Inflammation and Immune Dysregulation in Inborn Errors of Immunity

39th API ANNUAL MEETING

together with the 16th Meeting of the
Working Party Pediatric Immunology
of the German Society of Immunology (DGfI)

MAY 4th – 6th 2023

KLOSTER BANZ, BAD STAFFELSTEIN

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Invited Speakers

Petter Brodin (Stockholm/London)
Ulrich Baumann (Hannover)
Stephan Ehl (Freiburg)
Michael Lenardo (Bethesda)
Min-Ae Lee-Kirsch (Dresden)
Jo Spencer (London)
Holm Uhlig (Oxford)
Antigoni Triantafyllopoulou (Berlin)

Meeting Chair

Henner Morbach
Universitätsklinikum Würzburg

Congress Organization

b4c solutions

DGfI German Society for Immunology

ARBEITSGEMEINSCHAFT PÄDIATRISCHE IMMUNOLOGIE

Welcome

Dear API-members, colleagues and friends,

we are very delighted to invite you to the API annual meeting 2023.

Inborn errors of immunity are genetic disorders with broad clinical manifestation, ranging from susceptibility to infections to significant immune dysregulation. Chronic inflammation is a common paradigm in these diseases, which may result from impaired immune responses to pathogens or elicited as a primary symptom of the disease itself. Chronic inflammation is also the common symptom of many patients with inborn errors of immunity who may be cared for in different subspecialties aside clinical immunology (e.g. rheumatology, gastroenterology, pulmonology etc.). This is why we have chosen “Inflammation and immune dysregulation in inborn errors of immunity” as the main theme for this year’s API meeting.

We have invited distinguished keynote speakers who will present latest findings on immune dysregulation and (auto)inflammation in the context of impaired or disturbed immunity.

As in the past years we are looking forward to your active participation and exciting presentation of your most recent clinical, translational and basic research findings.

We are pleased to welcome you to the beautiful “Kloster Banz” in Bad Staffelstein for an inspiring and interactive 39th annual API meeting.

Kind regards,

PD Dr. Henner Morbach

Meeting Chair

Prof. Dr. Stephan Ehl

API Chairman

General information

Date

May 4th – 6th 2023

Venue

Bildungszentrum Kloster Banz
Kloster-Banz-Straße
96321 Bad Staffelstein
Germany

Scientific Society

Arbeitsgemeinschaft Pädiatrische Immunologie (API) e.V.
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Scientific Organizer

Henner Morbach
Kinderklinik und Poliklinik
Universitätsklinikum Würzburg

Organizing Committee

Johannes Dirks
Jonas Fischer

Congress Organization

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Congress language

Congress language is English.

Gefördert durch
DFG Deutsche Forschungsgemeinschaft

Program

Thursday, May 4th

8:30 – 12:00 **Pre-meeting: Educational Workshop**

Rita Beier (Hannover), Christian Klemann (Leipzig),
Henner Morbach (Würzburg), Catharina Schütz (Dresden)

12:00 – 12:45 **Registration**

12:45 – 13:00 **Welcome address**

13:00 – 15:00 **Session 1: Immune dysregulation I**

Chairs: Catharina Schütz (Dresden), Manfred Hönig (Ulm)

13:00 – 13:40 **JAK/STAT signaling in inborn errors of immunity**
Stephan Ehl (Freiburg)

13:40 – 13:55 (10'+5') Genome-wide CRISPR screening approach to study molecular mechanisms of human lymphocyte cytotoxic function
Artem Kalinichenko (Wien)

13:55 – 14:10 (10'+5') Polymorphism or risk allele? *PRF1* A91V *in trans* with a "severe" *PRF1* mutation
Oliver Wegehaupt (Freiburg)

14:10 – 14:25 (10'+5') Chronic active EBV Infection – immune dysregulation beyond "monogenetics"
Sujal Ghosh (Düsseldorf)

14:25 – 14:40 (10'+5') Diagnostic evaluation of pediatric autoimmune lympho-proliferative primary immunodeficiencies: the AL-PID study
Pauline Hägele (Freiburg)

14:40 – 14:50 (6'+4') Ruxolitinib in primary hemophagocytic lymphohistiocytosis
Seraina Prader (Zürich)

14:50 – 15:00 (6'+4') Late diagnosis of atypical HLH-5 with a homozygous *STXBP2* splice site mutation
Mirjam Völler (Berlin)

15:00 – 15:30	**Coffee break and Poster Session 1**
15:30 – 17:25	**Session 2: Immune dysregulation II** *Chairs: Jana Pachlopnik Schmid (Zürich), Fabian Hauck (München)*
15:30 – 16:10	**Gene identification in congenital immune disorders leads to precision therapies** ***Michael Lenardo (Bethesda)***
16:10 – 16:25 (10'+5')	Development of a disease activity score to assess treatment success in patients with *NFKB1* variants *Katharina Thoma (Freiburg)*
16:25 – 16:40 (10'+5')	Integrated multi-omics analyses of *NFKB1* patients B cells points towards an up regulation of NF-kB network inhibitors *Nadezhda Camacho-Ordonez (Freiburg)*
16:40 – 16:55 (10'+5')	Interim analysis of safety and hematological parameters of an ongoing long-term open-label extension study of leniolisib *Catharina Schütz (Dresden)*
16:55 – 17:10 (10'+5')	A *STAT1* variant beyond the gain- or loss-of function dichotomy offers an opportunity for targeted intervention *Anna Wolfers (Freiburg)*
17:10 – 17:25 (10'+5')	Epigenetic immune cell quantification for diagnosis and monitoring of patients with inborn errors of immunity *Bodo Grimbacher (Freiburg)*
17:30 – 18:00	**Meeting of the Working Party Pediatric Immunology (DGfI)**
18:30	**Dinner**

Friday, May 5h

9:00 – 10:30		**Session 3: Immune development** *Chairs: Sybille Landwehr-Kenzel (Hannover), Christian Klemann (Leipzig)*
9:00 – 9:40		**Systems-level analyses of human immune system development** ***Petter Brodin (Stockholm/London)***
9:40 – 9:55	(10'+5')	Immune dysregulation in children with complex heart disease *Anna Gieras (Hamburg)*
9:55 – 10:10	(10'+5')	A novel biallelic *LCK* variant resulting in profound T-cell immune deficiency *Anna-Lisa Lanz (München)*
10:10 – 10:20	(6'+4')	*BCL11B* mutations and immunodefiency *Lea Graafen (Düsseldorf)*
10:20 – 10:30	(6'+4')	Peculiarities of T cell numbers and function in neonates – implications for SCID confirmatory testing *Jonas Fischer (Würzburg)*
10:30 – 11:00		**Coffee break and Poster Session 2**
11:00 – 12:30		**Session 4: B cell Immunology** *Chairs: Shahrzad Bakhtiar (Frankfurt), Horst von Bernuth (Berlin)*
11:00 – 11:40		**Human B cells in mucosal space and time** ***Jo Spencer (London)***
11:40 – 11:55	(10'+5')	Sequencing the B cell receptor repertoires of antibody-deficient individuals with and without infection susceptibility *Bodo Grimbacher (Freiburg)*
11:55 – 12:10	(10'+5')	A multimorphic mutation in *IRF4* causes human autosomal dominant combined immunodeficiency *Ulrich Pannicke (Ulm)*

12:10 – 12:20	(6'+4')	Development of mature B cells despite lack of B cell receptor expression in a family with a complex *IGHM* variant *Johannes Dirks (Würzburg)*
12:20 – 12:30	(6'+4')	λ5 deficiency – newborn screening gives insight into a rare immunodeficiency *Maarja Soomann (Zürich)*

12:30 – 13:30 **Lunch**

13:30 – 15:00 **Session 5: Mucosal Inflammation I**

Chairs: Kaan Boztug (Wien), Gregor Dückers (Krefeld)

13:30 – 14:10		**A taxonomy of monogenic inflammatory bowel disease – implications for precision medicine** ***Holm Uhlig (Oxford)***
14:10 – 14:25	(10'+5')	Sceening for common inborn errors of immunity in patients with very early onset inflammatory bowel disease *Vasil Toskov (Freiburg)*
14:25 – 14:40	(10'+5')	Patients with CTLA-4 insufficiency have distinct intestinal microbiome signatures *Máté Krausz (Freiburg)*
14:40 – 14:50	(6'+4')	Transcriptome analysis of regulatory T cells from *CTLA4* mutation carriers *Sara Posadas Cantera (Freiburg)*
14:50 – 15:00	(6'+4')	Regulatory T cell subset in LRBA deficient patients and the effect of abatacept treatment on natural Treg *Shahrzad Bakhtiar (Frankfurt)*

15:00 – 15:30 **Coffee break and Poster Session 3**

15:30 – 17:00	**Session 6: Mucosal Inflammation II** *Chairs: Maria Faßhauer (Leipzig), Leif Hanitsch (Berlin)*
15:30 – 16:10	**How to manage bronchiectasis and interstitial lung disease in inborn errors of immunity** ***Ulrich Baumann (Hannover)***
16:10 – 16:25 (10'+5')	Establishment of an animal model to investigate pulmonary injury and repair in STAT3-hyper-IgE syndrome *Verena Häfner (München)*
16:25 – 16:40 (10'+5')	ARPC5 deficiency leads to severe early onset systemic inflammation and early mortality *Elena Sindram (Freiburg)*
16:40 – 16:50 (6'+4')	Functional analysis of *TCF3* variants in patients with Primary Antibody Deficiency *Aishwarya Saxena (Freiburg)*
16:50 – 17:00 (6'+4')	Targeted treatment approaches: biologic treatment in monogenic inborn errors of immunity with Th2-type-inflammation *Julia Körholz (Dresden)*
17:00 – 19:00	**API members' general assembly**
19:00	**Dinner**

