδ T cell phenotypes and clonotypes in a 10-year longitudinal cohort of four Malian children (aged 4 - 5 years at enrollment) exposed to yearly seasonal malaria transmission. Repeated infection drove clonal expansion of two distinct Vδ1$^+$ effector populations with either regulatory (CX3CR1, PDCD1, and IL10) or cytotoxic (CX3CR1, GNLY, INFG, and PRF1) phenotypes, alongside the establishment of a previously unrecognized precursor-like Vδ1$^+$ subset co-expressing self-renewal (TCF7) and exhaustion-associated markers (TOX, PDCD1, TIGIT, and HAVCR2). Clonal tracking revealed that the dominant Vδ1$^+$ clonotypes in each individual persisted over the decade with stable effector phenotypes, maintained by self-proliferation and involving a precursor-like memory pool. Together, these findings define mechanisms of durable adaptive γδ T cell immunity during recurrent malaria, instructing the development of γδ T cell–based immunotherapies for infectious diseases and cancer.

10 HLA-independent Chimeric Ligand Receptor (CLR)-T cells recognizing the HCMV immune evasion protein UL18 for universal HCMV immunotherapy

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Introduction: Human cytomegalovirus (HCMV) remains a major cause of morbidity in immunocompromised transplant recipients. Treatment options include antiviral therapy, reduction of immunosuppression and adoptive T-cell therapy (ACT). However, in solid organ transplantation (SOT), HLA mismatches complicate adoptive cell therapy (ACT) donor selection, as the infection can affect both the graft and the recipient. Here, novel chimeric ligand receptors (CLR), based on the interaction between the host inhibitory receptor Ig-Like Transcript 2 (ILT2) and the HCMV-encoded surface-expressed HLA-I homolog UL18 were developed to treat HCMV-associated complications.
Methods and Materials: Two ILT2-CLR constructs were generated, which are composed of the ILT2 extracellular domain and intracellular signaling domains, with differing length of the hinge region. These ILT2-CLRs were lentivirally transduced into human primary $CD3^+$ T cells (ILT2-CLR-Ts). Functionality of ILT2-CLR-Ts was assessed by live cell imaging or 2-photon microscopy using mCherry-expressing MRC-5 cells recombinantly expressing UL18 (MRC-5mCherry_UL18), as well as MRC-5 cells or human precision cut lung slices (PCLS) infected with HCMV encoding a fluorescent reporter (wt-HCMVeGFP) or a corresponding UL18 deletion mutant (ΔUL18-HCMVeGFP).
Results: Exposure of MRC-5mCherry_UL18 cells to ILT2-CLR Ts revealed their specific recognition and elimination, as determined by live cell imaging. In an in vitro HCMV infection model, ILT2-CLR-Ts displayed targeted cytotoxicity towards wt-HCMVeGFP- but not ΔUL18-HCMVeGFP-infected MRC-5 cells. Furthermore, 2-photon microscopy confirmed their ability to eliminate wt-HCMVeGFP-infected cells within PCLS.
Discussion: ILT2-CLR-Ts are effective in specifically eliminating wt-HCMVeGFP-infected MRC-5 cells or PCLS. Since ILT2-CLR targeting bypasses HLA-dependent antigen processing and presentation, these constructs offer universal applicability and are thereby presenting a promising novel therapeutic strategy for the treatment of HCMV infection and reactivation in immunocompromised SOT patients.
Outlook: Future validation of ILT2-CLR-Ts will unleash their full therapeutic potential through cutting-edge in vitro, ex vivo, and in vivo models to confirm safety and efficacy.
Patent: EP25226849: Effector cells for treatment of CMV infections.

11 Targeted degradation of viral RNA by Cas13d enables strong antiviral activity against both positive- and negative-sense RNA viruses

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RNA viruses remain a major global health threat, with their high mutation rates enabling rapid escape from conventional therapies. To address this challenge, we developed a versatile CRISPR-Cas13d-based antiviral strategy leveraging the high specificity and adaptability of the RNA-targeting Cas13d system. By designing crRNAs that target conserved regions of positive-sense RNA coronaviruses – including SARS-CoV-2 – we achieved potent inhibition of viral replication in vitro. Using lipid

nanoparticles (LNPs) to deliver Cas13d mRNA and virus-specific crRNAs, we demonstrated effective suppression of viral replication both before and after infection. This concept has also been demonstrated in negative-sense RNA viruses. In addition, the approach enables multi-locus targeting, significantly reducing the risk of viral escape and enhancing therapeutic resilience. This adaptable platform offers a promising, rapidly deployable solution for combating a broad spectrum of RNA viruses, with potential for rapid response to emerging pathogens.

SELECTED ABSTRACTS

PRESENTED AS POSTER

12 Genetic determinants of immune responses across diverse populations link variation in immunity to disease

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Immune responses are highly diverse within and across populations. The genetic causes of this diversity, which underlie immune-mediated diseases, are yet to be fully characterized. To study the effect of host genetics on immune function, we established ImHoGen (Immune Host Genetics), a multi-ancestry consortium encompassing twelve independent cohorts of individuals with Asian, African, and European ancestries, with matched genotype and immune function profiling. Performing quantitative trait locus mapping of immune cell cytokine responses (cQTL) in 5,629 individuals, we identified 57 cQTLs ($p < 5\times10\text{-}8$). A majority of cQTL were absent in steady-state proteomic studies, and we found significant colocalizations between cQTL and disease phenotypes. We found a link between African genetic ancestry and higher pro-inflammatory responses, and European genetic ancestry and anti-inflammatory cytokines. We developed polygenic scores for cytokine responses (cPGS), which were replicated in an independent cohort and were predictive for COVID-19 severity. Collectively, we present the first results of a multi-ancestry consortium studying the effect of host genetic variation in dynamic immune responses.

13 MoReHealth Niedersachsen: A Best Practice for Standardized Multi-omics Health Research in Personalized Medicine in Lower Saxony

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Multi-omics technologies have become a cornerstone of personalized medicine, offering unprecedented opportunities to uncover molecular disease mechanisms and derive diagnostic, prognostic, and therapeutic insights. Yet, the translational impact of multi-omics remains limited by heterogeneous acquisition protocols, inconsistent quality assurance, and fragmented data infrastructures – factors that directly impede the integrability of data across omics layers, sites, and time points. This lack of integrability, in turn, constrains the performance, robustness, and interpretability of AI-driven multi-omics analyses, often amplifying noise and reducing reproducibility. MoReHealth Niedersachsen, initiated within a Lower Saxony-wide, cross-site consortium coordinated by Hannover Medical School and the Hannover Unified Biobank (HUB), addresses this bottleneck by developing a best-practice framework for standardized, quality-assured, and integrable multi-omics health data. The project pursues three tightly linked objectives: (1) establish best-practice procedures (SOPs and QA) for standardized acquisition and processing of multi-omics data across technologies and participating sites; (2) develop a prototypic, modular, FAIR- and GDPR-compliant multi-omics data platform for Lower Saxony with defined processes for governance of metadata, users, projects, and data storage – explicitly designed to improve cross-omics and cross-site data integration; and (3) implement a comprehensive AI analysis toolbox for single- and multi-omics integration and interpretation, leveraging improved data integrability to enable more robust, reproducible, and biologically meaningful inference. As a first, scalable use case, MoReHealth builds on the DFG-funded Excellence Cluster RESIST (EXC 2155) cohorts, specifically the RESIST Senior Individuals (SI) cohort and the associated VZV/herpes zoster (HZ) patient cohort. HZ serves as a model of age-related susceptibility to severe viral disease, enabling the development of knowledge-driven research questions on inter-individual heterogeneity, constitutional risk factors, molecular signatures of severe HZ courses/complications, and potentially druggable pathways. Multi-layer molecular profiling – including (long-read) genomics, mitochondrial DNA sequencing, quantitative metabolomics, transcriptomics, high-dimensional immunophenotyping, and cytokine profiling – will be conceptually harmonized to maximize data comparability and integrability. Rather than prioritizing early biological findings, MoReHealth is designed as an end-to-end blueprint: standardized data generation and governance enable integrable multi-omics datasets, which in turn unlock more powerful and reliable AI-based integration, biomarker discovery, and mechanistic modeling. The resulting framework is intended to be transferable beyond the RESIST cohorts and to serve as a cornerstone for a sustainable molecular health data platform in Lower Saxony.

