SCANDINAVIAN JOURNAL OF
Immunology
AN INTERNATIONAL MONTHLY JOURNAL

The 51st Annual Meeting of the Scandinavian Society for Immunology

Bergen | 7-10 September 2026

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Velkommen til Bergen!

On behalf of the Norwegian Society of Immunology, it is our great pleasure to welcome you to the 51st SSI Meeting in Bergen 2026.

Over the next four days, we will enjoy exploring the latest advances in immunology, exchange ideas, and engage with cutting-edge research that is shaping the future of our field. Equally important, SSI 2026 is a place to create new friendships, strengthen existing collaborations, and build the professional networks that drive scientific progress. We hope the meeting will foster conversations that spark innovative ideas, lasting partnerships, and future breakthroughs.

We also encourage you to set aside some time to enjoy Bergen. Take a stroll through the historic Bryggen district, the atmospheric neighbourhoods in Nordnes or enjoy the panoramic views from Fløyen. With its unique combination of culture, history, and spectacular nature, Bergen provides the perfect setting for both scientific discovery and new friendships.

We are deeply grateful to our exhibitors and sponsors for making this meeting possible, and encourage you to visit their stands during breaks.

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Conference chair

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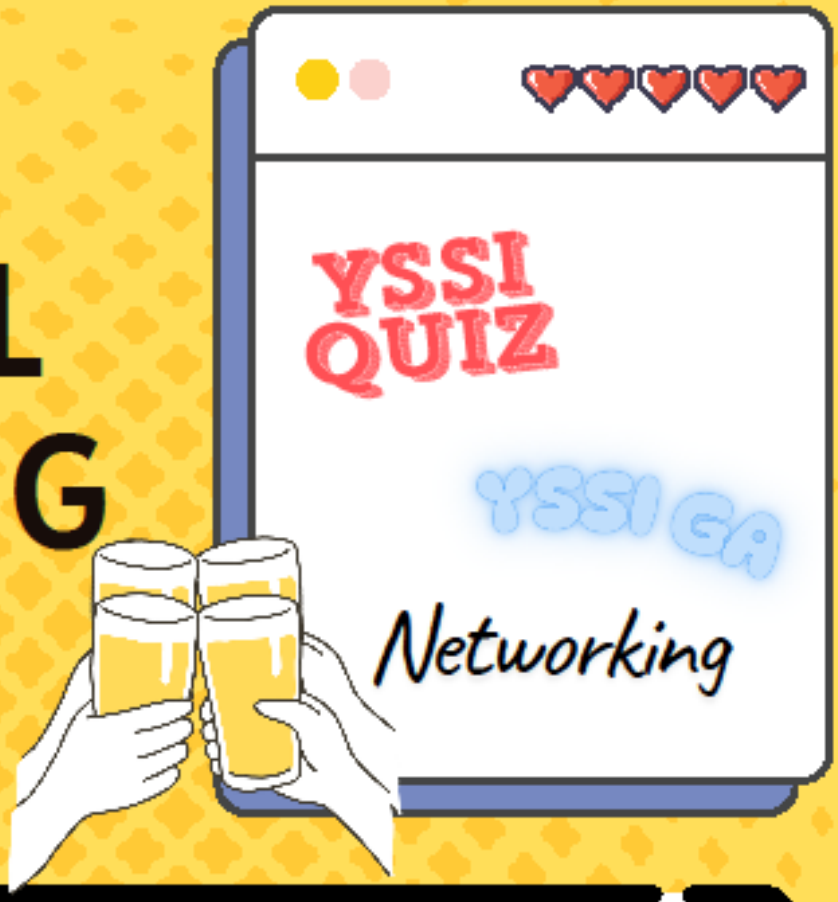
YSSI SOCIAL EVENING



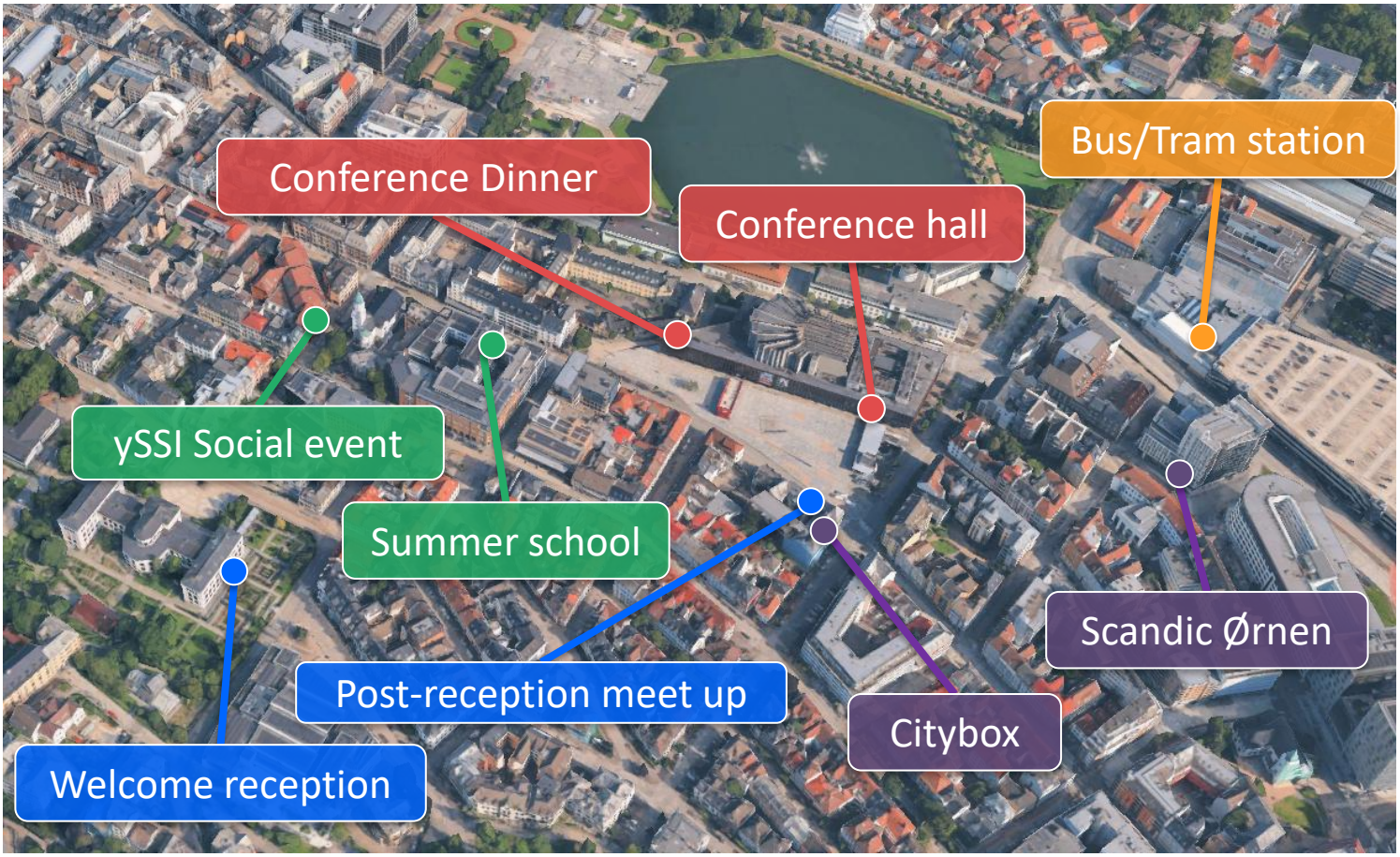
TUESDAY 8TH SEPTEMBER 2026

6 PM - 11 PM

'BERGEN STREET FOOD'

REGISTER TO JOIN US FOR YOUNG IMMUNOLOGISTS SOCIAL EVENING!

Map of conference venues



Venue	Address	Event
University of Bergen AULA	Muséplass 1	Opening ceremony & reception (Mon)
Nedre Nygaard	Nygårdsgaten 31	Optional meet up after the welcome reception (registration not required) (Mon)
UiB Læringsarena	Nygårdsgaten 5	Summer School (Mon)
Grieghallen	Edvard Griegs plass 1	Main conference (Tue-Thu) Conference dinner (Wed)
Bergen Street Food	Christies gate 13	ySSI Social event (registration required)
Scandic Ørnen	Lars Hillesgate 18	Hotel
Citybox Hotels	Nygårdsgaten 31	Hotel

Conference hall - Grieghallen

Level	Function
0	Klokkeklang, Gjendine
1	Entrance and coats (unattended)
2	Plenary room, exhibition, lunch, refreshments
3	Poster rooms

Main conference floor (2nd)

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Silver 1: Biolegend
Silver 2: Vectorbuilder
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Silver 5: Bionordika
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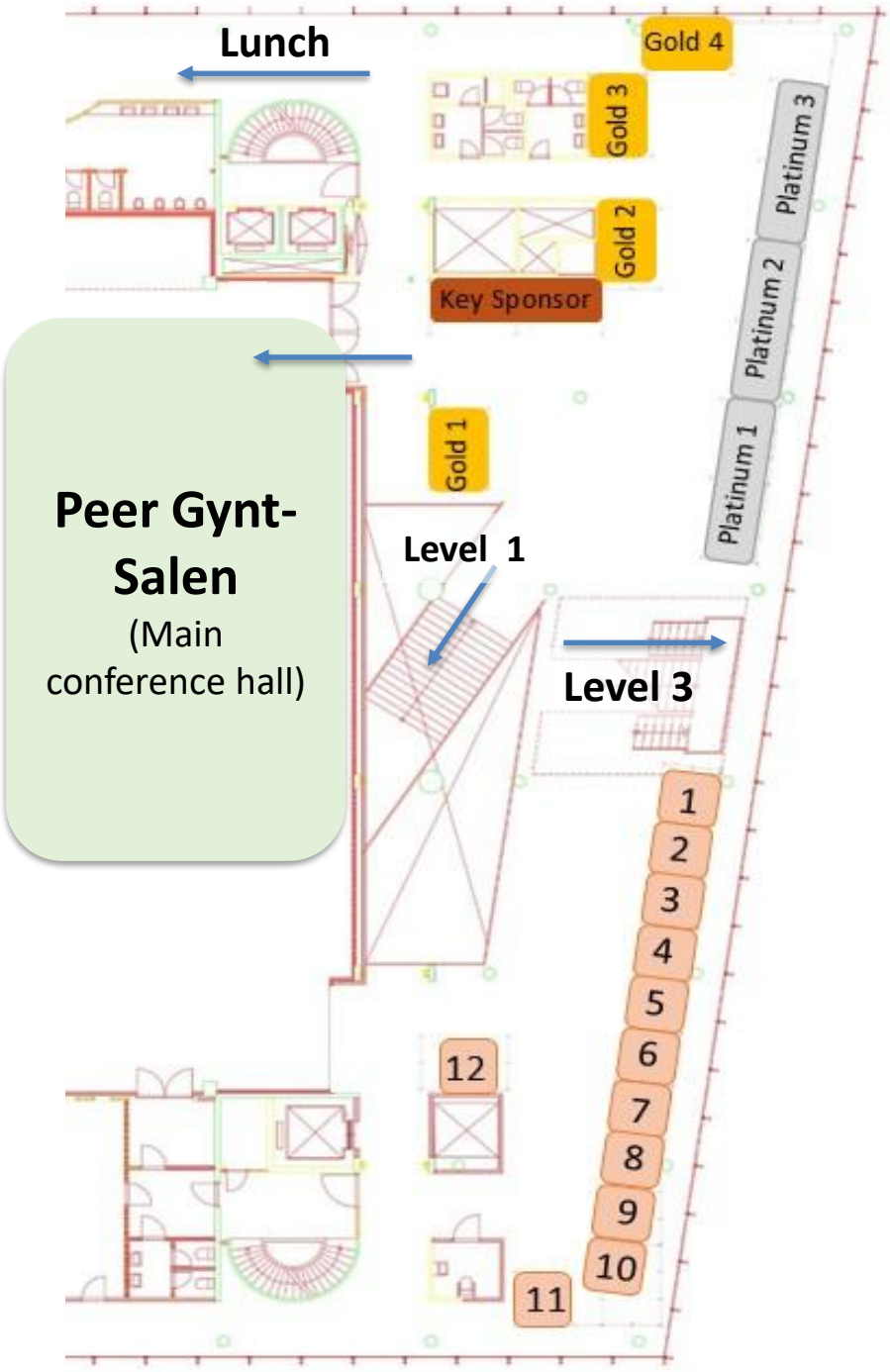
Silver 11: Bio-Techne
Silver 12: Genscript Biotech



Visit our exhibitors and collect stamps on your stamp card throughout SSI2026. Submit your completed card at the ySSI booth to enter the prize draw at dinner

PROGRAM

MONDAY SEPTEMBER 7th | UiB Learning Arena – Nygårdsgaten 5

Summer School | Emerging Concepts in Immunology

Chair: Piotr Mydel, Marta Kaminska and Jørn Skavland, University of Bergen

08:30	*Registration*	
09:00	**Anette S.B. Wolff** University of Bergen	Welcome
09:05	**Emmet McCormack** University of Bergen	Animal models for immunology studies
09:35	**Gyri Teien Haugland** University of Bergen	Comparative models for immunology: How can the fish be exploited?
10:05	*Break*	
10:25	**Rebecca Cox** University of Bergen	Virus and vaccine responses
10:55	**Victor Greiff** University of Oslo	T and B cell rearrangement and how to study it
11:25	**Flash Talk Competition**	Arranged by young SSI
12:00	*Lunch*	
13:00	**Gerhard Krönke** German Rheumatology Research Center	Cellular therapies in the treatment of autoimmune diseases
13:30	**Fatima Dhalla** University of Oxford	Thymus and T cell development
14:00	**Sonia Gavasso** University of Bergen	Recent Advances in Immunotherapy for Multiple Sclerosis
14:30	*Break*	
14:45	**Stein-Erik Gullaksen** Haukeland University Hospital	From single cells to early clinical decisions by precision pharmacodynamics
15:15	**William Agace** University of Copenhagen/ Lund University	Immune compartments of the human intestine
15:45	**Piotr Mydel** University of Bergen	Closing remarks

MONDAY SEPTEMBER 7th | University Museum of Bergen

Opening ceremony | Aula - University Museum of Bergen		
17:00	*Registration*	
17:30	*Welcome*	Bergithe Oftedal (Conference chair) and Stefanía Bjarnarson (SSI President)
17:45	**Federica Sallusto** ETH Zurich *EMBO Keynote Lecture*	Mapping the antigen specificity of human T cells in health and disease
18:30	*University Museum*	Tour the Natural History Museum or visit the café
19:30	**Reception**	Finger food and drinks (hosted by Bergen kommune)
21:00 -	*Nedre Nygaard (optional)*	Continue the evening if you wish

TUESDAY SEPTEMBER 8th | Grieghallen (Registration from 08:00)

Session 1: Peer Gynt-Salen Chairs: Kristin Greve-Isdahl Mohn and Seppo Meri		
08:30	**Hans-Gustaf Ljunggren**	Scandinavian Journal of Immunology
08:50	**Rahul Biradar and Martin Gonzalez**	The Young Scandinavian Society for Immunology
09:00	**Gerhard Krönke** Charité University Hospital Berlin *EFIS-IL Lecture award*	Resetting autoimmunity in immune-mediated inflammatory disease
09:30	**Florian Krammer** University of Natural Resources and Life Sciences, Vienna	Development of a broadly protective neuraminidase based influenza virus vaccine
10:00	**Sponsor lecture**	Stemcell Technologies
10:10	*Coffee & Exhibition*	

Session 2: Peer Gynt-Salen Chairs: Kristin Fenton and Hans-Gustaf Ljunggren		
10:45	**William Agace** University of Copenhagen and Lund University	Mapping alterations in the human intestinal CD4+ T cell compartment in Crohn's disease
11:15	**Riitta Lahesmaa** Turku Bioscience Centre and University of Turku	New regulators of human Tregs
11:45	**Niklas Björkström** Karolinska Institutet and Karolinska University Hospital	Tissue homeostasis of human NK cells
12:15	**Sponsor lecture**	Sony Biotechnology
12:30	***Lunch & Exhibition***	*SSI Council Meeting (Gjendine)- Only Council members*

TUESDAY SEPTEMBER 8th | Grieghallen

Session 3a: Workshops Chairs: Marta Kaminska and Rahul Biradar (I)/ Jona Freysdottir and Helka Kaunisto (II)		
13:30	**Workshops I & II**	Peer Gynt-Salen (I)/ Klokkeklang (II)
14:30	***Short break***	

Session 3b: Workshops Chairs: Helena Erlandsson Harris and Markus Ojanen (III)/ Johan Fernø and Adrianna Jebrzycka (IV)		
14:45	**Workshops III & IV**	Peer Gynt-Salen (III) / Klokkeklang (IV)

Time	**Session**	**Location**
15:45-17:30	**Poster Session 1 (even numbers)**	3rd floor / Refreshments & Exhibitions, 2nd floor

Time	**Event**	**Location**
18:00-23:00	**ySSI Social Event (separate registration)**	Bergen Street Food

WEDNESDAY SEPTEMBER 9th | Grieghallen

Session 4: Peer Gynt-Salen
Chairs: Alexandre Corthay and Eliisa Kekäläinen

08:30	**Marie Wahren-Herlenius** Karolinska Institutet and University of Bergen	Characterization of an unknown gene associated with multiple autoimmune rheumatic diseases
09:00	**Mariana Borsa** University of Basel	Organelle heterogeneity en route to T cell diversity
09:30	**Fatima Dhalla** University of Oxford	FOXN1 schedules thymic epithelial cell fitness during thymus ontogeny
10:00	**Sponsor lecture**	Pixelgen Technologies
10:10	***Coffee & Exhibition***	

Session 5: Peer Gynt-Salen
Chairs: André Sulen and Ranveig Braathen

10:45	**Bjørn Tore Gjertsen** University of Bergen and Haukeland University Hospital	Single Cell immune and signaling profiles in early response evaluation of cancer therapy

Session 6: Workshops
Chairs: André Sulen and Preeti Moar (V)/ Ranveig Braathen and Kenneth Green (VI)

11:25	**Workshops V & VI**	Peer Gynt-Salen (V) / Klokkeklang (VI)
12:25	***Lunch & Exhibition***	

Time	Session	Location
13:30	**SSI General Assembly**	Peer Gynt-Salen – *For all SSI members*
14:30	***Short break***	

Session 7: Peer Gynt-Salen
Chairs: Tapio Lönnberg and Julia Karen Demtröder (VII)/ Trevor Owens and Martín González-Rodríguez (VIII)

14:45	**Workshops VII & VIII**	Peer Gynt-Salen (VII) / Klokkeklang (VIII)

Time	**Session**	**Location**
15:45 – 17:30	**Poster Session 2 (odd numbers)**	3rd floor / Refreshments & Exhibitions, 2nd floor

Time	**Event**	**Location**
19:00	**Conference Dinner**	Grieghallen – Main entrance

THURSDAY SEPTEMBER 10th | Grieghallen

Session 8: Peer Gynt-Salen

Chairs: Karine Chemin and Siggeir Brynjolfsson

09:00	**Ludvig Sollid** University of Oslo	T-Cell and B-Cell Crosstalk in the Development of Autoimmunity
09:30	**Charles-Antoine Dutertre** IMRB, Université Paris-Est Créteil	Heterogeneity and spatial organisation of Tumour-Associated Mononuclear Phagocytes
10:00	**Sponsor lecture**	NMAS in collaboration with Beckman Coulter Life Sciences
10:10	***Coffee & Exhibition***	

Session 9: Peer Gynt-Salen

Chairs: Christopher Sundling and Thea Sjøgren

10:45	**SSI Young Investigator Awards**	*The award recipients are announced at the conference dinner*
10:50	- **Sweden**	
11:10	- **Denmark**	
11:30	- **Norway**	
11:50	**Closing remarks**	
12:00	***Grab & Go Lunch***	

WORKSHOP DETAILS

TUESDAY 13:30-14:30 | Workshops I & II

Workshop I: Peer Gynt-Salen

Immune Cell Development & Differentiation

Chairs: Marta Kaminska and Rahul Biradar

13:30	**Saumya Sinha** University of Turku	A novel regulator controls human regulatory T cell differentiation and function **(p.43)**
13:42	**Lukas Hoen** University of Bergen	The Overlooked Cellularity of the Human Germinal-Centre Mantle Zone: Beyond the Naive B-Cell Rim **(p.53)**
13:54	**Merel Sijbranda** Karolinska Institutet	Investigating metabolic adaptations of tissue-resident memory T cells in inflammatory joints in rheumatic diseases **(p.34)**
14:06	**H. Toprak Dogramaci** University of Bergen	Spatial Mapping of Breast Cancer during Anti-HER2 Treatment **(p.119)**
14:18	**Tracer Yong** Karolinska Institutet	Single cell analysis reveals molecular traits of pediatric lymphoma resistant subclones **(p.118)**

Workshop II: Klokkeklang

Autoimmunity & Immune Regulation / Infectious Diseases & Vaccination

Chairs: Jona Freysdottir and Helka Kaunisto

13:30	**Ingeborg Yddal** University of Bergen	Influenza vaccination induces robust antibody responses in pregnant women with efficient transfer to the fetus **(p.72)**
13:42	**Natalia Guzniczak** Aarhus University	Skin-draining lymph nodes: sentinels at the crossroad between localized cutaneous immune responses and systemic autoimmunity **(p.2)**
13:54	**Panteha Khaledi** QIMR Berghofer and University of Tasmania	Latent EBV-specific $CD8^+$ T cells Exhibit Disease-specific Transcriptional Programming in Multiple Sclerosis **(p.1)**
14:06	**Karine Chemin** Karolinska Institutet	CD49a tissue-resident memory T cells are enriched in ACPA+ rheumatoid arthritis and exhibit enhanced cytotoxicity and activation **(p.39)**
14:18	**Kristian Svastrup Kastberg** Aarhus University	Age-associated B cells drive CD8+ T-cell hyperactivation in systemic lupus erythematosus **(p.10)**

TUESDAY 14:45-15:45 | Workshops III & IV

Workshop III: Peer Gynt-Salen

Immune Intervention, Therapy & Novel Technologies + Lymphocyte Activation & Function

Chairs: Helena Erlandsson Harris and Markus Ojanen

14:45	**Kathrine Pedersen** Aarhus University	Curbing Autoimmunity: Inhibition of the Complement Amplification Loop & Autoreactive B-Cell Development Utilizing Nanobodies & Antibody Fragments **(p.55)**
14:57	**Demo Y. Tesfaye** Oslo University Hospital	Leveraging chemotactic properties of targeting units to enhance APC targeted DNA cancer vaccines **(p.61)**
15:09	**Christina Gerstner** Karolinska Institutet	Immune activation and cytokine production in neonates exposed to maternal anti-Ro/La autoantibodies **(p.40)**
15:21	**Paolo Fernando Cirillo** University of Bergen	DUSP4 acts as a novel regulator of NK cell functionality **(p.110)**
15:33	**Sara Jensen** Aarhus University	Thymic TREG-derived T follicular regulatory cells prevent severe humoral autoimmunity in response to TLR7-driven inflammation **(p.19)**

Workshop IV: Klokkeklang

Autoimmunity & Immune Regulation

Chairs: Johan Fernø and Adrianna Jebrzycka

14:45	**Marina Galesic** Karolinska Institutet	Effects of complete B cell depletion by CD19 CAR T-cell therapy on T cells in myositis **(p.21)**
14:57	**Thea Sjøgren** Haukeland University Hospital	The cytokine autoantibody landscape across systemic and organ specific autoimmune diseases **(p.6)**
15:09	**Mai Nguyen** University of Helsinki	Single-cell multi-modal profiling reveals transcriptome–BCR uncoupling and impaired B cell maturation in Common Variable Immunodeficiency **(p.27)**
15:21	**Yigit Koray Babal** University of Helsinki	Deep cervical lymph nodes of patients with multiple sclerosis show increased double negative B cells and their depletion by anti-CD20 therapy is incomplete **(p.13)**
15:33	**Marlene Oesterle** Lund University	Cataloging salamander phagocytosing immune cell diversity through image-guided sorting and orthogonal transcriptomic approaches **(p.107)**

WEDNESDAY 11:25-12:25 | Workshops V & VI

Workshop V: Peer Gynt-Salen

Infectious Diseases & Vaccination + Innate Immunity & Inflammation

Chairs: André Sulen and Moar Preeti

11:25	**Kristin Lunder Klausen** University of Oslo and Oslo University Hospital	Impaired Germinal Center Dynamics in mice following Influenza Vaccination during Diet-Induced Obesity **(p.27)**
11:37	**Taissa de Matos Kasahara** University of Oslo and Oslo University Hospital	Antibody and B-cell development in the context of repeated influenza vaccinations **(p.86)**
11:49	**Matthew Maasdorp** Tampere University	NHLRC2 deletion in Drosophila melanogaster replicates clinical features of the inherited childhood syndrome FINCA **(p.97)**
12:01	**Anja Hjelt** University of Turku	Investigating the treatment duration-dependent effects of estrogen receptor signaling: long-term estradiol treatment aggravates dextran sulphate sodium-induced colitis in female mice **(p.95)**
12:13	**Anne Mari Rokstad** Norwegian University of Science and Technology	Polystyren micro and nanoparticles trigger thromboinflammatory responses through complement, coagulation and leukocytes cross-talk **(p.96)**

Workshop VI: Klokkeklang

Autoimmunity & Immune Regulation

Chairs: Ranveig Braathen and Kenneth Green

11:25	**Andrea Ponzetta** Karolinska Institutet	State-resolved mapping of human double-negative αβ T cells reveals clonal architecture, tissue specialization and clinical relevance **(p.104)**
11:37	**Shiying Lin** Karolinska Institutet	FcRn-mediated antibody recycling promotes inflammation and tissue remodeling associated with atherosclerotic plaque vulnerability **(p.30)**
11:49	**Julia Karen Demtröder** Aarhus University	Monovalent antigens with defined physicochemical properties induce B-cell activation independently of receptor oligomerization **(p.111)**
12:01	**Preben Boysen** Norwegian University of Life Sciences	A farm-type natural habitat to increase the translational value of mucosal immunology experiments in the mouse **(p.33)**
12:13	**Karine Chemin** Karolinska University Hospital	Immune cell profiles in checkpoint inhibitor induced arthritis compared with rheumatoid arthritis **(p.25)**

WEDNESDAY 14:45-15:45| Workshops VII & VIII

Workshop VII: Peer Gynt-Salen

Lymphocyte Activation & Function

Chairs: Tapio Lönnberg and Julia Karen Demtröder

14:45	**Eylem Baysal** University of Bergen	PI3K/Akt Signaling Contributes to Treg-Derived Extracellular Vesicle-Mediated Osteogenesis in Bone Marrow Mesenchymal Stromal/Stem Cells **(p.109)**
14:57	**Francesca La Gualana** University of Gothenburg	Architecture of the B-cells surface: nanoscale protein interaction networks as a regulatory layer of B-Cell Antigen Receptor (BCR) signalling and cell fate **(p.112)**
15:09	**Chimezie Harrison Umeano** Lund University	Adaptive immunity is dispensable for salamander appendage regeneration **(p.108)**
15:21	**Lea Mikkola** University of Turku	Identification of antigen-presenting fibroblasts in human hip osteoarthritis **(p.31)**
15:33	**John Rizk** University of Copenhagen	Restrained Met1-linked ubiquitination prevents immune pathology and safeguards T cell development and function **(p.113)**

Workshop VIII: Klokkeklang

Infectious Diseases & Vaccination

Chairs: Trevor Owens and Martín González-Rodríguez

14:45	**Jenny Lorena Molina Estupinan** University of Iceland and Landspitali University Hospital	Mucosal pneumococcal immunization with mmCT accelerates neonatal B cell responses compared with parenteral immunization in neonatal mice **(p.76)**
14:57	**H. Craig Morton** Institute of Marine Research	Generation of IgM+ B cell-deficient Atlantic salmon **(p.51)**
15:09	**Mai-Chi Trieu** University of Bergen and Haukeland University Hospital	Broadly cross-reactive antibody responses after influenza B infection in adults **(p.79)**
15:21	**Camilla Tysnes Fauskanger** University of Bergen and Haukeland University Hospital	Human Immune Memory and Humoral Responses to Avian Influenza (H5) Vaccination **(p.87)**
15:33	**Simone Costagli** University of Siena and Oslo University Hospital	Immunocompromised individuals exhibit reduced immune imprinting after SARS-CoV-2 Omicron exposure compared with healthy individuals **(p.75)**

Index of presenting authors

Exhibitors

Latent EBV-specific CD8⁺ T cells Exhibit Disease-specific Transcriptional Programming in Multiple Sclerosis

Panteha Khaledi[1,2]; Oyime Poise Aula[2]; Jessica Engel[2]; Yuanhao Yang[3]; Nicholas Blackburn[1]; Chhavi Asthana[1]; Eleanor Sabey[1]; Natasha Stevens[1]; Yi Chao Foong[1,4]; Kathryn Burdon[1]; Jacob Gratten[3]; JianJun Liu[5]; Xin Lin[1]; Rajiv Khanna[2,6]; Bruce Taylor[1]; Yuan Zhou[1†]; Corey Smith[2†]

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[3]*Mater research, Translational Research Institute, University of Queensland, Woolloongabba, Brisbane, Queensland, Australia*

[4]*Royal Hobart Hospital, Tasmanian Health Service, Hobart, Tasmania, Australia*

[5]*Genome Institute of Singapore, Singapore*

[6]*Queensland Immunology Research Centre, University of Queensland, Brisbane, Australia*

† These authors jointly supervised this work.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease marked by immune-mediated damage in the central nervous system, with Epstein-Barr virus (EBV) strongly implicated in its development. Effective control of EBV requires coordinated CD8⁺ T cell responses across latent and lytic stages, yet the points of immune failure in MS remain unclear.