Saturday, May 6th

9:00 – 10:30 **Session 7: Autoinflammation**

Chairs: Ursula Holzer (Tübingen), Markus Seidel (Graz)

9:00 – 9:40 **Type I-interferonopathies**
Min Ae Lee-Kirsch (Dresden)

9:40 – 9:55 (10'+5') A novel hereditary autoinflammatory disorder due to defective isoprenoid biosynthesis
Jakob Berner (Wien)

9:55 – 10:10 (10'+5') Type I interferonopathy due to a truncating *RELA* mutation
Timmy Strauß (Dresden)

10:10 – 10:20 (6'+4') The recessive inheritance of STING-associated vasculopathy with onset in infancy
Sandra von Hardenberg (Hannover)

10:20 – 10:30 (6'+4') Optical Genome Mapping – a promising method for the detection of disease-causing structural variants in inborn errors of immunity
Isabel Klefenz (Hannover)

10:30 – 11:00 **Coffee break**

11:00 – 12:30 **Session 8: Innate immunity and inflammation**

Chairs: Almut Meyer-Bahlburg (Greifswald), Sujal Ghosh (Düsseldorf)

11:00 – 11:40 **Macrophage differentiation in chronic inflammatory diseases**
Antigoni Triantafyllopoulou (Berlin)

11:40 – 11:55 (10'+5') Extended clinical phenotype and treatment modalities in thirty JAGN1 deficient patients - an ESID/EBMT IEWP multi-center study
Julia Fekadu (Frankfurt)

11:55 – 12:10 (10'+5') CGD patient cohort analysis: an update
Myriam Lorenz (Ulm)

12:10 – 12:20 (6'+4') Lack of Efficacy of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) in Patients with JAGN1 Deficiency

Susan Farmand (Hamburg)

12:20 – 12:30 (6'+4') A forward rescue mutation in *ELANE* can revert the phenotype of severe congenital neutropenia

Svea Böhm (Hamburg)

12:30 – 12:45 Farewell

Poster Session 1

Chair: Kai Lehmberg (Hamburg)

Unraveling the molecular causes of polyautoimmunity including alopecia areata as a common feature
Buket Basmanav (Bonn)

IKAROS-deficiency in father and son with progressive B-cell lymphopenia and hypogammaglobulinemia
Renate Krüger (Berlin)

IKAROS associated disease as rare differential diagnosis of small vessel vasculitis and hypogammaglobulinaemia
Gregor Dückers (Krefeld)

Rapidly evolving anti-drug antibodies in a ADA2-deficient patient
Leif Hanitsch (Berlin)

EBV-positive mucocutaneous ulcers in a patient with combined immunodeficiency
Geraldine Engels (Würzburg)

Poster Session 2

Chair: Carsten Speckmann (Freiburg)

Heterozygous pathogenic *FOXN1* variant causes nail dystrophy and low CD8+ T cells
Olga Staudacher (Berlin)

HSCT in a two-year-old with IL2RB-Defect
Ommo Mauss (Ulm)

Compound heterozygous *DOCK8* mutation - time for transplantation?
Ursula Holzer (Tübingen)

A novel *SIK3* mutation presenting with combined immune deficiency (CID) in the context of severe skeletal dysplasia
Andrea Meinhardt (London)

Identification of a New Variant in *RFXAP* in a Patient with Hypomorphic Bare Lymphocytes Syndrome and Short Stature
Sybille Landwehr-Kanzel (Hannover)

Poster Session 3

Chair: Helmut Wittkowski (Münster)

A Toolkit for Monitoring Immunoglobulin G Levels from Dried Blood Spots of Patients with Primary Immunodeficiencies

Bodo Grimbacher (Freiburg)

The ABACHAI clinical trial protocol: Safety and Efficacy of abatacept (s.c.) in patients with CTLA-4 insufficiency or LRBA deficiency – establishment of a disease-specific scoring system

Máté Krausz (Freiburg)

A 10-year old girl with X-linked CGD presenting with polyarthritis and cheilitis granulomatosa

Nina-Christine Knopf (Dresden)

Gastric Adenocarcinoma due to immunodysregulation and chronic gastritis

Marcus Jakob (Regensburg)

Severe autoinflammatory syndrome/ CINCA with pathogenic de novo mutation in the NLRP3 gene with prenatal anemia with intrauterine blood transfusion, Ascites, generalized perinatal edema and massive splenomegaly

Anna Raab (Hannover)

Thursday, May 4th

13:00 – 15:00	**Session 1: Immune dysregulation I** *Chairs: Catharina Schütz (Dresden), Manfred Hönig (Ulm)*
13:00 – 13:40	JAK/STAT signaling in inborn errors of immunity *Stephan Ehl (Freiburg)*
13:40 – 13:55 (10'+5')	Genome-wide CRISPR screening approach to study molecular mechanisms of human lymphocyte cytotoxic function *Artem Kalinichenko (Wien)*
13:55 – 14:10 (10'+5')	Polymorphism or risk allele? *PRF1* A91V *in trans* with a "severe" *PRF1* mutation *Oliver Wegehaupt (Freiburg)*
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14:25 – 14:40 (10'+5')	Diagnostic evaluation of pediatric autoimmune lympho-proliferative primary immunodeficiencies: the AL-PID study *Pauline Hägele (Freiburg)*
14:40 – 14:50 (6'+4')	Ruxolitinib in primary hemophagocytic lymphohistiocytosis *Seraina Prader (Zürich)*
14:50 – 15:00 (6'+4')	Late diagnosis of atypical HLH-5 with a homozygous *STXBP2* splice site mutation *Mirjam Völler (Berlin)*

Genome-wide CRISPR screening approach to study molecular mechanisms of human lymphocyte cytotoxic function

Jakob Huemer[1,2,3,*], Artem Kalinichenko[1,2,3,*], Matthias Haimel[1,2,3], Celine Prakash[1], Maximilian von der Linde[1], Julia Pazmandi[7], Cheryl van de Wetering[1], Anton Kamnev[4], Sarah Giuliani[1], Martin Jäger[2], Elisa Hahn[2], Christina Rashkova[1], Birgit Höger[1,2,3],Georg Winter[2], Loic Dupre[4], Kaan Boztug[1,2,3,5,6]

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Human lymphocyte-mediated cytotoxicity against malignant and virus-infected target cells is fundamental to immune defense. It requires a tightly controlled molecular program to avoid collateral damage to healthy cells. Impairments in this process underly a group of diseases with dramatic hyperferritinemic inflammation termed hemophagocytic lymphohistiocytosis (HLH). Although genetic and functional studies of HLH have identified proteins controlling distinct steps of CG exocytosis, the molecular mechanisms that spatiotemporally coordinate CG release are only partially understood. To study the molecular machinery controlling regulated exocytosis in human lymphocytes and predict potential genetic factors predisposing to severe diseases like HLH we have developed an unbiased approach based on genome-scale screening using an NK-92 model cell line. Degranulation assays performed with STX11, UNC13D, and RAB27A KO NK-92 cells confirmed that the severity of exocytosis and killing defects recapitulate natural genetic defects observed in patient-derived primary NK cells, validating this model cell line for further functional and mechanistic studies. Using this cell line for the genome-wide loss-of-function screen we have discovered a complex network of genes from distinct pathways essential for exocytosis. We have further validated selected pathways using conventional molecular biology approaches and performed a mechanistic investigation of their molecular role in exocytosis.

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Institute name: St Anna Children's Cancer Research Institute

Polymorphism or risk allele? PRF1 A91V *in trans* with a "severe" PRF1 mutation

Oliver Wegehaupt[1,2], Oleg Borisov[3], Kai Lehmberg[4], Florian Oyen[4], Jasmin Mann[1], Despina Moshous[5], Geneviève de Saint Basile[5], Kimberly Gilmour[6], Wenying Zhang[7], Rebecca Marsh[7], Sharon Choo[8], Gillian Griffiths[9], Anna Köttgen[3], and Stephan Ehl[1]

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[7]Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA.

[8]Department of Allergy and Immunology, The Royal Children's Hospital, Melbourne, VIC, Australia.

[9]Cambridge Institute for Medical Research, University of Cambridge, Cambridge Biomedical Campus, UK.

Background: FHL2 is caused by mutations in PRF1. Null mutations lead to absent cytotoxicity and predispose to HLH. Hypomorphic variants allow residual cytotoxicity and can cause later onset of HLH, neuroinflammation or lymphoma. PRF1 A91V, carried by 4% of the population leads to reduced perforin expression and cytolytic activity, but does not affect health or longevity even in homocygocity. Uncertainty remains whether this is also true if A91V pairs with a loss-of-function PRF1 allele ("X"). Since FHL2 is an attractive target for newborn screening, we study the clinical significance of the A91V/X constellation.

Methods: (1) We recruit A91V/X individuals through our networks and literature; (2) We gather epidemiological data on A91V/X carriers using the UK Biobank; (3) We investigate the functional consequences of A91V/X for cytotoxicity.

Results: Perforin expression in A91V/X lymphocytes is reduced variably depending on the "X". Consequences for cytotoxicity remain to be evaluated. Of 29 PRF1 A91V/X individuals reported by the HLH network, 20 were identified because of FHL-2 related symptoms (12 HLH, 5 neuroinflammation, 4 lymphoma) with variable disease onset (mean 23.5y; range: neonatal–52y). Surprisingly, all 9 individuals identified by family screening of early-onset FHL2 patients were asymptomatic (mean 36.3y; range: 4-81y). In the UK Biobank, we identified 15/9691 A91V/X carriers among individuals with splenomegaly/cytopenia, neuroinflammation or lymphoma versus 414/ 460106 among control individuals (OR 1.72; p=0.058).