14 Integrative Multi-omics Predicts Immune Aging Across Health and Disease

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Aging is a complex, multi-level process, that represents a leading risk factor for most chronic diseases. Age-related alterations of the immune system emerge as a central and integrative hallmark. In this study, we built a multi-omics model integrating epigenomics, proteomics, metabolomics, cytokine responses upon stimulation, microbiomes and immune cell counts data in large-scale healthy cohorts (n=848), and validated it in independent elderly (n=384) and people with HIV cohorts (n=1912). The results revealed that epigenomics contributes to aging the most (Cumulative adjusted R2=0.746), following by proteomics (Cumulative adjusted R2=0.032) and metabolomics (Cumulative adjusted R2=0.031). Within these layers, age-associated trajectory analyses further uncovered nonlinear and decoupled immune aging dynamics, with inflammaging and immunosenescence shifting at distinct ages (approximately 45 and 60 years, respectively). We evaluated multiple modeling approaches and found that TabPFN foundation model (test R2 = 0.957 ± 0.002) consistently outperformed traditional machine learning methods, including ElasticNet (test R2 = 0.912 ± 0.004; ANOVA, p= 3.07 ×10-6), particularly in capturing nonlinear associations with aging. Moreover, cross-layer network analysis revealed trans-ferulic acid and its downstream metabolite ferulic-4-sulfate as central hubs connecting diet, epigenetic regulation (e.g. cg16867657), microbiome activity (e.g. LYSINE-DEG1-PWY), and immune inflammation (e.g.CDCP1), underscoring the integrative complexity of the aging process. Mendelian randomization analysis further supported causal effects of ferulic-4-sulfate on aging, including intrinsic epigenetic age acceleration IEAA (p= 1.91×10-155), PhenoAge acceleration (p= 1.52×10-3), and Hannum age acceleration (p= 2.80×10-3). Finally, immune age acceleration predicted by our parsimonious model was associated with clinical phenotypes in people living with HIV (such as CD4 counts, p= 2.97×10-3), highlighting the usefulness of multi-omics models in capturing aging in disease contexts. Together, this integrative multi-omics study describes a systems-level view of immune aging, capturing nonlinear trajectories, putative causal molecular drivers, and their relevance to age-related disease.

15 GENSEC: Your Unified Hub for Advanced Multi-Omics Research

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We introduce a newly established core facility designed to facilitate high-throughput, next-generation biological discovery. GENSEC offers a centralised, high-throughput solution for end-to-almost-end cutting edge multi-omics research, including state-of-the-art spatial and single-cell technologies.

16 Latent cytomegalovirus infection accelerates immune aging across the human lifespan

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Latent cytomegalovirus (L-CMV) infection is a highly prevalent lifelong exposure that reshapes human immunity and has been implicated in immunosenescence. However, its impact on immune transcriptomic aging trajectories at single-cell resolution remains poorly defined, largely because L-CMV serostatus is unavailable for most large scRNA-seq datasets. Here, we integrate L-CMV-status imputation with lineage-resolved single-cell aging clocks to quantify L-CMV-associated immune biological aging across the human lifespan. We developed scDeepCMV, a multi-head neural network trained on ~980,000 immune cells from 206 donors to infer latent CMV status directly from single-cell transcriptomes. In an independent

validation cohort, scDeepCMV achieved donor-level AUC=0.815 and recall=0.703, enabling a robust donor-level CMV index. Applying scDeepCMV to a curated meta-cohort of >5 million PBMC profiles from six studies (~1,700 donors), we identified high-confidence L-CMV− and L-CMV+ groups. L-CMV-associated genes formed coherent, age-dependent modules, with a transition around ~60 years in T, NK, and B cells from receptor-proximal signaling/differentiation toward effector and inflammatory states. To quantify immune biological age, we developed scDeepiClock, deep-learning, lineage-specific transcriptomic aging clocks trained on 2.61 million cells from 1,150 donors aged 18-90 years. scDeepiClock accurately predicted chronological age across five immune lineages and revealed significant L-CMV-associated immune age acceleration, with non-linear effects: divergence emerged by ~50 years and peaked around ~55-60 years. Intersection of scDeepiClock age-informative genes with L-CMV-associated genes implicated convergent effector and antigen-presentation programs. Finally, L-CMV status modified drug-associated effects on immunological ageing: antiretroviral therapy was associated with immune age rejuvenation that was more pronounced in CMV-negative than CMV-positive individuals with HIV, whereas metformin reduced immunological age more strongly in CMV-negative healthy donors. Together, our framework enables scalable inference of L-CMV status from single-cell transcriptomes and identifies L-CMV as a major, context-dependent modulator of immune biological aging, providing a foundation for patient stratification and for evaluating anti-aging and antiviral interventions using single-cell readouts.

17 Ageing reshapes genetic regulation of cytokine responses

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Ageing remodels immune function, yet whether it alters the genetic control of cytokine responses remains poorly understood. Here, we performed genome-wide, age-stratified cytokine quantitative trait locus (cQTL) mapping across five stimulation conditions in the RESIST Senior Individuals cohort (n = 650; n_{old}= 550, 61–94 years; n_{young}=100, 20–39 years). Fourteen independent loci reached genome-wide significance in older adults, of which all except one were undetectable in young individuals despite adequate statistical power, indicating age-dependent genetic control of inducible immune responses. A prominent example mapped to the CCL2 (MCP-1) locus, where the lead variant markedly influenced LPS-induced CCL2 secretion in older adults (β = −0.44, P = 3.8× 10−10) but not in young individuals (P = 0.67); genotype × age-group interaction confirmed effect heterogeneity (P = 0.046). The association replicated in immune-altered cohorts, but not in generally healthy populations. Cross-layer analyses across genetics, DNA methylation, immune cell composition, and stimulus-induced transcriptomics demonstrated that this response-cQTL is genetically distinct from baseline CCL2 regulatory variants, is independent of cell composition and local methylation, and is embedded within stimulus-induced inflammatory transcriptional programs. Fine-mapping across cohorts (total N = 1,786) identified a single credible set and revealed colocalisation with Parkinson's disease risk (PP4 = 0.82), linking age-dependent inflammatory regulation to neurodegenerative susceptibility. Together, these findings show that aging reshapes the immune regulatory landscape in a manner that unmasks context-specific genetic effects, highlighting age-stratified cQTL mapping as a strategy for understanding immunosenescence and age-related disease susceptibility.

18 The human gut microbiome as a transmissible component within families

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Introduction: The human gut microbiome is home to thousands of microbial species and trillions of microbial cells. Far from being static, our gut microbiomes are shaped from birth throughout life by continuous microbial acquisition and transmission, affecting health beyond infectious diseases.
Methods and Materials: Here, we analyze a cohort of 534 individuals from 141 households of the German LoewenKIDS study, each consisting of a mother (mean age 41.65 ± 5.18), a father (44.16 ± 5.24) and one to four children (8.32 ± 2.68). A stool sample was collected from each individual and analysed using deep shotgun metagenomic sequencing to investigate how household structure shapes microbiome transmission.
Results: Consistent with previous findings, household members share significantly more species than unrelated individuals. Using strain-level data, we demonstrate that 91% of shared species include strains transmitted within households, indicating cohabitation as a key driver of gut microbiome transmission. Notably, the strain-sharing rate (defined as the number of shared strains divided by the number of shared species) varies by relationship types: siblings share 60% of strains, followed by mother-child (35%), father-child (25%), and spousal pairs (25%). Furthermore, transmission efficiency varies across microbial species, with distinct species preferentially transmitting between specific family members following specific transmission direction. Notably, Segatella copri and Phocaeicola vulgatus were more likely to transmit from children to parents within households, with probabilities of 68.9% and 57.5%, respectively.
Discussion: These findings underscore the gut microbiome as a transmissible component of human biology and highlight its potential role in microbiome-associated noncommunicable diseases.
Outlook: Ongoing analyses explore the longitudinal microbiota composition of children within these households to pinpoint the time points in life when distinct groups of microbes are first observed, thereby clarifying infection and colonization dynamics.