Aims

To determine whether MS involves stage-specific dysregulation of EBV-specific CD8⁺ T cell immunity by resolving transcriptional and T cell receptor (TCR) features across latent and lytic programs.

Methods

Peripheral blood mononuclear cells from 21 treatment-naïve people with MS (pwMS) and 19 controls were analysed using HLA-matched peptide–MHC multimer enrichment with paired single-cell transcriptomic/TCR profiling. Samples then underwent autologous EBV lymphoblastoid cell line (LCL) stimulation to assess antigen-driven responses.

Results

MS was associated with stage-specific dysregulation of EBV immunity, characterised by selective expansion of latent-specific CD8⁺ T cells (PFDR = 0.04), largely driven by EBNA1- and EBNA3A-specific responses. These cells showed enhanced cytotoxic programming (PFDR = 0.03) and clonal expansion, while other stages were unchanged. Following LCL stimulation, pwMS exhibited selective amplification of a *GZMK*⁺ interferon-associated program within late effector memory cells (PFDR < 0.05), including *IFI6, HLA-B, IL7R, JAK1*, and *CXCR4*. Integration with MS GWAS highlighted overlap with an immune-regulatory module involving *IL7R, JAK1,* and *HLA-B*.

Conclusion

These findings identify impaired regulation of latent EBV infection as a key feature of MS, linking persistent antigen recognition to pathogenic CD8⁺ T cell activation and highlighting latent antigen-specific responses as therapeutic targets.

Skin-draining lymph nodes: sentinels at the crossroad between localized cutaneous immune responses and systemic autoimmunity

Natalia Guźniczak[1]; Kristian S Kastberg[1,2]; Layla Pohl[1]; Lasse F Voss[1]; Søren E Degn[1,2]

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[2]*CellPAT Center for Cellular Signal Patterns, Aarhus University, 8000 Aarhus C, Denmark.*

Aim

To characterize the cellular and microanatomical changes in the secondary lymphoid organs in a Toll-like receptor 7 (TLR7)-driven model of Systemic Lupus Erythematosus (SLE).

Methods

Immune responses were induced in mice using repeated topical application of the TLR7 agonist resiquimod (R848) to the ear skin. After 4 weeks, draining auricular lymph nodes and spleens were collected and analyzed using high-dimensional multiplex imaging (MACSima). Spatial organization of B cell, T cell and myeloid populations were assessed.

Results

R848 treatment induced lymphoid organ remodeling. We observed an increase in the numbers of B cells, especially IgM+ and CD138+ cells, indicating B-cell activation and plasma cell differentiation driven by extrafollicular and germinal center responses on the treated side. CD11c+, CD4+ and PD-1+ cells also expanded. Foxp3+ Helios+ thymus-derived regulatory T cells were significantly decreased on the treated side, indicating impaired tolerance. The contralateral lymph node and spleen also showed signs of immune activation. The spleen exhibited severe architectural disruption, including loss of marginal zone integrity and reduction of marginal zone macrophage and B cell layers, indicating breakdown of a key tolerogenic niche.

Conclusion

TLR7-driven inflammation initiates a localized immune response in the draining lymph node that progressively spreads systemically. Activated lymphocytes and inflammatory mediators promote widespread immune activation. Disruption of the splenic marginal zone further impairs tolerance, reinforcing autoreactive responses. Together, this establishes a feed-forward loop that drives the transition from local immune activation to systemic lupus-like autoimmunity.

Screening for transglutaminase activity among bacterial strains from human intestinal commensal flora, associated with dysbiosis in active celiac disease or ascribed with probiotic properties

Kapczyńska, Katarzyna[1]; Freitag, Tobias[2]; Krzyżewska-Dudek, Eva[1,2]

[1]Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

[2]Translational Immunology Research Program, Department of Bacteriology and Immunology, Faculty of Medicine, University of Helsinki, Finland

Aim

Celiac disease (CD) is a T cell mediated autoimmune disease driven by the ingestion of dietary gluten in genetically susceptible individuals bearing HLA-DQ2 or DQ8 alleles. However, as only a minority of HLA-DQ2/DQ8-positive individuals who regularly consume gluten develop the disorder, it is likely that other environmental factors play a role in the disease onset. Deamidation of gluten peptides by tissue transglutaminase 2 (TG2) enhances the binding affinity to HLA-DQ2/DQ8 molecules in antigen presenting cells. Representatives of the transglutaminase family of enzymes can be found also in the bacterial domain (microbial transglutaminases, mTG), as exemplified by *Streptomyces mobaraensis*. The aim of this study was to test whether specific bacterial strains from the intestinal commensal flora, associated with dysbiosis in active CD or with probiotic properties can produce mTG.

Methods

Bacterial strains (n=40) were screened for mTG activity with a direct detection method (filter paper disc assay). An mTG production medium was optimized, and enzyme production was quantified using an in house hydroxamate assay.

Results

The qualitative and quantitative screening revealed that among the tested strains, bacterial species producing mTG could be identified and compared (e.g. *Staphylococcus* spp., *Pseudomonas* spp.).

Conclusion

Bacterial strains present in the human gut and/or delivered to the intestine could potentially contribute to the development of CD by the production of mTG, provided that gluten may serve as substrate. The substrate specificities of mTG(s) produced by the identified strains may be characterized in future.

Immune cell metabolic profiling in patients with endocrine autoimmune diseases

Gunn Elise Børset[1]; Bergithe E. Oftedal[1,2]; Eystein S. Husebye[1,2]; Alexander Hellesen[1,2]; Thea Sjøgren[2].

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Metabolic processes such as aerobic glycolysis, fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) are important for immune regulation. These processes affect immune cell phenotype and function, exemplified by T cells undergoing a metabolic switch to aerobic glycolysis following activation. Metabolic imbalance and/or dysfunction have been reported in pathogenesis of autoimmune diseases, like systemic lupus erythematosus and rheumatoid arthritis, but whether such disturbances are present and play role in in the pathogenesis of endocrine autoimmune disorders is unknown.

We hypothesise that patients with autoimmune primary adrenal insufficiency (PAI) and autoimmune polyendocrine syndrome type 1 (APS-1) have impaired cellular metabolic function and aim to test this hypothesis by performing metabolic profiling of immune cell subsets with a single-cell flow cytometry-based assay (SCENITH), as well as measuring mitochondrial membrane potential and cellular uptake of sugar and fat.

As of now, we have profiled unstimulated and stimulated peripheral blood mononuclear cells in six patients with PAI and six controls by SCENITH. The results show that patients with PAI have similar metabolic profiles to that of controls when it comes to glucose and mitochondrial (OXPHOS) dependence, as well as fatty and amino acid oxidation capacities. Assays for measuring mitochondrial membrane potential and sugar and fat uptake have been optimised and will together with SCENITH be applied to larger cohorts of patients with PAI and APS-1 to allow for thorough metabolic scrutiny of individual samples.

Systemic immune composition and activation-dependent B cell responses in Autoimmune Addison's Disease

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Background

Autoimmune Addison's disease (AAD) is a rare, organ-specific endocrine condition caused by immune-mediated destruction of the adrenal cortex. It remains unclear whether AAD is associated with persistent systemic immune alterations over time or whether immune differences primarily emerge upon immune activation. The aim of this project was to characterize longitudinal immune cell composition and activation-dependent immune responses in AAD patients.

Methods

We applied three complementary immune profiling methods in partially overlapping AAD patient cohorts from the Norwegian registry of AAD (ROAS) with independent sex- and age-matched healthy control cohorts. Longitudinal immune cell composition was quantified using DNA methylation-derived estimates from frozen whole blood AAD patients' samples spanning up to 25 years (*n=22*, 107 samples, 4-7 samples per patient) and healthy controls (*n=45*, one sample per individual). Cross-sectional analyses were performed using CyTOF of resting PBMCs (*n=42* AAD, *n=40*controls) and flow cytometry on *in vitro* ionomycin-stimulated PBMCs (*n=20* AAD, *n=20* controls).

Results

CyTOF profiling of resting PBMCs revealed a reduced frequency of NK cells in AAD patients compared to controls, consistent with longitudinal analyses showing persistently lower NK cell frequencies across age. Stimulated PBMC flow cytometry showed elevated CD19+ B cells in AAD patients compared to stimulated controls.

Conclusions

Systemic immune cell composition in AAD appears largely stable over time under resting conditions, aside from persistently reduced NK cells. However, immune stimulation unmasks distinct functional differences, particularly exaggerated B cell responses, highlighting activation-dependent immune dysregulation in AAD that standard resting-state analyses might miss.

The cytokine autoantibody landscape across systemic and organ specific autoimmune diseases

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Cytokine autoantibodies target signalling molecules that immune cells use to communicate, coordinate responses and maintain tolerance. They have been described in disorders ranging from monogenic, including autoimmune polyendocrine syndrome type 1 (APS1), to systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), as well as in a fraction of the general population, with frequencies increasing with age.

In APS1, autoantibodies targeting type I interferons (IFN) are present in nearly all patients, have been shown to exert neutralizing effects and are used as diagnostic markers in the clinic preceding genetic analysis. However, the prevalence and functional significance of cytokine autoantibodies across autoimmune diseases remain incompletely understood.

We aimed to determine the frequency and distribution of autoantibodies targeting 16 different cytokines (IFN-*ω*, IFN-*α2*, IFN-*β*, IFN-*γ*, TNF-*α*, IL22, IL17A/F, IL2, IL1a, IL12p40, GM-CSF, G-CSF, BAFF, IL10 and IL6) using a multiplex assay. We included patients with APS1 (N=49), Graves' disease (N=114), autoimmune primary adrenal insufficiency (N=316), mixed immunodeficiencies (N=20), *STAT1* (N=19) and dominant *AIRE* (N=20) mutations, systemic sclerosis (N=163), SLE (N=362), mixed connective tissue disease (N=134), patients with anti-neutrophil cytoplasmic antibodies (N=89), type 1 diabetes (N=354) and blood donors (N=708).

Cytokine autoantibodies were detected across all disease groups, including blood donors. The majority of APS1 patients were positive for IFN-*ω,* IFN-*α2*, and IL-22. Notably, IFN-*α2* autoantibodies were also detected in patients with systemic autoimmune diseases with values comparable to those observed in APS1.

These findings indicate that cytokine autoantibodies are a shared feature across autoimmune diseases, warranting further investigation of their pathogenic role.

Enhanced proliferation and effector functions in in-vitro activated T cells from patients with autoimmune polyendocrine syndrome type 1 (APS-1)

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Self-reactive T cells drive autoimmune pathology in autoimmune polyendocrine syndrome type 1 (APS-1), caused by breach of autoimmune regulator (*AIRE*)-mediated central T cell tolerance. Although resting peripheral blood mononuclear cells (PBMCs) and T cells from APS-1 patients have been extensively profiled, phenotypical or functional T cell abnormalities have not been consistently reported. We here hypothesized that *in vitro* activation would reveal dysregulated pathways that are either inconsistently- or not detected in resting immune cells.

PBMCs from APS-1 patients (n=4) and matched healthy donors (n=4) were used for single-cell profiling of transcriptomes and epitopes (CITE-seq) at baseline and after 72 h anti-CD3/CD28 stimulation. Additional T cell cultures (n=6 per group) were stimulated and analyzed at baseline and every 24h for five days for activation, proliferation, exhaustion, and apoptosis markers by spectral flow cytometry.

APS-1 T cells had similar gene expression profiles to blood donors in resting state, but an exaggerated response to polyclonal stimulation in the transcriptomic experiments. In APS-1 T cells, activation induced a stronger proliferative signature, higher expression of co-stimulatory and inhibitory immune checkpoints, increased expression of cytotoxic molecules and antigen presenting cell (APC)-recruiting chemokines. These changes were accompanied by strongly elevated expression of glycolytic enzymes, as well as decreased expression of quiescence/stem-like markers.

Our findings indicate that APS-1 T cells have a lower activation threshold and exaggerated effector programming upon stimulation, despite appearing phenotypically normal at rest.

Angiotensin II Type 2 Receptor Activation Promotes Central Nervous System Repair in Progressive Multiple Sclerosis-Like Pathology

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Aim

Progressive multiple sclerosis (PMS) is characterized by chronic neuroinflammation, persistent glial activation, and demyelination, with limited therapeutic options. Age-related decline in central nervous system (CNS) repair capacity further contributes to disease progression. The angiotensin II type 2 receptor (AT2R) mediates anti-inflammatory and reparative effects in the CNS. This study investigated whether activation of this receptor with the selective agonist Compound 21 (C21) modulates glial responses, reduces neuroinflammation, and promotes repair in PMS-like pathology.

Methods

Two experimental models were used: a two-photon laser-induced cortical lesion model to mimic subpial inflammation and cortical demyelination, and a cuprizone-induced demyelination model to study mechanisms of myelin loss and repair. Histological and immunofluorescent analyses were performed to assess lesion size, immune cell infiltration, astrocyte activation, blood-brain barrier integrity (BBB), and microglial phenotypes following treatment.

Results

C21 significantly reduced cortical lesion size, attenuated astrocyte activation, decreased immune cell infiltration, improved BBB integrity, and reduced demyelination.

Microglia shifted towards a reparative phenotype (reduced MHC II expression and increased CD163 and P2RY12 expression), without changes in total cell numbers. In cuprizone-treated mice, demyelination was observed in both young and aged animals. Ongoing studies suggest that C21 reduces demyelination more effectively in young mice, while still promoting immunomodulatory and neuroprotective responses in aged animals, indicating preserved therapeutic potential across aging.

Conclusion

AT2R activation via C21 suppresses neuroinflammation and promotes reparative glial responses supporting CNS repair. These findings suggest therapeutic potential in both disease progression and established pathology in PMS-like conditions.

Autoantibody specificity towards neuronal targets in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies targeting nuclear antigens. A large proportion of patients are affected by neuropsychiatric manifestations of the disease. However, the mechanisms by which dysregulation of the peripheral immune system affects the central nervous system are not yet understood. Our aim is to investigate autoantibody specificity towards neuronal targets in SLE, with a focus on the mechanistic link between autoantibody reactivity and neuropsychiatric manifestations in systemic autoimmune disease.

Previous observations from DNA-PAINT super-resolution microscopy in our group indicated the presence of intraneuronal antibodies in a pharmacologically induced mouse model of SLE. Using flow cytometry, we found that cellular uptake of autoantibodies was significantly higher compared to other antibody controls, suggesting that autoantibodies may have intrinsic properties involved in facilitating cellular entry. Extending the imaging-based approach, confocal microscopy was used to study the binding specificities of autoantibodies and their cellular distribution. Surprisingly, SLE-associated autoantibodies exhibited pronounced and selective reactivity towards neuronal targets in brain tissue ex vivo. This pattern of reactivity indicates preferential recognition of a highly enriched antigen target present in neuronal somas, outcompeting nuclear antigens in microglia and astrocytes. Collectively, these findings support that SLE-associated autoantibodies may directly interact with neuronal targets, providing a potential mechanistic framework for neuropsychiatric manifestations in SLE.

Age-associated B cells drive CD8+ T-cell hyperactivation in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disease characterized by loss of tolerance, autoantibodies, and immune complex formation. SLE is associated with increased age-associated B cells (ABCs) and proinflammatory T cells. ABCs express T-bet and CD11c and contribute to disease by producing autoantibodies, secreting inflammatory cytokines, and activating self-reactive CD4+ T cells. However, no interplay between ABCs and CD8+ T cells in SLE has been identified so far.

In this study, epicutaneous application of resiquimod, a TLR-7 agonist, induced a SLE-like autoimmune response in mice, a hyperactivated CD8+ T-cell phenotype and an increase in ABCs. Single cell sequencing corroborated the hyperactivated CD8+ T-cell phenotype and revealed an interactome correlation between ABCs and CD8+ T cells. Genetic models revealed that the hyperactivation of CD8+ T cells depended on B-cell binding of self-antigen and IFN-γ, but was independent of antigen presentation on MHC-I. Restriction of the TCR repertoire to a non-cognate antigen permitted early activation of CD8+ T cells but prevented persistent activation. The hyperactivation of CD8+ T cells was dependent on T-bet expression specifically in B cells. We observed a correlate of this phenomenon in the blood of SLE patients, where the frequency of ABCs inversely correlated with CD8+ T-cell levels. Coculturing T-bet-positive or T-bet-negative B cells with CD8+ T cells under ABC-inducing conditions confirmed the direct activation of CD8+ T cells by ABCs and provided mechanistic insight.

This novel link between ABCs and CD8+ T-cell hyperactivation provides a new target for therapeutic intervention in autoimmunity.

Heterogeneous synovial fluid T-cell landscape in patients with juvenile idiopathic arthritis

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Aim

Juvenile idiopathic arthritis (JIA) is a heterogeneous autoimmune disease of childhood characterized by inflammation of the synovium, the tissue lining the inner surface of the joint. Previous studies have demonstrated that synovial fluid T cells reflect the T-cell composition of inflamed synovial tissue in JIA patients. Here, we aimed to comprehensively characterize the synovial fluid T-cell landscape in JIA at a single-cell resolution.

Methods

Paired synovial fluid and peripheral blood samples from pediatric JIA patients with active synovitis were analyzed using two complementary single-cell approaches: multicolor flow cytometry (n=28) and single-cell multiomics (CITE-seq, n=14).

Results

Compared with paired peripheral blood samples, synovial fluid was enriched for a wide range of memory and effector T-cell subpopulations, including activated regulatory T cells, CD8+ tissue-resident memory T cells expressing multiple granzymes, and polyfunctional CD4+ and CD8+ T cells expressing multiple cytokines. Through single-cell multiomics, we also identified two distinct PD-1-expressing CD4+ T cell populations exclusively present in synovial fluid: IL-21+CXCL13+TIGIT+BTLA+ T peripheral helper cells and a second population lacking the expression of these markers, but rather expressing EOMES, granzyme K, IL-10, and CD38. This latter T-cell population has been identified in other autoimmune diseases, such as systemic lupus erythematosus, but has not previously been characterized in JIA synovial fluid or tissue.

Conclusion

The T-cell landscape of synovial fluid in JIA patients is heterogeneous, and dominated by various effector and inflammatory T-cell subsets. Our results provide new insights into cellular immune mechanisms underlying JIA pathogenesis.

Proinflammatory Memory CD4+ T Cell Markers Are Elevated in the Circulation of Patients with Juvenile Idiopathic Arthritis

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Aim

Juvenile idiopathic arthritis (JIA) is an autoimmune disease in children characterized by chronic inflammation of the joints. In patients with JIA, pro-inflammatory CD4+ helper T cells have been suggested to play an important role in driving synovial inflammation. However, in JIA patients, only limited data on peripheral blood immune cell changes exists.

Methods

We studied peripheral blood mononuclear cells (PBMCs), particularly the memory CD4+ T-cell compartment, using multiparameter flow cytometry to compare patients with JIA (n=63) to healthy age- and sex-matched controls (n=45). Additionally, we utilized two Olink Target 48 proteomics panels to analyze cytokine and chemokine plasma levels in JIA patients compared with healthy controls.

Results

We detected an elevated frequency of circulating CD4+CXCR5-PD-1hi T peripheral helper (Tph) cells ($p<0.01$) in patients with JIA compared with controls. Moreover, increased frequencies of tumor necrosis factor-alpha (TNF-α) ($p<0.001$) and interleukin (IL)-2 ($p<0.001$) producing CD4+ memory T cells were detected in JIA patients compared with controls, when PBMCs were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin. The levels of several plasma proinflammatory cytokines and chemokines, such as IL-1β, IL-6, TNF-α and CXCL9, were also elevated in patients with JIA.

Conclusion

In summary, the memory CD4+ T cell compartment in patients with JIA showed alterations characterized by T-cell signatures linked to proinflammatory functions and enhanced T-cell-B-cell interactions. These signatures could potentially be utilized in the development of T-cell based biomarkers to monitor JIA disease activity.

Deep cervical lymph nodes of patients with multiple sclerosis show increased double negative B cells and their depletion by antiCD20 therapy is incomplete

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Aim

We have previously reported increased double negative (DN) B cells with lytic Epstein Barr virus (EBV) signatures in the deep cervical lymph nodes (dcLNs) of newly diagnosed patients with multiple sclerosis (pwMS). Here we explore the effect of anti-CD20 treatment on the B cell compartment of the dcLNs and on EBV-targeting CD8 T cell responses using single-cell transcriptomics.

Methods

Fine needle aspiration (FNA) of dcLNs of pwMS was performed prior to initiation of any immunotherapy (n=13) and after anti-CD20 therapy (rituximab, n=3 and ofatumumab, n=2). Comparison to PBMC before and after therapy and to FNA of healthy controls (n=3) were also performed. Single-cell suspensions were run on 5' scRNAseq coupled with V(D)J repertoire profiling.

Results

Significantly increased proportions of DN and IFNg-stimulated B cells were seen in the dcLNs of pwMS compared to controls. Tbet+CD11c+ and IFNg-stimulated B cells along with switched (SM) and unswitched (USM) memory B cells were significantly depleted by anti-CD20 in both dcLNs and circulation, whereas DN B cells of the dcLNs were not. Expanded CD8+ T cells of pwMS showed markedly high EBV epitope targeting, which was reduced by anti-CD20 therapy.

Conclusions

DN B cells are relatively spared by anti-CD20 therapy in the dcLNs compared to other B cell subgroups. Given the implications of a possible disease-driving role of these cells in autoimmune diseases, a complementary therapy to anti-CD20 could be implicated.

Immune signatures and T cell function in patients with rare immune gene variants and associated cytokine autoantibodies

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Background

Autoimmune diseases result from failure of immune tolerance, allowing self-reactive immune cells to damage tissues. Autoimmune polyendocrine syndrome type-1 (APS-1), caused by mutations in the *AIRE* gene, represents a prototypical monogenic disorder of immune tolerance, characterised by multi-organ autoimmunity and neutralising cytokine autoantibodies, particularly against type I interferons (IFNs). Similar IFN autoantibodies and rare variants in immune regulatory genes, including *LCK*and *NF-κB2*, have been identified in other immune dysregulatory conditions. The functional impact of such variants on immune signalling and cellular phenotypes remains incompletely understood.

Aim

To investigate how variants in *AIRE, LCK*, and *NF-κB2*affect interferon signatures, TCR signalling, and T-cell phenotypes.

Methods

Whole-blood interferon stimulated gene expression was assayed by qPCR in APS-1 patients (n=24), *NFKB2* (N=1) and *LCK* (N=2) variant carriers, and controls (n=46). TCR signalling was assessed in five of the patients using phospho-flow cytometry, and T-cell phenotyping by spectral flow cytometry.

Results

APS-1 patients revealed a distinct IFN signature, with upregulation of B2M, BAFF, CXCL10, STAT1, and SERPING1, and downregulation of LY6E, IFI44L, USP18, and MX1. *LCK* and *NF-KB2* variant carriers showed similar patterns. TCR signalling was largely preserved, though ERK phosphorylation was reduced in some individuals. T-cell composition was mostly similar to controls, with patient-specific alterations in subsets and activation markers. Results from complementary *in vitro* experiments using prime-edited Jurkat cells of *LCK* variants are currently pending.

Conclusion

Individuals with rare variants in *AIRE, LCK* and *NFKB2* showed signs of IFN dysregulation, supporting their role in immune dysfunction.

BANK1 Polymorphisms and Systemic Sclerosis: Insights from an Algerian cohort

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Aim

Systemic sclerosis (SSc) is a heterogeneous autoimmune disease characterized by distinct clinical and serological subsets. BANK1 (B-cell scaffold protein with ankyrin repeats 1) is a regulator of B-cell receptor signaling previously associated with systemic lupus erythematosus, but its role in SSc remains unclear. This study aimed to investigate the association of BANK1 polymorphisms (rs10516487 and rs3733197) with SSc susceptibility, clinical subtypes, and autoantibody profiles in an Algerian cohort.

Methods

A case-control study was conducted in an Algerian cohort including 91 SSc patients and 97 healthy controls. Genotype and haplotype distributions were analyzed according to disease status, clinical subtype, and autoantibody profile.

Results

No significant association was found between BANK1 polymorphisms and overall SSc susceptibility or clinical subsets. However, haplotype analysis showed a nominal association between the AA haplotype and anti-Scl70 status, with a higher frequency in anti-Scl70-negative patients than in positive patients (24% vs 8.5%, p = 0.041, OR = 0.26). Given the strong association of anti-Scl70 antibodies with fibrotic disease and interstitial lung disease, this finding may indicate a modest role of BANK1-related B-cell signaling pathways in autoantibody expression rather than disease susceptibility.

Conclusion

BANK1 polymorphisms do not appear to contribute to SSc susceptibility or clinical subtype distribution in this Algerian cohort. However, the observed association with anti-Scl70 status suggests a potential influence on serological endotypes. Larger multi-ethnic studies are needed to confirm these findings and further clarify the role of B-cell–mediated immunity in SSc.

The combined effects of variant allele biology and haploinsufficiency may contribute to clinical heterogeneity in NFKB1-deficient patients

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Introduction

NFKB1 encodes two proteins of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. It regulates inflammatory responses and loss of function (LOF) mutations in *NFKB1* can contribute to autoimmunity, common variable immunodeficiency (CVID) and hyperinflammation.

Objectives

We investigated the effects of NFKB1 haploinsufficiency caused by four heterozygous *NFKB1* variants found in six patients. We focused on charting the effect of *NFKB1* variants on patients' immune cell functions and inflammatory responses

Results

All variants were associated with altered lymphocyte profiles unique to individuals. Western blot analysis of PBMC-derived NFKB1 proteins (p105/p50) showed a comparable reduction across all variants, while flow cytometry revealed significant p50 decrease only in B lymphocytes. BioID analysis identified variant-specific protein interactions within the NF-κB pathway. Functional assays demonstrated increased IL-1β production after LPS-ATP stimulation, indicating enhanced inflammasome activity but IL-1β receptor antagonist therapy benefited only one family. Meanwhile, IgG supplementation reduced fever episodes in two patients without hypogammaglobulinemia. A previously reported defect in neutrophil oxidative burst was observed in two variants and three variants showed increased IL-8 secretion upon LPS-ATP stimulation.

Conclusions

These findings support association between NFKB1 haploinsufficiency and impaired B cell development, while highlighting its contribution to hyperinflammation. Although the role of anti-IL-1 treatment appears crucial, we recommend considering IgG substitution treatment against resistant fever despite the unaffected B cells or immunoglobulin levels. Finally, we believe that the alterations in interactions may contribute to the immunological condition; further attention should be paid to the biological effects of the variant allele products

Spatial and single-cell characterization of the tonsillar immune landscape in PFAPA syndrome

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Aim

PFAPA (Periodic fever, Aphthous stomatitis, Pharyngitis, Adenitis) is considered to be an autoinflammatory syndrome and it represents the most common cause of periodic fever in childhood. Tonsillectomy or tonsillotomy is a well-established curative treatment for the syndrome. However, the pathogenesis of PFAPA and how the tonsillar immune system is involved in it is poorly understood. Here, we aimed to comprehensively characterize the tonsillar immune cell landscape in children with PFAPA syndrome utilizing spatial transcriptomics.

Methods

Tonsillar tissue was collected during routine tonsillectomy/tonsillotomy from eight children with PFAPA syndrome and eight children with benign tonsillar hyperplasia at Kuopio and Oulu University Hospitals (Finland). Spatial transcriptomic analysis of FFPE tonsil sections was performed using the 10x Genomics Xenium platform.

Results

Preliminary spatial analysis of tonsils displays an immune cell landscape enriched for various B- and T-cell subsets consistent with the prominent germinal center-dominated architecture of the tonsillar tissue. Macrophages, pDCs and LAMP3+ DCs are also detected, albeit at lower frequencies compared with B and T cells. Ongoing analyses focus on detailed characterization of the transcriptomic profiles of the main immune cell subsets and the spatial architecture of the tonsils from the children with PFAPA syndrome in comparison to healthy controls.

Conclusion

Spatial transcriptomic profiling of tonsils from children with PFAPA syndrome allows a deeper characterization of the immune cells that could potentially be involved in the pathogenesis of PFAPA syndrome.

Human immune responses after surgical trauma

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Aim

Surgical trauma induces systemic immune responses that are required for tissue repair but may also contribute to excessive inflammation and postoperative complications. The cellular dynamics underlying this response remain incompletely characterized. This study aims to define longitudinal perioperative immune remodeling after surgical trauma at single-cell resolution.

Methods

The POSINI cohort includes individuals undergoing prostatectomy with peripheral blood sampling before surgery and at 24 hours, 14 days, and 6 months after surgery. Peripheral blood mononuclear cells were profiled using TotalSeq-based single-cell sequencing, enabling paired assessment of transcriptomic states and antibody-derived surface protein signals. Major immune compartments were annotated, followed by sub-clustering of selected lineages to resolve functionally distinct subsets.

Complementary Olink proteomics and flow cytometry data collected 6 hours after surgery from a related translational cohort were used to assess early systemic inflammatory responses.

Results

Preliminary analyses identified major immune populations, including CD4 and CD8 T cells, NK cells, B cells, monocytes, dendritic cells, and other myeloid subsets. Longitudinal comparison across perioperative timepoints revealed marked immune remodeling during the early postoperative phase. These changes were particularly evident within the myeloid compartment, where CD14+ classical monocytes showed pronounced transcriptional heterogeneity and distinct postoperative cell states.

Subset-level analyses further suggested rapid state-specific remodeling within 24 hours after surgery, followed by partial resolution at later timepoints.

Conclusion

This study provides a longitudinal single-cell framework for characterizing perioperative immune dynamics and highlights rapid monocyte remodeling as a central feature of the early systemic immune response to surgical trauma.