Conclusions: Our preliminary data indicate that PRF1 A91V/X does not significantly predispose to FHL2-related disease manifestations. Completion of this study will help guide clinical decision-making in A91V/X individuals.

Chronic active EBV Infection – immune dysregulation beyond "monogenetics"

Stavrieta Soura[1], Benjamin Fournier[2,3], Anne-Laure Roupie[3], Prasad T Oommen[1], Maximilian Seidl[4], Hans-Jürgen Laws[1], Fabian Hauck[5], Arndt Borkhardt[1], Bénédicte Neven[2], Sylvain Latour[3], Sujal Ghosh[1]

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Background: Chronic active Epstein-Barr virus (CAEBV) infection is a rare EBV-related disorder, in which the virus usually manifests with T and NK lymphoproliferation. Besides prolonged infectious mononucleosis-like symptoms, patients suffer from hydroa vacciniforme, hypersensitivity to mosquito bites, hemophagocytic lymphohistocytosis, or NK/T cell malignancies. Most patients described are of East Asian or Latin American origin, however monogenetic causes are only detected in a minority of cases.

Methods: We report of a 17-year-old boy of non-consanguineous Moroccan descent who developed ulcerative skin infections (lower arms / legs) at the age of 6 years. These were associated with mosquito bites and were paralleled by fever and malaise. At age 14, acne-like facial lesions were accompanied by further facial swelling. The patient described fatigue and muscle weakness.

Results: Blood investigations revealed highly elevated creatinine kinase, LDH and IgE levels. Blood count showed mild leukopenia (with T and B lymphopenia) and platelets on the lower end. EBV-PCR was performed on sorted lymphocyte DNA and revealed viral DNA in NK and T cells. Similarly, EBER Flow-FISH showed significant proportions of circulating EBER+ NK cells. Biopsy of inflamed muscle parts and epiglottis showed EBV associated T-cell infiltration. Exome sequencing did not identify a causative monogenetic defect.

Conclusion: The clinical course of CAEBV is heterogenous and unpredictable. Our case reflects the therapeutic dilemma in patients without suitable donor. Given the poor prognosis of a possible T/NK malignancy and severe current EBV manifestations a MMRD-SCT has to be discussed.

Diagnostic evaluation of pediatric autoimmune-lymphoproliferative primary immunodeficiencies: the AL-PID study

Pauline Hägele[1], Paulina Staus[2], Raphael Scheible[2], Annette Uhlmann[1], Maximilian Heeg[1], Christian Klemann[3], Sarah Salou[4], Julian Thalhammer[4], Oliver Wegehaupt[1,4], Vasil Toskov[4], Maria Elena Maccari[1,4], Miriam Gross[1], Myriam Ricarda Lorenz[5], Klaus Schwarz[5,6], Martin Wolkewitz[2], Carsten Speckmann[4], Henrike Ritterbusch[1], Anne Rensing-Ehl[1]*, Stephan Ehl[1]*, and the AL-PID consortium

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Background: Lymphoproliferation and autoimmune cytopenia characterize autoimmune-lymphoproliferative syndrome (ALPS). Other conditions sharing these manifestations have been termed “ALPS-like”, although they frequently have a more severe disease course. Better definition of the genetic landscape and the clinical and immunological features of these disorders may improve diagnostic algorithms and benefit rational disease classification.

Methods: We performed a 12-year prospective study on 431 pediatric patients referred for ALPS evaluation with lymphoproliferation and autoimmune cytopenia (n=236), lymphoproliferation and another sign of PID (n=148), or autoimmune cytopenia and sign of PID (n=47). ALPS biomarkers were determined in all, while sequencing sufficient to establish or largely exclude currently defined diseases (PID panels, WES or WGS) was performed in 230 patients.

Results: ALPS was diagnosed in 71 patients with sFASL as most predictive biomarker. Fifty-four patients had mostly autosomal-dominant autoimmune-lymphoproliferative PID (AD AL-PID) affecting CTLA4/LRBA or JAK/STAT, PI3K, NFkB or RAS signaling. Nineteen had rare other IEI, 17 had other diagnoses and 79 had no diagnosis despite extended genetics. Multivariate clustering of clinical and laboratory manifestations did not discriminate the latter from AD AL-PID patients, but they had later onset and 80% were male. Re-classification of patients fulfilling CVID or Evan’s syndrome criteria did not increase the proportion of genetic diagnoses.

Conclusion: ALPS clearly separates from similar conditions that frequently show more complex disease manifestations. We propose to classify these diseases as autoimmune-lymphoproliferative primary immunodeficiencies (AL-PID) instead of “ALPS-like”. This “umbrella” term is useful as 50% currently remain without genetic diagnosis despite extensive overlap with defined IEI.

RUXOLITINIB IN PRIMARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Seraina Prader[1], Maarja Soomann[1], Johannes Trück[1], Tayfun Güngör[3] and Jana Pachlopnik Schmid[1,2]

(1) Division of Immunology, University Children's Hospital Zurich, Zurich, Switzerland (2) Pediatric Immunology, University of Zurich, Zurich, Switzerland (3) Division of Stem Cell Transplantation, University Children's Hospital Zurich, Zurich, Switzerland

Background: Ruxolitinib, a Janus kinase (JAK) 1/2 inhibitor, has shown promising results in patients with primary and secondary hemophagocytic lymphohistiocytosis (HLH).

Method: We describe four infants with primary HLH treated with ruxolitinib, two as second-line monotherapy in palliative situations, two as part of a modified HLH-2004 protocol as bridging therapy to curative hematopoietic stemcelltransplantation (HSCT).

Results: P1 presented with 3 months with a first HLH episode due to homozygous pathogenic variant in PRF1. Despite control of systemic HLH with methylprednisolone, alemtuzumab and ciclosporin A (CSA), concomitant CNS-HLH progressed to severe encephalopathy with seizures. Because of her impaired neurologic status HSCT was not pursued. Nevertheless, treatment with ruxolitinib was initiated and clinical course was stable for 5 months.

P2 presented with HLH-like disease due to compound heterozygous pathogenic variants in ZNFX1. Initial treatment with corticosteroids, CSA and etoposide, followed by antithymoglobulin had limited success and clinical course was complicated by renal failure. Salvage treatment with ruxolitinib was initiated and partial remission was achieved for 7 weeks.

P3 and P4 were diagnosed with primary HLH due to homozygous pathogenic variants in PRF1 and UNC13D respectively. HLH was triggered by CMV infection in one of the patients. Remission without viral reactivation could be achieved in both with a modified HLH-2004 protocol with ruxolitinib instead of CSA with aimed area under the curve (AUC) of 700 ng.h/ml.

Conclusion: We describe four patients with severe forms of primary HLH who showed a stabilization of clinical courses under mono- or combination treatment with ruxolitinib. Ruxolitinib should therefore be further evaluated as effective replacement for CSA or as monotherapy the treatment of HLH.

Late diagnosis of atypical HLH-5 with a homozygous *STXBP2* splice site mutation

Mirjam Völler[1], Sarah Dinges[1], Uwe Kölsch[2], Anna Stittrich[3], Olga Staudacher[1,2], Nadine Unterwalder[2], Christian Meisel[2,4], Horst von Bernuth[1,2,5,6]

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Background: Familial hemophagocytic lymphohistiocytosis (HLH) is a group of genetic disorders characterized by uncontrolled activation of the immune system leading to inflammation and tissue damage. HLH-5 is caused by mutations in the *STXBP2* gene encoding the protein munc 18-2, which is essential for exocytosis of cytotoxic granules, thus, immune cell function. Symptoms of familial HLH-5 include recurrent fever, splenomegaly, cytopenia, colitis, bleeding disorders, and sensorineural defects.

Methods: We report about a 32-year-old woman with recurrent fever, chronic diarrhea, pancytopenia, splenomegaly, lymphadenopathy, a history of severe EBV-infection, and eczema. A comprehensive medical assessment including whole exome sequencing was conducted, while continuing pre-existing immunosuppressive therapy with cyclosporine.

Results: Clinical chemistry was remarkable for elevated liver enzymes and increased levels of soluble interleukin 2 receptor (>2400 U/mL) and ferritin (>500 ng/mL). Flow cytometry revealed a significant expansion of NK and CD8+ T cells in peripheral blood with strong skewing towards an effector memory phenotype. CD107 expression on NK cells upon stimulation with K562 cells was low normal with partial rescue upon IL-2 stimulation. Bone marrow analysis showed trilineage hematopoiesis with pronounced T cell infiltration, without hemophagocytosis. Next-generation sequencing revealed a homozygous *STXBP2* splice site mutation (c.1247-1G>C) in exon 15 which is known to cause hypomorphic HLH-5.

Conclusions: Our patient highlights the broad spectrum of clinical manifestations and diagnostic challenges of HLH-5. Patients with splice site mutations in *STXBP2* typically present with later onset and milder course of disease. We advised the patient to consider an allogenic stem cell transplantation for definite cure.