19 Multi-seasonal Systems Analysis of Influenza Vaccination Reveals Molecular Signatures Associated with Seroprotection

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Despite annual vaccination being the most effective strategy to prevent influenza, substantial inter-individual variability in vaccine responsiveness persists. We systematically analysed serological, genetic, and multi-omics profiles from 286 individuals across four influenza seasons, covering multiple viral strains and both un-adjuvanted and adjuvanted vaccines. To quantify individual-level protection, we developed a seroprotection-based classification framework integrating pre-existing and vaccine-induced antibody responses. Across seasons, strains, and vaccine formulations, we identified robust molecular signatures associated with enhanced seroprotection. Elevated pre-vaccination plasma levels of CD83 and cysteine (C3H7NO2S) were consistently associated with enhanced antibody responses and were independently selected as predictive features by machine-learning models trained on multi-omics data. Functionally, CD83 enhanced antibody production by increasing the cytokine-producing capacity of lymphocytes prior to vaccination, as demonstrated in vitro. In parallel, amino-acid metabolic pathways supporting anaplerotic metabolism were linked to improved vaccine responsiveness. At the genetic level, we identified a genome-wide significant association between the variant rs67211229 and antibody levels (abQTL), which is also linked to the regulation of GPT2 expression in immune cells, suggesting a genetic predisposition influencing mitochondrial metabolic regulation and seroprotection. Finally, using pre-vaccination serological and multi-omics profiles, we developed machine-learning models that accurately predict post-vaccination seroprotection. Together, these findings define robust, multi-layered determinants of vaccine responsiveness and provide foundation for data-driven strategies for personalised vaccination.

20 Generation of Human Alveolar Macrophages to Model Lung Innate Immunity and RSV Infection

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Introduction: Alveolar macrophages (AMs) are key regulators of lung immunity and homeostasis. Although animal-based models have provided valuable insights into AM biology, they differ substantially from human AMs, raise ethical challenges and provide only a limited number of primary cells. Human-specific models remain constrained by scarcity of primary human AMs. Surrogate systems, such as monocyte-derived macrophages (MDMs), fail to recapitulate the unique phenotype and functional specialization of human AMs.
Methods and Materials: To address the gap in the ex vivo generation of human AM-like cells, we applied advanced differentiation strategies that convert MDMs and human induced pluripotent stem cells (iPSC)-derived macrophages (iMac) into AM–like cells (AML and iAML respectively). For that, MDMs or iMacs were cultured for six days in surfactant-supplemented medium to mimic aspects of the alveolar microenvironment.
Results: Targeted qPCR analyses demonstrated AM-typical gene regulation in both AML and iAML, including upregulation of SPI1, PPARG and MRC1, alongside downregulation of MMP7 and MMP9. These findings were further supported by bulk transcriptomic profiling, which revealed a global shift toward an AM-associated gene expression pattern. Importantly, AMLs and iAMLs maintained basic macrophage functions, such as phagocytosis and reactive oxygen species (ROS) production. To underscore the functional relevance of tissue macrophages for human lung infection models, we compared respiratory syncytial virus (RSV) susceptibility across distinct human macrophage populations, including MDMs, AM–like cells, and iMacs. While MDM populations were highly permissive to RSV, iMacs exhibited pronounced resistance associated with elevated baseline expression of antiviral and innate immune signaling pathways. These observations reveal ontogeny-dependent differences in antiviral responses.
Discussion: Together, our results suggest that both AML and iAML generated by our technique share key molecular features with tissue-resident human AMs and point towards the beneficial use of this model which reflects aspects of human AM biology in functional and infection contexts.

21 Immune response activation and dynamic profiling of human iPSC-derived macrophages in tuberculosis infection models

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Introduction: Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a leading cause of infectious mortality worldwide. Antibiotic treatment is limited by long regimens and the emergence of multidrug-resistant strains. Disease outcome is critically shaped by interactions between Mtb and macrophages, the primary host cell niche and key mediators of antimicrobial immunity. Blood monocyte-derived macrophages (MDMs) are widely used to study TB but are constrained by donor variability, limited scalability, and finite lifespan. Induced pluripotent stem cell–derived macrophages (iMacs) offer a complementary, reproducible, scalable, and donor-independent alternative. Comparing immune responses between iMacs and MDMs is essential to assess iMacs as a TB model and as a potential platform for cell-based immunotherapy and translational development.
Methods and Materials: Human iMacs were generated from two healthy donors and compared with MDMs. Cells were challenged with the live attenuated Bacillus Calmette-Guérin (BCG) strain or heat-killed Mtb (HKMT). Migration, phagocytosis kinetics, autophagy and apoptosis-related responses, reactive oxygen species production, cytokine profiles, and phagosome maturation and acidification were assessed.
Results: Both macrophage types efficiently phagocytosed BCG and HKMT; however, iMacs showed enhanced migration and faster phagocytosis. Upon infection, iMacs displayed increased production of autophagy- and apoptosis-related proteins, higher reactive oxygen species production, and pronounced phagosome maturation and acidification, indicated by co-localization with V-ATPases and LAMP-1. Furthermore, iMacs demonstrated a stronger pro-inflammatory cytokine response to BCG and HKMT, followed by rapid resolution to baseline activation.
Discussion: The faster and stronger functional responses of iMacs indicate that macrophage ontogeny critically influences host–mycobacteria interactions, supporting iMacs as a physiologically relevant and complementary model for TB research.
Outlook: These findings establish iMacs as a robust in vitro platform for TB research and, by utilizing genetically-defined and scalable cells, highlighting their potential for precise modeling of TB pathogenesis and the development of novel cell-based immunotherapeutic strategies.

22 Detecting the individual transcriptional viral load and diversity in human cell lines

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Cell lines stemming from individual patients are frequently used as models for infection and disease. Just as primary human cells, cell lines transcribe a repertoire of viral nucleic acids from latent and endogenous viruses. Cell lines can harbour viruses acquired during establishment and cultivation that are absent from the donor. These may have infiltrated cell cultures through various means including deliberately introducing viruses for cell immortalization or contaminated FBS. However, genetic modification with vectors harbouring elements of viral origin will lead to detectable viral transcripts. Since viral transcripts and their products influence cellular processes, their reliable detection and quantification is key to knowing our in vitro disease models and their artefacts.
In order to examine the viral transcripts, RNA-seq data from 166 human cell lines were evaluated using FusionCatcher, covering tumour types such as leukemia, lymphoma, breast cancer (BC), neuroblastoma (NB), and retinoblastoma (RB), and cross-checking with ViralCellDetector. A total of 16% of these cell lines were tested positive for viral transcripts such as human herpesvirus 8 (HHV8), human herpesvirus 4 (HHV4, EBV), simian virus 40 (SV40), and murine leukemia viruses (MLV). Among cell lines of hematopoietic origin, 18 were associated with HHV4, with NC-NC, JVM-13, JVM-2, JVM-3 being established by HHV4-transformation for immortalisation. The B cell lymphoma cell lines BC-3 and BCBL-1 showed active transcription by HHV8 but not HHV4, whereas BCL lines CRO-AP2 and CRO-AP5 exhibited detectable levels of both HHV4 and HHV8 transcripts. Three BC cell lines ETCC-006, ETCC-007, and KPL-1 tested positive for SV40 RNA, while EVSA-T (BC), KELLY (NB), RBL15 (NB), and DEL (lymphoma) were positive for MLV.
When using human cell lines for investigating virus infections and immune responses, pre-existing viral transcription and its impact should be considered.
Systematic evaluations of cell lines for virus presence will follow, and viral activity will be assessed.