Thymic TREG-derived T follicular regulatory cells prevent severe humoral autoimmunity in response to TLR7-driven inflammation

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T follicular regulatory cells (TFR) are a follicle-resident subset of regulatory T cells (TREG) that constrains germinal center (GC) responses and maintain humoral tolerance. While TFR are known to direct antibody responses toward foreign antigens, their GC-specific regulatory role during sterile inflammation remains unclear. Therefore, this study aimed to investigate the role of TFR in GC regulation during sterile inflammation, using Resiquimod (R848)-treated transgenic mouse models and human blood samples from a systemic lupus erythematosus (SLE) patient cohort. Adoptive transfer experiments, serial-intravital two-photon microscopy, single-cell RNA sequencing, flow cytometry, and TRIFMA analyses were performed.

Selective loss of TFR resulted in severe autoimmunity and increased mortality in R848-treated mice, driven by inflammation-induced expansion of autoreactive B- and T-cell clones and autoantigen-directed epitope spreading. Following treatment cessation, autoimmunity resolved in TFR -sufficient mice, whereas TFR -deficient mice exhibited persistent inflammation.

Mechanistically, TFR restricted the establishment and expansion of spontaneous GCs during inflammation, with responding TFR displaying transcriptional, phenotypic and clonal characteristics consistent with a thymic TREG origin. These findings where further corroborated in the SLE patient cohort, in which patients exhibited expanded TFR compartments and frequencies of thymic TREG -derived TFR positively correlated with disease activity.

Collectively, our findings suggest thymic TREG-derived TFR as a distinct regulatory lineage that preserves GC integrity during sterile inflammation and protects against autoimmune diseases in both mice and humans.

Autoimmunity-associated DIORA1 binds the MRCK family of serine/threonine kinases and controls cell motility

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Aim

Genetic studies have linked *FAM167A*, encoding Disordered Autoimmunity 1 (DIORA1), to several autoimmune rheumatic diseases, including Sjögren's disease, systemic lupus erythematosus and rheumatoid arthritis. However, the cellular function of DIORA1 has remained unclear. We aimed to define the molecular role of DIORA1 and determine how it may connect to disease-relevant cellular phenotypes.

Methods

We combined proximity biotinylation, co-immunoprecipitation, interaction mapping and AlphaFold-guided structural modelling to identify and characterize DIORA1 protein interactions. Functional consequences of DIORA1 knockdown were assessed using CRISPR-based perturbation, phosphoproteomics, RNA sequencing, proteomics and cell motility assays in human cell lines.

Results

DIORA1 interacted with the MRCK family of serine/threonine kinases, key regulators of actomyosin contractility and cell movement. Interaction mapping showed that DIORA1 binds three distinct MRCK regions, including the kinase inhibitory motif, C1-PH domain and citron homology domain. DIORA1 knockdown altered global phosphorylation patterns and reduced phosphorylation of known MRCK targets. Transcriptomic and proteomic profiling revealed induction of epithelial-mesenchymal transition-associated programs, and functional assays demonstrated increased invasive capacity following DIORA1 depletion.

Conclusion

These findings reveal a previously uncharacterized role for DIORA1 as an interactor of a small but specific group of kinases and a regulator of cell motility. The data provide a foundation for future studies exploring the importance of DIORA1 in autoimmune pathogenicity and other motility-driven biological processes, such as cancer metastasis.

Effects of complete B cell depletion by CD19 CAR T-cell therapy on T cells in myositis

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Aim

Myositis is an autoimmune disease affecting skeletal muscle often accompanied by disappointing results to available treatments. Removal of autoantibodies and B cells has demonstrated clinical improvements, although persisting cytotoxic T cells are suggested to drive relapses. Complete B-cell depletion via CD19-targeting chimeric antigen receptor (CAR) T-cells induces remission in treatment refractory cases, but its impact on T cells remains unclear. We aimed to investigate changes in the T-cell compartment upon CAR T-cell therapy.

Methods

We performed a deep analysis of T-cell phenotype by single cell and T-cell receptor sequencing from three patients with myositis at baseline and three months after CD19 CAR T-cells treatment, before B-cell repopulation, included in the CASTLE trial.

Results

We observed a decrease in the frequency of cytotoxic effector memory (EM) T-cell clusters in all patients 3 months after therapy, before B-cell repopulation. T cells post-treatment showed a decreased cytotoxic signature in all patients whereas downregulation of type-I IFN signature was observed in one patient. Although more naïve-like phenotype was detected in T cells post-treatment, T-cell clones were shared between pre- and post-treatment time points. Identical clones persisted in the same EM clusters in one patient, whereas in another they had a different phenotype post-treatment.

Conclusion

Our results show how B-cell depletion affects the T-cell population in patients with myositis, although limited to three patients. This points towards the role of autoreactive B cells in modulating peripheral T cells, licensing them towards a more cytotoxic, interferon-stimulated phenotype.

Modulation of Immune Checkpoint Gene Expression by Immunosuppressive Agents in Human Peripheral Blood Mononuclear Cells

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In this study, our aim was to investigate whether long-term immunosuppressive therapy directly modulates immune checkpoint expression, potentially contributing to tumor development in transplant patients.

In our pilot study, human peripheral blood mononuclear cells (PBMCs) from healthy individuals were isolated using Ficoll density gradient centrifugation. As a validation control immune activation was performed using PMA and ionomycin. Cells were treated with four different immunosuppressive agents (tacrolimus, cyclosporin-A, methylprednisolone, and mycophenolate), both individually and in triple combinations that included one of the two calcineurin inhibitors. Samples were collected after 72 hours of incubation, modeling the effects of prolonged immunosuppression. Following RNA isolation and cDNA synthesis, gene expression levels were analyzed using a chip-based, high-throughput qPCR platform.

Our preliminary results show that, as expected, the expression of activation markers such as IL-2, TNFα, and CD25 increases following T-cell activation. In addition, several immune checkpoints reported in the literature to change upon activation showed similar patterns in our model: CD40L was strongly upregulated, while CTLA-4 was also increased, acting as an early negative feedback marker. Among the immunosuppressive treatments, the triple-drug combination caused the most pronounced changes in gene expression. Methylprednisolone alone induced notable alterations, and in some cases calcineurin inhibitors also affected the expression of immune checkpoint molecules.

These preliminary results suggest that immunosuppressive agents can modulate immune checkpoint expression patterns, potentially contributing to tumor development under long-term immunosuppression.

nhlrc2 is required for normal development in zebrafish

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NHL repeat containing 2 (NHLRC2) is evolutionally conserved, ubiquitously expressed gene with incompletely characterized function. In humans, pathogenic variations in NHLRC2 cause fibrosis, neurodegeneration and cerebral angiomatosis (FINCA), a severe childhood-onset multiorgan disease. In addition to FINCA, studies have connected NHLRC2 to several cellular functions, e.g. deficient phagocytosis, apoptosis and formation of fibrosis. NHLRC2 is required during early development, as it has been connected to viability of oocytes, embryonic lethality and developmental deformations in mammals.

In zebrafish (*Danio rerio*), a widely used model organism in biomedical research, *nhlrc2* is expressed from 2-cell stage onwards. Using a zebrafish knockout model, we have studied the role of *nhlrc2* in the context of early development and immunity.

Null mutant zebrafish fail to develop beyond five days post-fertilization, whereas mosaic zebrafish with a 52-95% mutation rate reach adulthood but exhibit failure to thrive and morphological abnormalities on histopathological analysis. In the offspring, partial loss of the gene is tolerated in heterozygous individuals. By injecting *in vitro*-transcribed *nhlrc2*mRNA into single-cell stage zebrafish embryos, we were able to delay the onset of lethal phenotype of the homozygous mutants.

Together, our findings support the conclusion of the crucial role of *nhlrc2* both during both early development and in adults and demonstrate the suitability of zebrafish model in resolving the function of *nhlrc2*. We hope that this will provide means to understand the mechanisms underlying FINCA disease.

Clinical study of the microbiome-immune axis in progression of Systemic Lupus Erythematosus

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The gut microbiome has revealed itself as a potential disease-driving factor in many autoimmune diseases, yet its dynamic character, mechanistic properties, and impact in Systemic Lupus Erythematosus (SLE) remain to be sufficiently investigated. This gap needs to be addressed, as SLE remains a heterogeneous disease with high morbidity, diagnostic delay, and limited treatment options.

In this explorative study, we aim to elucidate the gut microbiome-immune axis on a descriptive and functional level in SLE patients in a dynamic manner. For this purpose, we use a strong two-cohort foundation by collecting blood and feces samples from a longitudinal two-time-point cohort of treatment-naive SLE patients and a cohort of discordant twins. Using a multi-omic approach with spectral flow cytometry, single-cell RNA sequencing, and comparative metagenomic sequencing, we will be able to identify disease-relevant bacteria and/or bacterial networks with high immunophenotypic resolution and specificity. In parallel, through advanced culturing methods and in vitro experiments, we will demonstrate the mechanistic interplay between identified taxa and the immune system.

While we are currently still recruiting patients, we expect to identify disease-relevant bacterial species and their impact on disease progression, using human data, paving the way for microbiome-centred treatment regimes.

Immune cell profiles in checkpoint inhibitor induced arthritis compared with rheumatoid arthritis

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Aim

Immunotherapy with immune checkpoint inhibitors (ICI) improves cancer treatment but frequently induces adverse events, including inflammatory arthritis that may become chronic. The mechanisms driving ICI-induced arthritis remain poorly understood. We aimed to characterise the transcriptional signatures of immune cells in synovial fluid (SF) and peripheral blood (PB) from patients with ICI-arthritis and compare them with in-house datasets of classical rheumatoid arthritis (cRA).

Methods

We performed single-cell RNA-seq with paired TCR and BCR profiling on CD45+ cells from SF and PB of patients with newly diagnosed ICI-arthritis (n=5), generating 92,000 immune cells. Data were analyzed using Seurat with reference-based annotation and gene-module scoring.

Results

We identified 33 stable immune clusters across myeloid, dendritic cell, B cell, and T cell compartments. In ICI, SF was enriched for activated and cytotoxic CD8+T cell states and pro-inflammatory macrophages, whereas the frequency of dendritic cells was lower than in cRA. Distinct cytotoxic CD8+T cell subsets showed differential tissue distribution, including a TIGIT+ SF-enriched population. In SF, we also detected two states of CXCL13+ peripheral helper CD4+T cells and CD8+T cells with tissue-resident memory T cells characteristics. Altogether, our data suggest that ICI-arthritis shares features with cRA but also present distinct cell populations.

Conclusion

ICI-induced arthritis displays a distinct immune architecture with synovial enrichment of activated and cytotoxic T cells, pro-inflammatory macrophages, and tissue-resident memory-like T cells, which may contribute to chronicity as proposed in other autoimmune diseases. While sharing features with cRA, ICI-induced arthritis also shows disease-specific immune populations and inflammatory programs.

Tissue-resident autoantigen-recognizing CD8+ T cells are present in the cerebrospinal fluid of patients with Narcolepsy Type 1

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Narcolepsy Type 1 (NT1) is a sleep disorder characterized by the loss of hypocretin-producing neurons in the hypothalamus, possibly through an autoimmune attack. The autoimmune hypothesis for NT1 is based on associations between NT1 development and multiple factors, including (i) the predisposing HLA Class II DQB1*06:02 allele, (ii) vaccination with the "Pandemrix" H1N1 influenza vaccine, (iii) naturally occurring H1N1 Influenza A infection, and (iv) other genes related to the immune system. Despite this, the autoimmune hypothesis is not fully established.

Here, we applied DNA-labelled peptide:MHC (pMHC) multimers in a droplet-based single-cell sequencing setup on paired cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMCs). In the CSF compartment, NT1 patients exhibited increased clonal expansion of CD49a+ CCR5+ tissue-resident T cells compared with controls. Importantly, autoantigen-specific CD8+ T cells were exclusively detected in the CSF of patients with NT1. Moreover, when querying our CSF-derived TCR dataset for pMHC annotation using the immune epitope database, we found an enrichment of CD8+ T cells recognizing Influenza A epitopes in patients with NT1 compared with controls. In the PBMC compartment, we identified clonally-expanded autoantigen-specific CD8+ T cells which were almost exclusively identified in patients compared to controls.

Together, our data support the autoimmune hypothesis for NT1 and the potential contribution of influenza A to NT1 development.

Single-cell multi-modal profiling reveals transcriptome–BCR uncoupling and impaired B cell maturation in Common Variable Immunodeficiency

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Aim

Common Variable Immunodeficiency (CVID) is the most prevalent symptomatic primary antibody deficiency, yet the molecular mechanisms underlying defective antibody production remain incompletely understood. We aimed to define B cell-intrinsic abnormalities in CVID using integrated single-cell multi-modal profiling.

Methods

Peripheral blood B cells from 7 CVID patients and 8 healthy controls were analyzed using single-cell transcriptomics, B cell receptor (BCR) repertoire sequencing, and a computational framework integrating enhancer activity, transcriptional programs, and differentiation trajectories.

Results

CVID samples exhibited expansion of age-associated B cells, reduced memory B cell diversity, depletion of IgA and double-negative memory subsets, and expansion of unswitched memory B cells. Trajectory analysis revealed impaired progression toward class-switched memory states. BCR repertoire analysis demonstrated reduced clonal expansion and somatic hypermutation frequencies, consistent with impaired germinal center activity. Surprisingly, transcriptional programs characteristic of IgG and IgA memory B cells remained detectable despite profound defects in antibody production. Integration of transcriptomic and BCR data revealed that many cells transcriptionally classified as class-switched memory B cells retained IgM BCR sequences, a phenomenon significantly enriched in CVID relative to healthy controls. Flow cytometry independently validated the absence of corresponding surface IgG expression.

Conclusion

Our findings identify impaired B cell maturation and a previously unrecognized uncoupling between memory B cell transcriptional identity and immunoglobulin isotype status in CVID. This multi-modal framework provides new insight into the molecular basis of defective humoral immunity and establishes a foundation for identifying regulatory mechanisms underlying impaired class-switching.

Targeting T follicular helper cells in the Salivary Glands of Sjögren's Disease Patients

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Introduction

Sjögren's disease (SjD) is an autoimmune disorder causing salivary and lacrimal gland impairment, disproportionately affecting women (>90%). The golden-standard diagnostic tool includes microscopic evaluation of the minor salivary glands (MSG), where ectopic germinal centre (GC)-like structures including T follicular helper (Tfh) cells occur in approximately 25% of cases. In SjD, such structures may increase the risk of B-cell lymphoma development later in life.

Aim

Given the limited understanding of the Tfh cell-associated signals within SjD patients' MSG, we aimed to address this gap by utilizing novel RNAscope in-situ-hybridization technology.

Methods

A unique cohort (n=40) consisting of equal proportions of male and female MSG biopsies from SjD patients and non-sicca controls, was integrated into a tissue micro-assay (TMA) for cell analysis using RNAscope in-situ-hybridization, and extracellular tissue characterization.

Results

The spatial mRNA expression of the Tfh cell markers IL-21R, CXCL13, and CXCR5 was predominantly localised within focal infiltrates of the patients' MSG, with significantly higher transcript abundance observed in the GC+-group (p<0.001). When SjD patients and non-sicca controls were analysed together, BAFF-positive ductal epithelial cells were associated with hyposalivation (p<0.05). Patients of higher age showed a greater level of atrophy than those diagnosed earlier in life, suggesting a potential age-related shift in tissue pathology (r=0.455, p=0.044). Interestingly, none of these characteristics differed between the sexes.

Conclusion

Ongoing experiments incorporate additional Tfh cell markers to link the tissue composition with patients' clinico-serological profiles. This could offer new insights into previously uncharacterized biological processes in SjD within male and female target organs.

Haplotype-Aware Proteomics to Investigate Regional Differences in Prevalence of Autoimmune Diseases in Norway

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Aim

Autoimmune diseases (ADs) exhibit differences in prevalence across regions of Norway. While environmental factors have been proposed, genetic variation likely plays a role in this geographic disparity. Effects of genetic variants on the phenotype are mediated by the proteome, and understanding this relationship will bring novel insights on the effects of population-specific variation. Integrating genomic and proteomic data from the Norwegian Childhood Diabetes Registry (NCDR) and the Registry of Organ-Specific Autoimmune Diseases (ROAS), we aim to dissect the molecular basis of differences in AD prevalence.

Methods

We have developed haplotype-aware proteomics, an approach using mass spectrometry to investigate protein sequences encoded by individual haplotypes. Using the tool ProHap, we generate haplotype-resolved sequence databases capturing observed combinations of protein-altering variants. Mass spectrometry data are searched against these customized databases to quantify peptides. Using proteomics of serum samples from NCDR and ROAS with whole-exome sequencing and genotyping, we will map genetic risk onto the proteome.

Results

We used ProHap to publish population-specific databases from widely-used genomic reference panels, revealing that over 9% of the proteome in all major populations may align to variant peptides, which would otherwise be undetectable. We then used ProHap to construct sequence databases for 955 participants from NCDR, capturing over 230,000 protein-altering variants.

Conclusion

Haplotype-aware proteomics provides a powerful framework to map genetic variation onto protein landscapes. Our work will establish whether there is a substantial genetic driver of AD prevalence patterns in Norway and whether proteomics can provide further explanations of the molecular mechanisms.

FcRn-mediated antibody recycling promotes inflammation and tissue remodeling associated with atherosclerotic plaque vulnerability

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Aim

Atherosclerotic plaques represent a chronic inflammatory niche enriched with low-density lipoprotein (LDL)-derived antigens and antigen-specific antibodies, particularly those targeting apolipoprotein B100 (apoB). The mechanisms by which plaque-resident myeloid cells regulate antibody and immune complex dynamics within the atherosclerotic microenvironment remain unclear. We investigated the role of the neonatal Fc receptor (FcRn) in immune complex handling and macrophage-mediated inflammatory responses in atherosclerosis.

Methods

Carotid endarterectomy specimens from atherosclerotic patients were analyzed for apoB-specific antibodies and apoB–IgG immune complexes. Integrated single-cell transcriptomic analyses were performed to map FcRn expression across plaque-resident cell populations. Functional studies were conducted in human myeloid cells and ex vivo carotid plaque cultures using FcRn blockade.

Results

Symptomatic patients exhibited increased levels of free apoB-specific IgG accompanied by reduced apoB–IgG immune complexes. Single-cell analyses identified FcRn as highly expressed in plaque-resident macrophages. Mechanistically, FcRn promoted the dissociation of apoB–IgG immune complexes and facilitated extracellular IgG recycling, resulting in increased local free antibodies. FcRn expression increased with age and correlated with inflammatory and tissue-remodeling pathways associated with plaque vulnerability. Pharmacological FcRn blockade inhibited immune complex dissociation, reduced extracellular IgG accumulation, and attenuated matrix metalloproteinase-9 (MMP-9) production in human myeloid cells.

Similar effects were observed in ex vivo plaque cultures.

Conclusion

These findings identify FcRn as a key regulator of apoB–IgG immune complex processing and IgG homeostasis in plaque macrophages. FcRn-mediated antibody recycling contributes to inflammation and tissue remodeling associated with plaque vulnerability, highlighting FcRn as a potential immunomodulatory target in atherosclerosis.

Identification of antigen-presenting fibroblasts in human hip osteoarthritis

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Osteoarthritis (OA) is increasingly recognized as a disease involving immune-mediated mechanisms in addition to tissue degeneration. Antigen-presenting fibroblasts (APFs) have recently emerged as important regulators of immune responses in cancer, autoimmune diseases, and tissue fibrosis, but their presence and potential role in OA remain unknown. We aimed to identify and characterize APFs in human hip OA tissues.

Samples from synovium, femoral head ligament, and fibrous joint capsule were collected from patients undergoing total hip arthroplasty. Fibroblast populations were characterized using single-nucleus and high-resolution spatial transcriptomics performed on the same tissue specimens. Candidate APFs were mapped based on expression of the *CIITA–RFX* transcriptional module regulating *MHC-II* expression and validated by multiplex immunofluorescence.

Single-nucleus transcriptomics identified fibroblast populations expressing the *CIITA–RFX*gene module across multiple hip joint tissues. Spatial transcriptomics demonstrated co-expression of the *CIITA–RFX*-co-stimulatory molecule module and stromal fibroblast markers. Multiplex immunofluorescence confirmed co-expression of PDGFRA, CD74, and HLA-DR proteins in fibroblasts, providing evidence for antigen-presenting fibroblasts in osteoarthritic hip synovial tissue.

We provide evidence for the presence of antigen-presenting fibroblasts in human hip OA. These findings expand current understanding of stromal cell heterogeneity in OA and suggest that fibroblasts may actively participate in local immune regulation.

Application and optimization of protein extraction methods to study tissue-bound IgA in Dermatitis herpetiformis

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Dermatitis herpetiformis (DH) is an autoimmune disease affecting mainly the skin but also small intestine. Patients suffer from a blistering, itchy rash, and most also have atrophy of the small intestine. DH is a sub-type of celiac disease, with both diseases sharing genetic HLA predisposition, and being driven by consumption of a food antigen, gluten. DH patients present with IgA deposition in the papillary dermis of the skin, which disappears after introduction of gluten-free diet. To understand the role IgA deposition plays in pathogenesis of DH, and whether it originates from the small intestine, this study aims to extract and study the properties of skin deposited IgA in DH patients using mass spectrometry.

Utilizing OCT-embedded skin punch biopsies from DH patients, the minimum amount of material was first determined. 500 µm thickness of cryosections was found to be sufficient to visualize IgA on a western blot. Noteworthily, total protein extracts from healthy controls showed no noticeable difference in IgA amount to DH patients. From these extracts, IgA pulldown using peptide M/agarose gel slurry was carried out, but the protein yield was not sufficient to be visualized on an SDS page gel even after concentrating the pulldown eluates.

The signal from papillary dermis deposited IgA may be overpowered by disease-unrelated IgA present in the dermis, as demonstrated by healthy controls having comparable amounts of detected IgA. Therefore, laser-capture microdissection of papillary dermis will be carried out as the next step of the project, followed by mass spectrometry analysis.

A farm-type natural habitat to increase the translational value of mucosal immunology experiments in the mouse

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Mice in conventional laboratory settings only reach a limited microbiome diversity and maintain a naïve immune system, limiting their translational value. To address this, we have established a housing system for C57BL/6 mice containing soil and domestic animal excrements, providing a habitat more closely resembling natural conditions. These “feralized” mice developed a diverse gut microbiota and an antigen-experienced gut immunity. While co-housing with wild or pet-store mice further matured systemic immunity, the farm-like environment was sufficient to strengthen gut barrier function and confer resistance to colorectal cancer.

In our recent study, we hypothesized that feralization would enhance the fiber-degrading capacity of the gut microbiome. We compared susceptibility to low-grade dextran sulfate sodium (DSS)-induced colitis in mice fed diets high or low in fermentable fibers, and different hygiene levels. Feralized mice were protected against colitis, exhibiting lower disease scores, reduced inflammatory biomarkers in feces, plasma, and liver, and altered colonic gene expression. Protection was most pronounced with a fiber-rich diet, which paradoxically worsened colitis in conventional mice. Transfer of fecal microbiota from feralized to conventional mice confirmed that colitis protection was microbiome-dependent. Metagenomic analysis revealed that a fiber-rich diet enriched the microbiome with genes encoding fiber-degrading enzymes, while low-fiber diet promoted mucin-degrading enzymes.

Overall, we present a more translatable mouse model achieved by enhancing environmental microbial exposure. Specifically shown here, dietary fibers modulated intestinal inflammation in a microbiota-dependent manner. Along with previous results this demonstrates the value as a more human-relevant platform to investigate complex interactions between diet, microbiota and disease.

Investigating metabolic adaptations of tissue-resident memory T cells in inflammatory joints in rheumatic diseases

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Aim

Our group and others have identified tissue-resident memory (TRM) cells as potential drivers of pathology in rheumatic diseases, such as Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA) and Myositis, where they persist in the tissue after immunosuppressive treatment and are hypothesized to drive flares. Until now, it remains largely unknown how TRM cells function in rheumatic diseases, and how their metabolic adaptation supports their survival in inflammatory tissues.

Methods

We investigated metabolism of TRM cells ex vivo using the flow cytometry method SCENITH on synovial fluid samples from patients with PsA (n=12). Additionally, we performed 10X single-cell RNA sequencing on synovial fluid samples from 4 PsA patients and re-analyzed our published dataset on synovial T cells in RA.

Results

Across synovial CD8+ T cells, we observed that TRM cells were more dependent on glycolysis, reflecting an activated T cell state, compared to non-resident T cells, which exhibited more mitochondrial metabolism. We identified pro-inflammatory Th17-like TRM cells to be highly dependent on glycolysis compared to cytotoxic TRM cells. We confirmed the enrichment of genes related to the glycolysis pathway in TRM cells on a transcriptional level in both PsA and RA.

Conclusion

Altogether, we show that TRM cells, especially Th17-like TRM, exhibit glucose-dependent metabolism in inflammatory joints, suggesting an active role in contributing to disease. The presence of glycolytic-related genes signatures in TRM cells in both RA and PsA points to sharing of this metabolic pathway across several rheumatic diseases. This knowledge could help to develop therapies targeting TRM cells that contribute to disease chronicity.

Plasma proteome reflects tissue damage and clinical manifestations in patients with inflammatory myopathies

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Aim: Tissue-specific biomarkers that are reliable and associated with clinical manifestations of idiopathic inflammatory myopathy (IIM) are lacking. The blood circulation in individuals serves as a central conduit, allowing communication between tissues and facilitating clearance and recycling of tissue-derived proteins. Thus, we applied a dual antibody-based proximity extension assay to profile >1000 proteins in in subtypes of patients with IIM.

Methods: Plasma samples from 201 patients diagnosed with IIM at Karolinska University Hospital were analyzed using Olink Proximity Extension Assay technology. Clinical manifestations, disease activity measurements, and laboratory results were collected. First, we evaluated differential protein abundance across IIM subtypes. Then we calculated tissue scores for each disease subtype using tissue gene expression databases. Finally, interferon (IFN) pathway scores were calculated.

Results: Plasma from patients with dermatomyositis was enriched for IFN signalling and skin associated proteins, anti-synthetase syndrome (ASyS) for lung associated proteins, immune-mediated necrotizing myopathy (IMNM) for muscle associated proteins, and inclusion body myositis for T cell response proteins. Patients with IMNM showed the highest tissue score for skeletal muscles, and ASyS for lung tissue score. Skeletal muscle scores correlated strongly with muscle disease activity visual analog scale scores, creatine kinase levels, and muscle weakness score. Patients with interstitial lung disease along with anti-MDA5 and those with anti-Jo1 autoantibodies showed differential enrichment of surfactant proteins and IFN pathway activation.

Conclusions: Circulatory proteome is a promising tool to capture distinct tissue-specific responses that correlate with disease activity and tissue injury in patients with IIM.

Autoantibodies towards protein epitopes may better inform clinical outcomes compared to full-length or complexed proteins in SLE patients

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Objectives

Systemic lupus erythematosus (SLE) presents a vast array of clinical manifestation, with patients having hugely diverse autoantibody repertoires. Previous studies demonstrate clustering of SLE patients based on clinical presentation and autoantibody positivity - patients with nucleic acid-binding autoantibodies are more likely to have kidney involvement, whereas patients with anti-phospholipid-antibodies have higher risk of cardiovascular events. In this pilot study, we used a new multiplex platform to explore epitopes of known autoantigens to better link disease manifestation and course prediction to autoantibody repertoire.

Methods

Serum from SLE patients (n=237) and matched controls (n=162) within a highly-characterised cross-sectional cohort were tested for autoantigen binding in a custom Luminex bead-based array. For every autoantigen (n=37), several 'variants' were included: Protein complexes (e.g. RNP-Sm), full-length & truncated proteins, (e.g. beta2GP1 domains), and linear peptides. In total, 100 'variants' were used and validated (where possible) against pre-existing clinical tests.

Results

Autoantibody binding to 'variants' but not full proteins correlated with clinical phenotype. For example, we observed autoantibody binding to RNPA correlating with lupus nephritis (OR 2.6, *p=0.0028*), but not RNP-Sm complex (OR 1.5, *p=0.159*).

Conclusions

Here we demonstrate that epitope specificity of autoantibodies is a key determinant in SLE clinical manifestation, to a greater extent than full-length protein. This is perhaps due to the variety of exposed epitopes *in vivo*, which can be difficult to replicate *in vitro*. By utilising multiplex assays, we can continue to investigate the correlation between epitope-specific autoantibodies and clinical manifestation to improve disease path prediction and better treat patients.

PD-1: A promising pathomarker for Predicting Type 1 Diabetes Progression

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Aim

Type 1 diabetes (T1D) is an autoimmune disorder characterized by T cell-mediated destruction of insulin-producing pancreatic β-cells. Our previous finding linked immune checkpoint molecules to disease progression, in particularly PD1. Based on our previous finding, this study aims to clarify the role of PD1 on T1D.

Methods

We combined a retrospective study and prospective study, in which we recruited: autoantibody-positive (AAb+) subjects, onset of T1D (T1D) subjects and healthy children (HC). Through flow cytometry and Luminex analysis, we evaluated the frequency and the relationship between soluble PD1(sPD1) and membrane PD1 (mPD1), in CD4+ T lymphocytes population, at different activation time points. In parallel, we performed transcriptional analysis of both the membrane-bound (full-PD1) and the soluble forms of PD-1 (Δ3-PD1).