Thursday, May 4th

15:30 – 17:25	**Session 2: Immune dysregulation II**
	Chairs: Jana Pachlopnik Schmid (Zürich), Fabian Hauck (München)
15:30 – 16:10	Gene identification in congenital immune disorders leads to precision therapies *Michael Lenardo (Bethesda)*
16:10 – 16:25 (10'+5')	Development of a disease activity score to assess treatment success in patients with *NFKB1* variants *Katharina Thoma (Freiburg)*
16:25 – 16:40 (10'+5')	Integrated multi-omics analyses of *NFKB1* patients B cells points towards an up regulation of NF-kB network inhibitors *Nadezhda Camacho-Ordonez (Freiburg)*
16:40 – 16:55 (10'+5')	Interim analysis of safety and hematological parameters of an ongoing long-term open-label extension study of leniolisib *Catharina Schütz (Dresden)*
16:55 – 17:10 (10'+5')	A *STAT1* variant beyond the gain- or loss-of function dichotomy offers an opportunity for targeted intervention *Anna Wolfers (Freiburg)*
17:10 – 17:25 (10'+5')	Epigenetic immune cell quantification for diagnosis and monitoring of patients with inborn errors of immunity *Bodo Grimbacher (Freiburg)*

Development Of A Disease Activity Score To Assess Treatment Success In Patients With NFKB1 Variants

Katharina Thoma[1], Pia Hassunah[1], Manfred Fliegauf[1], Andres Caballero Garcia de Oteyza[1], Pavla Mrovecova[1], Helene Kraus[1], Marlon Grodd[2], Prerana Agarwal[3], Maximilian Frederik Russe[3], Birgit Sawitzki[4], Nadezhda Camacho Ordonez[1], Aleksandra Hirsch[1], Christoph Geier[1], Mate Krausz[1], Sigune Goldacker[1], Stephan Ehl[5], Sarah Salou[6], Ansgar Schulz[7], Nina Brauer[8], Shahrzad Bakhtiar[9], Maria Fasshauer[10], Oliver Hausmann[11,12], Anja-Dorothee Hüfner[13], Martin Stanulla[14], Anna Partheil[14] Georgios Sogkas[15], Ulrich Salzer[1], Klaus Warnatz[1], Bodo Grimbacher[1]

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Background: NFKB1 encodes for the p105/p50 nuclear factor-kappa-B (NF-kB1) transcription factors. Heterozygous mutations in NFKB1 may lead to NF-kB1 insufficiency, which may result in a multiorgan disease. Here, we aimed to develop a Disease Activity Score to assess treatment success in patients with NF-kB1 insufficiency.

Methods: Based on eight publications on 230 NFKB1 patients with 62 distinct NFKB1 variants, characteristic organ manifestations of NFKB1 insufficiency were identified. With consideration of established scores and clinical expert knowledge, 43 parameters representing 13 disease manifestations were included in a first score draft. Clinical data from 40 patients with damaging NFKB1 variants was collected at 1-6 time points to calculate the score. Using a leave one out analysis, less relevant score parameters were identified.

Results: With a cut-off differential value of 3,24 the leave one out analysis identified the 20 most relevant score parameters: IgG, IgA, IgM and sIL2 receptor level, number of lymphocytes and CD4+ T cells, percentage of CD4+CD45RA+ T cells, HLA-DR+ CD4+ cells, HLA-DR+ CD8+ cells and switched memory B cells, spleen size, number of non-respiratory and respiratory infections, antibiotic courses to treat respiratory infections, need for therapy of non-respiratory infections, MRC Scale value, need for therapy of skin manifestation and gastrointestinal manifestation, percentage of affected skin, stool frequency and quality. Considering all collected data, three score parameters were adapted: i) chronic infections, ii) liver manifestations, and iii) a novel CT scoring algorithm.

Conclusion: At the meeting we will present for the first time an adapted NFKB1 Disease Activity Score (NFKB1-DAS).

Integrated Multi-omics Analyses of NFKB1 patients B cells points towards an up regulation of NF-κB network inhibitors

Nadezhda Camacho-Ordonez[1,2,3], Neftali Ramirez[1,2*], Sara Posadas-Cantera[1,2*], Andrés Caballero-Oyteza[1,2], Manfred Fliegauf[1,2,4], Fangwen Zhao[5], Guarini Maria[5], Gernedl Victoria[5], Mateo Pecoroaro[6], Klaus Warnatz[1,2,7], Christoph Bock[5,8,9], Esteban Ballestar[10], Roger Geiger[6,11], Michele Proietti[1,2,12,13], and Bodo Grimbacher[1,2,4,7,13,14].

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Background: The transcription factor NF-κB plays a pivotal role in the adaptive immune response. Pathogenic variants in NFKB1 are the most common genetic etiology of common variable immunodeficiency (CVID). Patients frequently present with impaired terminal B cell differentiation, autoimmunity, and hyperinflammatory immune dysregulation. NF-κB signaling and target gene expression are expected to be dysregulated in NFKB1-mutated patients.

Methods: Here, we performed a multi-omics characterization of B cells from a cohort of clinically affected and unaffected NFKB1 mutation carriers.

Results: Our analysis identified specific epigenetic dysregulation and gene expression differences on B cells from NFKB1-mutated patients. We observed an aberrant expression of negative regulators of NF-κB signaling in NFKB1 mutation carriers, which may be a key factor for the autoinflammatory phenotype of these patients. Moreover, our analysis points towards a dysregulation of XBP1 and BCL3, key players of B cell activation and proliferation at different stages of B cell differentiation. The reduced expression of negative regulators of the NF-κB network is likely to be one of several mechanisms responsible for the aberrant NF-κB signaling, which impairs the maintenance of a normal humoral immune response.

Conclusion: Our findings highlight epigenetic and gene expression changes in B cells associated with NFKB1 mutations. Our data give insight into future therapeutic opportunities for patients with NFKB1 (haplo)insufficiency.

Interim Analysis of Safety and Hematological Parameters of an Ongoing Long-Term Open-Label Extension Study of Investigational PI3Kδ Inhibitor Leniolisib for Patients with Activated PI3K Delta Syndrome (APDS)

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Background Variants in the *PI3Kδ* genes cause the activated PI3Kδ syndrome (APDS) with frequent infections, lymphoproliferation, enteropathy, autoimmune cytopenias and increased risk of lymphoma. We previously reported results from a 12-week randomized placebo-controlled trial of 31 patients with APDS (Rao VK, *et al. Blood* 2023). Here, we describe interim outcomes from the ongoing open label, single arm, long-term extension study (NCT02859727).

Methods 37 patients with APDS aged ≥12 years were enrolled globally; 20 patients have been on leniolisib for nearly 2, and 5 patients for 5 years. The primary objective was to evaluate long-term safety of leniolisib. Spleen and index lymph node size were measured at extension days (ED) 168 or 252. Presence of lymphoma, cytopenias and infections were also assessed.

Results Lymph node size decreased with a mean change from baseline in the sum of product diameters of index nodes (cm^2) of -7.73 (SD of baseline 6.47) on ED168, and -11.70 (15.97) on ED252. Spleen 3D volume (cm^3) also decreased: ED168, -198.48 (SD of baseline 124.23); ED252, -236.64 (165.20). 3 patients with a history of B-cell lymphoma remained in remission. There was a statistically significant decrease in infection rates under leniolisib, despite a concomitant reduction in immunoglobulin replacement therapy usage. As for autoimmune cytopenias, 17/31 improved or resolved. Leniolisib was overall well tolerated; no serious adverse events were suspected related to lenolisib.

Conclusion Long-term PI3Kδ pathway modulation with leniolisib administration was well tolerated in patients with APDS, with continued improvement in lymphoproliferation and cytopenias and no recurrence of lymphoma.

A STAT1 variant beyond the gain- or loss-of function dichotomy offers an opportunity for targeted intervention

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Background: Immune cells fine-tune their responses to infection to avoid uncontrolled inflammation. STAT1 is a central signalling molecule engaged by receptors of type I and type II Interferons to exert their antimicrobial and immunomodulatory function. Human genetic deficiency (loss of function) of STAT1 results in susceptibility to severe viral and mycobacterial infection, while increased STAT1 activity (gain of function) leads to hyperinflammation. Here, we identified a rare novel patient mutation that does not follow this simple LOF/GOF paradigm and illustrates an important layer in the molecular fine-tuning of interferon signalling.

Methods: We worked with patient-derived T and EBV B cells and with STAT1-deficient U3C cells reconstituted with WT or mutated STAT1 alleles. The different cell systems were used to study STAT1 expression levels, phosphorylation kinetics, transcription of target genes, DNA binding and antiviral response.

Results: A 4 year-old boy presenting with disseminated mycobacterial infection was diagnosed with a homozygous germline STAT1 I248F mutation. The mutation results in 50-fold reduction of STAT1 protein and poor transcription of interferon-stimulated genes (ISG) early after type I IFN stimulation. Unexpectedly, late after stimulation ISG production was excessive and sufficient to induce an antiviral response. We did not observe these effects of late upregulation after stimulation with type II IFN. Consistently, the patient had uneventful seroconversion to at least 5 different viruses. However, he did develop rubella granulomas in the brain.

Mechanistically, reduced STAT1 expression alone was not sufficient to reproduce this phenotype of excessive late pathway activation. Instead, we found that STAT1 I248F specifically impairs desensitization of the type I IFN pathway by mechanisms unrelated to known pathway regulation by USP-18, resulting in continuous IFNα signalling in the patient. This was associated with severe autoinflammatory episodes that could paradoxically be controlled by the JAK inhibitor ruxolitinib, successfully used to treat STAT1 GOF disorders.

Conclusions: This study shows that a simple GOF and LOF classification does not cover the complexity of STAT1 protein function, its regulation and associated clinical phenotypes with important consequences for therapy.