23 Identifying antiviral activity of human PBMCs in an autologous co-culture model post lung transplantation

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Introduction: Iatrogenic immunosuppress-sion is required to prevent adverse allogeneic immune responses such as graft rejection or graft-versus-host disease in transplant

recipients, but it frequently predisposes patients to opportunistic infections. Cytomegalovirus (CMV) re-activation represents one of the most common and clinically relevant infections in lung transplant recipients, particularly in CMV-seronegative recipients of lungs from seropositive donors. In these patients, viral dissemination can lead to severe and potentially life-threatening disease. Notably, CMV does not reactivate or cause overt disease in all recipients, and the mechanisms underlying these divergent outcomes remain poorly understood. It is widely assumed that interindividual differences in immune responses contribute to variable control of CMV. To directly assess antiviral immune activity, we developed a co-culture system combining CMV-infected target cells with peripheral blood mononuclear cells (PBMCs) from lung transplant recipients.
Methods: Primary lung stromal cells, which are highly permissive for CMV infection, were isolated from explanted lung tissue and infected with a recombinant CMV expressing green fluorescent protein (GFP) under control of the major immediate-early promoter. These infected stromal cells were co-cultured with PBMC fractions obtained from recipients at multiple time points following transplantation. Viral replication was monitored by time-lapse fluorescence microscopy. GFP signals were normalized to infected stromal cells cultured in the absence of PBMCs to control for innate and cell-intrinsic antiviral effects.
Results: PBMCs reduced CMV replication in all conditions; however, the magnitude of viral control varied markedly between individuals and, in some cases, across longitudinal samples from the same recipient. CMV-seropositive recipients generally exhibited stronger antiviral activity. Increased PBMC-mediated control was also observed in recipients with documented CMV reactivation, as well as in some individuals without detectable viremia by routine clinical monitoring. Flow cytometric analysis of PBMC composition and functional assessment using a 50-plex inflammatory cytokine panel revealed that effective viral control correlated with elevated frequencies of effector T-cell populations and increased Th1-associated cytokines.
Discussion and Outlook: This study presents the first isogenic assay enabling direct measurement of leukocyte-mediated antiviral activity against CMV. The identified immune correlates may support improved risk stratification of lung transplant recipients. While the current findings are correlative, causality will be addressed in a validation cohort using sorted T-cell populations in co-culture assays.

24 Characterizing anti-HCMV immunity in an infection model of explanted human lungs

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Introduction: Cytotoxic T lymphocytes (CTLs) play a crucial role in eliminating infected cells and are therefore a prime target for developing treatments, including the adoptive transfer of HCMV-specific CTLs to control post-transplant HCMV reactivation. However, the efficiency of HCMV-specific CTLs in protecting the host at the individual level and the underlying mechanisms of action involved in virus clearance remain unclear. An ex vivo, human tissue–based imaging approach was established to study CTL function in a 3D context.
Methods and materials: Human precision-cut lung slices (PCLS) were generated from healthy tissue surrounding tumor resections, cryopreserved, and HLA-A*02:01-typed by PCR. PCLS were infected with HCMV. Unfractionated PBMCs were stimulated with the HLA-A*02-restricted immunodominant CMVpp65495-503 (NLVPMVATV; “NLV”) peptide, yielding cytolytic NLV-specific CTLs. CTL behavior and target interactions were analyzed by two-photon imaging. Integrin involvement was investigated by CRISPR/Cas9-mediated Talin-1 knockout in CTLs and LFA-1 inhibition using BIRT377.
Results: Immunohistology showed broad HCMV tropism, including fibroblasts, endothelial cells, and epithelial cells. Imaging showed infected targets were eliminated only in HLA-A*02:01-matched lungs, whereas no killing was detected in mismatched tissue. Upon contact with infected cells, CTLs reduced migration speed and prolonged contact duration. Talin-1-deficient and LFA-1 inhibited CTLs failed to activate LFA-1, showed decreased degranulation and reduced cytotoxicity, but retained migratory capacity.
Discussion: Human PCLS provided a relevant scaffold for the generation of infected targets and CTL migration, enabling direct visualization and quantification of HCMV-specific CTL–target dynamics in intact human tissue. Although integrin signaling (Talin-1/LFA-1) shaped degranulation efficiency, cytotoxic function against infected targets remained detectable, indicating modulatory rather than absolute control.
Outlook: Extension to further HLA types, coupled with targeted pathway modulation, can clarify mechanisms of viral clearance; infection with additional clinically relevant viruses can benchmark antiviral CTL efficacy and inform virus-specific T-cell product development.

25 Modulation of cytotoxicity and inflammatory response by co-infection of S. aureus with S. mitis on human bronchial epithelial cells

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Background: Staphylococcus aureus is a major pulmonary pathogen implicated in pneumonia and exacerbations of chronic lung diseases. Emerging evidence suggests that commensal bacteria can modulate airway inflammation and barrier function. Among these, Streptococcus mitis is a frequent commensal of the upper airways and oral cavity and is also regularly detected in the lower airways.
Objective: This study investigates the interaction of S. mitis with S. aureus during co-culture for observation of growth interplay as well as co-infection on normal human bronchial epithelial (NHBE) cells to assess the effects on cytotoxicity and inflammation.
Methods and Results: To investigate whether the response of NHBE cells to S. aureus infection is modulated by presence of S. mitis, we performed co-infection experiments. Our results showed that in mono-infection S. mitis does not induce IL 8 release from NHBE cells, compared to S. aureus, although showing profound cytotoxic effects. In co-infection with S. aureus, interestingly a reduction in cytotoxicity and inflammatory response in the presence of S. mitis was observed. We observed that the presence of S. mitis in co-culture experiments is able to reduce the growth of S. aureus, depending on the culture media used (Bronchial Epithelial Growth Media or Columbia Broth).
Conclusion: Our data shows that S. mitis is able to modulate the response of NHBE to S. aureus during co-infection. This highlights the potential of airway commensals to shape the immunological tone and infection response in the human respiratory epithelium.
Funding by German Research Foundation (DFG) within the Excellence Strategy (RESIST)-EXC 2155 project number 390874280.

26 Metabolic control of RSV-induced syncytia formation through STAT1-dependent cholesterol regulation

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Respiratory syncytial virus (RSV) remains a major cause of severe respiratory tract infections, leading to significant morbidity, hospitalizations, and mortality among infants, older adults, and immunocompromised individuals. Beyond immunological susceptibility, recent clinical data suggest that metabolic conditions, such as dyslipidemia, may significantly increase the risk of severe RSV infection. Despite the recent introduction of vaccines and monoclonal antibodies, treatment options for RSV infection remain limited. Detailed understanding of the molecular mechanisms underlying virus-host interactions associated with disease severity may aid the development of novel intervention strategies. STAT1 is a pivotal regulator of interferon-mediated antiviral responses, which, beyond its canonical role in innate immune responses, is increasingly recognized as a metabolic modulator of cholesterol and lipid biosynthesis. To elucidate the role of STAT1 in metabolic regulation during viral infections, we performed CRISPR-Cas9-mediated *STAT1* knockout in HEp-2 cells and characterized cellular responses to infection using transcriptomics, confocal microscopy, live-cell imaging, and biochemical assays. Under baseline conditions, STAT1 depletion disrupted transcriptional regulation of the SREBP pathway, leading to intracellular cholesterol accumulation and disruption of cholesterol homeostasis. Consequently, expression of key cholesterol biosynthesis genes decreased, while expression of cholesterol efflux mediators increased. Upon RSV infection, $STAT1^{-/-}$ cells demonstrated an increased formation of syncytia, a hallmark of severe RSV infection. Pharmacological depletion of cholesterol with 25-hydroxycholesterol or gemfibrozil reduced syncytia formation in infected $STAT1^{-/-}$ cells, thereby confirming that cholesterol availability governs RSV F protein maturation and cytopathology. These findings establish STAT1 as a critical transcriptional regulator of oxysterol-mediated antiviral responses, linking impaired oxysterol synthesis to disturbed cholesterol metabolism and enhanced pathogenesis. Given the clinical prevalence of dyslipidemia and age-related alterations in interferon signaling, our results propose cellular cholesterol as both a mechanistic determinant and a potential biomarker for risk stratification, and support lipid-lowering therapy, such as statins, as a complementary antiviral strategy.