Results

We observed an imbalance between sPD1 and mPD1 on T Lymphocytes cells, indeed the expression of sPD1 is significantly higher in AAb+ compared to the others. Furthermore, we found that mPD1 expression was reduced in AAb+'s CD4+ T lymphocytes, compared to the other groups; specifically in regulatory T cells (Tregs), both in freshly isolated cells and after activation. Also, the analysis of the cell culture supernatants revealed increased levels of sPD-1 in AAb+ children compared with other. In addition, after TCR stimulation, the expression of Δ3-PD1 was increased in AAb+ children than HC, whereas expression of the full-PD1 was reduced.

Conclusion

Our findings revealed that the presence of this imbalance (sPD1/mPD1) in the early stage of T1D disease, may be a critical driver of T cell dysregulation before T1D onset.

Whole blood DNA methylation differences precede type 1 diabetes progression in children at risk

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Type 1 diabetes (T1D) is an autoimmune disease preceded by a variable preclinical phase during which disease-specific islet autoantibodies emerge. A better understanding of early changes that precede disease onset, including those occurring before autoantibody appearance, may improve disease prediction and enable earlier preventive interventions. In this study, we aim to assess early methylation changes in whole blood that predate autoantibody seroconversion and clinical diagnosis.

We performed reduced representation bisulfite sequencing (RRBS) on longitudinal whole blood samples from 16 matched case–control pairs enrolled in the Type 1 Diabetes Prediction and Prevention (DIPP) study. The case samples were collected before and after islet autoantibody seroconversion and permanently islet autoantibody-negative controls were matched by date of birth, sex and HLA risk class.

We identified 292 differentially methylated CpG sites (DMCs) and 44 differentially methylated regions (DMRs) associated with progression to T1D. All DMRs showed hypomethylation in autoantibody-positive individuals compared with controls. Moreover, several DMRs mapped near immune-regulatory loci, suggesting early epigenetic remodelling of pathways relevant to immune activation and inflammatory signalling. The methylation differences observed by RRBS were validated in multiple candidate regions using targeted pyrosequencing.

Our findings demonstrate that DNA methylation differences can be detected in whole blood prior to the clinical diagnosis of T1D. These results suggest that early epigenetic changes accompany disease progression and, if validated in larger independent cohorts, may support the use of whole-blood methylation profiling as a useful approach for exploring the preclinical phase of T1D.

CD49a tissue-resident memory T cells are enriched in ACPA+ rheumatoid arthritis and exhibit enhanced cytotoxicity and activation

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Aim

Tissue-resident memory T cells (TRM) are associated with disease chronicity and poor outcomes in autoimmune diseases, such as rheumatoid arthritis (RA). We previously reported that clonally expanded CXCL13+ peripheral helper CD4+ T cells display a TRM phenotype in anti-citrullinated protein antibodies (ACPA)-positive RA, that was less prominent in autoantibody negative disease. Here, we aimed to investigate whether CD8+ T cells also display RA subtype-specific features.

Methods

We characterised tissue-infiltrating and resident CD8+ T cells in synovial fluid of ACPA+ and ACPA- RA using single-cell RNA sequencing. Additionally, we confirmed our RNA signatures on protein level using flow cytometry.

Results

We identified ten distinct CD8+ T-cell clusters, including four phenotypically distinct TRM clusters, present in both disease subgroups. *ITGA1*, encoding the TRM-associated receptor CD49a, showed higher expression in CD8+ T cells from ACPA+ RA compared to ACPA-RA, which was confirmed at the protein level using flow cytometry. CD49a expression associated with cytotoxic features and activation markers including elevated granzyme B and HLA-DR, respectively. Finally, the frequency of CD49a+ CD8+ T cells positively correlated with the frequency of peripheral helper CD4+ T cells, suggesting shared co-regulation within the synovial joint.

Conclusion

Altogether, these data suggest that cytotoxic CD8+ TRM expressing CD49a are preferentially activated in the ACPA+ RA joint and may contribute to sustained inflammation and disease chronicity.

Immune activation and cytokine production in neonates exposed to maternal anti-Ro/La autoantibodies

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Aim

Autoimmune congenital heart block develops following placental transfer of maternal anti-Ro/La autoantibodies. Recent studies demonstrated a correlation between maternal and neonatal interferon (IFN)-scores and elevated neonatal plasma IFNa levels. However, whether fetal immune cells produce IFNa or other pro-inflammatory cytokines remains unclear. We therefore characterize immune cells regarding phenotype, effector functions and cytokine production in anti-Ro/La-exposed neonates.

Methods

Full-spectrum flow cytometry of cell surface markers and intracellular cytokines was used to analyze major innate and adaptive immune cell populations at steady state in cord blood from anti-Ro/La-exposed (n=23) and non-exposed (n=14) newborns.

Results

Anti-Ro/La-exposed neonates had increased activation of multiple monocyte subpopulations and conventional DCs, marked by elevated Siglec-1 and HLA-DR as well as reduced galectin-9 expression. B cells were also found to be more activated, with decreased CD19 and increased HLA-DR expression. Notably, several antigen-presenting cell populations, including monocytes, DCs, and B cells displayed higher frequencies of IL-6- and TNFa-producing cells in anti-Ro/La-exposed neonates. Some anti-Ro/La-exposed neonates also had increased frequencies of IFNa-producing monocytes, although not significant at a group level. Anti-Ro/La-exposed neonates further exhibited increased proportions of proliferative CD62L+ NK cells and higher expression of activation markers on several T-cell subpopulations. Select anti-Ro/La-exposed neonates even harbored cytokine-producing CD8+ and NKT cells.

Conclusion

Our data demonstrate broad immune activation in anti-Ro/La-exposed neonates, involving both innate and adaptive compartments and characterized by pro-inflammatory cytokine production. This study provides valuable insight into the effects of anti-Ro/La autoantibody exposure in utero and advances the understanding of immunological mechanisms underlying neonatal lupus.

Linker 2 Emerges as an Immunodominant Target of HMGB1 Autoantibodies in Systemic Lupus Erythematosus

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Background

High mobility group box 1 (HMGB1) is a nuclear protein released to the extracellular space during cell stress, necrosis, and NETosis, where it acts as an alarmin driving inflammatory signaling. Elevated HMGB1 levels and HMGB1 autoantibodies are detected in patient sera and correlate with disease activity. The epitope specificity of these autoantibodies remains unexplored and may have functional consequences, for example - antibodies targeting different HMGB1 domains could either block receptor engagement protectively or promote inflammation through immune complex formation.

Aim

To investigate the presence and epitope specificity of IgG HMGB1 autoantibodies in sera from patients with SLE (n=210) and healthy donors (n=100).

Methods

Sera were screened by ELISA for HMGB1 autoantibodies using recombinant HMGB1 domain constructs: full-length, box A+B, box A, and box B. Top reactive samples were selected for high-resolution epitope mapping using a peptide microarray incorporating cyclic epitopes spanning the full HMGB1 sequence (38 SLE, 10 HC).

Results

ELISA demonstrated higher anti-HMGB1 reactivity in SLE versus healthy controls across all domain constructs. Epitope mapping revealed that 29/38 SLE samples (76%) reacted to the Linker 2 region — 22 exclusively to Linker 2, and 7 with additional reactivity to the C-terminal tail or box B.

Conclusion

These findings identify Linker 2 as an underappreciated immunodominant region for HMGB1 autoantibodies in SLE. Epitope heterogeneity of HMGB1 autoantibodies may indicate the potential for pathogenic and protective autoantibody responses, however functional studies are required to determine the biological consequences of autoantibody binding and their clinical relevance.

Spatial single-cell profiling reveals immune–stromal heterogeneity in orbital tissue from Graves' orbitopathy

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Aim

Graves' orbitopathy (GO) progresses from an active inflammatory phase to a chronic fibrotic phase, but the cellular composition of these disease stages remains incompletely understood. This study aimed to characterize the cellular landscape of orbital tissue using spatial single-cell profiling.

Methods

Orbital adipose tissue from 18 individuals (4 active GO, 7 chronic GO, and 7 healthy individuals) was analyzed using imaging mass cytometry (Hyperion) with a 35-marker panel. Forty-five regions of interest were segmented into single cells and analyzed in R using Harmony batch correction, Phenograph clustering, and UMAP visualization. Marker expression patterns and cluster distributions were evaluated using heatmaps and exploratory statistical analyses.

Results

Harmony-corrected imaging mass cytometry identified multiple distinct cellular populations within orbital tissue from patients with active GO, chronic GO, and controls. Unsupervised clustering revealed immune-associated, stromal/remodeling, and vascular-associated populations across all sample groups. Several clusters demonstrated marker-expression profiles consistent with inflammatory and extracellular matrix-remodeling processes. Spatial single-cell profiling highlighted cellular heterogeneity within the orbital microenvironment and suggested potential differences in the distribution of cellular populations between disease states.

Conclusions

Spatial single-cell analysis revealed a complex cellular landscape characterized by immune-associated, stromal/remodeling, and vascular populations in Graves' orbitopathy. These findings provide a framework for further characterization of cellular populations and their potential roles in inflammation and tissue remodeling in GO.

Spatial single-cell profiling of the adrenal cortex stratifies autoimmune Addison's disease into diffuse and focal immune infiltrating patterns.

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Aim

Autoimmune Addison's disease (AD) displays immune reactivity towards adrenal steroidogenic enzymes, with immune infiltration leading to destruction of the adrenal cortex. Since adrenal biopsies are unavailable for technical and ethical reasons, little is known about the pathology within the tissue. To bridge this gap, we performed single-cell spatial analysis on rare paraffin embedded adrenal autopsies.

Methods

Imaging mass cytometry (IMC), which allows spatial detection of dozens of protein markers in tissues, was used with a 31-marker antibody panel covering immune cells and adrenal biology. AD (n=5) was compared with controls without known adrenal disease (n=3). IMC images underwent spillover correction followed by preprocessing using the Penguin pipeline. Normalized images were segmented in Steinbock using Mesmer. Single-cells were graph clustered in Seurat with the "Louvain" algorithm and visualized with uniform manifold approximation and projection.

Results

The AD group was enriched for CD4+ and CD8+ T-cells, B-cells, and CD11c+ macrophages previously described as newly recruited monocytes. AD cases formed two patterns when performing principal component analysis on centered log-ratios of cluster proportions. One displayed diffuse immune infiltration associated with a distinct adrenal cortex phenotype, while the second showed dense focal immune infiltration associated with presence of B-cells.

Conclusions

Spatial single-cell profiling identifies two distinct autoimmune AD disease patterns where the presence of B-cells correlates with degree of immune infiltration. Future studies should investigate whether these patterns represent stages of disease activity or subgroups with distinct pathologies.

Spatial transcriptomics identify subgroup specific immune cell niches in juvenile idiopathic arthritis

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Aim

Juvenile Idiopathic Arthritis (JIA) is the most common inflammatory rheumatic disease in children. JIA is a highly heterogenous disease, with seven clinically defined subtypes. However, pathophysiology at the molecular level is poorly understood. The aim of this study was to identify subgroup specific immune cell niches in JIA-affected synovial tissue, using gene expression data.

Methods

Spatially resolved gene expression data from synovial tissue of 11 JIA patients and 4 controls was collected using the Visium platform from 10x Genomics. Gene expression data was analysed using Python packages Scanpy and Harmony to assign cell types. All H&E stained tissues were examined by a pathologist.

Results

Unsupervised clustering separated the transcriptomes from synovial tissues into four groups. 1) The systemic JIA patient had a distinct cell composition dominated by neutrophils dispersed throughout the tissue, as well as monocytes and macrophages, but with few lymphocytes compared to the other JIA samples. 2) The majority of oligoarticular JIA patients showed a 10-fold increase in B-cells, which were located deep in the sublining, forming tertiary lymphoid structures (TLS). 3) In contrast, the Rheumatoid Factor-positive polyarticular JIA sample showed immune cell infiltration of both lining and sublining, but lacked clear TLS. Instead, macrophages appeared to be more involved. 4) In synovial tissue from controls immune cells were located near blood vessels.

Conclusions

Spatial transcriptomics revealed immune cell infiltration of the JIA synovium, with clear cell type distinctions across different JIA subtypes.

Local immune responses following DNA plasmid injection in Atlantic salmon

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Aim

DNA plasmid-based approaches are promising tools for modulating antiviral immunity in Atlantic salmon. This study aimed to investigate local and systemic immune responses following intramuscular injection of plasmids encoding salmon interferons and viral open reading frame proteins.

Methods

Atlantic salmon pre-smolts were injected intramuscularly with plasmid formulations containing interferon- and/or viral ORF-encoding constructs. Fish were maintained under controlled freshwater conditions and sampled at selected time points after injection. Injection-site muscle, lymphoid tissues, and serum were collected. Local tissue responses were assessed by histology, immune-related gene expression was analyzed by quantitative PCR (qPCR), and serum antibody responses were evaluated by ELISA.

Results

DNA plasmid injection induced measurable local immune responses at the injection site, including cellular changes consistent with inflammatory and immune activation. Gene expression analysis showed modulation of antiviral and adaptive immune markers in both local and systemic tissues, with responses varying between plasmid formulations and sampling time points. Serum analysis indicated that plasmid injection may also contribute to systemic humoral immune reactivity.

Conclusion

Intramuscular delivery of interferon- and viral ORF-encoding plasmids induced local and systemic immune responses in Atlantic salmon. These findings support further investigation of plasmid-based immunostimulation and DNA vaccine strategies for improving antiviral protection in salmonid aquaculture.

Toward Characterizing Dendritic Cells in APECED: Developing an Induced Pluripotent Stem Cell Model and a Multiplex Tissue Imaging Approach

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Background & Aims

Mutations in *AIRE* (*Autoimmune Regulator*) cause APECED (Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal-Dystrophy, also known as APS-1), a multiorgan autoimmune disease characterized by high-titer autoantibodies, especially against type I interferons. While AIRE's role in thymic central tolerance is well established, its peripheral functions remain unclear. The "extra-thymic AIRE-expressing cells" often appear to share features with dendritic cells (DCs). Germinal centers (GCs), crucial for antibody production via DC, T cell, and B cell interactions, are deemed dysregulated in autoimmunity. The aim is to investigate whether peripheral AIRE-dependent mechanisms in DCs relate to GC activity.

Methods

To model AIRE's function in human peripheral cDC2s, APECED patient and healthy control derived induced pluripotent stem cells (iPSCs) were differentiated into AIRE-deficient and AIRE-sufficient cDC2s, respectively. The differentiation was conducted using an embryoid body -formation method, harvesting the emerging precursor cells for the sequential steps. After differentiation, the cells were stimulated with R848, LPS, Poly(I:C) and *Candida Albicans* and phenotypes were assessed by spectral flow cytometry. In parallel, we are developing multiplex, spatially resolved immunofluorescence imaging of human lymphoid tissues to map DC-related phenotypes and their microanatomical context within GC-containing regions.

Results

The early flow cytometric analysis suggests that APECED iPSC-derived DCs acquire a more inflammatory, DC3-like phenotype, compared to healthy control. Different serum types strongly affect the cell marker expression.

Conclusions

Optimization of the differentiation, staining and imaging protocols is currently ongoing, and the updated results will be presented on the poster.

2xCPET-Induced Serum Metabolomic Changes in ME/CFS: clues for impaired immunological response

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Introduction
Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a condition characterized by post-exertional malaise (PEM), suggesting an impaired bioenergetic metabolism. To date, reliable diagnostic tools and treatments remain unavailable, and no definitive etiology has been identified; however, recent evidence suggests an autoimmune mechanism.

Aims
To characterize metabolomic dynamics during and after repeated cardiopulmonary exercise testing (2xCPET) in ME/CFS patients compared to healthy controls, investigating impaired immunological response to exercise stress.

Methods
Participants (18 ME/CFS patients and 15 healthy controls, women only) completed repeated cardiopulmonary cycling exercise testing over two consecutive days. Blood serum samples were collected at rest, during peak power output, and at 15 and 60 minutes post-exercise. A Bayesian Random Intercept Model analyzed 23 metabolites (amino acid metabolites, acylcarnitines, and nucleosides) to describe comparative dynamics and estimate effect sizes for Group (ME or healthy), Day (1 and 2), and Timepoint (1 to 4).

Results
Notable differences emerged between ME/CFS and healthy controls: elevated octanoylcarnitine (a metabolite needed for neutrophil-induced repair) during peak exercise on day 1, with ADP and ATP following the same pattern, suggesting possible DAMP signaling. Additionally, lower hypoxanthine in ME patients during 15 minutes post-peak activity, particularly on day 2, may lead to reduced hypoxic stress-response and impaired immune-recovery from exercise.

Conclusion
These findings provide fine-granularity evidence of exercise-induced metabolomic alterations in ME/CFS patients, deepening understanding of impaired immunological responses and contributing valuable information for improved experimental design and development of predictive models.

B-cell characterization in a patient with STAT1 gain-of-function reveals T-bet-associated skewing and impaired IgA switching

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STAT1 gain-of-function (GOF) causes immune dysregulation and is a well-established cause of susceptibility to chronic mucocutaneous candidiasis, but its B-cell-intrinsic effects remain incompletely defined.

Here, we identified a heterozygous STAT1 GOF variant (p.R274W) in a 35-year-old male with chronic mucocutaneous candidiasis and pan-hypogammaglobulinemia. Immunophenotyping revealed elevated frequencies of T-bet-positive lymphocytes, particularly within the B-cell compartment, with marked expansion of DN2 B cells and absence of CD27+ memory B cells and plasmablasts. In human inducible germinal center B-cell cultures, however, purified naïve B cells from the patient showed accelerated differentiation into memory B cells and plasmablasts, yet reduced proliferative capacity, supporting a B-cell-intrinsic effect of chronic STAT1 activation. Notably, patient B cells showed robust antibody secretion and class switching *in vitro*, except for switching to and secretion of IgA.

These findings link STAT1 GOF to chronic T-bet-associated B-cell skewing and altered B-cell differentiation dynamics, while suggesting a selective defect in IgA class switching. This work supports a role for sustained STAT1 activity and T-bet-associated programming in reshaping human B-cell fate during immune dysregulation.

A novel regulator controls human regulatory T cell differentiation and function

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Regulatory T cells (Tregs) are key components of the immune system that maintain immune homeostasis, preventing excessive immune responses in conditions such as autoimmunity, cancer, and infection. Treg differentiation, stability, and suppressive function involve multiple cooperating factors. Long intergenic noncoding RNAs (lincRNAs) have emerged as important regulators of gene expression through epigenetic, transcriptional, and translational mechanisms. Although several studies have highlighted the significance of lincRNAs in immune cell differentiation, their role in human Treg biology remains poorly explored.

In the present study, we identified a novel iTreg (*in vitro* induced Treg) associated lincRNA, which is highly enriched in iTregs compared to other T cell subsets. Loss of this candidate lincRNA resulted in substantial alterations in Treg-associated genes, including reduced expression of the key regulatory molecule FOXP3 and CTLA4, alongside marked upregulation of the Th17 lineage-specific transcription factor RORC.

To investigate its functional targets, we performed integrated multi-omics analyses of candidate lincRNA-deficient iTregs at the transcriptomic, proteomic, and epigenomic levels. Results indicate that candidate LincRNA plays a critical role in preserving Treg identity and suppressive function while restricting Th17-associated inflammatory programs. Furthermore, our data demonstrate that LincRNA contributes to maintaining the delicate Treg/Th17 balance, which is essential for immune tolerance and immune homeostasis.

Collectively, we have discovered a previously unrecognized lincRNA-dependent regulatory mechanism in human Tregs that provides a basis for developing new strategies for modulating Treg function.

Unveiling a novel regulator of human Th17 differentiation

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T helper 17 (Th17) cells are important for maintaining immune homeostasis and defending against pathogens, but their dysregulation is closely linked to autoimmune and chronic inflammatory diseases. Although Th17 differentiation and effector function are governed by highly complex regulatory networks, many aspects of these pathways remain unexplored, emphasizing the need to identify additional regulators that elucidate how Th17 responses are controlled and maintained.

In this study, we identify NDRG1 as a novel negative regulator of human Th17 differentiation and begin to explore the mechanisms through which it operates. To investigate this, we differentiated primary human naive $CD4^+$ T cells under Th17-polarizing conditions in vitro and combined RNAi-mediated gene silencing with bulk RNA sequencing at 24 h and 72 h to capture transcriptional changes during early differentiation. Using these approaches we found that NDRG1 suppresses Th17 differentiation by downregulating key lineage-defining genes including IL17A, CCR6, STAT3 and RORA, as well as additional Th17-associated targets indicative of broad repression of the Th17 transcriptional program. We further observed a similar inhibitory effect on Th17 differentiation by SGK1, an upstream regulator of NDRG1. Importantly, we identified TIMP1 as a common downstream target of NDRG1 and SGK1, revealing an unrecognized component of the Th17 regulatory network.

Overall, our results define the SGK1–NDRG1 axis as an important inhibitory pathway controlling human Th17 differentiation, providing new mechanistic insights into how Th17-mediated immune responses are restrained and identifying potential targets for modulation of autoimmunity and inflammation.

Generation of IgM+ B cell-deficient Atlantic salmon

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Teleost fish like Atlantic salmon express only three classes of immunoglobulins: IgM, IgD, and IgT. However, the roles of these three Ig isotyes in B-cell development and antibody-mediated immune responses in still not very well understood. In teleosts, IgM is the most abundant antibody class in serum and mucosal fluids and is considered to be the most important isotype involved in systemic immune responses. IgT, on the other hand, is expressed mainly in mucosal fluids, but at much lower concentrations than IgM. IgT and IgM are considered to play key roles in the maintenance of microbial homeostasis at mucosal surfaces.

In order to better understand the role of IgM in teleost B-cell development and systemic and mucosal immune responses, we used CRISPR/Cas9 technology to knock out both IgM genes in Atlantic salmon. High-throughput sequencing revealed an average mutagenesis efficiency of 97% across both loci, with a predominance of frameshift mutations. Gene expression analysis demonstrated significantly reduced mRNA levels of membrane-bound IgM in head kidney and spleen tissue. Flow cytometry analysis revealed a 78% reduction in IgM+ B cells in peripheral blood, which corresponded with severely decreased levels of soluble IgM in serum. Interestingly, an upregulation of IgT mRNA was also observed in these fish, suggesting a potential compensatory mechanism involving this isotype.

This study has implications for advancing our understanding of the immune system of Atlantic salmon and other teleosts, and for developing strategies to design better vaccines to improve the health and welfare of fish in Norwegian aquaculture.

Satb1 integrates cohesin mediated genome organization and transcriptional regulation during T cell development

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Aim

To define the mechanisms driving dynamic 3D genome folding, cell fate transitions, and transcriptional regulation during T cell development.

Methods

We analyzed genomic co-occupancy, physical protein interactions, and chromatin contacts in double-positive (DP) and single-positive (SP) T cells using wild-type and Satb1-deleted models, alongside in vitro liquid-liquid phase separation assays.

Results

Satb1 co-occupies genomic regions with the cohesin complex and Ctcf in DP thymocytes, where chromatin interactions are highly enriched. Satb1 physically interacts with the cohesin subunit Smc1a. Deletion of Satb1 causes aberrant Smc1a binding and diminishes chromatin contacts at co-occupied sites. This disrupts cooperative gene regulation at the Cd3 locus and functionally impairs proper T cell activation and cytokine signaling in both DP and immature CD4 SP cells. Furthermore, Satb1 exhibits properties consistent with liquid-liquid phase separation in vitro, which are actively impaired by disease-associated mutations.

Conclusion

Satb1 governs spatiotemporal transcriptional regulation during T cell development by facilitating hierarchical chromatin looping. This organization is mechanistically driven by Satb1's direct physical interaction with the cohesin complex and its ability to form functional nuclear condensates.

The Overlooked Cellularity of the Human Germinal-Centre Mantle Zone: Beyond the Naive B-Cell Rim

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Background

Germinal centres are dynamic structures that generate high-affinity B-cell memory and long-lived plasma-cell responses. While extensive research has mapped the Germinal centre into light and dark zones, the surrounding mantle zone is often dismissed as a passive barrier. However, its anatomical position at the interface between the germinal centre and the surrounding follicular environment suggests that the mantle zone may represent a more complex immune compartment.

Methods

We analysed FFPE palatine tonsil tissue from children and adults undergoing tonsillectomy as part of a live attenuated influenza vaccine immune-profiling study. Samples were collected between 2-22 days post-

vaccination, comprising 14 children, 6 adults, and 8 unvaccinated controls. Spatial proteomic profiling was performed using Hyperion imaging mass cytometry with a germinal-centre-focus to resolve follicular architecture, B-cell differentiation, T-cell subsets, myeloid populations, regulatory phenotypes, and activation/exhaustion-associated states. In parallel with

a CosMx whole-transcriptome panel providing spatial single-cell RNA-based annotation of mantle-zone and follicular cell states. Germinal-centres and mantle-zones were segmented using follicular and B-cell markers, with single-cell phenotypes mapped relative to the germinal-centre border and mantle-zone architecture.

Results

Spatial protein and transcriptomic analysis showed that the human mantle zone is not a uniform naive B-cell boundary, but a heterogeneous region with variable architecture and immune-cell composition. In the context of the LAIV, the mantle zone may participate in the organization of local follicular immune responses after mucosal antigenic challenge.

Conclusion

Our data support a broadened view of the human germinal-centre mantle zone as an immunologically engaged compartment rather than a passive naive B-cell rim.

Modulating the immune response in vivo via functionalized nanodiamond-coated polymer scaffolds

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Macrophages (Mø) and regulatory T cells (Treg) modulate immune responses that are crucial during tissue repair to prevent excessive inflammation while promoting regeneration. In bone tissue engineering polycaprolactone (PCL) scaffolds are widely used, while nanodiamond particles (nDP) exhibit functionalization potential to drive pro-regenerative immune responses. We aimed to modulate the immune response by coating PCL scaffolds with different functionalized nDP and compared in vivo immune responses.

PCL scaffolds were coated with pristine nDP (nDP), biotin-functionalized nDP (nDPBio), or nDP functionalized with positively- (nDP+) or negatively- (nDP–) charged dipeptides, with uncoated scaffolds as control. Scaffolds were implanted subcutaneously into rats and harvested at weeks 1 and 4, for immune cell analysis using spectral flow cytometry. Protein and gene expressions were analyzed using Bio-Plex immunoassay and bulk RNA-Seq, respectively.

Myeloid, T and B cells infiltrated scaffolds at weeks 1 and 4, with reduced frequencies in nDP_PCL at week 1. The CD163–CD86+/CD163+CD86– ratio increased at week 1 in PCL and nDP–_PCL, shifting toward CD163+CD86– at week 4. CD25highFoxP3+ Treg frequencies increased at week 1 and were maintained at week 4 only in nDP–_PCL. Inflammatory cytokine levels were elevated at week 1, with distinct profiles between groups. Gene expression patterns also differed between groups and timepoints.

Functionalized nDP-coated PCL scaffolds influenced immune responses through modulation of Mø and Treg populations. Resolution of pro-inflammatory responses by week 4 indicates appropriate scaffold integration and absence of adverse reactions. Nanodiamond coating holds promise for promoting a pro-regenerative microenvironment through targeted immune modulation.

Curbing Autoimmunity: Inhibition of the Complement Amplification Loop & Autoreactive B-Cell Development Utilizing Nanobodies & Antibody Fragments

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Dysregulation of autoantibody-producing B cells and excessive inflammation resulting from complement activation are pivotal factors driving the pathogenesis in Systemic Lupus Erythematosus (SLE).

Current treatment modalities focus on targeting either broad inflammation or implementing pathway-specific therapies. Our aim is to introduce a dual-targeting strategy, targeting two of the core mechanisms of the disease simultaneously; the innate (complement system) and the adaptive (B cells) immune system. By using smaller, safer, and more precise therapeutic molecules, we aim to develop more efficient and better tolerated treatments than current therapies.

We have developed an anti-CD40L Fab fragment for the inhibition of B-cell activation as well as a nanobody targeting Factor B (FB), an essential part of the alternative pathway responsible for around 80 % of complement activation. The anti-CD40L Fab fragment, Fab20, binds strongly to the CD40L trimer (KD = 70 nM) in a 3:1 manner. Fab20 sterically blocks the CD40 binding site and efficiently inhibits B-cell proliferation, activation, and differentiation *in vitro*. The anti-FB nanobody strongly binds FB (KD = 15.1 nM) and potently inhibits the alternative pathway of the complement system without affecting the classical pathway *in vitro*. Additionally, we also show that by modifying the anti-FB nanobody by addition of an albumin-binding nanobody, the half-life *in vivo* is drastically prolonged.

Both therapies represent promising novel therapeutic approaches. Interestingly, our future aim is to demonstrate how these therapies operate in a dual-targeting strategy. This approach could represent a paradigm shift in the treatment of autoimmune diseases.

VISTA, a new-generation immune checkpoint: expression in heart failure and cardiovascular safety of inhibition

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Introduction

VISTA is a next-generation cancer immune checkpoint undergoing clinical testing, with limited cardiovascular safety data. Beyond T cells, VISTA modulates innate immune responses and shows maximum inhibitory activity in acidic environments, a critical immunological consideration for cancer patients receiving checkpoint blockade.

Aim

To characterize myocardial VISTA expression in human heart failure and evaluate the cardiovascular safety of VISTA checkpoint blockade in healthy and acutely injured hearts.

Methods

Myocardial tissue was obtained at transplantation from patients with ischemic and non-ischemic heart failure (screening: n = 7/group; validation: n = 80) and non-failing donors (n = 6). VISTA and co-inhibitory checkpoint expression profiles were quantified by Western blot. In vivo safety was assessed in aged C57BL/6 mice and an isoprenaline-induced injury model, with anti-VISTA blocking antibody or isotype control administered before, during, and after injury. Cardiac function was assessed by echocardiography, strain, and ECG; injury by histology.