Epigenetic Immune Cell Quantification for Diagnosis and Monitoring of Patients with Inborn Errors of Immunity

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Purpose: Early detection and monitoring of patients with Inborn Errors of Immunity (IEI) requires quantitative determination of their cellular immune system, from fresh blood analyzed by flow cytometry. However, epigenetic immune cell quantification allows cell profiling from fresh, frozen, or dried blood. Here, we demonstrate the utility of epigenetic immune cell quantification compared to flow cytometry for patients with IEI.

Methods: Using an *in vitro* diagnostic test for epigenetic quantification of T-, B- and NK lymphocytes, 259 whole blood samples of patients with IEI were analyzed and compared to flow cytometric data. Furthermore, we extended the immune cell panel to regulatory T cells (Treg), Th17 cells, Tfh cells, PD-1+ cells, CCR6+ cells and memory B cells. In addition, epigenetic immune cell profiling was compared between venous EDTA and capillary dried blood (self-sampling).

Results: Spearman correlation of > 0.9 was observed for T- and B- lymphocyte populations and a robust correlation of 0.73 for NK cells. Extended epigenetic immune cell profiling showed quantitative trends in patients grouped into primary antibody defects, other primary immunodeficiencies, and secondary immunodeficiency. However, individual epigenetic immune cell profiles varied substantially within these groups. Epigenetic analysis of dried blood was equivalent to EDTA blood and non-distinguishable between professional- and self-sampling.

Conclusion: Epigenetic immune cell quantification is suitable for broad immune cell profiling in patients with IEI. The approach allows self-collection of a dried blood spot (DBS) sample, which enables patient management over long distances and in cases where venous blood drawing is difficult or not possible.

Friday, May 5h

9:00 – 10:30		**Session 3: Immune development** *Chairs: Sybille Landwehr-Kenzel (Hannover),* *Christian Klemann (Leipzig)*
9:00 – 9:40		Systems-level analyses of human immune system development *Petter Brodin (Stockholm/London)*
9:40 – 9:55	(10'+5')	Immune dysregulation in children with complex heart disease *Anna Gieras (Hamburg)*
9:55 – 10:10	(10'+5')	A novel biallelic *LCK* variant resulting in profound T-cell immune deficiency *Anna-Lisa Lanz (München)*
10:10 – 10:20	(6'+4')	*BCL11B* mutations and immunodefiency *Lea Graafen (Düsseldorf)*
10:20 – 10:30	(6'+4')	Peculiarities of T cell numbers and function in neonates – implications for SCID confirmatory testing *Jonas Fischer (Würzburg)*

Immune dysregulation in children with complex congenital heart disease

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Background: Congenital heart disease (CHD) is the most common birth defect worldwide affecting about 1 % of newborns. Patients with CHD suffer from frequent infections and have an increased risk of infant morbidity and mortality. Recently, lower levels of T cell receptor excision circles (TRECs) have been observed in newborns with CHD. This suggests that CHD negatively affects early T cell development with possible long-term health consequences.

Methods: We investigated T cell development and clinical profile in a cohort of 58 CHD patients who underwent early-life thymectomy during heart surgery. Patients were categorized according to the complexity of their CHD. To evaluate the relationship between CHD complexity and thymic output we have analyzed different maturation stages of thymocytes, recent thymic emigrants (RTEs) and a series of biomarkers that are associated with either cardiac defects, stress or inflammation.

Results: Our analysis revealed a unique thymocyte signature in CHD patients who suffered from complex CHDs. We observed elevated cortisol levels in all patients, while increased levels of NT-proBNP and the inflammatory cytokine IL-6 were associated with thymic atrophy in children with highly complex CHDs. Furthermore, those children showed a decreased thymic output characterized by reduced levels of RTEs.

Conclusions: Our findings revealed that highly complex CHDs are associated with thymic atrophy. Further studies are needed to investigate whether these immunological alterations are transient or if there is a causal connection of CHD and immune deviations that might lead to an increased risk of developing immune-mediated diseases.

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A novel biallelic LCK variant resulting in profound T-cell immune deficiency

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Lymphocyte-specific protein tyrosine kinase (LCK) is an SRC-family kinase critical for initiation and propagation of T-cell antigen receptor (TCR) signaling through phosphorylation of TCR-associated CD3 chains and recruited downstream molecules.

Until now, only one case of profound T-cell immune deficiency with complete LCK deficiency caused by a biallelic missense mutation (c.1022T>C, p.L341P) and three cases of incomplete LCK deficiency caused by a biallelic splice site mutation (c.188-2A>G) have been described. Additionally, deregulated LCK expression has been associated with genetically undefined immune deficiencies and hematological malignancies.

Here we describe the second case of complete LCK deficiency in a 6 months old girl born to consanguineous parents presenting with profound T-cell immune deficiency. Whole exome sequencing (WES) revealed a novel pathogenic biallelic missense mutation in LCK (c.1393T>C, p.Cys465Arg), which led to absence of LCK protein expression and phosphorylation, and consecutive decrease in proximal TCR signaling. Loss of conventional CD4+ and CD8+ αβT-cells and homeostatic T-cell expansion was accompanied by increased γδT-cell and Treg percentages. Surface CD4 and CD8 co-receptor expression was reduced in the patient T-cells, while the heterozygous mother had impaired CD4 and CD8 surface expression to a lesser extent.

We conclude that complete LCK deficiency is characterized by profound T-cell immune deficiency, reduced CD4 and CD8 surface expression, and a characteristic TCR signaling disorder. CD4 and CD8 surface expression may be of value for early detection of mono- and/or biallelic LCK deficiency.

BCL11B mutations and immunodeficiency

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Background: The transcription factor encoded by BCL11B is essential for development and functionality of CNS, skin and immune system. Facial dysmorphism, neurodevelopmental disorders, atopic manifestations and T cell abnormalities have been described in patients harboring mutations in BCL11B. However, it remains challenging to predict the clinical course and give standardized treatment recommendations.

Methods: We report three cases of patients with heterozygous BCL11B mutations in Germany. Clinical data was collected retrospectively from three large pediatric immunological departments.

Results: All observed patients are female and now 5 months, 3.5 years and 8.5 years of age (patient 1-3). Patients 1 and 2 were detected by TREC newborn screening; severe T and B cell lymphopenia was subsequently confirmed by flow cytometry confirmatory testing. Patient 1 showed a remarkable increase in T and B cell counts at the age of six weeks and has not required any further treatment yet. Due to persistent severe lymphopenia Patient 2 received a matched-unrelated-donor HSCT at the age of 4 months and cell counts normalized several months post-HSCT. Patient 3 presented with combined immunodeficiency, as well as allergic inflammation of the respiratory and gastrointestinal tract, which responded to anti-IL4Rα treatment. Global developmental delay and dystonic movement disorders were observed in patients 2 and 3. Despite the favorable clinical course, psychomotor development of patient 1 still needs to be observed.

Conclusions: BCL11B mutations can be detected by TREC newborn screening due to postnatal severe T cell lymphopenia. Immunological courses can differ highly, and so far, no standardized treatment recommendations exist.

Peculiarities of T cell numbers and function in neonates – implications for SCID confirmatory testing

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Background: Confirmatory testing of newborns with abnormal results in the T-cell receptor excision circle (TREC)-based newborn screening (NBS) for severe combined immunodeficiencies (SCID) involves assessment of T cell numbers and function. In approximately 20% of abnormal NBS results SCID diagnosis is confirmed. Interpreting the other 80 % remains a challenge, since age-adapted reference values for T cell subsets in neonates are not yet established.

Methods: Lymphocyte subpopulations were analysed in 68 healthy neonates/infants (age 0 - 50 days) by flow cytometry. Additionally, we compared T cell function between neonates and adult controls by assessing expression of activation markers and proliferation following TCR stimulation as well as analysing the cytokine expression profile of T cells.

Results: Absolute T cell numbers increase considerably during the first 50 days of life with doubling of the mean T cell counts within the first 10 days of life. Compared to adults, neonatal CD4+ and CD8+ T cells showed higher proliferation rates and increased expression of activation markers upon TCR stimulation. Proliferating CD4+ T helper cells were capable of expression of cytokine upon re-stimulation with higher levels of IL-8 and similar levels of IL-2, TNF-α and IFN-γ compared to adult controls.

Conclusion: T cell activation and proliferation upon TCR stimuli differs between neonates and adults with lower TCR activation thresholds and increased proliferation capabilities of neonatal T cells. High variance and age-dependency of neonatal T cell subsets must also be considered when interpreting TREC-NBS confirmatory testing results, particularly for those infants with Non-SCID T cell lymphopenia.

Friday, May 5h

11:00 – 12:30	**Session 4: B cell Immunology** *Chairs: Shahrzad Bakhtiar (Frankfurt), Horst von Bernuth (Berlin)*
11:00 – 11:40	Human B cells in mucosal space and time *Jo Spencer (London)*
11:40 – 11:55 (10'+5')	Sequencing the B cell receptor repertoires of antibody-deficient individuals with and without infection susceptibility *Bodo Grimbacher (Freiburg)*
11:55 – 12:10 (10'+5')	A multimorphic mutation in *IRF4* causes human autosomal dominant combined immunodeficiency *Ulrich Pannicke (Ulm)*
12:10 – 12:20 (6'+4')	Development of mature B cells despite lack of B cell receptor expression in a family with a complex *IGHM* variant *Johannes Dirks (Würzburg)*
12:20 – 12:30 (6'+4')	λ5 deficiency – newborn screening gives insight into a rare immunodeficiency *Maarja Soomann (Zürich)*

Sequencing the B cell receptor repertoires of antibody-deficient individuals with and without infection susceptibility

Yoong Wearn Lim[1,*], Neftali Jose Ramirez[2,3,*], Michael A. Asensio[1], Yao Chiang[1], Gabriele Müller[2,3], Pavla Mrovecova[2,3], Noriko Mitsuiki[2,3,4], Máté Krausz[2,3,5], Nadezhda Camacho-Ordonez[2,3,5,6], Klaus Warnatz[3,5], Adam S. Adler[1,^], and Bodo Grimbacher[2,3,5,7,8,9,^]

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Purpose: Most individuals with antibody deficiency (hypogammaglobulinemia) need immunoglobulin replacement therapy (IgG-RT) from healthy plasma donors to stay clear of infections. However, a small subset of hypogammaglobulinemic patients do not require this substitution therapy. We set out to investigate this clinical conundrum by asking whether the peripheral B cell receptor repertoires differ between antibody-deficient patients who do and do not need IgG-RT.