27 Metabolites in cerebrospinal fluid as biomarkers for improved diagnosis and understanding of pathogenesis of central nervous system infections

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Introduction: Cerebrospinal fluid (CSF) is in intimate contact with the brain, the target organ of central nervous system (CNS) infections. Neurotropic pathogens interact intimately with cells in CNS and are well known to alter synthesis and release of metabolites into CSF, both by reprogramming metabolism of affected cells and by release of cell membrane constituents as part of a cytopathic effect. We have, therefore, searched for

metabolites in CSF that will hone treatment-relevant diagnosis, most notably to discriminate between viral and bacterial infections, and between viral infections and non-infectious neuroinflam-mation.
Methods and Materials: We have been applying a targeted metabolomic screen to CSF samples from >200 patients with infectious (bacterial meningitis, viral meningitis/encephalitis) and non-infectious neuroinflammation (multiple sclerosis, anti-NMDA receptor autoimmune encephalitis). Diagnostic performance was compared to that of CSF parameters used in routine clinical diagnosis (e.g., CSF cell count).
Results: This ongoing cohort study has, this far, identified membrane phospholipids such as phosphatidylcholines (reflecting release from compromised cell membranes) as sensitive biomarkers for the discrimination between bacterial and viral CNS infection and elevated levels of short-chain acylcarnitines (reflecting virus-induced mitochondrial dysfunction) as accurate biomarkers for the differentiation between viral CNS infections and autoimmune neuroinflammation.
Discussion and Outlook: These results reveal the potential of CSF metabolites to improve patient stratification and understanding of pathogenesis. As next steps, we are planning to recruit additional patients for external validation studies and to expand analyses to include high-sensitivity proteomics and single-cell RNA sequencing to further improve diagnostics and to build more comprehensive models of pathogenesis.

28 Epigenetic clocks indicate accelerated aging in individuals with hyperuricemia and patients with gout

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Epigenetic clocks are powerful tools in aging research that use DNA methylation at CpG sites to estimate biological age, providing insights that may differ from chronological age. Hyperuricemia is a metabolic disorder that plays a key role in the development of gout and may influence biological aging. This study investigates whether hyperuricemia and gout are linked to accelerated epigenetic aging by analyzing DNA methylation patterns in whole blood from individuals with normouricemia, asymptomatic hyperuricemia, and patients with gout. Materials and methods. DNA methylation was profiled using the Infinium EPIC v2 array in 145 normouricemic controls, 119 individuals with asymptomatic hyperuricemia, and 140 patients with gout. Beta values were extracted with preprocessFunnorm and blood cell proportions were inferred using FlowSorted.Blood.EPIC. Epigenetic age was estimated using Horvath, Hannum, and PhenoAge clocks in accordance with previously published literature describing these methods. Epigenetic age acceleration (EAA) was calculated as residuals from regressing DNAmAge on chronological age. Results. All clocks showed strong correlations with chronological age across groups. We next assessed the association between serum urate and epigenetic age acceleration, observing a significant positive correlation with PhenoAge EAA. EAA was also significantly increased in hyperuricemic individuals and patients with gout compared with controls across multiple DNA methylation clocks. Because gout patients were predominantly male and groups differed in immune cell composition, adjusted models were applied to account for these potential confounders. Although adjustments reduced effect sizes, group differences in EAA persisted, indicating that these factors only partly explain the observed epigenetic age acceleration. Conclusion. Elevated serum urate and gout are associated with accelerated epigenetic aging, suggesting that urate-related inflammatory processes may contribute to faster biological aging. Outlook. Future analyses should incorporate additional methylation clocks and independent cohort validation to further investigate the mechanisms linking urate metabolism and epigenetic aging.

29 Cell type heterogeneity in the autophagy of Salmonella

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Introduction: Salmonella enterica serovar Typhimurium (STm) is a food-borne, facultative intracellular pathogen and a major cause of gastroenteritis in humans and animals. Upon ingestion, STm first invade the intestinal epithelial cells (IECs) and subsequently infect macrophages for systemic dissemination. Within IECs, Salmonella can either remain enclosed within a Salmonella-containing vacuole (SCV) or escape into the

cytosol, giving rise to two heterogeneous intracellular populations. In contrast, within the macrophages, Salmonella predominantly reside within the SCV. The intracellular fate of Salmonella is tightly regulated by autophagic pathways, which are controlled by the post-translational modifications ubiquitinatination and deubiquitination. OTU deubiquitinase ubiquitin aldehyde-binding protein 1 (OTUB1) is a deubiquitinating enzyme that selectively removes K48-linked ubiquitin chains, thereby preventing proteasomal degradation of target proteins.
Methods: To study the in vivo function of OTUB1, LysM-Cre OTUB1fl/fl and Vil1-Cre OTUB1fl/fl mice, lacking OTUB1 specifically in macrophages and IECs, respectively, were infected with S. Typhimurium strain SL1344. To obtain mechanistic insights on OTUB1 function, primary bone marrow-derived macrophages (BMDMs), primary intestinal epithelial cells, and Caco-2 cells were used.
Results: OTUB1 is essential for the control of S. Typhimurium in macrophages but dispensable in IECs. In macrophages, Salmonella within the SCV damage the vacuolar membrane triggering autophagy. This protective autophagic response is dependend on OTUB1 mediated deubiqutination of DEPTOR, a negative regulator of mTOR and modulator of autophagy. OTUB1-mediated deubiquitination stabilizes DEPTOR, leading to enhanced autophagy efficient clearance of Salmonella. In contrast, in IECs, cytosolic Salmonella are ubiquitinated and targeted by ubiquitin-mediated xenophagy. This pathway is OTUB1-independent and bypasses DEPTOR-mediated autophagy.
Conclusion: Distinct intracellular niches elicit heterogeneous host responses to S. Tm that are cell type-specific. OTUB1 critically regulates autophagy in macrophages but is dispensable for autophagy in IECs, highlighting the cell type-specific heterogeneity of immune response.

30 CD16⁺ γδ T Cells Mediate Antibody-Dependent Cellular Cytotoxicity and Associate with Viral Control in Chronic Hepatitis B Virus Infection

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Background: Chronic hepatitis B virus (HBV) infection is characterized by immune dysfunction. While conventional T-cell responses are well studied, the contribution of γδ T cells, which are cytotoxic, innate-like lymphocytes enriched in the liver, remains poorly understood.
Objective: To characterize γδ T-cell subsets in HBV infection and assess their association with viral control and antibody-dependent cellular cytotoxicity (ADCC).
Design: Peripheral blood from patients with chronic (n=83) and acute (n=16) HBV, healthy controls (n=24), and cord blood donors (n=3) was analyzed using multiparameter flow cytometry, single-cell RNA sequencing, and in vitro ADCC assays.
Results: A distinct subset of CD16⁺ γδ T cells negatively correlated with plasma hepatitis B core-related antigen (HBcrAg), a surrogate of intrahepatic viral replication. Transcriptomic and phenotypic profiling revealed a cytotoxic CD16⁺ γδ T-cell signature with high expression of granzyme B, perforin, and granulysin, whereas CD16⁻ cells exhibited inflammatory, non-cytotoxic profiles. Upon HBsAg-specific antibody stimulation, CD16⁺ γδ T cells mounted potent ADCC responses, mainly mediated by Vδ2⁺ cells expressing the activating receptor CD226, while Vδ1⁺ cells preferentially expressed the inhibitory receptor TIGIT, indicating a functional dichotomy. CD16⁺ γδ T cells were expanded and highly cytotoxic in acute HBV but reduced and partially impaired in chronic HBV. Neonatal cord blood γδ T cells lacked CD16 expression and failed to mediate ADCC.
Conclusion: CD16⁺ γδ T cells are previously unrecognized mediators of antibodydependent antiviral immunity in HBV. Their quantitative and functional integrity associates with viral control, suggesting that therapeutic enhancement of γδ T-cell-mediated ADCC may support functional HBV cure strategies.