Results

PD-L1 was increased in failing myocardium and correlated with LVEF ($r = -0.403$, $p = 0.002$). VISTA was uniformly reduced across both heart failure etiologies with no association with LVEF ($r = 0.016$, $p = 0.904$) or hemodynamics. VISTA checkpoint blockade did not alter cardiac function in healthy mice (LVEF: 61.52 ± 10.04% vs. 61.14

± 8.28%, $p = 0.994$) or exacerbate ischemic injury.

Conclusion

VISTA checkpoint blockade induced no adverse cardiac effects, addressing a critical gap in cardio-oncology immunotherapy and supporting further clinical evaluation as a next-generation checkpoint target.

Bead-based affinity measurements for the multiplexed characterization of antibody-antigen interactions

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High-throughput discovery and engineering of therapeutic antibodies require precise affinity quantification across large variant libraries, yet existing platforms force a trade-off between throughput and accuracy. Here, we introduce a set of multiplexed assays that enable quantitative, high-throughput affinity measurements that require only common flow cytometers and widely available reagents, minimizing cost and complexity.

By arraying either antibodies or antigens on an established microsphere-assisted proteomics (MAP) platform, we are able to perform parallel equilibrium-binding experiments across entire libraries on a single plate. Controlled immobilization on microspheres yields homogeneous antibody display and eliminates surface-expression and avidity artifacts that limit the accuracy and controllability of yeast surface display-based methods such as Tite-Seq. By reducing the number of titrations and combining direct-binding with competitive-binding assays we extended the scope from one-dimensional library screenings to a two-dimensional library-on-library readout. We demonstrate our system's capability by rapidly inferring binding affinities for multiple viral proteins across a library of ~70 affinity-matured antibodies (> 400 affinity measurements) while maintaining strong correlation with SPR and BLI reference data ($r > 0.9$). Our assays can be run in a single day, demonstrating order-of-magnitude higher throughput than conventional biosensor-based measurements.

Restoring Antiviral Defense in Heart Failure: Pharmacological Regulation of IFITM3 in Human Cardiomyocytes

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Introduction

Viral infections are major risk factors for heart failure exacerbations and contribute substantially to mortality in affected patients. A key host defense mechanism against viral infections is the interferon (IFN)-mediated antiviral pathway, regulated by interferon regulatory factors (IRFs) and viral restriction factors such as IFITM3, BST2, SAMHD1. IFITM3 plays an important role in inhibiting viral replication, and its deficiency has been associated with increased susceptibility to severe viral infections.

Aim and Methods

We investigated the expression of antiviral factors in cardiac tissues from patients with heart failure with reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF), and assessed whether antiviral pathways can be modulated pharmacologically. mRNA expression levels were analyzed by RT-qPCR in HFrEF samples with ischemic (ICM) and dilated cardiomyopathy (DCM) etiology, as well as HFpEF samples, compared with healthy controls. Protein expression was evaluated by Western blot. Human cardiomyocytes (AC16 cells) were treated with drugs commonly used in heart failure and comorbidity management, followed by RT-qPCR and Western blot analyses.

Results and Conclusion

IFITM3 expression was significantly reduced in both HFrEF and HFpEF samples compared with controls, which was confirmed at the protein level. SAMHD1 and BST2 expression were also decreased in HFrEF. Most investigated drugs had minimal effects on antiviral factor expression; however, carvedilol significantly increased IFITM3 expression in cardiomyocytes. These findings suggest that impaired antiviral defense may contribute to increased viral susceptibility in heart failure, while carvedilol-mediated IFITM3 upregulation may represent a potential therapeutic strategy to improve antiviral resilience.

Optimized MELOE-1 TCR expression in primary T-cells leads to enhanced clearance of metastatic melanoma

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Among cellular immunotherapies in the treatment of metastatic melanoma, T-cell Receptor (TCR) therapy is one of the most promising. However, two longstanding challenges remain; the proper expression of the TCR by the T cells and selection of a tumor-specific antigen, both crucial for the success of such a therapy. In this study, we focused on a TCR specific of MELOE-1 -an immunogenic non-conventional melanoma antigen- derived from a peripheral blood CD8+ T cell clone.

Primary T cells were retrovirally transduced with natural or murinized versions of this TCR. Results showed that murinization of both α and β-constant domains improved TCR expression (fold change>4). Functional assays demonstrated that increased TCR expression was consistent with an increased response against endogenous MELOE- 1 antigen in melanoma cell lines showing superior cytotoxicity capacity. More interestingly, although this TCR is CD8-dependent, the combined CD4 and CD8 TCR transduced T cells were as effective as the CD8 alone, reinforcing the potential of this therapeutic strategy. This contribution of CD4 T-cells in the co-culture appears to rely on both cellular and molecular mechanisms. Finally, TCR efficacy was assessed in vivo in NXG mice injected with 2 melanoma cell lines, and engineered combined CD8 and CD4 TCR-T cells were able to control tumor growth. Additionally, no toxicity against melanocytes was detected, suggesting that our optimized TCR remains melanoma-specific and safe.

In summary, these data support the further development of an encouraging TCR-based T-cell therapy for metastatic melanoma treatment and can be combined with other therapeutic approaches.

Comparing spatial transcriptomic technologies for resolving immune infiltration in arthritic human hip joint tissue

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Osteoarthritis (OA) and rheumatoid arthritis (RA) are chronic joint disorders characterised by cartilage degradation, joint dysfunction, pain and inflammation, which can lead to physical incapacitation. Disentangling how immune cells locally play part in the pathogenesis of the joint microenvironment may open doors for new and improved therapies. Thus, we applied two new high-resolution spatial transcriptomic technologies to determine which one is more suitable for studying immune infiltration in synovial membrane of human hip joint from OA and RA patients undergoing total hip arthroplasty.

We compared Visium HD by 10X Genomics and Curio Seeker by Curio Bioscience on tissues from the same patients, as FFPE and OCT blocks, respectively. Favourably, Visium HD allowed histological analysis of the exact same tissue section, confirming cell clustering and inflammation morphology. The transcript capture area was also four times larger (6.5x6.5 *vs* 3x3 mm). Visium HD also readily captured myeloid clusters, while Curio did not, and some Curio clusters were enriched in ribosomal- and pseudogenes. However, Visium HD for FFPE does not capture certain highly variable genes, such as HLA genes, while Curio Seeker does. For both technologies, immune cell populations were reflected in the top biological processes revealed by gene set enrichment analysis, such as lymphocyte, B cell and immunoglobulin mediated immunity. In conclusion, both technologies successfully captured T and B/plasma cells, in both OA and RA samples, but only Visium HD identified myeloid clusters. Thus, Visium HD was technically more robust and informative for studying synovial immune cell infiltration.

Leveraging chemotactic properties of targeting units to enhance APC targeted DNA cancer vaccines

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DNA cancer vaccines are attractive due to their flexibility, rapid production, stability and ease of storage. Their immunogenicity can be enhanced by directing their encoded protein to antigen-presenting cells (APCs). Because effective cancer vaccine should induce robust cytotoxic CD8+ T and CD4+ Th1 cell responses, precise targeting of the right APC subset with superior cross-presentation and T cell priming is highly desirable.

To investigate optimal APC targets, we designed plasmid DNA constructs encoding tumor-specific neoantigens derived from a preclinical multiple myeloma (MM) model fused to APC-targeting units. A panel of vaccines targeting 13 distinct surface molecules expressed on murine APCs was generated and evaluated for its ability to confer protection against MM tumor challenge.

Of the 13 targeting units tested, only four conferred tumor protection: the chemokines MIP-1α (CCL3), RANTES (CCL5), mXCL1, and β-defensin 2. These physiological targeting units promoted chemotaxis and activation of conventional dendritic cells type 1 (cDC1), and induced durable cytotoxic CD8+ T cell responses. In contrast, non-protective constructs including those targeting CD11c, MIP3α and hXCL1, failed to elicit these effects. Interestingly, the requirements for antiviral immunity appeared less stringent, as all 13 targeting units, when fused to hemagglutinin (HA), conferred protection against influenza virus challenge. This findings suggests that the ability to recruit and activate cDC1s is a key determinant of anti-tumor efficacy, but less critical for antiviral immunity.

Thus, efforts in DNA cancer vaccine should focus on APC targeting strategies that promote cDC1 chemotaxis and activation, and in turn enable generation of long-lasting cytotoxic CD8+ T cell responses.

Bacteria-targeting vaccine in prevention of cardiovascular diseases

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Cardiovascular diseases (CVDs) are the leading cause of death in the world, causing ~18 million cases annually. Although high levels of LDL-cholesterol and unhealthy lifestyle are known risk factors for CVDs, also human commensal bacteria have been demonstrated to induce atherogenesis and platelet aggregation in mouse models. In addition, we have detected oral bacteria in atherosclerotic plaques and thrombus aspirates of patients with myocardial infarction or acute ischemic stroke. Here, our aim is to develop bacteria-targeting vaccines that could prevent bacteria-associated cardiovascular complications.

Target species were selected based on metagenomic and immunohistochemical findings from human coronary artery plaques and thrombus samples. Total of five bacterial antigens were produced in *E. coli* and their immunogenicity was studied using BALB/c mice. Antigen-specific IgG and cytokine levels were analyzed with ELISA and FluoroSpot, respectively. Protein purity (~100%), endotoxin (<1.5 EU/µg) and DNA levels (<10 ng/dose) were acceptable for preclinical studies. After the immunization protocol, we did not detect antigen-specific T cell responses but obtained high IgG titers with three antigens (endpoint titer >100,000) and detectable levels of antibodies with the other two antigens (endpoint titer >1000). Next, we aim to improve the immunogenicity of the antigens using VLP display and establish an infection model in mice to study whether the formation of atherosclerotic plaques or thrombi can be prevented by vaccination.

Our study can lead to novel bacteria-targeting vaccine against cardiovascular diseases and has potential to have significant socioeconomical impact around the world.

Anti-tumour properties of CD20-targeting BiTEs in an in vitro model of canine lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common hematopoietic tumour in dogs. Dogs with DLBCL are usually treated with multi-agent chemotherapy protocols including cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). We aim to develop novel bispecific T-cell engagers (BiTEs) targeting the canine transmembrane B-cell receptor CD20. Our BiTEs consist of two tandem single-chain variable fragments (scFv), one binding to canine CD20 and the other binding to CD3 on T cells. We will produce BiTEs using a mammalian expression system and purify them using affinity tag chromatography. The BiTEs' ability to bind to CD20 and CD3 will be assessed by flow cytometry on peripheral blood mononuclear cells (PBMCs) and a canine B-cell lymphoma cell line (CLBL-1). We will assess the BiTEs' ability to induce anti-tumour cell-mediated cytotoxicity using a luminescence- and flow cytometry-based assay. CLBL-1 cells will be cocultured with PBMCs from healthy dog donors in the presence of CD20-targeting BiTEs, or relevant isotype controls. We will also measure T-cell activation by measuring IFN-γ and IL-2 in the supernatant. We expect to find that CD20-targeting BiTEs can induce a strong cell-mediated anti-tumour cytotoxic response in canine lymphoma cells. We also expect to find high levels of T-cell activation markers.

We anticipate to present results of the anti-tumour cytotoxicity effect of the BiTE protein in an *in vitro* model of canine lymphoma (CLBL-1 cell line).

CRISPR approaches to understanding and treating autoimmune disease

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Dysregulation of the immune system can have a devastating impact on human health, both through overactivation leading to autoimmune diseases and through insufficient activity resulting in immunodeficiencies. CRISPR-based gene-editing technologies offer powerful tools for both research and therapeutic applications. In particular, base and prime editing enable the correction of disease-causing mutations without introducing double-strand DNA breaks. In addition, high-throughput CRISPR screening approaches allow the selective targeting of hundreds to thousands of genes within a singular experiment, enabling systematic investigation of the effects of gene knockout, knockdown, or activation in relevant cellular contexts. In this poster, I will provide an overview of these technologies and present a practical case study illustrating our planned future application of massively parallel interrogation of gene regulatory networks in immune cells in the context of an immune disorder.

γδ T Cell dynamics during Immune Checkpoint Blockade in melanoma: emerging biomarkers of therapeutic response

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Aim

Immune checkpoint blockade (ICB) has transformed the treatment of melanoma, yet biomarkers capable of predicting therapeutic response remain limited. This study aimed to investigate the role of γδ T cells during ICB therapy and evaluate their potential as biomarkers of treatment efficacy.

Methods

Immune checkpoint receptor (ICR) expression and regulation were analyzed across human γδ T-cell subsets. Functional characterization, flow cytometric analyses, and single-cell RNA sequencing were performed on samples from melanoma patients receiving anti–PD-1 therapy.

Results

Distinct patterns of constitutive and inducible ICR expression were identified among γδ T-cell subsets, including constitutive expression of TIGIT and PD-1 and inducible expression of TIM-3, LAG-3, and CTLA-4. Single-cell RNA sequencing revealed that circulating Vδ1 cells exhibited elevated expression of inhibitory KIR genes, TOX, and multiple ICR transcripts, suggesting a phenotype reminiscent of T-cell exhaustion. Despite this transcriptional profile, patient-derived γδ T cells remained functionally competent. Furthermore, successful ICB therapy was associated with reduced KIR gene expression, indicating dynamic remodeling of the γδ T-cell compartment during treatment. Importantly, Vδ1 cells from patients responding to anti–PD-1 therapy bound substantially higher levels of therapeutic antibody than those from non-responders.

Conclusion

These findings identify γδ T cells as a previously underappreciated component of the anti-tumor immune response and suggest that therapeutic anti–PD-1 antibody binding may serve as a novel biomarker of treatment efficacy. Ongoing studies aim to validate these findings in larger patient cohorts and further characterize the phenotype, function, and transcriptional dynamics of γδ T cells during ICB therapy.

Ultra-rapid elimination of B cells directly after intravenous infusion of anti-CD20 therapy in multiple sclerosis

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Background

Ocrelizumab VErsus Rituximab Off-Label at the Onset of Relapsing Multiple Sclerosis Disease (OVERLORD-MS) was a double-blinded non-inferiority trial comparing the efficacy and safety of off-label rituximab to ocrelizumab in treatment-naïve relapsing-remitting multiple sclerosis (RRMS) patients. Anti-CD20 therapies are widespread in the treatment of multiple sclerosis, but pharmacodynamic effects have not been well established.

Objective

To gain a deeper understanding of how rapidly the anti-CD20 drugs deplete B cells and how they affect overall lymphocyte composition.

Methods

Whole blood samples were taken prior to first intravenous infusion of anti-CD20 therapy and immediately after the medication was fully administered. The samples were fixed using Proteomic Stabilizer (SMART Tube Inc) and promptly stored at -80°C.

Forty treatment-naïve RRMS patients were selected for further immune cell analysis by flow cytometry. A standardized six-marker panel was used to determine the lymphocyte composition and bead-based absolute cell counts (BD Biosciences, cat: 337166).

Results

Post-infusion samples demonstrated the immediate effect of anti-CD20 therapies in both treatment groups. The data showed a significant reduction of CD19+ B cells in both groups after the 4-hour infusion (Wilcoxon signed-rank test, $p < 0.0001$).

At baseline, CD19+ B cells accounted for 9.68% (IQR 7.51-12.70%) of lymphocytes. After infusion, the B cell population was reduced to 0.25% (IQR 0.14-0.69%). No significant difference was observed between rituximab and ocrelizumab treated groups (Mann–Whitney U test, $p = 0.23$).

Conclusion

In all MS patients, both rituximab and ocrelizumab led to a significant elimination of B cells 4 hours after first infusion. Most patients displayed a relatively similar immune response after first infusion irrespective of treatment group. These findings suggest that lymphocyte percentages could be a reliable measurement in cryopreserved whole blood, whereas absolute cell counts should be interpreted with caution.

Exposure-dependent PfEMP1 antibody breadth and kinetics revealed in asymptomatic and acute Plasmodium falciparum infections in a malaria non-transmission setting

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Background

Antibodies to the malaria parasite *Plasmodium falciparum* proteins erythrocyte membrane protein 1 (PfEMP1) contribute to clinical immunity, yet the breadth and kinetics of PfEMP1-specific responses in symptomatic and asymptomatic infection remain incompletely defined. Non-transmission settings such as Sweden allow assessment without frequent reinfection and ongoing antigenic boosting.

Methods

Plasma IgG to 27 recombinant *P. falciparum*-derived PfEMP1 constructs was quantified by multiplex bead-based Luminex. Participants in Stockholm included symptomatic travelers with acute *P. falciparum* malaria sampled at diagnosis and 10 days, 1, 3, 6 and 12 months post-treatment, stratified as primary infection or previously exposed, and individuals with asymptomatic infection detected via migrant screening with baseline and follow-up samples.

Results

Asymptomatic infections showed higher PfEMP1-specific IgG levels and breadth than symptomatic malaria at baseline and follow-up. PfEMP1 IgG levels were increased for antigenic sub-groups, including both CIDR and DBL domains. In symptomatic cases, exposure history shaped kinetics, with previously exposed individuals having higher titers at diagnosis and an earlier post-treatment antibody peak at day 10, consistent with recall, whereas primary infections showed lower acute titers with delayed boosting, peaking around 1 month after diagnosis. In asymptomatic individuals, PfEMP1-specific titers and breadth declined with time spent in Sweden.

Conclusion

Broad PfEMP1-directed IgG is most pronounced in asymptomatic infection, while prior exposure accelerates and amplifies responses during symptomatic malaria. The time-dependent decline in a malaria-free environment suggests PfEMP1 immunity requires repeated exposure for maintenance and supports PfEMP1 domains as informative markers and potential vaccine targets.

Next generation proteomics links morphotype-specific protein secretion to virulence in macrophage-like cells during Mycobacterium abscessus infection

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Mycobacterium abscessus (Mabc) is an opportunistic pathogen predominantly affecting patients with underlying lung conditions and is difficult to treat due to intrinsic drug resistance. Its pathogenicity is characterized by the transition from a smooth to rough morphotype, driven by the loss of surface glycopeptidolipids (GPL), which is associated with increased virulence and poorer clinical outcomes. However, the molecular mechanisms underlying the increased virulence of rough morphotypes remain poorly understood.

Here, we investigated two clinical smooth and rough morphotypes isolated from the same patient to identify secreted bacterial proteins potentially contributing to differential host responses in macrophage-like cells. Whole genome sequencing confirmed >99% sequence similarity, with a truncating mutation within the GPL locus of the rough isolate. To identify proteins associated with the hypervirulent rough morphotype, we performed LC-MS/MS-based secretome profiling of bacterial culture supernatants and time-resolved proteomics of infected macrophage-like cells at 24, 48, and 72 hours post infection (hpi). In addition, size-exclusion chromatography coupled to mass spectrometry (SEC-MS), enabling detection of proteins co-migrating with larger molecular complexes, was performed at 72 hpi to explore potential host-pathogen interactions (HPI).

More than 600 bacterial proteins were detected in culture supernatants, with a subset unique to the rough morphotype. Integration of secretome, temporal infection proteomics, and SEC-MS datasets prioritized secreted bacterial proteins enriched during infection and associated with host protein complexes as potential candidates for further study.

Together, these results highlight how morphotype-dependent protein secretion contributes to differential macrophage responses and provides a systems-level framework to study virulence in Mabc.

Influenza H1N1 Strains Differ with Respect to their Potential for Dominating Immune Formation during Secondary Viral Exposures

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Antigenic drift may render antibody responses generated against earlier variants of influenza viruses inefficient against new strains. Unfortunately, a new viral exposure may preferentially raise antibodies with pre-existing specificities at the expense of new and efficient antibodies. While this phenomenon of immunological imprinting has been described at the serological level, little is known of the underlying cellular mechanisms. The diminution in reactivity against emergent strains has implications for vaccine efficacy, and understanding the dynamics of an imprinted immune repertoire upon novel encounters is essential for predicting vaccine outcomes amid diverse immunological histories.

In this study, we demonstrated that a primary infection with selected influenza H1N1 strains will result in an unsymmetrical imprinting of mice and subsequent secondary responses to heterologous strains. We observed variances in the generation of new hemagglutinin (HA) specific serum IgG responses and virus neutralization capacity. Importantly, this unsymmetrical serological imprinting was mostly absent in either memory B cells or B cells in draining lymph nodes.

Delving into the mechanics of B cell selection, we performed single-cell B-cell receptor (BCR) sequencing of antigen-specific germinal center (GC) B cells post-secondary challenge, and juxtaposed these findings with bulk BCR sequencing of the bone marrow plasma cell (PC) repertoire, bridging the understanding between GC dynamics and antibody-producing cells. Our results underscore the necessity of analyzing antigen-specific BCRs to comprehensively evaluate the immune response within an imprinting-compromised system amidst fluctuating viral landscapes.

APC-targeted, bivalent hemagglutinin and neuraminidase antigens combined in one DNA vaccine results in broader protection against influenza viruses

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The immunogenicity of DNA vaccines can be improved by designing plasmids that encode secreted vaccine fusion proteins that (i) target antigen-presenting cells and (ii) display bivalent antigen. Antigen bivalency enhances B cell receptor cross-linking in antigen-presenting cell-B cell synapses, thus improving B cell activation and subsequent antibody responses.

We extended this principle to a combinatorial and simultaneous delivery of two influenza virus antigens, hemagglutinin and neuraminidase. Conventional inactivated influenza virus vaccines focus on the induction of hemagglutinin-specific immunity, but eliciting effective responses towards both viral surface proteins hemagglutinin and neuraminidase could provide an additional and independent layer of protection. Using distinct dimerization motifs prevented mixing of the two antigens in the endoplasmic reticulum of transfected cells, maintaining antigen-presenting cell targeting and bivalency of each. Such a combinatorial DNA vaccine induced B and T cell responses against hemagglutinin and neuraminidase in mice at levels comparable to single-antigen vaccines, and increased protection against homologous influenza virus. In addition, vaccinated mice showed enhanced resistance to challenge with heterologous H1N1 and H5N1 viruses compared with both single-antigen and, importantly, conventional inactivated virus vaccines.

This combinatorial DNA vaccine technology can be expanded to more influenza antigens as well as other pathogens. These results highlight the importance of crosslinking of B cell receptors for efficient antibody responses and therefore aid in the development of more potent and broadly reactive DNA vaccines.

Impact of Aging on CD8+ T-cell Immunity to Circulating and Pandemic Viruses

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Introduction

Age is a major factor in determining disease outcome during seasonal epidemic and pandemic outbreaks, including influenza and SARS-CoV-2. CD8 T-cells recognize conserved viral proteins, resulting in broad-reactivity. They protect against severe disease, particularly in the absence of neutralizing antibodies, making them an attractive target for vaccine strategies. However, mechanisms underlying age-related changes in virus-specific CD8 T-cell immunity and their impact on T-cell-based vaccine effectiveness remain ill defined. We investigate how virus-specific T-cell immunity develops across the human lifespan.

Methods

Ex vivo CD8 T-cell responses toward the prominent human influenza epitope, HLA-A*02:01-M158-66, were analysed across four age groups at phenotypic, transcriptomic, clonal and functional levels. Heterotetramer combinatorial coding allowed in-depth* ex vivo *profiling of 35 SARS-CoV-2-specific CD8 T-cell populations.

Results

Highly-functional public (shared) clonotypes dominated the young and adult influenza-specific CD8 T-cell repertoires, whereas less-functional private (not shared) clonotypes dominated older CD8 T-cell populations. Both public and private influenza-specific CD8 T-cells were long-lived, but gradually decline with age, eventually resulting in the loss of highly-functional public clonotypes. The SARS-CoV-2 pandemic presented a unique opportunity to examine the impact of immunosenescence and CMV related decline in naïve CD8 T-cells on the generation of *de novo*SARS-CoV-2-specific CD8 T-cell responses. We demonstrated that age and CMV status did not impact the formation of robust SARS-CoV-2-specific CD8 T-cell responses.

Conclusion

Our findings suggest that T-cell-based influenza vaccines are likely more effective in younger individuals, whereas T-cell-based vaccines against newly emerging viruses may induce robust T-cell immunity in both young and older individuals.

Influenza vaccination induces robust antibody responses in pregnant women with efficient transfer to the fetus

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Aim

Pregnant women and young infants are at increased risk of severe influenza. Although influenza is a vaccine-preventable disease, vaccination during pregnancy is rarely implemented in low- and middle-income countries (LMICs), partly due to limited region- specific immunogenicity data. This study aimed to assess immune responses to the inactivated quadrivalent influenza vaccine (QIV) in pregnant women in Bangladesh and evaluate transplacental antibody transfer to newborns.

Methods

We conducted a cluster-randomized controlled trial in 20 rural villages in Bangladesh. Pregnant women in their third trimester were randomized by village to receive either QIV (n=37) or inactivated polio vaccine (IPV; n=38) as a control. Blood samples were collected at baseline (day 0), day 28, and 6, 12, and 18 months post-vaccination, as well as maternal and cord blood at delivery. Antibody responses were measured using the hemagglutination inhibition (HI), hemagglutinin (HA)-specific IgG ELISA, and microneutralization (MN) assays.

Results

QIV induced significant increases in HI, HA-specific IgG, and MN titers against all four vaccine strains by day 28, with most participants achieving protective HI titers (≥40). Antibody levels declined over time but generally remained above the protective threshold for at least six months. At delivery, maternal antibody titers remained elevated and were comparable to cord blood titers, indicating efficient transplacental transfer. No significant antibody changes were observed in the IPV group.

Conclusion

Maternal QIV vaccination elicited robust and durable immune responses and effective antibody transfer to infants, supporting influenza vaccination during pregnancy in LMIC settings.

Cross-protective immune responses to seasonal and zoonotic influenza across the ages

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Aim

The emergence of novel zoonotic influenza viruses poses a threat for future pandemics. Immunity against zoonotic influenza in the Norwegian populations is unknown. There is no data on cross-reactive immune responses against influenza viruses and their role in protection against emerging seasonal and zoonotic influenza viruses across the ages in Norway. We will investigate immune responses to vaccination and infection in children (<5 years old), adult health care workers (HCW) (<65 years old), and the elderly (≥65 years old), contributing to a better understanding of the breadth of cross-reactive immunity and the age-related differences.

Methods

Biobanked serum samples were collected pre- and post-vaccination or during acute and convalescent phases across multiple influenza seasons. We will assess cross-reactive antibodies against historical, current and future yet-to-circulate seasonal strains, as well as pandemic and zoonotic viruses using the hemagglutination inhibition (HI) assay.

Results

Preliminary HI results show that both the HCW and elderly vaccinees developed cross-reactive antibodies against multiple previously circulating seasonal viruses and a future strain. We further divided the HCWs and elderly into two groups based on whether they developed cross-reactive antibodies against seasonal viruses. These groups will be used to investigate potential cross-reactivity against zoonotic viruses.

Conclusions

This project will aid future vaccine development and strategies to induce broadly cross-protective immunity in all age groups, which is essential in the unpredictable event of vaccine mismatch such as the subclade K variant or a future pandemic.

mRNA-LNP and adenoviral vaccines benefit from encoding antigens targeted to antigen-presenting cells

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We wanted to explore whether antigen-presenting cell (APC)-targeting of antigens could further enhance immunogenicity of mRNA-lipid nanoparticle (LNP) and adenoviral delivery of vaccines. Especially, mRNA-LNP vaccines proven highly effective during the COVID-19 pandemic. Experiments were performed in mouse models for malaria (*Plasmodium falciparum* using reticulocytes-binding protein homolog 5, PfRH5, antigen) and influenza (hemagglutinin, HA, antigen). We designed genetic constructs encoding bivalent fusion proteins that target antigens to MHC class II (MHCII) molecules on professional APCs. We demonstrated that MHCII-targeted fusion proteins bound to professional APCs. Such APC-targeting increased antibody and T cell responses against both PfRH5 and HA. Importantly, a single immunization with mRNA-LNP vaccine showed complete protection against influenza virus infection. The improved responses elicited by the MHCII-targeted vaccines suggest that the injected mRNA-LNP and adenoviral vectors resulted in secretion of fusion proteins that are capable of targeting APCs. Thus, employing the APC-targeting principle could improve the efficiency of adenoviral and mRNA-LNP vaccines against a variety of diseases.

Immunocompromised individuals exhibit reduced immune imprinting after SARS-CoV-2 Omicron exposure compared with healthy individuals.

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Aim

Immune imprinting, whereby prior antigenic exposures influence immune responses to antigenically related strains, constrains variant-specific immune responses in healthy individuals primed with SARS-CoV-2 mRNA vaccines encoding the wild-type (wt) spike. Since its impact in immunocompromised individuals is unclear, this study investigates their ability to generate *de novo* Omicron-specific antibody and memory B cell (MBC) responses compared with healthy individuals.

Methods

Blood samples were collected two years post-priming from 69 immunocompromised and 33 healthy individuals enrolled in the PatoVac_COV and IMMUNOCOV clinical studies (Siena University Hospital), all primed with wt mRNA vaccines and subsequently exposed to Omicron antigens. Frequencies and phenotypes of MBC specific only for the Omicron BA.2 RBD and pre-existing MBC recognising both wt and BA.2 RBDs (double reactive) were characterised by flow cytometry. BA.2 RBD-specific IgG were assessed by ELISA, live virus neutralization and avidity assays, upon magnetic depletion of wild-type RBD-binding antibodies.

Results

Immunocompromised individuals displayed higher ratios of new BA.2 RBD-specific to pre-existing double reactive MBC compared to healthy individuals, indicating reduced immune imprinting. BA.2 RBD MBC phenotypes differed between groups, with healthy individuals displaying a greater contribution of extrafollicular and/or less mature phenotypes. BA.2-specific IgG levels were higher in healthy individuals before depletion of wt RBD-binding antibodies, while BA.2-specific IgG, neutralization and avidity were comparable after depletion.