Methods: We sequenced and analyzed IgG and IgM heavy chain B cell receptor repertoires from peripheral blood mononuclear cells (PBMCs) isolated from patients with low serum IgG concentrations who did or did not require IgG-RT.

Results: Compared to the patients who did not need IgG-RT, those who needed IgG-RT had higher numbers of IgG antibody clones, higher IgM diversity, and less oligoclonal IgG and IgM repertoires. The patient cohorts had different heavy chain variable gene usage, and the patients who needed IgG-RT had elevated frequencies of IgG clones with higher germline identity (i.e., fewer somatic hypermutations).

Conclusion: Antibody-deficient patients with infection susceptibility who needed IgG-RT had more diverse peripheral antibody repertoires that were less diverged from germline and thus may not be as optimal for targeting pathogens, possibly contributing to infection susceptibility.

Keywords: Antibody repertoire sequencing, hypogammaglobulinemia, infection susceptibility

A multimorphic mutation in IRF4 causes human autosomal dominant combined immunodeficiency

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Background: Interferon regulatory factor 4 (IRF4) is a transcription factor and key regulator of immune cell development and function.

Methods: To determine disease causing genetic variants whole-exome and Sanger sequencing of patient and family members' DNAs was performed. A plethora of cellular and molecular methods (e.g. flow cytometry, CyTOF, RNA-seq, ChIP-seq and EMSA) were used to characterize the immunological phenotype of the patients and to reveal the underlying pathomechanism of their diseases.

Results: We report a recurrent heterozygous mutation in IRF4, p.T95R, causing an autosomal dominant combined immunodeficiency (CID) in 7 patients from 6 unrelated families. The patients exhibited profound susceptibility to opportunistic infections, notably Pneumocystis jirovecii, and presented with agammaglobulinemia. Patients' B cells showed impaired maturation, decreased immunoglobulin isotype switching and defective plasma cell differentiation, whereas their T cells contained reduced Th17 and Tfh populations and exhibited decreased cytokine production. The $IRF4^{T95R}$ variant maps to the transcription factor's DNA-binding domain, alters its canonical DNA-binding specificities, and results in a simultaneous multimorphic combination of loss-, gain- and new-functions for IRF4. $IRF4^{T95R}$ behaved as hypermorph by binding to DNA with higher affinity than $IRF4^{WT}$. Despite this increased affinity for DNA, the transcriptional activity on IRF4 canonical genes was reduced, showcasing a hypomorphic activity of $IRF4^{T95R}$. Simultaneously, $IRF4^{T95R}$ functions as neomorph by binding to noncanonical DNA sites to alter the gene expression profile, including the transcription of genes exclusively induced by $IRF4^{T95R}$ but not by $IRF4^{WT}$.

Conclusions: An IRF4 mutation with multimorphic impacts on DNA-binding specificity and activity reveals a new disease-causing mechanism in humans.

Development of mature B cells despite lack of B cell receptor expression in a family with a complex *IGHM* variant

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INTRODUCTION Expression of a functional B cell receptor (BCR) is considered essential for B cell development and survival of mature B cells. Additionally, BCR signaling strength is involved in B cell fate decision and – at least in mice – weak, intermediate and strong BCR signals are linked to the development of marginal zone, follicular and B1 B cells, respectively. Agammaglobulinemia in humans is mainly caused by genetic defects of components of the (pre)BCR or its signaling cascade and loss of a functional BCR-complex in these patients, results in the absence or severe reduction of mature B cells in the periphery

RESULTS We identified three children with agammaglobulinemia from a consanguineous family of Kurdish origin without a history of immunodeficiency. Whole genome sequencing of all family members (Bavarian Genomes project) revealed a complex structural variant in the *IGHM* locus (Chr14:g.[106312683_106322504dup; 106320626_106322627del]) compatible with *IGHM* deficiency, which segregated with the disease in a homozygous state. Analysis of bone marrow B cell development revealed a partial block at the pro-B to pre-B transition. The patients displayed reduced but not absent numbers of peripheral blood B cells without evidence of memory B cell formation. BCR expression was completely absent on all of the B cells and B cells did not respond to IgM but to CD40 or TLR9 triggering. Of note, peripheral blood and bone marrow B cells showed normal expression of the remaining components of the (pre-)BCR signaling complex (e.g. CD79A and CD79B, CD19, λ5, VpreB). Strikingly, circulating B cells displayed a phenotype of mature naïve B cells and histological analysis of intestinal biopsies showed formation of B cell follicles with regular architecture. Remarkably, the majority of mature B cells expressed $CD45RB^{MEM\text{-}55}$ assigning the cells to the marginal zone developmental pathway. Furthermore, with increasing age the patients developed a significant proportion of B cells with a $CD21^{lo}CD38^{-}CD11^{+}$ phenotype challenging the essential role of BCR signals in the differentiation of these B cells.

CONCLUSION These patients appear to be a suitable model to analyze B cell development and differentiation trajectories in humans in the absence of ligand-induced BCR triggering events. Exclusive tonic BCR signaling impinges on B cell development but may be sufficient for differentiation into mature B cells. Interestingly, B cell differentiation seems to be skewed towards the marginal zone B cell trajectory without additional ligand-induced BCR triggering.

λ5 deficiency – newborn screening gives insight into a rare immunodeficiency

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Background: Since the introduction of newborn screening (NBS) using the combined quantification of T-cell receptor excision circles and kappa-deleting recombination excision circles (KREC), several patients have been diagnosed with λ5 deficiency, a rare autosomal-recessive form of agammaglobulinemia. We present the largest case series to date to describe the clinical and immunological phenotype and its development over the first years of life.

Methods: Contacts from countries with NBS programmes using the combined screening method were approached. Data on patients with genetically confirmed λ5 deficiency were systematically collected between August and November 2022.

Results: Eight patients were identified. Detailed data was available in five with a median follow-up of 23 months. All patients had unmeasurable or very low B-cell, IgM and IgA levels but normal IgG levels (maternal) in their initial investigations. IgG levels fell below the age-appropriate reference at the median age of 5 months in all patients. Some IgA production was seen in all patients. All but one patient showed transient spikes of IgM production. CD19+ B-cell counts varied between 0 and 30/µL of peripheral blood at 3 to 4 weeks, showed some increase but remained low throughout the entire follow-up period. All patients remained well without relevant infections under regular immunoglobulin substitution therapy.

Conclusions: NBS programmes quantifying KREC can identify patients with λ5 deficiency. Our series highlights the variable immunophenotype of this condition with residual B-cell counts and function indicating that genetic analysis of IGLL1 should be considered also in older patients with hypogammaglobulinemia and reduced number of B-cells.

Friday, May 5h

13:30 – 15:00	**Session 5: Mucosal Inflammation I** *Chairs: Kaan Boztug (Wien), Gregor Dückers (Krefeld)*
13:30 – 14:10	A taxonomy of monogenic inflammatory bowel disease – implications for precision medicine *Holm Uhlig (Oxford)*
14:10 – 14:25 (10'+5')	Sceening for common inborn errors of immunity in patients with very early onset inflammatory bowel disease *Vasil Toskov (Freiburg)*
14:25 – 14:40 (10'+5')	Patients with CTLA-4 insufficiency have distinct intestinal microbiome signatures *Máté Krausz (Freiburg)*
14:40 – 14:50 (6'+4')	Transcriptome analysis of regulatory T cells from *CTLA4* mutation carriers *Sara Posadas Cantera (Freiburg)*
14:50 – 15:00 (6'+4')	Regulatory T cell subset in LRBA deficient patients and the effect of abatacept treatment on natural Treg *Shahrzad Bakhtiar (Frankfurt)*

Sceening for common inborn errors of immunity in patients with very early onset inflammatory bowel disease: a case-report illustrating a single referral center experience

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Background and aims: There is increasing awareness that inborn errors of immunity (IEI) can present as isolated very early onset inflammatory bowel disease (VEO-IBD). However, data on the diagnostic detection rate of known IEI in an unselected group of patients are limited. We present the case of a VEO-IBD patient diagnosed with XIAP deficiency. The case is discussed in the context of a cohort of further VEO-IBD patients referred for immunological evaluation to our immunological diagnostic laboratory over a 10-year period.

Methods: We examined the immunological screening results of 125 patients (78 males, 47 females) with VEO-IBD. The recommended diagnostic algorithm included lymphocyte subsets with naïve T cells and neutrophil oxidative burst in females, supplemented by percentage of FOXP3+ regulatory T cells and XIAP expression in males. L-18 MDP stimulation was used as a confirmatory test for suspected XIAP deficiency. LPS stimulation in untreated versus IL-10 pretreated monocytes was recommended in children presenting under 6 months of age or with fistulating disease.