31 HDV-specific CD8+ T cells are imprinted into a dysfunctional effector phenotype in chronic HDV infection

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Immune dysfunction drives the development of chronic viral hepatitis and contributes substantially to progressive liver disease. Hepatitis D virus (HDV) represents the most severe form of viral hepatitis, yet the immune mechanisms underlying viral control and clearance remain poorly defined. Here, we aim to identify HDV-specific CD8$^+$ T-cell clonotypes associated with HDV control using multi-condition single-cell profiling. We performed CITE seq-based multi-modal analysis of antigen-specific and global CD8$^+$ T cells from 43 individuals with distinct clinical stages of chronic HDV infection (high viremia, low viremia, undetectable viremia) compared to HDV clearance (HBsAg loss), alongside HBV mono-infected patients and healthy controls. In total, more than 150,000 CD8$^+$ T cells were analysed to resolve cellular heterogeneity across conditions. Differential abundance analysis revealed redistribution of CD8$^+$ T-cell subsets dependent on viremia levels. HDV-specific CD8+ T cells from patients with high HDV viremia were predominantly enriched within effector populations, whereas HDV-specific CD8+ T cells from individuals with HBsAg loss were preferentially enriched in memory and activated states, resembling influenza-specific CD8+ T cells. Importantly, HDV-specific CD8+ T cells from HBsAg loss patients had increased GZMK expression, indicating an increased proliferative capacity. Integration of T-cell receptor sequencing further demonstrated increased clonality within effector populations of patients with high HDV viremia compared with memory compartments in HBsAg loss patients. Motif and clonotype modularity analyses suggested that expanded clonotypes preferentially express CD56, indicating that they may have an innate-like activation state. Together, these findings provide an initial framework for understanding how distinct CD8$^+$ T-cell states are associated with viral persistence versus clearance in chronic HDV infection.

32 Non-selective beta blockers reduce bystander CD8+ T cell activation in decompensated liver cirrhosis

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Background & Aims: Liver cirrhosis is linked to immune dysfunction, involving both immunodeficiency and systemic inflammation. Several studies have shown that bystander-activated CD8+ T cells substantially contribute to this inflammation. Non-selective beta blockers (NSBB) are commonly used in cirrhosis to lower portal pressure and reduce decompensation risk. However, recent evidence suggests that NSBB have also anti-inflammatory effects.
Methods: Beta adrenergic receptor expressing CD8$^+$ T cells were characterized by single-cell sequencing, and the impact of NSBB therapy was assessed in paired blood and ascites from patients with decompensated cirrhosis (n=31) using high-dimensional phenotyping and functional assays. Bulk RNA sequencing further profiled NSBBassociated transcriptomic changes in CD8$^+$ T cells.
Results: CD8$^+$ T cells expressed ADRB1 and ADRB2, with notably high levels of ADRB2 on effector/memory subsets and particularly on bystander compared to antigen-specific CD8$^+$ T cells. In vitro propranolol treatment reduced the frequency of bystander-activated CD69+CXCR6+ and NKG2D+ CD8+ T cells and reduced the production of pro-inflammatory cytokines upon interleukin stimulation, without affecting antigen-specific responses. In line with this, bulk RNA sequencing following in vitro propranolol treatment unraveled a downregulation of the interferon signaling pathway through STAT1. This was further corroborated by lower frequencies of bystander-activated CD8+ T cells in patients on NSBB therapy compared to those without, within our study cohort (n=31), in line with

clinical data from a retrospective cohort of 624 patients with cirrhosis showing reduced WBC counts and ALT levels under NSBB treatment.
Conclusions: Propranolol suppresses bystander-activated CD8+ T cells in decompensated cirrhosis while maintaining antigen-specific functions. This highlights NSBB therapy as a potential strategy to mitigate systemic inflammation in cirrhosis.

33 Role of UL36 in protective T-cell responses in HCMV-infected individuals

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Introduction: Infection with or reactivation of human cytomegalovirus (HCMV) remains a major problem in immunocompromised patients, mainly due to insufficient T-cell immunity. Knowledge of immunodominant viral targets is crucial to classify T-cell responses, improve monitoring of high-risk patients and optimize antiviral T-cell therapy. This study aims to evaluate the suitability of the anti-apoptotic HCMV protein UL36 to expand the spectrum of immunogenic targets for anti-HCMV immunotherapy.
Methods and Materials: T-cell responses to an overlapping peptide pool covering the entire UL36 protein sequence were quantified by IFN-γ EliSpot assay and the resulting frequencies were analyzed in relation to frequencies of T-cell responses to the immunodominant HCMV targets pp65 and IE1. Futhermore, UL36-specific T cells were characterized by evaluating their activation state and cytokine production following antigen exposure using flow cytometry. To further assess their functionality, UL36-specific T cells were isolated via IFN-γ Cytokine Secretion Assay (CSA), and the cytotoxic effect towards autologous UL36-loaded peripheral blood mononuclear cells (PBMCs) as well as partially Human Leukocyte Antigen (HLA)-matched HCMV-infected MRC-5 cells was analyzed by flow cytometry and live cell imaging, respectively.
Results: IFN-γ EliSpot assay revealed overall strong T-cell responses towards UL36 in healthy individuals, with T-cell frequencies comparable to those directed against the immunodominant antigen pp65 and higher than IE1. The cytotoxic effect of UL36-specific T cells was confirmed by specific elimination of UL36-loaded PBMCs alongside target-specific activation. In accordance with these results, UL36-specific T cells successfully controlled HCMV infection in vitro.
Discussion: UL36-specific T cells substantially contribute to anti-HCMV immunity, positioning UL36 as a promising novel antigen to broaden the immunogenic target repertoire for enhanced immune monitoring and antiviral therapies.
Outlook: Assessment of UL36-specific T-cell responses in transplant patients and patients with immunodeficiencies are required to confirm the role of UL36 in HCMV-specific T-cell immunity in immunocompromised patients. Such studies will establish UL36 as a clinically relevant target.

34 Single cell RNAseq-guided identification of a BKV protein VP1- and HLA-B*07:02-restricted TCR for personalized gene-engineered T cell therapy

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Introduction: In kidney transplant patients (KTx), BK Virus (BKV) infects and destroys renal proximal tubule epithelial cells (RPTECs) in the usually HLA-mismatched graft, increasing the risk of graft loss. Treatment entails adjusting immunosuppression to restore cellular immunity, but also increases the risk of rejection. T cell receptor (TCR)-engineered autologous T cells emerge as a new approach to treat BKV infection while avoiding graft rejection.
Methods and Materials: Sequences of a TCR were identified by scRNAseq from healthy donor BKV-specific memory T cells restimulated with BKV peptide pools (LT and VP1), then transduced into primary CD8+ T cells

(TCR-Ts). These TCR-Ts were functionally validated in vitro against donor-derived B-LCLs, partially HLA-match PBMCs pulsed with LT or VP peptides, and BKV-infected RPTECs. Target cell recognition was assessed by T-cell activation and cytotoxic effects via flow cytometry. The VP1-RNA levels in supernatants of BKV-infected RPTECs were quantified by qPCR.
Results: TCR-Ts demonstrated specific activation and cytotoxicity against VP1- but not LT-pulsed B-LCLs. When exposed to peptide pool pulsed partially HLA-matched PBMCs, TCR-Ts showed targeted activation and killing exclusively against HLA-B07:02+ (but not HLA-B07:02-) VP1-pulsed targets. Their infection-relevant functionality was confirmed by their potent cytotoxicity against BKV-infected HLA-B*07:02+ RPTECs, accompanied by substantial VP1-RNA reduction in culture supernatants.
Discussion: Here, an HLA-B*07:02/VP1-specific TCR exhibiting potent activation and cytotoxicity in an in vitro BKV-infection model was identified. The high abundancy of the VP1 protein within the BKV capsid, the restriction to a common HLA allele, and the specificity and functionality in an in vitro infection model support the high therapeutic potential of this TCR, making it a promising candidate for TCR-engineered T-cell therapy.
Outlook: Epitope fine-mapping and alloreactivity profiling represent the critical next steps to advance this TCR toward clinical trials, with potential to revolutionize BKV management and graft preservation in kidney transplant patients.