Conclusion

Immunocompromised individuals appear less affected by immune imprinting than healthy individuals, who preferentially recall pre-existing responses at the expense of new variant-specific immune responses. These findings may inform future vaccination strategies against antigenically evolving pathogens.

Mucosal pneumococcal immunization with mmCT accelerates neonatal B cell responses compared with parenteral immunization in neonatal mice

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Introduction

Immaturity of neonatal immune system contributes to increased susceptibility to infections and suboptimal vaccine responses, highlighting the need for improved early-life vaccination strategies. Adjuvants can enhance the magnitude and durability of immune responses and mucosal immunization offers the advantage of inducing protection at pathogen entry sites.

Methods

We assessed the effects of immunization routes and adjuvant mmCT on B cell activation and survival pathways following neonatal immunization with a pneumococcal conjugate vaccine Pn1-CRM197. Neonatal mice were immunized intranasally (i.n.) or subcutaneously (s.c.) and responses assessed 4, 8 and 14 days later. B cell subsets, plasmablasts/plasma cells and expression of BAFF-R, TACI and BCMA were analyzed by flow cytometry.

Results

I.n. immunization with Pn1-CRM197+mmCT enhanced early BAFF-R expression in splenic marginal zone, follicular and transitional B cells compared with s.c. immunization, while TACI expression increased following both routes. I.n. immunization also led to a greater early expansion of splenic plasmablasts/plasma cells and higher BCMA expression. Notably, i.n. delivery preferentially increased plasmablasts/plasma cells co-expressing TACI and BCMA, suggesting enhanced survival potential. In the bone marrow, i.n. immunization promoted earlier homing of plasmablasts/plasma cells with higher BCMA expression and increased frequencies of APRIL$^+$ cells compared with s.c. immunization. These effects were associated with enhanced induction of vaccine-specific antibody-secreting cells and higher systemic and mucosal antibody responses.

Conclusion

Mucosal immunization including mmCT accelerates B cell activation, differentiation and establishment of plasma cell survival niches, thereby overcoming key limitations of neonatal humoral immunity and promoting more rapid and effective protective responses.

Comparative Effectiveness of mRNA and Protein Subunit XBB.1.5 Booster Vaccines in Highly Vaccinated Older Adults

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Aim

Despite repeated COVID-19 booster vaccination, protection against SARS-CoV-2 infection wanes rapidly in older adults, and the effectiveness of different vaccine platforms remains unclear. We aimed to compare the immunogenicity and clinical effectiveness of XBB.1.5-updated mRNA and subunit booster vaccines administered as an eighth dose to older adults in Sweden.

Methods

In a cohort of nursing home residents (n=51), venous blood samples were collected longitudinally up to 180 days-post-vaccination. Spike-specific-IgG levels were measured by ELISA, antibody function by neutralization assay, and memory B-cell responses by flow cytometry. In a second cohort (n=889), capillary blood samples were used to measure antigen-specific antibody levels using a multiplex serology assay. Clinical outcomes were evaluated using national registry data (n=33,913). Vaccine effectiveness against COVID-19 hospitalization was assessed using a matched case-control design with conditional logistic regression. Antibody kinetics were analyzed using longitudinal models with half-life estimation.

Results

mRNA boosters induced higher peak spike-specific-IgG and stronger neutralizing antibody responses, whereas subunit boosters showed minimal boosting but slower antibody decay. Both platforms predominantly recalled cross-reactive immune memory with limited expansion of variant-specific memory B-cells. Infection risk was initially similar, followed by a transient increase among subunit booster recipients between 110-160 days-post-vaccination. Only mRNA vaccine was associated with additional protection against COVID-19-related hospitalization. Both boosters reduced overall mortality compared with no booster.

Conclusion

mRNA and subunit boosters elicit distinct immune responses but confer limited and short-lived clinical benefit in highly vaccinated older adults, highlighting the need for improved vaccination strategies in this vulnerable population.

Intestinal granuloma sterilization by combined CD4+ and CD8+ T cell activity

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Granulomas are immune aggregate structures that form across different tissues in response to several infections. They act as barrier to pathogen dissemination and further restrict inflammation within specific areas. Although protective, granulomas can also provide a niche for pathogens to persist within the host. Initially, innate immunity, mainly neutrophils and macrophages, have been shown to play an important role in granuloma establishment and restriction of the bacterial burden within these structures. However, the exact mechanism of how granulomas resolve in the long term remains elusive.

To investigate this process, we established an in-vivo enteric infectious model by employing the bacterium *Yersinia pseudotuberculosis* (Yp), a known inducer of granulomas in the host. Our preliminary data indicated that T cells cover an important role in later stages of the resolution. Hence, we further developed an in-vivo infection-transfer model by orally infecting Rag1-/- mice, lacking adaptive immunity, with Yp. Reconstitution of the T cells via transfer pre-infection showed that they were sufficient in enhancing the survival rate of the Rag1-/- mice similar to wild-type level. Furthermore, T cells supported granuloma resolution, with CD4+ or CD8+ subsets alone being sufficient for bacterial clearance in deeper tissues as spleen. On the contrary, in the small intestine, both CD4+ and CD8+ T cells were necessary for successful resolution.

In conclusion, T cells provide a sufficient protective role in combating oral Yp infection and supporting granuloma clearance, with both helper and cytotoxic subsets needed in the small intestines, albeit the precise mechanism involved is currently under investigation.

Broadly cross-reactive antibody responses after influenza B infection in adults

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Aim

Influenza B viruses cause substantial morbidity and mortality annually, especially affecting children and older adults. Of the two lineages of this virus, B/Victoria and B/Yamagata, only B/Victoria has continued to circulate in recent years. In this study, we aim to investigate the cross-lineage antibody responses following infection with one lineage and to assess the extent of sero-protection in adults (< 65 years) and older adults (≥ 65 years).

Methods

The study included hospitalised patients with laboratory-confirmed influenza B during the 2017/18 season (n=29) and healthcare workers (HCWs) identified by serology as infected between the 2010/11 and 2013/14 seasons (n=25). Serum samples were collected during acute and convalescent phases in patients and yearly before influenza season in HCWs. Antibody titres were measured using the hemagglutination-inhibition (HI) assay against a panel of historical, current, and future yet-to-circulate strains.

Results

Following infection with one lineage, HI titres significantly increased against multiple previously circulating strains from both lineages, with higher titres observed against the infecting lineage (all above the protective threshold HI ≥ 40) compared to the non-infecting lineage. This pattern was consistent in both adults and older adults. While infection boosted cross-reactive antibodies against future strains, these titres remained below the protective levels (HI < 40), suggesting only partial protection in subsequent seasons.

Conclusion

Influenza B infection generates broadly cross-reactive antibodies. However, the resulting sero-protection from cross-lineage infection and for future seasons may be insufficient. Our findings highlight the importance of continued seasonal influenza vaccination, particularly among high-risk groups.

Magnitude, kinetics and breadth of cross-reactive antibody responses after homologous or heterologous prime and boost of influenza H5 vaccines

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Aim

The highly pathogenic avian influenza (HPAI) H5N1 viruses have evolved into multiple clades with divergent antigenicity. The recently emerged clade 2.3.4.4b virus has spread globally and spilled over into a wide range of mammalian hosts. HPAI H5 virus causes severe disease and high case fatality rates in humans, underscoring the mounting threat to public health. Vaccination is the most cost-effective measure to combat influenza virus. The knowledge of immune responses after different H5 vaccination regimes and schemes is crucial for future vaccine development and pandemic preparedness.

Methods

Biobanked serum samples of 112 participants from two clinical trials were used in the study. Of whom, 31 received the virosomal H5N1 clade 1 vaccine in 2009; 52 received the split antigen H5N8 clade 2.3.4.4b vaccine in 2025; and 29 received both vaccines. We developed a pseudotyped-virus panel covering multiple (sub)clades of H5HAs to characterize the magnitude, kinetics and breadth of cross-(sub)clades neutralizing antibodies.

Results

Both H5 vaccines elicited potent neutralizing antibody responses. Significant antibody increases were detected as early as 7 days after the first dose vaccination. Cross clade neutralizing antibodies were measured after H5 clade 1 and clade 2.3.4.4b vaccines, with the group receiving heterologous prime and boost inducing the most rapid and broadly cross-reactive responses.

Conclusion

Both H5 clade 1 and clade 2.3.4.4b vaccines elicited potent neutralizing antibody responses, and the heterologous prime and boost achieved most rapid and broadly cross-reactive responses.

Evaluating the immunogenicity of the quadrivalent influenza vaccine immunization in European infants, children and elderly

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On behalf of the European Union–INCENTIVE consortium

Background

Influenza remains a major public health concern, particularly among vulnerable populations such as young children and the elderly. Although vaccination is the most effective preventive strategy, vaccine effectiveness varies across age groups. The INCENTIVE consortium aimed to characterise age-related differences in vaccine responsiveness by conducting phase IV clinical trials in Europe to assess immune responses to quadrivalent inactivated influenza vaccine in infants, children and elderly.

Methods

A total of 120 participants were enrolled across three European sites between 2021and 2023; 25 infants (6-8 months), 46 children (3-11 years) and 49 elderly (60-79 years). Participants received one dose (elderly and children ≥ 9 years) or two doses (infants and children <9 years) of QIV. Hemagglutination inhibition and microneutralization assays were performed against four vaccine strains using samples collected at baseline and post-vaccination.

Results

At baseline, most elderly were primed, whereas infants were largely naïve. QIV significantly increased HI titres across all age groups. Children mounted the strongest responses, achieving the highest post-vaccination GMT against A/H3N2 (477; 95% CI: 288 - 791). Elderly participants reached protective HI levels for all strains, although responses were more modest, particularly for the B/Phuket (GMT190; 95% CI:62.6-236.7). Infants responded well to B/Phuket (GMT 98.5; 95% CI: 67-145) but showed weaker responses to A/H1N1. Neutralizing antibody responses follow similar age-dependent pattern.

Conclusions

QIV elicited robust responses in children and the elderly, whereas infants showed variable immunogenicity. These findings underscore important age-dependent differences relevant for optimising vaccination strategies and guiding the development of next-generation influenza vaccines.

Divergent inflammatory and neurology-related protein levels in long COVID following primary and breakthrough SARS-CoV-2 infections

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Aim

While persistent inflammation has emerged as an important feature of long COVID, it is unclear whether immune responses from COVID-19 vaccination or SARS-CoV-2 re-infection exacerbate or mirror the initial inflammatory responses.

Methods

We quantified 182 inflammatory and neurology-related proteins in plasma using multiplexed affinity proteomics. Plasma samples from the COVID PROFILE cohort conducted in Victoria, Australia, were collected 6-9 months after first infection, but before COVID-19 vaccination from individuals who had recovered from COVID-19 (*n*= 21) or from individuals with long COVID (*n*= 12). To establish baseline plasma profiles, protein levels were benchmarked against unvaccinated, SARS-CoV-2 naive individuals (*n*= 24). In addition, we performed longitudinal analysis in a subset of individuals (*n*= 34), where paired samples collected 2-4 weeks after a third COVID-19 vaccine dose and after SARS-CoV-2 breakthrough infection were available to assess inflammatory and neurology protein plasma levels after antigen exposure in these contexts.

Results

In this cohort, Boruta feature selection and lasso regression models identified IL-20, HAGH, NAAA, CLEC10A, LXN, MCP-1, TRAIL, G-CSF, NBL1, and CCL23 as best discriminating proteins when comparing the long COVID group to groups of either healthy or COVID-19 recovered. Notably, longitudinal analysis indicated differences in the levels of a subset of plasma proteins following primary infection compared to after COVID-19 booster vaccination and breakthrough infection within the groups.

Conclusions

These findings suggest that there is an altered immune response outcome primarily observed in individuals with long COVID upon re-exposure.

Identification of biomarkers in blood for early diagnostics of tuberculosis

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Tuberculosis (TB) is the deadliest infectious disease. Only 5–10% of those infected progress from TB infection (TBI) to active TB disease (TBD), yet current TBI diagnostics such as the interferon-gamma release assay (IGRA) cannot identify those at high risk of progression. Since early-stage TB is transmissible but often asymptomatic, early-detection biomarkers are urgently needed. We therefore aimed to identify blood biomarkers associated with early TB.

We analysed a multi-omics dataset from peripheral blood in a Swedish TB cohort comprising 20 symptomatic TBD patients, 7 early TBD patients with few or no symptoms (household contacts exposed within 6 months), 47 TBI patients (IGRA-positive), and 18 controls (IGRA-negative). Plasma proteomics (Olink and SomaScan) and whole-blood bulk RNA-seq were performed. Multi-omics integration was conducted using Multi-Omics Factor Analysis (MOFA) and Similarity Network Fusion (SNF), followed by differential protein abundance analysis and Leiden clustering. Candidate markers were further filtered using public RNA-seq datasets from lung TB lesions.

Multi-omics integration distinguished early TB from TBD, TBI, and controls. We then focused on plasma proteins as candidate biomarkers. A TBD-signature comprising 59 Olink proteins that distinguished early TB from controls and symptomatic TB stratified patients into four clusters. One cluster contained all early TB patients alongside recently exposed TBI patients, suggesting shared molecular features. Markers defining this cluster were identified and validated using SomaScan data. In the public lung TB dataset, thirteen of these genes showed high expression in central TB lesions and were enriched for apoptosis-related genes, potentially capturing early TB-associated molecular patterns.

Sequencing IGH from dried blood spots in a large malaria vaccine trial

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Aims

The recent rollout of malaria vaccines promises to reduce childhood disease and mortality in Sub-Saharan Africa. However, how immunity to malaria is acquired remains poorly understood, though it is hypothesised to depend on host and parasite genetic factors and their interactions. Motivated by this, we have sequenced thousands of parasites from dried blood spots (DBS) collected in the R21/Matrix-M Phase III trial (VAC078), and we now aim to determine whether IGH can also be profiled from the same DBS.

Methods

Study DBS were created by pipetting 50 µL whole blood onto filter paper and DNA was extracted using spin columns. We developed a PCR assay using IGHV leader-based primers. For each sample, 300-400 ng of DNA was split between two replicate reactions, each estimated to contain 500-2,500 functional templates. As a pilot, the assay was applied to 11 VAC078 samples. Amplicons were sequenced on Element Biosciences and Oxford Nanopore platforms. Data were assessed for clonotype recovery and expansion, and replicate consistency.

Results

We successfully amplified IGH from DBS-derived DNA. Clonotype counts were congruent with our estimates, and occasionally exceeded the predicted range, consistent with high B-cell counts in these malaria-infected children. V-gene usage was consistent across replicates. We also detected expanded clonotypes, defined as clonotypes recovered independently in each replicate.

Conclusion

We show that IGH repertoires can be profiled from DBS. We are applying this to thousands of VAC078 infections to explore how vaccination- and infection-induced immunity is shaped by host and parasite genetics.

Impaired Germinal Center Dynamics in mice following Influenza Vaccination during Diet-Induced Obesity

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Influenza vaccination is important for individuals with a high risk for severe disease, such as elderly and obese, but vaccine responses are often hampered in these groups. Obesity is associated with a chronic low-grade inflammation, characterized by immune cell infiltration into adipose tissue and pro-inflammatory cytokine secretion. We hypothesized that this inflammation drives immune formation to a less protective type. While dysregulation of T cells and reduced levels of neutralizing antibodies during obesity are well-documented, the mechanisms of immune formation remain less understood.

Here, we show that diet-induced obesity (DIO) in mice impairs germinal center (GC) responses following influenza vaccination, resulting in reduced affinity maturation, fewer memory B cells (MBCs), and weakened antibody-mediated protection. Obese mice exhibited significantly lower levels of IgG2a, whereas total IgG and IgG1 levels were unchanged. Transfer of sera from vaccinated normal-weight (NW) and obese mice into naïve mice demonstrated improved survival in NW recipients following viral challenge, despite similar in vitro neutralization capacities.

Although vaccine-specific GC B-cell numbers were comparable between groups, obese mice displayed reduced somatic hypermutation and clonal expansion after vaccination. In addition, T follicular helper (Tfh) cell numbers positively correlated with body weight. We speculate that increased Tfh abundance in severely obese mice permits positive selection of lower-affinity B cells that would otherwise be outcompeted in NW animals.

Together, these findings demonstrate that DIO compromises GC dynamics and memory formation, leading to altered humoral immunity and reduced long-term protection against influenza infection.

Antibody and B-cell development in the context of repeated influenza vaccinations

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The exposure history of an individual by infection or vaccination can influence its subsequent immune response. Although inactivated influenza vaccines were first approved for widespread use in 1945, we still do not know how their annual use influences immune formation. Understanding this process can improve the vaccine development against seasonal influenza and pandemic threats. Therefore, our goal is to address current knowledge gaps on the impact of repeated vaccinations against influenza. Here, we have collected blood samples before and after seasonal influenza vaccination from healthcare workers during three consecutive years. As expected, each vaccination led to an increase in the antibody neutralizing titers against the H1N1 and H3N2 virus variants present in the vaccines with the fold change being higher in individuals not vaccinated in the previous year. Pre-absorbed sera with a hemagglutinin protein present during earlier years was able to inhibit neutralization against closely related strains, but not more distant influenza variants, suggesting an induction of new response. Preliminary data from RNA sequencing of HA-specific B cells showed an increase in memory switched and exhausted phenotypes after vaccination. We are currently combining the transcriptional profile with the B cell receptor sequences present before and after vaccination. The aim is to shed light on germinal center activation in response to repeated antigenic exposures, as such providing information that is key for development of future vaccination strategies against variable viruses such as influenza.

Human Immune Memory and Humoral Responses to Avian Influenza (H5) Vaccination

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Avian influenza (H5) viruses pose a significant pandemic threat due to their high case fatality in humans, expanding host range, and potential for zoonotic transmission. Although human-to-human transmission has not been established, pandemic preparedness relies on evaluating immune responses to pre-pandemic vaccines to guide vaccination strategies for key populations, including healthcare workers. This study evaluated immune responses to a licensed, stockpiled influenza H5 vaccine (clade 2.3.4.4.b) by assessing reactogenicity, vaccine-induced antibody-response kinetics, and long-term immune memory in a clinical trial of naïve and revaccinated (previously primed) healthcare personnel.

A total of 81 participants (52 naïve, 29 primed) received two doses of a zoonotic H5 vaccine (clade 2.3.4.4.b) on days 0 and 28. Blood samples were collected longitudinally (days 0, 1, 7, 28, 29, 35, 56, and 6 months). Neutralizing antibody responses were analyzed using pseudotype-based neutralization assay to characterize homologous vaccine and cross-reactive H5 antibody responses over time. Responses to the clade 2.3.4.4.b H5 vaccine were compared between previously vaccinated (primed) individuals (clade 1 A/Vietnam/1194/2003, 2009 first-in-human trial) and immunologically naïve participants.

Preliminary results suggest that primed individuals had a faster, stronger neutralizing response to 2.3.4.4.b after one dose, whereas naïve individuals required two doses to achieve similar titers. Primed individuals also showed strong cross-reactivity to their priming strain (A/Vietnam/1194/2003), while responses in naïve individuals were limited. This study provides rare insight into long-term immune memory and recall responses to avian influenza vaccination and may inform vaccine-stockpiling strategies, dosing regimens, and pre-pandemic priming of high-risk groups including healthcare personnel.

Nutritional Status Does Not Alter HI Responses to QIV in Young Children in a Low-Resource Setting

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Introduction

Young children in LMICs are particularly vulnerable to influenza, and child malnutrition may impact the ability of influenza vaccines to induce protective immune responses. Approximately 30% of pre-school aged Bangladeshi children are malnourished. Child malnutrition can be regarded as a state of immunodeficiency. However, it is not clear how malnutrition might impact the development of protective antibody responses following influenza vaccination. We therefore aimed to compare the antibody response of well-nourished and malnourished children using samples from a large phase IV effectiveness trial of quadrivalent influenza vaccine (QIV) conducted in rural Bangladesh.

Methods

In this phase IV clinical trial (NCT04998344), 2,736 children aged 6–59 months were randomized by village to receive either QIV or inactivated polio vaccine (IPV) as control. Pre- and post-vaccination blood samples were collected to assess antibody responses. Nutritional status at time of vaccination was evaluated using WHO growth standards.

Results

Despite a high prevalence of malnutrition (36% of children met WHO criteria for stunting, wasting or underweight), QIV induced robust antibody responses to all vaccine strains, with post-vaccination serum protection rates ranging from 47.2% to 93.8% depending on the vaccine strain. We observed no differences in geometric mean antibody titers, fold induction or serum protection rates between well-nourished and malnourished children, including those classified as severely malnourished.

Conclusion

Quadrivalent influenza vaccination induced comparable antibody responses in well-nourished and malnourished children, including those with severe malnutrition. Our results indicate that malnutrition does not impair the ability to mount antibody responses following vaccination.

Establishing protocols and data processing pipelines for sequencing of antigen-specific B cells from influenza vaccination

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Effective influenza vaccine formulation and development require a mechanistic, sequence-level understanding of how B-cell receptors (BCRs) recognize and bind viral antigens. Specifically, there is a need to understand the BCR repertoire features that drives antigen recognition and, conversely, how changes in the antigen affect the generation of new antigen-specific BCRs.

Towards this goal, we have collected PBMC from healthcare workers following influenza vaccination to generate a large-scale resource of paired chain BCR sequences linked to different influenza antigens. By integrating BCR repertoire profiles at both bulk and single-cell levels, together with orthogonal information on gene expression and serological responses, this resource will enable fine-grained analyses of characteristics guiding BCR repertoire development, including somatic hypermutation trajectories, clonal lineage reconstruction, germline gene usage biases, and convergent sequence motifs across influenza types and lineages.

From this foundation, we will develop machine learning models capable of predicting antigen-specific BCR sequences from unlabelled repertoire data, with the aim to recover specificity determinants from sequence alone, generalize across cohorts and influenza strains, and provide interpretable features. The resulting resource and models have the potential to accelerate antigen selection, anticipate variability in vaccine responses, and ultimately enhance the effectiveness and durability of next-generation influenza vaccines.

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Characterizing microglial activation and spatial distribution in paraneoplastic cerebellar degeneration (PCD) using imaging mass cytometry

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Aim

Paraneoplastic cerebellar degeneration (PCD) is an immune-mediated neurodegenerative disorder characterized by rapid Purkinje neuron loss, but its underlying mechanisms remain poorly understood. We aim to characterize microglial distribution, activation states, and immune profiles in the cerebellum of PCD patients.

Methods

Formalin-fixed paraffin-embedded post-mortem cerebellar tissue from four PCD patients (>50 years) and three non-neurological controls was analyzed using imaging mass cytometry (IMC). This approach enables spatially resolved single-cell profiling of multiple immune and microglial markers. Tonsil tissue was included as a positive control for immune marker expression, and brain tissue with multiple sclerosis lesions served as a reference for microglial activation in neuroinflammation. A panel of 10 microglial markers was used to assess activation states, alongside markers for immune cell infiltration.

Results

PCD samples confirm marked loss of Purkinje neurons and showed reduced microglial numbers compared to controls. While healthy control samples predominantly displayed microglia in homeostatic states, homeostatic marker expression was largely absent in PCD tissue, indicating a shift in microglial phenotype. These findings suggest that the remaining microglia predominantly exhibit a proinflammatory activation state. Ongoing analyses focus on spatial distribution patterns, particularly in relation to the Purkinje cell layer, and comparisons with multiple sclerosis samples to define disease-specific microglial signatures.

Conclusion

These findings indicate altered microglial activation accompanied by reduced microglial numbers in PCD. Further characterization of microglial dynamics may provide insight into mechanisms of neurodegeneration and identify potential therapeutic targets.

Describing type 2 innate lymphoid cells in mouse skin and their role in atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease involving type 2 immune responses. Th2 cells and ILC2s are sources of type 2 cytokines, which lead to eosinophilia and tissue remodelling. ILC2s are increased in AD lesions and decrease upon successful treatment. However, the role of ILC2s in AD pathogenesis remains poorly understood, mainly due to a poor definition of skin ILC2s by surface markers.

Using an IL-5-reporter mouse, we have phenotypically described ILC2s. To study their role in AD, we use a calcipotriol mouse model. Topical administration of calcipotriol significantly induced ear thickening and expansion of CD25+ ICOS+ ILC2s, reaching the peak of expansion at day 3 post-treatment. We have previously shown that ILC2s exhibit immunological memory properties in the lung. To investigate whether skin ILC2s can acquire this phenotype, we performed a second calcipotriol challenge 2 weeks after the first treatment. Mice receiving the initial calcipotriol treatment showed a significant increase in ear thickness compared to previously untreated mice. Furthermore, pretreated mice showed a greater expansion of ICOS+ ILC2s in the skin compared to controls, indicating that previously activated ILC2s can respond stronger upon secondary challenge. Furthermore, skin ILC2s isolated 3 weeks after in vivo calcipotriol treatment, secreted higher levels of cytokines upon in vitro stimulation compared to naïve skin ILC2s.
Altogether, these findings suggest that ILC2s can acquire a memory phenotype in the skin.

Overall, understanding the role of ILC2s in AD pathogenesis may lead to the design of novel therapeutic targets to ameliorate the disease.

Investigating the dynamics of ILC2 during bone marrow fibrosis progression

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Group 2 innate lymphoid cells (ILC2s) are tissue resident lymphocytes present throughout the organism. While their roles in initiating and orchestrating type 2 immune responses at barrier surfaces is well established, the function of bone marrow (BM) resident ILC2s remains essentially unexplored. Type 2 inflammation, driven by IL-4 and IL-13, has recently been shown to promote BM fibrosis (BMF) While T cells are implicated, the role of ILC2s remains unclear.

We aim to understand the contribution of ILC2s to the inflammatory response that promotes the progression of BMF. We have established a mouse model of myeloproliferative neoplasm(MPN).

At early stages of the disease we observed an increase in circulating ILC2s, concomitant with reduced numbers in the BM, suggesting ILC2s egressing. Phenotypical characterization showed ILC2s in peripheral blood expressed KLRG1, associated with an activated phenotype. As disease progressed, ILC2s, in contrast to T cells, accumulate in the enlarging spleen. Interestingly, ILC2s retain an activated profile, with increased KLRG1 and CD25 expression. At later stages, once fibrosis is ongoing, we found that ILC2s in BM and spleen, become PD1+, suggesting an exhausted phenotype. Also true for the T cell compartment.

Analysis of peripheral blood from MPN patients showed a shift towards ILC2 phenotype within the ILC compartment. In vitro analyses showed that purified ILC2s from MPN patients are functional, producing IL-5,IL-13 and IL-4.

Together, our findings suggest a stage specific involvement of ILC2s in the progression of BMF, becoming activated at early stages of the disease followed by an exhaustion phenotype at fibrotic onset.

Investigating the treatment duration-dependent effects of estrogen receptor signaling: long-term estradiol treatment aggravates dextran sulphate sodium-induced colitis in female mice

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Aim

Epidemiological and experimental evidence suggest that ovarian hormones influence intestinal inflammation. However, contradictory effects of the main estrogen, estradiol, have been reported. We aimed to illuminate this divergence and answer how experimental setup influenced the pro- and anti-inflammatory effects of estradiol in colitis.

Methods

We investigated the divergent effects of estradiol in experimental colitis through a quantitative analysis of previous results. Also, we challenged female mice with dextran sulphate sodium for 7 days to study the effects of long-term ovarian hormones on induced inflammation. Treatments were initiated three weeks before colitis; mice with endogenous hormones, estrogen receptor α antagonist treatment, ovariectomy, and estradiol or progesterone supplementation were used. Localization of estrogen receptors was investigated by immunofluorescence. Signs of inflammation were graded. Effects on colon immune cell populations were analyzed using FACS, and gene expression responses by RNAseq.

Results

The analysis of previous literature suggested that short- and long-term estradiol treatment induces opposing effects in colitis. In our experiments, estrogen receptors were expressed in the colon, with estrogen receptor α observed in epithelial cells as well as in T cells, B cells, and macrophages. Ovariectomy protected from inflammation. Estradiol, but not progesterone, aggravated colitis, while estrogen receptor α inhibition alleviated inflammation. Estradiol altered inflammatory and homeostatic immune cell populations and the transcription of 791 genes, with enrichment in the antimicrobial defense and immune cell activation pathways.

Conclusion

Estrogen receptors are present in the colon, and the estradiol response is substantial. Our research strengthens the evidence that long-term estradiol treatment exacerbates inflammatory signaling in colitis.

Polystyren micro and nanoparticles trigger thromboinflammatory responses through complement, coagulation and leukocytes cross-talk

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Aim

Micro- and nanoplastics (MNPs) are increasingly detected in human circulation and associated with cardiovascular disease, yet their interactions with innate immune pathways regulating thromboinflammation remain poorly defined. We aimed to determine how polystyrene (PS) MNPs activate thromboinflammatory pathways in human whole blood and identify key mechanisms.

Methods

PS MNPs spanning nano- to microscale sizes (0.05-90 µm; 0.1-1000 µg mL^{-}1) were incubated (0.5-4 h) in hirudin-anticoagulated human whole blood *ex vivo*. Complement (sTCC), coagulation (prothrombin fragment 1+2), leukocyte activation (CD11b, monocyte tissue factor), and cytokines (Bio-Rad 27-plex) were assessed. Complement (C3c) and coagulation (FXII) deposition on particle surfaces were visualized by confocal microscopy. Mechanisms were probed using inhibitors targeting C3 (CP40), C5 (eculizumab), FXII (garadacimab), and tissue factor (TF).