Results: Among 125 patients, the proposed diagnostic algorithm was followed in 40,8%, while in other patients investigations remained incomplete. A compatible genetic diagnosis was established in 7 patients: 3 had XIAP deficiency, 2 IPEX and 2 IL-10R deficiency. The index case showed a lack of intracellular XIAP protein expression and reduced TNF production to L18-MDP. XIAP deficiency was confirmed by genetic testing. During the same period, we diagnosed 14 patients with CGD, 19 patients with XIAP deficiency, 4 patients with IPEX and 2 patients with IL-10R deficiency, but not in the context of an isolated VEO-IBD presentation.

Conclusion: Our real-life experience shows that of the “hit rate” of a genetic disease for patients referred for immunological evaluation of VEO-IBD is low. However, the diagnosis can have important consequences. In the patient with XIAP deficiency, therapy with infliximab was initiated, but discontinued due to poor clinical response. The patient was subsequently transplanted with hematopoietic stem cells from a matched unrelated donor.

Patients with CTLA-4 insufficiency have distinct intestinal microbiome signatures

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CTLA-4 insufficiency is a monogenetic condition caused by heterozygous mutations in *CTLA4* that is characterized by immune dysregulation, including autoimmune enteropathy. The disease presents with a reduced (~70%) penetrance, so we hypothesized that affected and unaffected *CTLA4* mutation carriers have distinct intestinal microbiome signatures compared to each other and to healthy controls, and that the microbiome may identify patients with disease-related organ involvements.

We collected stool samples and clinical data from healthy donors (HDs, n=178), affected (n=33) and unaffected CTLA-4 patients (n=8) and performed 16S rRNA sequencing. We also compared samples from patients with and without a history of specific organ involvement (enteropathy, splenomegaly, lymphadenopathy, GLILD).

Affected patients had a significantly decreased alpha-diversity (Shannon), compared to unaffected carriers and HDs. Moreover, CTLA-4 patients with a history of specific organ involvement had decreased alpha-diversity (not significant). Additionally, we identified significantly different taxa in affected and unaffected CTLA-4 mutation carriers. We found that the Proteobacteria were significantly enriched in affected patients, compared to unaffected carriers and controls. Furthermore, we could identify various taxa that are the main drivers of the differences between affected and unaffected mutation carriers. In affected individuals Veillonella, Escherichia, and Haemophilus were enriched, in unaffected individuals Ruminococcacease, Tenericutes, and Lachnospiraceae were expanded. Some of these taxa are known to be correlated with inflammatory bowel disease.

Here we show, that affected *CTLA4* mutation carriers have distinct intestinal microbiome structures, and that the microbiome may be a relevant disease modifier. Our results could serve as a basis for further interventional studies.

Keywords: CTLA4, microbiome, disease modifiers

Transcriptome analysis of regulatory T cells from CTLA4 mutation carriers

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Background Heterozygous germline mutations in CTLA4 can lead to CVID and lymphoproliferative and autoimmune manifestations with a penetrance of about 70%. The clinical presentation is highly variable even among individuals carrying the same variant. CTLA-4 deficiency results in an impairment of regulatory T cell function and uncontrolled T cell responses. Studying the transcriptomic dysregulation of regulatory T cells in CTLA4 mutation carriers is a step forward towards a better understanding of the pathogenic mechanisms of the disease.

Methods Regulatory CD4+ T cells from 4 CTLA4 mutation carriers and 4 CTLA4 wild-type carriers were isolated from PBMCs and bulk RNA-sequencing was performed.

Results Regulatory T cells from CTLA4 mutation carriers showed an enrichment of mTORC1 signaling, glycolysis and cell cycle pathways. TCF7 and CXCR5, encoding a typical transcription factor and surface marker of follicular regulatory and T helper cells, were amongst the most significantly upregulated genes, possibly indicating a skewing towards a follicular regulatory T cell phenotype.

Conclusions The enriched pathways in regulatory T cells from CTLA4 mutation carriers could contribute to the impairment of their suppressive function. In line with an expected higher signalling activity of CD28 in CTLA4 mutation carriers, circulating follicular regulatory T cells could be increased in these subjects.

Regulatory T-cell subsets in LRBA deficient patients and the effect of abatacept treatment on natural Treg

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Background: Mutations in the lipopolysaccharide-responsive beige-like anchor (LRBA) gene were identified to cause autoimmunity and immunodeficiency with a broad spectrum of clinical manifestations. A disturbed interplay between LRBA and the CTLA-4 protein due to an impairment of vesicle transendocytosis has been associated with a reduced suppressive capacity of Tregs.

Objective: We sought to investigate an extended Treg profile of patients with LRBA deficiency (N=6) compared to normal values for Treg subpopulations from an age-matched healthy cohort (N=39). We investigated the effect of abatacept treatment in LRBA deficient patients receiving biweekly intravenous infusions (N=6).

Methods: Using a flow cytometric approach with a pre-formulated antibody panel in peripheral blood samples, we analyzed Treg subsets including $CD4^{+}FoxP3^{+}$ Treg, $Helios^{+}$ natural Treg, $Helios^{-}$ induced Treg, $CD39^{+}$ Treg, $CD62L^{+}CD45RA^{+}$ naïve Treg, $CD62L^{+}CD45RA^{-}$ memory Treg, $FoxP3^{hi}CD45RA^{-}$ effector Treg as well as $CD4^{+}$ $CD25^{hi}CD127^{low}$ Treg. Longitudinal data were collected while patients received abatacept.

Results: The LRBA deficient cohort showed a significant alteration in distinct Treg subpopulations, not in the entire Treg population. A significant lack was found in natural Treg, naive Treg, and effector Treg, while memory Teg were significantly increased. abatacept treatment led to a significant increase of natural Treg (P=0.003) in all patients without having a measurable effect on the other subpopulations.

Friday, May 5h

15:30 – 17:00	**Session 6: Mucosal Inflammation II** *Chairs: Maria Faßhauer (Leipzig), Leif Hanitsch (Berlin)*
15:30 – 16:10	How to manage bronchiectasis and interstitial lung disease in inborn errors of immunity *Ulrich Baumann (Hannover)*
16:10 – 16:25 (10'+5')	Establishment of an animal model to investigate pulmonary injury and repair in STAT3-hyper-IgE syndrome *Verena Häfner (München)*
16:25 – 16:40 (10'+5')	ARPC5 deficiency leads to severe early onset systemic inflammation and early mortality *Elena Sindram (Freiburg)*
16:40 – 16:50 (6'+4')	Functional analysis of *TCF3* variants in patients with Primary Antibody Deficiency *Aishwarya Saxena (Freiburg)*
16:50 – 17:00 (6'+4')	Targeted treatment approaches: biologic treatment in monogenic inborn errors of immunity with Th2-type-inflammation *Julia Körholz (Dresden)*

Establishment of an animal model to investigate pulmonary injury and repair in STAT3-hyper-IgE syndrome

Verena Haefner[1,2,3,4], David Kutschke[3], Carola Voss[3,4], Christine Hollauer[3], Christine Wolf[1,2], Ali Önder Yildirim[3,4], Renate Effner[1,2], Ellen Renner[1,2,5,#], Tobias Stoeger[3,4,#], Beate Hagl[1,2,#]

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Background: Various inborn errors of immunity present with chronic pulmonary diseases, including STAT3-hyper-IgE syndrome (STAT3-HIES). STAT3-HIES is caused by heterozygous dominant-negative mutations in the STAT3 gene. STAT3HIES patients suffer from recurrent pulmonary infections leading to tissue destructive changes with pneumatocele formation and restrictive and obstructive lung disease. Little is known about the mechanism underlying pulmonary injury and the development of chronic lung disease in STAT3-HIES.

Objective: We aim to establish a lung injury STAT3-HIES mouse model to investigate molecular and cellular changes after pulmonary challenge.

Methods: We used bacterial lipopolysaccharide (LPS) administration to induce lung injury in a transgenic mouse model (mutStat3) carrying the dominant-negative Stat3 mutation Stat3-ΔV463, causing a STAT3-HIES like immunological phenotype (MGI:J:210877) and compared it to wildtype (wt) animals. We analyzed different parameters (such as diffusion capacity), lung tissue, and bronchoalveolar lavage (BAL) at different time points (1, 3 and 7 days) after LPS application by immunohistochemistry, quantitative PCR (qPCR), cytological staining, and ELISA.

Results: While no differences were observed between untreated wt and mutStat3 mice, LPS administration led to a reduced diffusion capacity, increased tissue inflammation and elevated levels of pro-inflammatory cytokines in BAL fluid in mutStat3 compared to wt animals. Quantification of alveolar epithelial cells revealed significant changes between mutStat3 compared to wt mice after injury.

Conclusions: Taken together, these results of a murine STAT3-HIES model suggest an increased local inflammatory response resulting in a higher susceptibility to pulmonary tissue damage with impaired pulmonary function and deficient epithelial recovery in STAT3-HIES after pulmonary injury.

ARPC5 deficiency leads to severe early onset systemic inflammation and early mortality

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The seven subunit Arp2/3 complex drives the formation of branched actin networks that are essential for many cellular processes including cell migration. In humans, the ARPC5 subunit of the Arp2/3 complex is encoded by two paralogous genes (*APRC5* and *ARPC5L*), resulting in proteins with 67% identity. Genetic mutations in proteins associated with the actin cytoskeleton can be lethal and affect both the innate and adaptive immunity. In 2017, the first actin-related primary immunodeficiency (PID) in the Arp2/3-complex was described: ARPC1B-deficiency. It has a very early clinical onset and patients suffer from recurrent infections, eczema and platelet abnormalities.

Through whole-exome sequencing, we identified a biallelic ARPC5 frameshift variant in a female child who presented with recurrent infections, multiple congenital anomalies, diarrhea, and thrombocytopenia and suffered early demise from sepsis. Her consanguineous parents had a previous child who died with similar clinical features.