35 Reshaping Adoptive T-cell Therapies with T Memory Cell Based Strategies, Control of Graft-Versus-Host Disease (GVHD), and Modulation of the Tumor Microenvironment (TME)

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Introduction: Targeting intracellular antigens, particularly tumor specific neoantigens in solid tumors with adoptive T cell therapies comes to the forefront of clinical interest. However different limitations should be considered, such as the loss of neoantigens, the modification of antigen peptide presentation, tumor heterogenicity, the immunosuppressive activity of the tumor environment, graft versus host disease and exhaustion of T-cells. This perspective highlights three interdependent pillars that together define the next generation of adoptive immune therapies: T memory cell–based strategies, control of graft-versus-host disease (GVHD), and modulation of the tumor microenvironment (TME).
Methods and Materials: Most promising T cell-based therapies targeting cell surface proteins or intracellular antigens/neoantigens use T cells expressing a chimeric antigen receptor (CAR) or a recombinant complete TCR (TCR T-cell), respectively.
Results: Seven CAR-T cell therapies for lymphomas and hematological cancers and two TCR T-cell therapies for solid tumors have been FDA-approved. Nevertheless many effects of T cell-based therapies are not durable and exhibit many toxicities.
Discussion: Consequences of low effectivity of T cell therapy is transplantation of memory T cells, targeting immune suppressive cells such as Tregs, M2 macrophages and MDSCs of the microenvironment and preventation of graft versus host disease.
Conclusion: Adoptive T cell therapies have to be optimized for effective, safe, and durable cancer immunotherapy with recombinant T cells. Particularly reprogramming immune-suppressive TME components, such as regulatory T cells, tumor-associated macro-phages, and myeloid-derived suppressor cells creates conditions that allow memory T cells to expand and exert durable cytotoxicity.

36 Essential roles of IgM+IgD+ regulatory B cells, non-switched memory B cells and plasmablasts in the progression of MASLD and HCC

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Introduction: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality. In addition to viral hepatitis, alcohol abuse, and aflatoxin exposure, metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as a major contributor to HCC development. Current therapies are limited, highlighting the need for more effective strategies. Immune factors are central regulators of MASLD progression to HCC. While B cells can mediate anti- and pro-tumor responses, their roles in MASLD and HCC progression remain poorly understood. Here, we aimed to characterize distinct B cell subsets and define their functional roles in precancerous (MASLD) and cancerous (HCC) liver diseases.

Methods and Materials: Established murine models of MASLD and HCC were analyzed using spectral multicolor flow cytometry and immunohistochemistry to profile B cell populations locally (in the liver) and systemically (in the blood). The results obtained in mice have also been verified in patients with MASLD and HCC.
Results: We identified several immuno-suppressive B cell subsets associated with disease progression. Regulatory B cells (CD19+B220+CD5+CD1d+ and CD19-B220+CD5+CD1d-) that express high levels of PD-L1, IL-10, IgM, and IgD promote liver inflammation and tumor growth. Additionally, non-switched memory B cells (CD19+B220+CD27+IgD+) and plasmablasts (CD19+B220+CD138+) exhibited pro-tumorigenic phenotypes. Therapeutic B cell depletion and a Listeria-based vaccine significantly reduced immunosuppressive B cells and tumor burden in mice.
Discussion: Detection of similar B cell phenotypes in human MASLD and HCC supports their clinical relevance as potential biomarkers for disease progression and therapeutic targeting. Overall, our findings reveal a critical role for B cells in shaping the liver tumor microenvironment and highlight new targets for immunotherapy.
Outlook: Future studies will integrate single-cell transcriptomics, proteomics, and spatial immune profiling to dissect B cell heterogeneity in liver disease. Additionally, mechanisms of hepatic B cell recruitment and B-T cell crosstalk will be investigated to enable personalized immunotherapy.

37 Aid-based engineering of B cells through the integration of HIV-receptor exons

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Antibody diversification plays a key role in fighting infections by producing specific antibodies of high affinity against pathogens. Rarely, B cells can diversify by incorporating pathogen receptors resulting from a genomic insertion in the switch region. Here, we aim to replicate this natural mechanism using AID and CRISPR-based integration of exon-inserts and their splicing into final antibody mRNAs.
To guide substrate design, we generated recombinant antibodies bearing HIV-specific Llama-VHHJ3, human wtCD4 and CD4v1 domains and confirmed their expression, breadth, and specificity. To enable optimal splicing, a GFP-based fluorescent screening system was developed to identify B cell-specific intronic splice-enhancers (ISE). Utilizing a high-throughput sequencing approach, we identified and validated novel intronic splice enhancer sequences specific to B and T cells.
Building upon this work, we engineered primary human and murine B cells with VHHJ3, wtCD4 and CD4v1 substrates employing AID and CRISPR knock-in strategies. We show successful in vitro engineering of primary human and murine B cells with stable expression of VHHJ3, wtCD4 and CD4v1, respectively. Furthermore, in vivo studies with adoptive transfer of AID and CRISPR-edited B cells exhibited germinal center recruitment and production of CD4-positive antibodies in response to a heterologous immunization with HIV-BG505-gp140 and HIV-Bal-gp140.
Prospectively, AID-based editing of B cells by the addition of receptor domains represents a promising alternative to the CRISPR-based replacement of V(D)J heavy and light chain genes, without affecting the fitness of a cell to take part in the immune response and reducing the chance of off-targets.

38 Towards B cell engineering in vivo - a lentiviral vector that can specifically target human and mouse B cells

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Introduction: Despite the highly diverse human repertoire of B cell receptors (BCR), too often only few B cells are induced to produce antibodies that can broadly neutralize rapidly evolving pathogens such as HIV. In such cases, genetic engineering of the BCR would enable the design of B cells are predisposed to develop into broadly neutralizing antibodies. However, B cells are notoriously difficult to genetically engineer and target specifically in vivo using existing vector platforms. Here, we aimed to a develop lentiviral vector that can specifically target human and mouse B cells.
Materials & methods: Lentivirus encoding eGFP was pseudotyped with a Sindbis virus spike protein that 1) has mutations that abrogate cell attachment without affecting fusion-potential 2) contains a protein A domain (ZZ) that is an anchor for cell-targeting antibodies (m168-ZZ). m168-ZZ lentiviral particles were coated with B cell-specific antibodies (anti-CD19, anti-CD21) or an aspecific antibody (anti-MERS-CoV S), and incubated with CD40L and IL-4 activated primary human or mouse B cells to read-out transduction efficiency by flow cytometry.

Results: m168-ZZ lentivirus coated with B cell-specific antibodies targeting CD19 or CD21 transduced up to 70-80% of human or mouse B cells ex vivo. Only B cell-specific antibodies increased transduction efficiency approximately 20-fold compared to lentivirus without antibody, whereas lentivirus coated with aspecific antibody did not. Additionally, B cell-specific antibodies did not increase transduction efficiency of HEK293T cells, suggesting B cell-specific enhancement of transduction.
Discussion & outlook: We have successfully developed a lentiviral vector that can target and transduce human and mouse B cells. Further experiments will be performed to assess specific targeting of B cells and vectors with cargo to engineer BCRs in more complex ex vivo models and in vivo models.

39 Lipid nanoparticle-encapsulated mRNA programs human myeloid cells to produce tick-borne encephalitis virus-neutralizing antibodies

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Monoclonal antibodies (mAbs) represent powerful tools for the prevention and treatment of viral infections, but their conventional development and production remain labor-intensive, time-consuming, and costly. Here we investigated lipid nanoparticle encapsulated mRNA (LNP-mRNA) as a platform for expression of mAbs in primary human immune cells. Following LNP-mRNA-GFP delivery to human peripheral blood mononuclear cells (PBMC), transgene expression was observed predominantly in $CD14^+$ monocytes. In monocyte-derived macrophages (moMφ) and dendritic cells (moDC) GFP expression was detectable within 3 h, reaching a plateau by 24 h. Extending this approach to mRNA-mediated expression of the neutralizing IgG1 mAb T028 against tick-borne encephalitis virus (TBEV), for which no specific antiviral therapy exists, resulted in robust secretion of functional antibody by both moMφ and moDC. The expressed mAb retained potent in vitro neutralizing activity, and LNP-mRNA-T028-treated moDC significantly reduced viral replication and infectious titers in a co-culture infection model. Importantly, treated cells exhibited minimal induction of activation markers such as CD80, CD86, and HLA-DR and did not elicit pro-inflammatory cytokine responses. Together, the immunologically inert profile of LNP-mRNA, rapid expression kinetics, and preservation of antibody functionality establish this platform as an efficient strategy for therapeutic mAb production in primary human myeloid cells. These findings provide proof-of-concept for LNP-mRNA-mediated delivery as a rapid, targeted approach for passive immunization against TBEV and potentially other emerging viral pathogens.