Results

PS MNPs induced dose-dependent thromboinflammatory activation. Normalized to surface area, responses were size-dependent, with microscale particles driving stronger coagulation and cytokine responses than nanoscale particles. Imaging confirmed complement and coagulation protein deposition on particles. Monocyte tissue factor was upregulated by microsized PS and less by nanosized particles. Inhibition revealed contributions from FXII- and TF-dependent coagulation pathways. Complement activation was primarily C3/C5-dependent and consistent with alternative pathway engagement. Complement inhibition reduced coagulation and leukocyte activation, while FXII inhibition reduced complement activation, indicating bidirectional cross-talk.

Conclusions

PS MNPs act as activation surfaces that couple complement, coagulation, and leukocyte responses. These data identify microplastics as non-biological integrators of thromboinflammatory signalling, with potential relevance for vascular inflammation and thrombosis.

NHLRC2 deletion in Drosophila melanogaster replicates clinical features of the inherited childhood syndrome FINCA

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The rare inherited childhood multi-organ disease FINCA (Fibrosis, Neurodegeneration, Cerebral Angiomatosis), caused by a mutation in the NHL repeat containing protein 2 (NHLRC2) gene, was first identified in three patients from two separate families in Finland using whole exome sequencing. FINCA is characterized by pulmonary disease, including recurrent infections, suggesting an immune component, as well as neurodevelopmental delay. The gene *NHLRC2* has been identified in a screen for involvement in phagocytosis, and as a potential biomarker for Alzheimer's disease. *NHLRC2* is conserved across animal species, and is present in plants. In this study, we develop a *Drosophila melanogaster* (fruit fly) model as a low cost, highly genetically tractable model for studying the role of *NHLRC2* in FINCA, and generally in immune and neurological processes. This complements ongoing work using patient-derived cells, and other animal models. We use a mutant *Drosophila*model, and genetic tools allowing genetic rescue experiments and examination of the role of NHLRC2 in different tissues.

While flies lacking NHLRC2 develop and propagate, they have increased susceptibility to bacterial infection, as well as changes in locomotion and behavior, suggesting a lack of NHLRC2 in the fly causes changes in neurodevelopment. By forced *NHLRC2* expression, we were able to alleviate both the immune and behavioral components observed in the mutant flies.

The *Drosophila* model recapitulates key aspects of FINCA syndrome, and therefore offers an important tool for studying FINCA, and the role of NHLRC2 in the immune response and neurological development.

TGFβ signaling regulates microglial immune resilience to myelin degeneration during aging

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As the resident innate immune cells of the central nervous system (CNS), microglia continuously survey the tissue microenvironment and regulate myelin integrity during development and adult homeostasis. However, the extent to which microglial immune responses to myelin are spatially and temporally specialized remains unclear.

Here, by analyzing spinal cord white matter tracts in mice, we identified a region-specific vulnerability to myelin degeneration within the dorsal column (DC) during physiological aging. This degeneration was associated with distinct transcriptional and functional reprogramming of DC microglia, including TGF-β signaling activity. Genetic disruption of microglial TGF-β signaling resulted in dysregulated, exaggerated immune activation and exacerbated myelin loss in the DC, accompanied by progressive neurological impairment during aging. Single-nucleus RNA sequencing revealed the emergence of a TGF-β-responsive microglial subset with an altered immune profile, alongside a spatially restricted, disease-associated oligodendrocyte population within the DC. Mechanistically, we demonstrate that microglia employ a TGF-β-dependent autocrine signaling axis to constrain local immune activation and preserve myelin integrity.

Together, these findings identify TGF-β signaling as a critical immunoregulatory checkpoint that enforces region-specific microglial homeostasis and limits immune-mediated myelin damage during aging. This work uncovers spatially restricted neuroimmune interactions between microglia and oligodendrocytes, providing new insights into how localized immune regulation shapes CNS tissue resilience.

Single-Cell Profiling of CD4+ T Cells Reveals Early-Stage Immune Alterations in Type 1 Diabetes

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Aim

The detection of pancreatic islet autoantibodies is the earliest indicator of an ongoing autoimmunity in Type 1 diabetes (T1D). Previously, we reported immune cell-specific changes prior to autoantibody appearance in a small cohort using bulk RNA sequencing. This study aims use single-cell transcriptome analysis to better understand the role of CD4+ T cell subtypes in the early stages of T1D, from birth to the appearance of autoantibodies in children.

Methods

We conducted a comprehensive analysis of 74 longitudinal CD4+ T cell samples from children who later developed type 1 diabetes (N=11) and matched controls (N=11) who remained autoantibody-negative. Using deep phenotypic characterization of over 99,000 cells, we examined gene expression patterns and cell type composition, followed by gene regulatory network analysis of single-cell RNA sequencing data.

Results

Our analysis revealed specific gene expression patterns associated with the progression of type 1 diabetes, with comparable cell type compositions between cases and controls. Notably, we identified increased in gene regulatory network activity in T cell types among children who progressed to the disease, indicating distinct cellular dynamics involved in early disease development.

Conclusion

This study provides novel insights into the cellular dynamics and gene regulatory mechanisms at play during the early stages of type 1 diabetes, enhancing our understanding of the disease's pathogenesis and potentially guiding future therapeutic strategies.

Regulators of innate immunity pathways in Drosophila melanogaster

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Drosophila melanogaster, commonly called the fruit fly, is a widely used model organism for studying many biological processes relevant to human health. Conservation of the innate immunity pathways between flies and humans as well as the lack of the adaptive immune system makes fruit flies a great model for innate immunity research. Evolutionarily conserved NF-κB signaling pathways, namely the Toll and Imd pathways, are mainly responsible for the fly humoral innate immune responses. Although important for defense, keeping these pathways in control is important to avoid harmfully strong immune reactions to the host.

The aim of our study was to investigate how recently identified *Induced by infection A* and *B*(*IbinA, IbinB*) genes regulate immune responses and lifespan. We obtained knockout mutants for *IbinA* and *IbinB*, generated using the Crispr/Cas9 method, and an *IbinA, IbinB* double knockout mutant, by crossing the two lines together. Lifespans of *Ibin* mutant flies and controls were studied with and without infection. Furthermore, expression of immune-induced genes was studied in flies at various timepoints after immune induction with bacteria.

The results show that the absence of *IbinB* significantly shortened the lifespan of flies, particularly under immune challenge, indicating a role for *IbinB* in controlling immune activity over time. In contrast, loss of *IbinA* alone had little effect on lifespan, even in immune-challenged conditions. The roles of *Ibin*genes in immune response against several selected infecting organisms are currently being investigated. Overall, our findings highlight the importance of functional immunoregulation to lifespan and well-being.

Effects of a pro-resolution cocktail on NK cell resolution activity *in vitro*

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Natural killer (NK) cells play an important role in resolution of inflammation and we have demonstrated that NK cell depletion abrogates resolution of antigen-induced inflammation in mice. This study aimed to investigate the effects of a cocktail (RES) of anti-inflammatory compounds and specialized pro-resolving mediators (SPMs) on potential pro-resolution function of NK cells.

Freshly isolated human NK cells were cultured for 18 hours and then stimulated with RES for 6 hours. For comparison the NK cells were stimulated with inflammatory molecules (positive control) or IL-15 only (negative control). The effects of RES on NK cell activity were evaluated by measuring secretion of SPMs, growth factors, and pro-inflammatory cytokines by ELISA, expression of SPM receptors, apoptosis-mediating molecules, and induction of neutrophil apoptosis by flow cytometry.

Treating NK cells with RES increased their secretion of the SPM lipoxin A4. However, it decreased their secretion of the growth factor GM-CSF without affecting secretion of the pro-inflammatory cytokine IFNγ. RES did not affect NK cell expression of the SPM receptors formyl peptide receptor 2 and chemerin receptor 23 or the apoptosis-related molecules FasL and TNF-related apoptosis-inducing ligand (TRAIL). On the other hand, RES increased NK cell induction of neutrophil apoptosis.

These results indicate that the SPMs and anti-inflammatory compounds in the RES cocktail induce changes in NK cells leading to their increased ability to mediate neutrophil apoptosis, possibly by increasing their secretion of SPMs and down-regulating their secretion of the survival factor GM-CSF.

Spatial and single-nucleus transcriptomic characterization of neuro-immune populations in the femoral head ligament of hip osteoarthritis patients

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Aim

Osteoarthritis (OA) pain is a complex phenomenon, and addressing gaps in current pain treatments requires a deeper understanding of its mechanisms. Multiple joint tissues contain nociceptive free nerve endings, and immune cells such as macrophages and mast cells within these tissues can further modulate pain. Among these tissues, the femoral head ligament (ligamentum teres) in hip OA remains poorly characterized. This study aims to map neuro-immune populations within femoral head ligament tissue from hip OA patients using high-resolution spatial transcriptomics and single-nucleus RNA sequencing (snRNA-seq), and begin to characterize their gene signatures and potential neuro-immune interactions.

Methods

Femoral head ligament tissue was collected from four patients undergoing total hip arthroplasty (3 OA, 1 rheumatoid arthritis (RA)). The RA sample was used as a positive control for immune infiltration. FFPE sections were analyzed using Visium HD spatial transcriptomics (10x Genomics) at 16 µm bin resolution. Unsupervised clustering was performed using Seurat. snRNA-seq was performed for detailed cell-type characterization. IHC staining was conducted to validate the presence of nerve structures and immune cells within the tissue.

Results

Unsupervised clustering revealed distinct cell populations within the femoral head ligament, including clusters expressing neuronal and immune-associated markers, whose spatial localization was confirmed through spatial transcriptomic mapping. IHC staining further validated the presence of nerve structures and immune cells at the protein level. Utilizing snRNA-seq enabled more detailed cell-type characterization.

Conclusion

Findings reveal distinct neuro-immune populations within the hip OA femoral head ligament, highlighting potential cellular mediators of joint pain.

Investigating the association between immunosenescence and long COVID

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Aim

Immunosenescence refers to age-associated remodelling of the immune system and is linked to reduced immune function and chronic low-grade inflammation. We aimed to assess whether Long COVID is associated with an immunosenescence-like immunophenotype, particularly in younger individuals, and to determine whether circulating and transcriptional signatures support this pattern.

Methods

We performed a cross-sectional analysis of peripheral blood samples from 85 participants, including Long COVID cases and age- and sex-matched controls. We profiled immune cell subsets and circulating cytokines using immunophenotyping and multiplex cytokine assay, complemented with targeted transcriptomics.

Results

Age-stratified analyses showed that younger Long COVID cases had higher frequencies of age-associated CD8+ T cells and lower frequencies of CD8+ recent thymic emigrants compared with matched controls, whereas these differences were less apparent in older participants. Similar age-related case-control differences were observed in monocyte and NK-cell subsets, together with elevated circulating pro-inflammatory cytokines, including CXCL9. Targeted transcriptomics suggested increased TNF/NF-κB-associated inflammatory signaling and altered responses to innate cytokine stimulation in Long COVID samples.

Conclusion

Our findings suggest that Long COVID is associated with an immunosenescence-like immunophenotype, most prominently in younger individuals. The combined cellular, soluble, and transcriptional data support the hypothesis that incomplete recovery after COVID-19 may be linked to premature immune aging and persistent inflammatory dysregulation.

State-resolved mapping of human double-negative αβ T cells reveals clonal architecture, tissue specialization and clinical relevance

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Aim

Human double-negative αβ T cells (DNTαβ) have been linked to inflammation, immune regulation and cancer, but whether they represent a discrete lineage or a heterogeneous T-cell compartment remains unresolved. We aimed to define their phenotypic, transcriptional, clonal and anatomical organization in homeostasis and disease.

Methods

We combined high-dimensional flow cytometry, bulk and single-cell RNA sequencing, paired single-cell TCR sequencing, repertoire-structure analyses and functional assays across peripheral blood, healthy tissues, hematopoietic stem cell transplantation (HSCT) and gastroesophageal adenocarcinoma (GEAC).

Results

Stringent exclusion of MAIT, iNKT and γδ T cells revealed that human DNTαβ are not a uniform innate-like population, but comprise reproducible states captured by both flow cytometry and scRNA-seq. A cytotoxic state showed clonal expansion, reduced repertoire evenness, preferential CD8 T-cell clonotype sharing and CD8-oriented TCR structural similarity. A Fas-controlled state was transcriptionally and clonally distinct, enriched in tonsils and progressively accumulated during HSCT reconstitution, whereas a quiescent state showed prominent tissue-residency features across solid tissues. Broad tissue profiling demonstrated marked anatomical skewing of DNTαβ abundance and subset composition. In GEAC, DNTαβ state composition and function were altered relative to blood, including reduced tumor-associated cytokine production, and circulating activated/cycling and effector DNTαβ states associated with clinical outcome and treatment response.

Conclusion

Human DNTαβ cells form a structured, anatomically specialized αβ T-cell compartment composed of discrete states with distinct clonal relationships, tissue distribution and clinical relevance. Resolving DNTαβ states may be more informative than measuring total DNTαβ abundance in transplantation, tissue immunity and cancer.

Transcriptional profiling at the single-cell level of γδ T cells across development, health and disease

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Gamma delta (γδ) T cells exit the thymus functionally competent and undergo rapid proliferation during neonatal life and in response to inflammation. However, it remains unclear how γδ T cells proliferating during development differ from those expanding during inflammation, and how the tissue environment shapes these responses during early life and neuroinflammation. To address this, we used single-cell transcriptomics to characterize γδ T cell heterogeneity in lymph nodes (LN) and brain during experimental autoimmune encephalomyelitis (EAE).

During EAE, IL-17-producing γδ T cells (γδT17) became highly activated and proliferated in the LNs 11 days after MOG35-55 immunization, with marked expansion of a Vγ4Vδ2-2 subset. Although rare in the healthy brain, this population accumulated during disease and adopted a transcriptional profile resembling activated LN γδ T cells. In contrast, non-γδT17 cells showed minimal early changes but underwent substantial transcriptional remodeling in the brain by day 21. Even in the absence of inflammation, brain γδ T cells exhibited a more activated signature than LN counterparts and included a small KLRG1$^+$ tissue-resident population.

To distinguish inflammation-driven proliferation from developmental expansion, we compared EAE-responsive cells with neonatal γδ T cells. Immunization-responsive cells expressed higher levels of TIM-3, musculin, and metallothionein 1, whereas neonatal γδ T cells showed elevated Igf2bp3 expression and included a type 2-skewed population absent in adults.

Together, these findings reveal spatial and temporal heterogeneity of γδ T cells across development, homeostasis, and disease.

Understanding the Development of Th2 and Th1 Immune Responses Against Heligmosomoides polygyrus

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Adaptive type 2 immunity against parasitic nematodes is thought to be initiated by type 2 classical dendritic cells (cDC2s), whereas cDC1s primarily drive type 1 immunity. Although cDC2s are highly plastic and can polarize diverse T helper (Th) lineages, they do not produce the key Th2 cell differentiation cytokine, interleukin (IL)-4. The precise cellular source of IL-4 in lymph nodes (LNs), as well as the bystander molecular events through which cDC2s drive Th2 cell responses, remain poorly defined.

Here, we show that infection of mice with the small intestinal nematode *Heligmosomoides polygyrus*induces a rapid increase of eosinophils, Mgl2-expressing cDC2s, and Mgl2+ infiltrating macrophages in the upper small intestinal lamina propria and the corresponding draining LNs as early as two days post-infection.

This early innate response is followed by an increase in GATA3-expressing CD4+ T cells by day six. Notably, a subset of these Th2 cells not only secretes the canonical type 2 cytokines IL-4 and IL-13 but also co-expresses T-bet and secretes IFN-γ. These dual Th2/Th1 effector cells, which we term 'hybrids', subsequently disseminate to distal tissues, with increased numbers detected in the lung and visceral adipose tissue two months after infection compared with non-infected controls.

Our findings suggest that hybrid Th2/Th1 effector cells are induced as early as the first week of *H. polygyrus* infection, and persist in distal tissues long-term. Whether specific antigen-presenting cells or soluble factors are required for hybrid cell differentiation, and the physiological functions of these hybrids, remains under investigation.

Cataloging salamander phagocytosing immune cell diversity through image-guided sorting and orthogonal transcriptomic approaches

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Invertebrate species such as Hydra and planarians exhibit exceptional regenerative capabilities. Among vertebrates, salamanders uniquely retain the ability to regenerate complex tissues, including limbs, throughout life. Regeneration depends on phagocytes (Godwin et al. 2013), although their precise role remains poorly understood. Progress in characterizing salamander immune cells has been limited by the lack of available antibodies and immunological tools. This study aimed to establish an antibody-independent strategy for identifying and characterizing salamander immune cell populations.

To overcome these limitations, we employed an image-guided cell sorting platform integrated with transcriptomic profiling. Using fluorescent *E. coli*bioparticles, we assessed phagocytic activity and applied high-content imaging combined with clustering algorithms to characterize cellular morphology, followed by Smart-seq3xpress sequencing (Hagemann-Jensen et al. 2022).

We identified and isolated distinct cell populations based on image-derived parameters and phagocytic capacity. Subsequent plate-based transcriptomic sequencing enabled phenotypic characterization and direct linkage of phagocytic function and morphology to gene expression profiles.

This work provides a framework for immune cell identification and characterization in non-model organisms without relying on antibody-based markers. The approach enables targeted cell isolation and downstream functional studies, facilitating future investigations of immune function and immune-mediated tissue repair in regenerative species.

Adaptive immunity is dispensable for salamander appendage regeneration

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Complex multi-tissue regeneration capacity varies across vertebrates. Mammals are amongst the least regenerative species, while salamanders can regenerate complex tissues such as limbs and tails throughout life. Previous studies have shown that innate and adaptive immune cells are present during salamander limb regeneration. While innate immune cells have been shown to promote limb regeneration, it is unknown whether adaptive immunity is responsive to amputation or plays a role in appendage regeneration.

Here we show that during limb regeneration in axolotls, the immune response is characterized by a coordinated immunoregulatory signature including the downregulation of antigen presentation, cytokine secretion, and T cell activation. To test the role of adaptive immune cells in regeneration, we generated *Recombination activating gene 1*deficient (*Rag1*-/-) newts. *Rag1*-/- newts lack antigen receptor recombination and show a marked reduction of adaptive immune cells. We find that *Rag1*-/- newts do not reject allografts, confirming their functional immunodeficiency. Finally, we demonstrate that both larval and adult newts regenerate appendages in the absence of adaptive immunity.

Our work demonstrates that the adaptive arm of the immune system is not required for appendage regeneration and establishes an important model for novel experimental approaches in comparative immunology and regenerative biology.

PI3K/Akt Signaling Contributes to Treg-Derived Extracellular Vesicle-Mediated Osteogenesis in Bone Marrow Mesenchymal Stromal/Stem Cells.

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Aim

Regulatory T cells (Treg), a specialized subset of CD4+ T cells, are central mediators of immune homeostasis and tissue repair. Emerging evidence suggests that Treg-derived extracellular vesicles (Treg-EV) recapitulate the immunomodulatory and regenerative properties of their parental cells. Current understanding of Treg-EV-mediated bone regeneration is mainly based on animal-derived systems, however their functional and molecular roles in human osteogenesis remain poorly understood.

Here, we investigated the impact of human Treg-EV on human BMSC osteogenesis, with particular emphasis on PI3K/Akt signaling.

Methods

Treg-EV were isolated and characterized by morphology, particle size and concentration, and expression of EV markers. Their cytokine and miRNA cargo were analyzed to identify potential osteoimmunomodulatory mediators. BMSC were treated with Treg-EV under osteogenic conditions, and osteogenesis was assessed using functional and molecular readouts. PI3K/Akt activation was evaluated by Akt phosphorylation, and BMSC were treated with a PI3K inhibitor to determine the contribution of this pathway to Treg-EV-mediated osteogenesis.

Results

Treg-EV were efficiently internalized by BMSC and significantly enhanced osteogenic differentiation. Potential contributors to these effects were identified within the Treg-EV cargo, including osteogenesis-associated mediators such as miR-21 and VEGF-A. Treg-EV also altered the cytokine milieu during osteogenic differentiation. Mechanistically, Treg-EV induced early PI3K/Akt activation, while PI3K inhibition attenuated Akt activation and partially reduced the pro-osteogenic effects of Treg-EV, suggesting involvement of additional regulatory pathways.

Conclusion

Together, these findings identify Treg-EV as osteoimmunological mediators linking immune regulation and bone regeneration, highlighting their potential in regenerative medicine and bone tissue engineering.

DUSP4 acts as a novel regulator of NK cell functionality

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Aim

Natural killer (NK) cells activity is carefully calibrated by exogenous cytokines and cell intrinsic feedback loops. Dual specificity phosphatases (DUSPs) play a critical role in fine-tuning lymphocytes functionality and can be triggered upon cellular activation. However, their role in NK cells remains unclear.

Methods

Via both RNA-seq and western blot we quantified DUSPs expression via freshly isolated and cytokine-stimulated human NK cells from healthy blood donors, and we found DUSP4 being strongly induced. To gain insights into the functional relevance of DUSP4 expression, we generated DUSP4-KO cells via CRISPR/Cas9-mediated genome editing of primary NK cells as well as NK cell lines of leukemic origin. Changes in functionality of engineered NK cells were examined via flow cytometry to assess proliferation, metabolism and phosphorylation of key signaling pathways.

Results

In NK cell lines, DUSP4-KO enhanced cell proliferation and metabolic fitness, identifying a previously underappreciated role as tumor suppressor gene. In contrast, primary NK cells lacking DUSP4 depicted reduced proliferative capacities. We also found increased phosphorylation of ERK and STAT3 in DUSP4-KO cell lines, and elevated STAT3 and STAT5 tonic signaling in primary NK cells.

Conclusion

Our findings suggest that DUSP4 may function as a novel regulatory node of NK cell signaling.

Monovalent antigens with defined physicochemical properties induce B-cell activation independently of receptor oligomerization

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B cells are essential for protective immunity but can also contribute to pathological responses, as exemplified by allergies, autoimmunity, and lymphoid malignancies. Despite extensive characterization of downstream signaling pathways, the molecular mechanisms linking antigen recognition by the B-cell receptor (BCR) to the initiation of intracellular signaling cascades that drive transcriptional activation remain incompletely understood.

We investigated how the physicochemical and structural properties of monovalent antigens regulate BCR activation using precision-engineered antigens, DNA origami nanotechnology, advanced flow cytometry, and super-resolution microscopy.

We show that cognate antigen recognition alone is insufficient to induce signaling, thereby excluding a purely allosteric mechanism. Notably, monovalent antigens were required to exceed a minimal steric threshold to trigger signaling. Antigen geometry and receptor-proximal steric occupancy emerged as key determinants of agonistic activity, supporting a model in which steric proofreading governs BCR activation. Furthermore, non-crosslinking ligands targeting the BCR light chain triggered signaling independently of canonical paratope engagement.

Together, these findings suggest that positioning sterically active ligands near the BCR Fab is sufficient for activation and support the hypothesis that spatial segregation of transmembrane phosphatases constitutes a central, and potentially shared, mechanism regulating both antigen-mediated and antigen-independent BCR signaling.

In conclusion, our findings demonstrate that reorganization of the BCR configuration is not the primary driver of B-cell activation and suggest that antigenicity is determined by the physicochemical properties of an antigen. These insights may inform the rational design of vaccines and facilitate the development of novel therapeutic strategies.

Architecture of the B-cells surface: nanoscale protein interaction networks as a regulatory layer of B-Cell Antigen Receptor (BCR) signalling and cell fate.

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Aim

The B-cell antigen receptor (BCR) initiates signalling cascades upon antigen recognition that are crucial for B-cell activation. BCR is organized in membrane nanodomains together with other co-receptors and signalling proteins. These nanodomains affect signalling and downstream responses. We aimed to define reference maps of surface protein organization across human B-cell subpopulations in health and disease, using malignant B cells from chronic lymphocytic leukaemia (CLL), characterized by aberrant BCR signalling, as a disease model.

Methods

Peripheral B cells were isolated from healthy donors (n=12) and CLL patients (n=2). Surface protein colocalization was profiled using the Pixelgen Proximity Network Assay, which enables simultaneous detection of proximities among 155 membrane proteins at single-cell resolution.

Results

Dimensionality reduction resolved major B-cell populations, including transitional, naïve, memory, atypical, and plasmablast subsets. Network analysis uncovered subset-specific co-localization patterns and identified CD19 as a central hub engaged with a diverse range of membrane proteins. Canonical CD19 interactions, including those with complement receptor CD21 and tetraspanin CD81, were conserved, while others showed subset-specific remodelling. Notably, IgM-BCR formed small, dispersed clusters, whereas IgD-BCR associated with co-receptors in larger, more structured nanodomains. In CLL, membrane organization differed markedly from healthy B cells: the inhibitory receptor CD22 showed increased co-localization with tetraspanins and other inhibitory receptors, suggesting rewired inhibitory network architecture in malignant cells.

Conclusions

Our data establish reference maps of human B-cell membrane nanodomains and reveal dynamic reorganization of BCR-associated protein networks across both B-cell differentiation states and disease.

Restrained Met1-linked ubiquitination prevents immune pathology and safeguards T cell development and function

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Methionine 1-linked ubiquitin (Met1-Ub) is a non-degradative protein-modification that is essential for immune regulation and T cell biology, and it is generated solely by the E3 ubiquitin ligase LUBAC. HOIP, the catalytic subunit of LUBAC is responsible for Met1-Ub conjugation, contains a PUB domain that interacts with regulators of LUBAC harbouring a PUB-interacting motif (PIM), including the deubiquitinases OTULIN and CYLD. However, the role of these PUB-PIM interactions in regulating immune responses is largely unknown.

Here, we reveal that the ablation of LUBAC's PUB-PIM interactions causes superfluous deposition of Met1-Ub, driving systemic immune pathology characterised by acute cytokine storm, emergency myelopoiesis, and a sharp reduction in splenic CD4+ T cells. Bone marrow chimeras revealed that this immune dysregulation was hematopoietic in origin; furthermore, while neutralization of TNF largely rescued the cytokine storm, it did not resolve the immune dysregulation or the reduction of CD4+ T cells. This indicates that the immune pathology, resulting from inhibition of the PUB-PIM interactions, is driven by cell-intrinsic defects across multiple immune populations. Indeed, ablation of the PUB-PIM interactions in αβ T cells selectively abated the generation of thymic regulatory T cells (Tregs) and skewed gut CD4+ T cells towards a T-helper (Th) 2 phenotype without affecting Th17 cells.

Thus, our data indicate that immune homeostasis, and T cell development and function relies on tight regulation of Met1-Ub via LUBAC PUB-PIM interactions.

Modulation of adaptive immunity: Signaling in experienced T cells

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T cell specific adaptor protein (TSAd) is coded by the *SH2D2A* gene, and is an adaptor protein. While it is well established that TSAd is interacting with Lck, identification of additional interaction partners of TSAd might give further insights into its role in T cell signaling. However, due to the transient nature of adaptor protein interactions, elucidating their interaction partners remains a challenge.

Recently our lab discovered two new TSAd interaction partners, SHP-2 and DOK2, using affinity purification [AS1] mass spectrometry. While this is an unbiased approach, pulldown methods like the one we have used are lysis-based, requiring the interaction to be stable in the lysis buffer. One of the tools recently developed to tackle this problem is *in vivo* [HC2] [PB3] proximity labeling, using a modified version of the biotinylation enzyme BirA (i.e. TurboID) fused to a protein of interest. In the presence of excess biotin, this will result in biotinylation of any lysine residue within 10 nm range of the enzyme. Biotinylated proteins, closely associated with the target protein *in vivo* can then be isolated using streptavidin beads and identified using mass spectrometry (MS). We have generated TSAd-TurboID constructs and successfully transduced them into primary CD8+ T cells.

So far, we have conducted one round of MS run on a single donor and currently, we are working on improving the experimental pipeline in order to analyze more donors (Data to be presented).

Assessing Immune Exhaustion as a Potential Contributor to Cancer Risk in BRCA2 Mutation Carriers

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Background

BRCA2 germline mutations significantly increase the lifetime risk of breast, ovarian, and other cancers. However, the immunological features that might contribute to this susceptibility remain poorly understood. Emerging evidence suggests that BRCA2 is involved in lymphocyte homeostasis and activation, raising the possibility that subtle immune dysfunctions arise before malignant transformation. In this work, we aim to refine and implement a comprehensive, exhaustion-focused immunophenotyping panel to analyze T-cell and NK-cell function in healthy BRCA2 mutation carriers. Preliminary analyses indicate that T-cell activation capacity is lower in BRCA2 mutation carriers than in matched controls following mitogenic stimulation, suggesting impaired proliferative or signaling responses. We also observe a significant decrease in the circulating NK-cell population, potentially indicating a defect in cytotoxic immune surveillance.

Aim

To further determine whether these differences reflect an intrinsically more exhausted immune state, we developed a multi-parameter flow cytometry panel incorporating key inhibitory receptors: PD-1, TIGIT, LAG-3, TIM-3, and CD95, alongside markers of activation, proliferation, and lineage differentiation.

Methods

Using the FACSDiscover S8 multi-color flow cytometer, we optimized fluorochrome selection and acquisition parameters to achieve high-dimensional resolution of lymphocyte exhaustion phenotypes.

Results

This refined panel enables detailed evaluation of exhaustion signatures across T-cell subsets and NK-cells with enhanced precision and sensitivity. This approach will provide important insight into whether BRCA2 mutation carriers exhibit a functionally or phenotypically exhausted immune system that could contribute to their elevated cancer risk.

Conclusion

Findings from this work may support future biomarker development and preventive immunological strategies.

Molecular rewiring leading to bladder cancer tumorigenesis

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Bladder cancer (BC) is the most common malignancy of the urinary tract. Around 80% of BC cases are non-muscle invasive at diagnosis; however, a significant proportion of patients relapse with aggressive muscle invasive disease. Emerging evidence indicates that deregulation of molecular pathways occurs prior to visible tumor formation. Our aim is to identify signatures that can anticipate BC recurrence and to demultiplex immune contribution.