40 Dual, antimicrobial and anticancer activity of Streptomyces ambofaciens (Myt 8) and S. globisporus ONU 1019 (Myt 11) secondary metabolites isolated from the Odesa Bay, the Black Sea: an in vitro study

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Primary liver cancer (PLC) is a leading cause of cancer-related mortality worldwide. Intrahepatic cholangiocarcinoma (CCA) accounts for around 15% of PLC cases. CCA risk factors include primary sclerosing cholangitis, chronic hepatitis B and C, and biliary tract inflammation frequently caused by bacterial pathogens, including Klebsiella, Escherichia, Enterococcus and Enterobacter. Current CCA treatments are insufficient. Furthermore, chemotherapy, such as gemcitabine, often leads to severe side effects, while cancer patients are at higher risk of infections that worsen outcomes and increase mortality. In this context, we investigated the antimicrobial and anticancer potential of secondary metabolites from six marine actinobacteria strains of the genus Streptomyces isolated from the Black Sea and identified using 16S rRNA gene sequencing. Antimicrobial activity was assessed using agar block-diffusion method against ten indicator microorganisms, including biliary tract pathogens. Streptomyces exometabolites were extracted with ethyl acetate and anticancer activity was evaluated on murine CCA cells, and normal mouse fibroblasts CBA-310 served as a control. Extracts were tested alone or combined with gemcitabine. Cell proliferation, viability, senescence and apoptosis were analyzed using crystal violet, cell counting kit-8, β-galactosidase assay and fluorescence-activated cell sorting, respectively. Liquid chromatography-mass spectrometry (LC-MS/MS) analysis was performed to identify key bioactive compounds. All Streptomyces strains exhibited antimicrobial properties against pathogenic microorganisms. S. ambofaciens (Myt 8) showed the strongest antagonistic activity against nosocomial pathogens, including Staphylococcus aureus and Candida albicans, while S. globisporus ONU 1019 (Myt 11) demonstrated consistent inhibitory activity against key bacterial pathogens associated with biliary tract infections, including Enterococcus faecalis, Escherichia coli, and Klebsiella pneumoniae. Furthermore, these strains also displayed time- and dose-dependent anticancer activity against CCA cells. LC-MS/MS identified daidzein, germicidin, staurosporine alpha-curcumene, and alpha-calacorene with known antibacterial and anticancer properties. Our in vitro findings highlight the potential of Streptomyces as dual-action therapeutic agents targeting both, biliary and nosocomial infections and CCA.

41 Course and clinical outcomes of chronic hepatitis delta: A longitudinal analysis of 565 patients from the D-SOLVE and HDV-1000 Consortia

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Background and aim: Chronic hepatitis Delta (CHD) is considered the most severe chronic viral hepatitis. However, some studies challenged this accelerated course and the predictors of CHD progression are scarcely defined. This study aims to examine the clinical course and outcomes of CHD patients in a large real-life cohort.
Materials and Methods: CHD patients with ≥3 years follow-up from the multicenter retrospective D-SOLVE and HDV-1000 database (6 European centers) were enrolled. Longitudinal changes in biochemical and laboratory markers were analyzed. Time-to-event analysis was performed, and predictors of liver-related events (LREs) were assessed with univariable and multivariable Cox regression analysis.
Results: Among a total of 1,004 patients, 565 (56%) with ≥3 years follow-up were included. Patients had a mean (SD) age of 45 (12) years, 55% men, 60% of European origin. During a median (IQR) follow-up of 55 (46-62) months, 48 patients progressed to cirrhosis at 1-, 3- and 5-year cumulative incidence of 1.8%, 5.6% and 13.6%, respectively. De-novo liver-related events (LREs) occurred in 47 (9%) patients at 1-, 3-, and 5-year cumulative incidence of 0.8%, 2.4% and 10.8%, respectively. Cox regression analysis showed that anti-HCV+ (aHR=1.72, 1.22-5.88) and elevated GGT (aHR=2.77, 1.22-6.27) at baseline significantly associated with cirrhosis onset, while older age (aHR=1.03, 1.00-1.07), elevated GGT (aHR=4.38, 1.81-10.57), detectable HDV RNA (aHR=10.32, 1.34-79.53) and cirrhosis diagnosis (aHR=2.23, 1.03-4.84) correlated with LREs. The risk for LREs increased from HDV RNA ≥1000 IU/mL, while HBsAg levels did not correlate with disease progression.

Conclusions: In a large real-life cohort of CHD patients, older age, GGT elevation, cirrhosis and detectable HDV RNA were the main determinants of liver-related outcomes, with worse prognosis noted from HDV RNA ≥1000 IU/mL.

42 Impact of specific bile acids on NK cell function in HDV patients

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Introduction and specific aims: Bile acids (BA) are important regulators of hepatic metabolism and inflammation . Indeed, BA-activated receptors were reported to regulate innate-immunity, implying a regulatory role of BAs on innate immune cells.
The antiviral therapy Bulevirtide (BLV) inhibits HBV/HDV entry by blocking Na+ taurocholate co-transporting polypeptide (NTCP) expressed on hepatocytes. This leads to increased bile acid levels in the blood of most BLV-treated patients. However, how elevated BA levels functionally impact NK cells is still unclear. Thus, the study aims to analyze the effects of specific BAs on NK cells.
Methods: Profiling of serum BAs was done using liquid chromatography and mass spectrometry (LC-MS). We selected specific BAs and analyzed their effects on NK cells by using high-dimensional flow cytometry and in-vitro functional assays. Serum cytokines and chemokines were measured using Bio-Plex Pro Human Cytokine Screening Panel.
Results: The preliminary clinical data shows the correlation of ALT levels, chemokine levels with total BA levels during the BLV therapy. Levels of deoxycholic acid (DCA) and its conjugated forms (Glyco- and Tauro-conjugated) were found to be frequently upregulated in patients' serum after 48 weeks of BLV therapy. The in vitro functional assay also showed upregulated levels of degranulation marker CD107a and cytotoxic granule Perforin in the IL-stimulated NK cells exposed to GDCA for 48 hours. While in the activated NK cells exposed with TCA, no change of cytokine levels was found, implying that GDCA but not TCA might upregulate the cytotoxicity of circulating NK cells
Conclusion: Our studies identified specific types of BAs upregulated in patients receiving BLV therapy. This study also highlights functional markers on activated NK cells that are affected by BAs. However, further in-vitro cytokine profiling and cytotoxicity assay will be performed to confirm the effects of BAs on NK cells.

43 The impact of rivers Nyamwamba, Mubuku and Nyamugasani flooding on infectious diseases in Uganda

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Uganda experiences dual crises of climate change and infectious diseases. Kasese district often experiences floods as a result of bursting river banks of Nyamwamba, Mubuku and Nyamugasani rivers. These floods leave people homeless, displaced, property destroyed and diseased due to infectious disease infections. The aim of this paper is to explore the impact of rivers Nyamwamba, Mubuku and Nyamugasani flooding on infectious diseases. The study utilized hospital-based surveillance and meteorological data with descriptive statistics, Spearman's correlation, and negative binomial regression to quantify disease burden Climate change impacts like floods lead to health-related concerns by exposing communities to vector and waterborne and diarrheal diseases like Meningitis, Malaria, Cholera, Typhoid Fever, Hepatitis A and E and Dysentery. The increased spread of the waterborne and diarrheal diseases exerts maximum pressure on the already strained health centres. Kasese district has poor access to WASH infrastructure with a water access rate at approximately 57% significantly lower than the national average at 70% and low sanitation access significantly contribute to waterborne and diarrheal diseases outbreaks. Kasese district is also extremely vulnerable to climate-related risks, with frequent severe weather events including floods, rising temperatures and droughts. Waterborne and diarrheal diseases exhibited strong positive correlations with floods ($\rho = 0.846$, $\rho = 0.99$) peaking during bursting of river banks. Regression analysis revealed floods increased waterborne and diarrheal diseases. Extreme weather events due to human-driven climate change have considerable implications for population health. Addressing infectious diseases induced by climate change events, needs to be integrated with climate change responses strategies so as to combat both crises. Ideally there is need to apply a holistic approach to building community resilience that can cope with the multifaceted effects of climate change.