We collected pre-cancerous and cancerous bladder tissues from carcinogen-induced BC mouse-model and analyzed using immunofluorescence and omics-based approaches. Patients' biopsies, analyzed by TMT-proteomics, were compared with mouse datasets using Ingenuity Pathway Analysis to identify conserved deregulated molecular pathways.

To characterize early oncogenic events, we performed short-term carcinogen (BBN) exposure and collected bladder tissues at multiple time points alongside age-matched controls. We observed rapid and cumulative proteomic changes. Early responses were characterized by enhanced lipid transport and fatty-acid metabolism, followed by immune cell infiltration. A marked activation of cell-cycle pathways was detected, with proliferating cells primarily localized to the basal urothelial layer.

Importantly, these molecular signatures closely resemble predictive profiles previously associated with rapid relapse in BC patients. To investigate immune involvement, immunodeficient NSG mice and immunocompetent C57BL/6 mice were treated with BBN. In long-term exposure, muscle-invasive BC developed only in immunocompetent mice, highlighting a potential role of immune cells in promoting tumor progression.

In short, molecular deregulation precedes macroscopic tumor formation and may be used to predict disease progression. Additionally, the immune cells appear to play a critical role in driving tumor progression.

Rasal1 impairment enhances natural killer cell-mediated anti-tumor immunity

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Aim

Natural killer (NK) cells are critical mediators of anti-tumor immunity; however, their effector activity is restrained by intracellular checkpoints that may limit their therapeutic potential. Rasal1, a Ras GTPase-activating protein, has emerged as a negative regulator of T cell-mediated immunity, but its role in NK cell biology remains undefined. Here we demonstrate that Rasal1 acts as an intracellular checkpoint in NK cells, and its impairment enhances NK cell-mediated tumor control.

Methods

We employed an endonuclease-mediated Rasal1-impaired (Rasal1i) mouse model to investigate tumor progression and NK cell function in vivo. Syngeneic tumor models of breast cancer (EO771), colorectal cancer (MC38), and melanoma (B16F10) were inoculated into wild-type and Rasal1i mice. Tumor growth was monitored longitudinally, and tumor-infiltrating NK cells (TINKs) were characterized using flow cytometry.

Results

Rasal1 impairment significantly delayed tumor growth across multiple tumor models, indicating a broad enhancement of anti-tumor immunity. Mechanistically, Rasal1i mice exhibited enhanced NK cell effector function, including increased activation and cytotoxic potential in the tumor microenvironment. These functional gains were associated with augmented Ras pathway signaling and context-dependent modulation of NK cell maturation states. Notably, in the B16F10 melanoma model, Rasal1 impairment strongly enhanced cytotoxic TINK responses.

Conclusion

Our ongoing findings identify Rasal1 as an intracellular checkpoint in NK cells. By relieving this checkpoint, Rasal1 impairment enhances NK cell effector responses and improves murine tumor control in vivo. These results provide a rationale for targeting Rasal1 to potentiate NK cell responses in cancer, warranting further validation in human translational models.

Single cell analysis reveals molecular traits of pediatric lymphoma resistant subclones

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Paediatric lymphoma accounts for 10-15% of all childhood cancer. Accumulating evidence underscores paediatric lymphoma being a unique entity from its adult counterpart. Current treatment for (R-CHOP) is associated with severe long-term side-effects. Moreover, refractory/relapsed (R/R) is associated with poor survival. Here, we aim to identify R/R associated lymphoma cells to ultimately eradicate R/R events.

We applied single cell RNA sequencing (scRNA-seq) and immune receptor sequencing (scVDJ-seq) on freshly acquired pediatric non-Hodgkin lymphoma (pNHL n=10), Hodgkin lymphoma (pHL n=5), and 10 reactive lymph nodes from adults or children (a/pReLy). In search of R/R seeding lymphoma subclones, we combined gene-count based chromosomal copy number variation inference (inferCNV) and sequence-sensitive single nucleotide variation (SNV, via SComatic) on cross-sectional scRNA-seq data to leverage longitudinal tumour evolution pattern. In addition, we applied spatial transcriptomics on 4 pHL samples to characterise tumor-host interaction.

We discovered NOTCH-pathway enrichment in progenitor-like B and T cells predominantly found in pReLy, whose transcriptome program was found utilised by pNHL subclones emerging late during clonal evolution, accompanied by loss of immune receptor expression. R-CHOP target gene expression indicated that these subclones may escape first-line treatment, and suggested MSI2, an RNA binding protein, as a potential target. In pHL, we showed relapsed Hodgkin cells adopt a myeloid-like status and reshape the tumour microenvironment by recruiting distinct T- cell populations through chemokine signalling pathways classically associated with myeloid cells.

Together, our work delineates cellular states underlying R/R events across pediatric lymphoma and highlights therapeutic opportunities beyond conventional therapy.

Spatial Mapping of Breast Cancer during of Anti-HER2 Treatment

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Aim

The study aims to investigate potential immune mechanisms underlying response to anti-HER2 therapy in primary breast cancer.

Methods

Samples were drawn for the PETREMAC trial, in which patients with HER2-positive breast tumors (n = 60) received dual anti-HER2 therapy plus chemotherapy. We analyzed samples collected pretreatment, 24-hour post-treatment initiation, and post-treatment. For comparison, we analyzed corresponding samples from the older EpiTax trial (n = 40) where patients only received chemotherapy. We used CosMx Spatial Molecular Imaging with a 64-marker Immuno-Oncology Protein Panel, including HER2 and Fcγ receptors, to characterize tumor and immune cell organization at single-cell resolution. By comparing chemotherapy-only and anti-HER2-based treatment cohorts, we assess spatial immune changes specifically associated with anti-HER2 therapy. We also integrate spatial protein data with existing WGS, WTS, and IHC data from the same cohorts to identify tumor microenvironment features associated with treatment response.

Results

RNA-seq and IHC data revealed that a general immune presence pre-treatment predicted response to anti-HER2 therapy, while a similar effect was not seen in patients only receiving chemotherapy. Further, distinct pretreatment immune cell distributions were associated with different treatment response patterns. Niche analyses also revealed response-associated spatial immune patterns, suggesting that the organization of immune and tumor cells within the tumor microenvironment may contribute to variability in treatment response.

Conclusion

Our findings suggest that immune-cell abundance and localization affects treatment response to anti-HER2 therapy, specifically, and that spatially resolved analyses can help identify tumor microenvironment features with potential relevance for future treatment decisions.

CD8+ T-cells, CD68+ macrophages, and PD-1+ cells as candidate biomarkers for risk classification in oral epithelial dysplasia.

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Oral epithelial dysplasia (OED) is characterized by the structural and cellular alteration of the oral mucosa epithelium and is associated with an increased risk of malignant transformation into oral squamous cell carcinoma (OSCC). Currently, clinicians rely on highly subjective histological grading systems to establish a follow-up protocol for OED patients. Therefore, reliable biomarkers able to stratify high-risk from low-risk cases of malignant transformation are urgently needed.

We applied multiplex immunostaining on 12 oral tongue dysplasia samples classified as high-risk (HR) and low-risk (LR) based on OSCC development within a 5-year follow-up period. We used a panel of 10 antibodies targeting immune (CD4, CD8, CD11b, CD68, CD56, FoxP3) and epithelial cells (PanCK), as well as proliferation marker (Ki67) and immune checkpoints (PD-1, PD-L1).

Our study revealed distinct immune cell distributions between HR and LR samples. HR samples exhibited a significantly higher density of CD8+ T-cells and CD68+ macrophages compared with LR lesions. HR samples also displayed an increased proliferation of PanCK+ epithelial cells and CD8+ T-cells, and a higher expression of PD-1 compared with the LR group. Moreover, the logistics regression model supports these findings by confirming the predictive value of individual markers and by identifying a multi-marker model with superior risk prediction performance.

Our findings demonstrate significant differences in the immune landscape between LR and HR OED cases, with CD8+ T-cells, CD68+ macrophages, and PD-1+ cells emerging as candidate biomarkers for risk classification.

Integrating immune repertoire profiling and antigen prediction in the identification of antibodies associated with pediatric lymphoma

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**These authors contributed equally to this work.*

Pediatric lymphoma accounts for 10-15% of all childhood cancer with the majority of non-Hodgkin lymphoma being associated with B cells malignancies. The antigenic drivers of malignant or reactive lymphocyte clones remain unknown.

Here, we developed a single-cell sequencing pipeline of paired transcriptomic and B/T-cell receptor (VDJseq) data from lymphoma biopsies combined with bioinformatics-based antigen prediction and functional characterization of candidate antibodies derived from the malignant immune repertoire.

From 3 Burkitt lymphoma samples we identified 5239 cells with hyperexpanded clonal BCR. Concatenated heavy chain from unique B cell receptor sequences were aligned with 176,894 paired antibody sequences from the PlabDab database. We identified sequences with identity scores ranging from 47.37 to 96.15% with previously described antibodies, with predicted binding to pathogens and self-antigens. To proceed with our antigen prediction pipeline, we selected a self-reactive sequence with 62.5% identity to a previously described anti-CD73 antibody. The 3D-structure was predicted and molecular dynamics interaction with human CD73 is ongoing to determine the residues involved in the binding. Confirming our *in silico* prediction, binding assays showed specificity of the recombinant antibody to CD73-expressing cells.

These results suggest that a combination of *in silico* and *in vitro* methods can contribute to the identification of antibodies involved in B cell malignancy development. The development of these pipelines can contribute to understanding pediatric lymphoma biology and drive the development of novel biotechnological tools and next-generation immunotherapies.

Single-cell based multiomics profiling of tumour infiltrating lymphocyte in adoptive cell therapy reveals signatures of relevance for clinical response

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Adoptive cell transfer (ACT) with tumour-infiltrating lymphocytes (TILs) can induce durable tumour regression, yet long-term clinical benefit remains inconsistent, and the determinants of T-cell persistence are poorly understood.

To address this, we developed a multimodal platform, capturing pMHC-specificity and paired TCR sequences, transcriptomes and surface proteins in single cells, allowing us to link specificity, phenotype, and clonal dynamics. Across nine patients with diverse clinical outcome, we investigated a total of 67 longitudinal samples, including the infusion product and pre- and post-infusion PBMCs. From these patients we profiled T cells recognising neoepitopes, shared tumour antigens and viral epitopes.

We often found several TCRs recognising the same tumour epitope. TCR specificity and functionality were experimentally validated by TCR knock-in, confirming both the TCR-pMHC pairing and the direct tumour reactivity of both neoantigen and shared tumour antigen-specific TCRs. Across the longitudinal samples from the nine patients, we observed varying persistence of the tumour-specific T cell clones from the infusion product samples, with examples of clones detected up to 6 years after infusion. Infusion products and early post-infusion samples were consistently enriched for tumour-specific responses. However, non-responders had fewer tumour-specific responses in blood prior to the TIL transfer, and had a more rapid decline of tumour-reactive clonotypes post ACT. Based on the clonal dynamics and the phenotype of the T cell clones, we related the persistence of the anti-tumour T-cell clones to clinical outcomes.

These results highlight persistence of tumour-reactive clonotypes as a key determinant for ACT response and show potential for biomarker discovery.

Spatial multi-omics profiling reveals immune- and tumour features associated with survival following BRAF/MEK-targeted therapy in metastatic melanoma

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Aim

Resistance to BRAF/MEK-targeted therapy remains a major challenge in metastatic melanoma, yet biomarkers capturing baseline tumour–immune states associated with treatment outcomes are limited. We aimed to identify spatially resolved tumour and immune features associated with survival and to determine whether immune-associated biomarkers define distinct phenotypes in adjacent tumour regions.

Methods

Pre-treatment skin and lymph node biopsies from metastatic melanoma patients treated with BRAF/MEK inhibitors (n=18) and other systemic therapies (n=39) were profiled using NanoString GeoMX spatial multi-omics. Immune- and tumour-enriched regions were analyzed using whole-transcriptome RNA profiling (>11,000 targets) and a 67-plex protein panel. External datasets were included for targets validation.

Results

Higher FOS expression in immune-enriched regions was consistently associated with prolonged overall survival, whereas tumour-compartment FOS showed no significant association, indicating spatial specificity. Using immune-compartment FOS as a central spatial node, we identified heterogeneous expression patterns in neighbouring tumour regions linked to immune FOS status, suggesting coordinated tumour–immune microenvironment states. Immune regions from short-term survivors demonstrated enrichment of pathways related to sustained T-cell receptor signalling, interferon responses, and T-cell exhaustion. Reanalysis of independent single-cell datasets identified FOS-positive CD8+ effector memory T cells with lower exhaustion signatures that were enriched in treatment responders before and during therapy.

Conclusion

Baseline immune states within the tumour microenvironment are associated with survival under BRAF/MEK-targeted therapy. Spatially resolved immune FOS expression emerges as a promising biomarker and provides insight into tumour–immune interactions associated with treatment outcomes in metastatic melanoma.

Highly activated and exhausted CD8+ T cells identified at the tumor site of myeloid neoplasms

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Aim

Immune dysregulation is a hallmark of chronic inflammation; however, it remains difficult to understand how the immune system tolerates slowly developing disease. We studied low-risk myeloid neoplasms (MN), slowly progressive tumors that develop in the bone marrow (BM) over decades before diagnosis. We sought to characterize CD8+ cells in the BM of patients compared with healthy donors (HD).

Methods

We performed 26-color full-spectrum flow cytometry on cryopreserved BM and peripheral blood (PB) mononuclear cells from patients with MN (n=22) and HD (n=10), using a BD FACSDiscover S8 cytometer.

Results

BM CD8+ T cells showed significantly increased expression of 4-1BB, HLA-DR, PD-1, TIGIT and granzyme-B (GZMB) in MN compared to HD. PD-1+TIGIT+ cells displayed increased expression of BLIMP1 and LAG-3, suggesting an exhausted phenotype. Activated tumor-reactive CD69+4-1BB+ cells were enriched in MN BM, with increased inhibitory receptor expression.

Conclusion

CD8+ cells in MN BM displayed a dysfunctional phenotype, characterized by enhanced activation and exhaustion, potentially contributing to the impaired anti-tumor response.

The impact of DNase I and LMW heparin on systemic lupus erythematosus on renal inflammation, immune complex deposition, and tubular epithelial responses in imiquimod-induced NZB/W-F1 mice and RPTEC/hTERT1 cells

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Systemic lupus erythematosus (SLE) is an autoimmune disease marked by production of anti-dsDNA autoantibodies and immune complex (IC) deposition, particularly in the kidneys, leading to lupus nephritis (LN). DNase I degrades extracellular DNA, and low molecular weight (LMW) heparin is suggested to support DNase I function and reduce IC formation. The aim was to investigate whether treatment with DNase I and/or LMW heparin influenced extracellular DNA availability, IC deposition, and renal inflammation in SLE. Lupus-prone NZB/W F1 mice with imiquimod (IMQ)-induced LN were analyzed by hematological analysis, ELISA, immunohistochemistry, qPCR, immuno-electron microscopy, and radial diffusion assay. Parallel in vitro experiments used 3D RPTEC/hTERT1 cultures to model kidney tubular epithelial cell involvement in LN progression.

IMQ induced lupus-like symptoms in NZB/W mice, including spleen enlargement, anemia, and thrombocytopenia. IMQ and treatments caused autoantibody formation. IgG deposition was observed in glomerular and mesangial ultrastructure across all groups, with reduced tubulointerstitial IgG deposition in DNase I– and combination-treated mice. Urinary DNase I activity was increased while serum activity decreased in all treated groups. Gene expression of FcγRIIb and TGF-β1 was reduced in treated mice, suggesting ongoing immune dysregulation. In vitro, RPTEC/hTERT1 cells responded to inflammatory and IMQ stimuli, and 3D cultures showed successful polarization and a diverse inflammatory profile.

Although DNase I and LMW heparin reduced tubulointerstitial IgG deposition, they did not prevent overall disease progression or anti-dsDNA production. The findings suggest a potential synergistic effect but highlight the complexity of LN pathogenesis. The RPTEC/hTERT1 model confirmed that 3D cultures are physiologically relevant for future investigations.

Exploring the blood-thymus barrier in human thymic organ culture

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Aim:

Allogenic hematological stem-cell transplantation (alloHSCT) is a curative treatment of hematological cancers but requires heavy preconditioning regime that destroys the malignant cell line and bystander immune cells and harms other tissues. De novo T-cell reconstitution after alloHSCT depends on the function of thymus. However, ageing, the precondition regime of alloHSCT and its complications, such as graft-versus-host disease and viral reactivations, may further damage the thymic epithelium. A special characteristic of the thymic cortex are endothelial tight junctions and tissue-resident macrophages that considered to form a blood-thymus barrier (BTB) and protect the cortex form blood-borne molecules. BTB has been demonstrated in mouse but it existence in human remains unestablished.

Methods:

We use pediatric human thymus samples obtained from open-heart surgery to develop a thymus organ culture model. A vessel in a thymic lobe is identified and cannulated of a vessel. A peristaltic pump provides the tissue continuous circulation in a culture chamber maintaining its viability without disrupting the physiological barriers and microanatomy.

Results:

First, we use intravascular tracers of different molecular weights to demonstrate the presence of BTB in human thymus. Second, we identify its molecular and cellular components using immunohistochemistry and electron microscopy. Third, we challenge the organ culture with anti-thymocyte globulin to mimic the damage related to preconditioning regime before HSCT.

Conclusion:

Our results allow refining a system to study and manipulate human thymic function in various conditions.

Germline-Encoded TCR Segments Shape allergen-specific CD4⁺ T Cell Responses

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T cells recognize antigens through highly diverse cell-surface receptors known as T cell receptors (TCRs). While TCR specificity is largely attributed to the random insertion of non-templated nucleotides within the complementarity-determining region 3 (CDR3), the contribution of genetically encoded TCR elements to antigen recognition remains poorly defined. We hypothesize that loss of a single germline allele can substantially alter immune responses to allergens and pathogens.

To address this, we characterized the CD4⁺ T cell response to house dust mite (HDM) allergen extract using MHC class II I-A^b tetramers presenting the immunodominant Der $p1_{117–127}$ peptide. Single-cell RNA sequencing revealed a consistent bias in TCR usage, with selection of Trbv31-expressing T cells across multiple mice. We validated that Der $p1_{117–127}$ peptide-restricted T cells expressed Vβ14, encoded by the Trbv31 gene, across multiple HDM experiments. Moreover, the biased expansion of Vβ14⁺ tetramer⁺ CD4⁺ T cells could be recapitulated following intraperitoneal immunization with Der $p1_{117–127}$ and lipopolysaccharide (LPS); this indicates that the peptide antigen selectively induces the activation and expansion of a very limited selection of T cell clones that primarily express Vβ14.

Further work on Trbv31-deficient mice will determine whether this biased response is essential or whether compensatory TCR repertoires can emerge in its absence. These findings raise the possibility that variation or loss of specific germline TCR alleles could meaningfully alter immune responsiveness to allergens and pathogens, potentially explaining inter-individual differences in disease susceptibility.

Integrated Strategies for Producing Functional Recombinant Proteins to Advance Autoimmune Disease Therapeutics

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Aim

Autoimmune drug discovery depends on recombinant proteins that retain native conformation, oligomeric state and biological activity. However, complex cytokines and receptors are often difficult to produce because of structural instability and heterogeneous assembly. We aimed to establish an integrated protein-engineering workflow for generating functional autoimmune therapeutic targets, using IL-17A/IL-17F and TL1A as representative proteins.

Methods

Structure-guided design, computational modelling and sequence comparison were combined with optimized expression and purification processes. IL-17A and IL-17F were co-expressed, and linker length was optimized according to the spatial relationship between their terminal regions to stabilize heterodimer formation. For TL1A, structural comparison with TNF-α and site-directed cysteine mutagenesis were applied to regulate trimerization and reduce nonspecific oligomerization. Protein identity and homogeneity were assessed using SDS-PAGE, HPLC and SEC-MALS. Functional activity and ligand binding were evaluated by ELISA and biolayer interferometry.

Results

The engineered IL-17A/IL-17F construct formed a stable heterodimer and retained ligand-binding and biological activity. Initial TL1A preparations displayed a heterogeneous equilibrium of monomeric, trimeric and higher-order oligomeric states. Structure-guided cysteine modification stabilized the desired trimeric assembly and generated a more uniform, monodisperse protein population.

Conclusion

Combining structural analysis, targeted protein engineering, process optimization and application-specific validation can overcome key production challenges associated with complex autoimmune targets. This integrated workflow enables reliable production of high-purity, bioactive recombinant proteins and may support mechanistic research, antibody discovery and the development of therapeutics for autoimmune and inflammatory diseases.

Abstract 129 *Exhibitor*

Optimizing LNP-Encapsulated IVT mRNA for Next-Generation Cancer Immunotherapies

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Lipid nanoparticle (LNP)-encapsulated in vitro-transcribed (IVT) mRNA has become increasingly popular for the development of cancer immunotherapies, including chimeric antigen receptor T cell therapy (CAR-T) and therapeutic cancer vaccines. Compared with traditional delivery systems, such as viral vectors, IVT mRNA offers enhanced safety and efficacy and has shown great promise in numerous preclinical and clinical trials. However, challenges remain, as the inherent instability of mRNA can compromise both manufacturability and expression levels, while targeted and efficient delivery can be difficult to achieve. Here, we demonstrate the power of a rational design approach, optimizing mRNA and LNP formulations to enhance stability, expression levels, and delivery. Importantly, we highlight the implications for therapeutic development, demonstrating that optimized LNP-mRNA enhances both ex vivo CAR-T and in vivo anti-tumor vaccine applications. Overall, these results underscore the potential of optimized LNP-encapsulated IVT mRNA as a versatile platform for developing safer and more effective cancer immunotherapies.

Abstract 130 *Exhibitor*

Clonal-seq enables high-throughput identification of proliferating immune clonotypes using the BD rhapsody™ system

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Identifying antigen-responsive T-cell clonotypes remains challenging due to low precursor frequencies and limited sensitivity of conventional single-cell TCR/BCR sequencing workflows. Here, we describe Clonal-seq, a single-cell multiomic workflow enabled by the microwell-based BD Rhapsody™ _Single-Cell Analysis System, which allows immune cell stimulation, culture, visualization, and sequencing library preparation to be performed directly within the same cartridge.

Clonal-seq leverages on-cartridge stimulation and multi-day culture to selectively enrich proliferating, antigen-responsive T-cell clones prior to sequencing. Using the BD Rhapsody™ _Scanner, cell survival and clonal expansion can be directly visualized during culture, providing immediate confirmation of functional responses. Expanded clonotypes exhibit increased RNA content and enrichment of activation- and cell-cycle–associated gene expression, enabling clear distinction between proliferating clones and non-responding single cells.

Compared with standard TCR/BCR workflows, Clonal-seq substantially increases the detection of diverse expanded clonotypes while reducing the total number of cells required for analysis. By pairing full-length TCR sequences with transcriptomic profiles in a multiomic context, Clonal-seq enables high-throughput functional immune screening and sensitive identification of clonotypes associated with specific stimuli. This approach is particularly well suited for studies of immune activation, antigen specificity, and clonal dynamics in translational and discovery immunology research.

Building a Better Cell Therapy Potency Assay: High-Throughput, Multiplex Approach to Characterize CAR-T and CAR-NK Cell Protein Secretion Profiles

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Chimeric antigen receptor (CAR) T and natural killer (NK) cell therapies require robust potency assays to ensure therapeutic efficacy and regulatory compliance[1]. Currently, most potency assays measure interferon-gamma (IFN-γ) secretion following overnight co-culture of CAR-T or CAR-NK cells with antigen-expressing target cells. Regulatory agencies are increasingly calling for deeper characterization of immune cell function to better define potency and therapeutic relevance[2].

We developed a high-throughput immunoassay workflow combining Luminex® multiplex screening capabilities with the fast, high sensitivity quantification provided by the automated Ella Simple Plex™ system to characterize the secretomes of CD19-CAR-T and CAR-NK cells. Primary human CD4+ and CD8+ T cells or NK cells from three healthy donors were engineered using the non-viral TcBuster™ transposon system.

Primary human T and NK cells from healthy donors were edited on the MaxCyte® platform, expanded, and co-cultured with $CD19^+$ Nalm-6 target cells. Supernatants were analyzed for 123 analytes using R&D Systems Luminex assays, revealing distinct cytokine and effector protein profiles between CAR-T and CAR-NK cells, including donor-specific heterogeneity. Key findings were then validated utilising Simple Plex assays on Ella™, demonstrating strong cross-platform correlation ($R^2 \geq 0.92$) and enabling a more sensitive quantification of low-abundance proteins such as perforin.

This study demonstrates an effective immunoassay workflow for characterizing the complex secretomes of activated CAR-T and CAR-NK cell. Together the Luminex and Simple plex technologies deliver a streamlined approach supporting the transition from broad discovery to focused, rapid, highly sensitive multi-analyte potency assays, offering a more holistic measure of immune cell function. The workflow is adaptable for diverse CAR targets and facilitates deeper characterization of cell therapy mechanisms of action.

Literature:

[1] Simon, C.G. et al. Mechanism of action, potency and efficacy: considerations for cell therapies J Transl Med. 2024 May 2;22(1):416. doi: 10.1186/s12967-024-05179-7.

[2] Potency assay in FDA approved CAR-T products. Food and Drug Administration. Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products. 2011. Available at: https://www.fda.gov/media/79856/download. Accessed 17 September 2025.

Abstract 132 *Exhibitor*

Large-scale protein–disease risk association analysis in the UK Biobank

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Aim

Large-scale proteomics offers opportunities to identify circulating biomarkers associated with future disease risk. We aimed to systematically characterize prospective protein–disease associations across a broad range of diseases using the UK Biobank Pharma Proteomics Project (UKB-PPP), and to make the resulting resource freely available to the research community.

Methods

Baseline plasma protein levels measured using the Olink Explore platform were analyzed in more than 50,000 UK Biobank participants. For 106 disease outcomes, incident cases occurring within 10 years after sampling were identified using hospital records, cancer registries and death registries. Cox proportional hazards models were used to assess associations between approximately 3,000 proteins and disease risk, adjusting for relevant demographic and lifestyle covariates.

Results

The analysis revealed extensive heterogeneity in the number and strength of protein associations across diseases. Several proteins showed broad associations across multiple outcomes, suggesting potential roles as general morbidity markers, while others displayed highly disease-specific patterns consistent with established biology. Examples included associations of growth differentiation factor 15 (GDF15) and WAP four-disulfide core domain 2 (WFDC2) with multiple disease outcomes. Overall, the results provide a systematic map of circulating protein levels associated with subsequent disease development.

Conclusion

This large-scale prospective analysis provides a resource for investigating disease biology, prioritizing candidate biomarkers and cross-referencing findings from proteomic studies. The complete results are freely available through Olink Insight, enabling researchers to explore protein–disease risk associations across 106 disease outcomes.

A Simple Workflow Produces Calibrated MFIs Consistent Between Non-Harmonized Spectral Flow Cytometers

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Calibration is increasingly important for ensuring reproducible and comparable flow cytometry data across instruments.

This study evaluated Antibody Binding Capacity (ABC) calibration across multiple Cytek spectral flow cytometers using CD4-stained cells and an 8-color TBMNK kit.

Data were acquired on Aurora Evo, Aurora, Northern Lights, and Aurora CS systems and calibrated using microCal ABC calibration beads.

Following calibration, fluorescence measurements were within 15% across instruments and gain settings for nearly all fluorochromes.

These results demonstrate that ABC calibration can provide consistent, biologically relevant fluorescence measurements across different Cytek spectral flow cytometers, independent of laser configuration or detector gain.

Mapping the Protein Interactome of Single Immune Cells using the Proximity Network Assay

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[3]Karolinska Institutet, Solna, Sweden

Background

The spatial distribution of cell surface proteins and the complexes they form are vital to their own function and communication with their environment. Conventional high-resolution microscopy is limited to low plex and few cells, and omics techniques such as CITE-seq, although high plex, reveal no spatial information.

Aims

DNA Microscopy was first discussed as a way of understanding molecular identity and distribution at high plex and high resolution in biological samples some 5 years ago, but had not been applied to proteins until 2023. Here we present data from a high plex assay (155/156 plex) and its use to characterise the cell surface protein interactome of CAR-T and other immune cells at high resolution, 1000 cells at a time.

Methods

The assay enables high-throughput mapping of the dynamic protein interactome of the cell surface of single cells. With nanoscale resolution averaging 50nm, individual proteins are connected together to form a spatial network of the location of cell surface targets. Using Pixelgen's open source Pixelator software, we have produced heatmaps for quantitative visualisation of protein colocalization and clustering, and reconstructed "images" of individual cells.

Results

We demonstrate changes in protein clustering on cell-cell interaction, and show how an apparent upregulation of ICAM1 on CAR-T cells after incubation with target cells is due to trogocytosis and not native upregulation.

Summary/Conclusion: The high plex panel used here is widely targeted to PBMCs and could be used in several applications, and we believe it can complement and enhance current techniques used from early research to clinic, and could provide a key potential biomarker of the future.

Abstract 135 *Exhibitor*

Precision in Every Color: Comprehensive Human Immune Profiling with an Optimized 46-Color Spectral Flow Panel

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Deep cell characterization is essential for unraveling the complexity of immune responses and driving progress in immunology. BioLegend's latest fluorophore innovations and extensive antibody portfolio unlock the full potential of conventional and spectral flow cytometry. Here, we present a 46-color panel designed for comprehensive characterization of human immune cell populations. We highlight approaches for streamlining complex panel design while maintaining data quality, accuracy, and reproducibility, and demonstrate how integrated tools and resources can support efficient assay development and accelerate discoveries in immunology, health, and disease.