To study the impact of the mutation in *ARPC5*, we generated ARPC5 knock-out (KO) cells using the CRISPR/Cas9-system and demonstrate that loss of ARPC5 affects cytoskeleton organization and function, as well as chemokine-dependent cell migration *in vitro*. Moreover, homozygous *ARPC5-/-* mice do not survive past embryonic day 9 due to severe developmental defects, including loss of the second pharyngeal arch which contributes to craniofacial and heart development.
In conclusion, we describe the first patient with a homozygous mutation in the ARPC5 subunit of the Arp2/3-complex and indicate that ARPC5 is important for both prenatal development and postnatal immune signalling, in a non-redundant manner with ARPC5L.

Functional analysis of TCF3 variants in patients with Primary Antibody Deficiency

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Background: Primary Antibody Deficiency (PAD) is the most common type of symptomatic primary immunodeficiency in humans. In most of the patients with PAD, the genetic diagnosis remains undetermined. We performed Whole Exome Sequencing in more than 400 patients with late-onset PAD.

We identified 24 patients with monoallelic variants in the Transcription Factor 3 (TCF3). TCF3 plays a crucial role in lymphopoiesis and especially in B cell development. Patients with homozygous loss-of-function TCF3 variants have shown to develop hypogammaglobulinemia and B-cell acute lymphoblastic leukemia.

Methods: In our study, we will systematically evaluate the functional impact of the TCF3 variants observed in our cohort. We performed class-switch recombination assay by stimulation of the naïve B cells isolated from five patients. We evaluated the percentage of switched B cells upon stimulation with CD40L and IL21 and compared it with the healthy donors. Further, we evaluated gene expression profile by performing quantitative real time PCR (qPCR) for AICDA, a gene that mediates AID induction which a crucial process for class switch recombination.

Results: Our preliminary results show that these patients show reduction in class switch recombination upon in-vitro stimulation of naïve B cells. Furthermore, gene expression profile shows reduction in the levels of AICDA expression in patients harboring variants in TCF3.

Conclusion: We would further perform additional in vitro studies by cloning the variant to functionally characterize the effect of these variants in B cell development and differentiation.

Targeted treatment approaches: biologic treatment in monogenic inborn errors of immunity with Th2-Type-inflammation

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Background: Dupilumab is a monoclonal antibody targeting IL4/13, used for treatment of atopic dermatitis and other inflammatory conditions[1]. In inborn errors of immunity (IEI), systemic manifestations of Th2-type inflammation are not uncommon, especially but not exclusively in Hyper-IgE syndromes[2,3]. Over the past years, biologic treatments have become "state-of-the art" in IEI clinical care[4].

Patients: We report 4 IEI patients from 3 different kindreds with prominent Th2-type inflammation. Patient 1 has classical Hyper-IgE syndrome (*STAT3*LOF) characterized by classic whole-body-eczema and bacterial superinfection besides chronic lung disease. Patients 2 and 3 are siblings diagnosed with CADINS disease (*CARD11*DN) in adulthood. Their atopic features include pronounced chronic eczema, asthma and multiple food allergies. Patient 4 was recently diagnosed with *CARMIL2*-deficiency. Since early childhood, he has been suffering from most severe psoriasiform eczema, erythrodermia, recurrent superinfection in addition to mucocutaneous candidiasis.

Results: All four patients were treated with dupilumab, which markedly improved severe eczema as indicated by decreased SCORAD. During the treatment period no severe side effects have been reported. In one patient, decrease in Th2-type inflammation was documented by intracellular cytokine measurement of IL-4 in CD45RO+ T cells.

Conclusion: Genetic and functional characterization of IEI is essential to identify targeted therapies for selected patients. Dupilumab has proven its efficacy in Hyper-IgE-syndromes[5,6]. We add to these reports, and expand the spectrum of patients in whom this biological has led to clinical response with limited side effects. Dupilumab may be considered as treatment option in IEI patients with Th2-type inflammation.

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Saturday, May 6th

9:00 – 10:30 **Session 7: Autoinflammation**

Chairs: Ursula Holzer (Tübingen), Markus Seidel (Graz)

9:00 – 9:40 Type I-interferonopathies
Min Ae Lee-Kirsch (Dresden)

9:40 – 9:55 (10'+5') A novel hereditary autoinflammatory disorder due to defective isoprenoid biosynthesis
Jakob Berner (Wien)

9:55 – 10:10 (10'+5') Type I interferonopathy due to a truncating *RELA* mutation
Timmy Strauß (Dresden)

10:10 – 10:20 (6'+4') The recessive inheritance of STING-associated vasculopathy with onset in infancy
Sandra von Hardenberg (Hannover)

10:20 – 10:30 (6'+4') Optical Genome Mapping – a promising method for the detection of disease-causing structural variants in inborn errors of immunity
Isabel Klefenz (Hannover)

A novel hereditary autoinflammatory disorder due to defective isoprenoid biosynthesis

Jakob Berner[1-3], Raul Jimenez-Heredia[1,3,4], Johannes G. Weiss[5,6], Alexandra Frohne[1,3], Sarah Giuliani[1,3], Sacha Ferdinandusse[7], Hans R. Waterham[7], Irinka Castanon[1,3], Jürgen Brunner[5,8 #], Kaan Boztug[1-4,9#]

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Background: Hereditary autoinflammatory disorders (AIDs) comprise a heterogenous group of monogenic disorders. Pathogenic variants in Mevalonate Kinase (MVK) have been shown to cause varying degrees of autoinflammatory disorders namingly, mevalonic aciduria (MA), hyper-IgD-syndrome (HIDS) and disseminated superficial actinic porokeratosis (DSAP) depending on the degree of enzyme deficiency. In the isoprenoid biosynthesis pathway, mevalonate is phosphorylated in two subsequent enzyme steps by mevalonate kinase (MVK) and phosphomevalonate kinase (PMVK) to generate mevalonate pyrophosphate that is further metabolized to produce sterol and non-sterol isoprenoids. So far, however, no patients with PMVK deficiency due to biallelic pathogenic variants in the PMVK gene have been reported.

Methods: We performed whole exome sequencing and functional studies in cells from a patient who, upon clinical and immunological evaluation, was suspected of an autoinflammatory disease.

Results: We identified a biallelic homozygous variant in the PMVK gene of the patient (NM_006556.4: c.392T>C, p.Val131Ala). Pathogenicity was confirmed by functional studies in patient cells, which revealed a markedly reduced PMVK enzyme activity due to a virtually complete absence of PMVK protein. Clinically, the patient showed various similarities, but also distinct features compared to MKD patients, and responded well to therapeutic IL-1 inhibition.

Conclusion: In this study we report the first patient with proven PMVK deficiency, including the clinical, biochemical and immunological consequences of a homozygous pathogenic variant in the PMVK gene. PMVK deficiency expands the genetic spectrum of the systemic autoinflammatory diseases (SAID), characterized by recurrent fevers, arthritis and cytopenia and thus should be included in the differential diagnosis and genetic testing for SAIDs.

Type I interferonopathy due to a truncating RELA mutation

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Background: We describe the case of a 7-year-old girl with autoinflammatory symptoms of unknown reason. We investigated the genetic cause and the underlying mechanism driving the disease.

Methods: Expression of IFN-stimulated genes and cytokines was assessed by qRT-PCR in patient PBMCs and fibroblasts. Serum cytokines were measured by FACS. p65 protein was examined by Western blot and immunofluorescence staining.

Results: The patient presented with recurrent fever, panniculitis, arthritis and positive ANA. Genetic analysis identified a heterozygous nonsense mutation in RELA, encoding the NF-κB family member p65. The previously unknown mutation is predicted to lead to a C-terminal truncation of p65 lacking the transactivation domain. The patient exhibited a strong IFN signature in blood accompanied by an elevated secretion of pro-inflammatory cytokines. The asymptomatic father, who also carried the RELA mutation, showed a strong IFN signature and increased cytokine levels. Western blot analysis of patient fibroblasts demonstrated the presence of a truncated p65 protein along with increased STAT1 phosphorylation, favoring constitutive NF-kB activation. In unstimulated patient fibroblasts, cytokine gene expression was downregulated. However, upon TNF-α stimulation, patient cells reacted hyperresponsive. In an overexpression model, truncated p65 was unable to dimerize with p50, the main p65 binding partner. Moreover, truncated p65 constitutively localized in the nucleus both in patient cells and in HEK293 cells.

Conclusion: Our findings indicate that a heterozygous truncating RELA mutation can cause type I IFN-driven autoinflammation and autoimmunity. We hypothesize that interference of mutant p65 with NF-κB signaling pathways poises cellular homeostasis towards constitutive type I IFN activation.

The recessive inheritance of STING-associated vasculopathy with onset in infancy

Rensheng Wan[1], Johannes Fänder[2], Ia Zakaraia[2], Min Ae Lee-Kirsch[3], Christine Wolf [3], Nadja Lucas[3], Lisa Isabel Olfe[1], Corinna Hendrich[1], Danny Jonigk[4], Dirk Holzinger[5], Mathis Steindor[5], Gunnar Schmidt[1], Christian Klemann[5], Nicolaus Schwerk[6], Matthias Griese[7], Brigitte Schlegelberger[1], Florian Stehling[5], Christine Happle[6], Bernd Auber[1], Doris Steinemann[1], Martin Wetzke[6]†*, Sandra von Hardenberg[1]†*

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† These authors contributed equally to this work and share senior authorship

Background: STING1 gain-of-function (GoF) pathogenic variants lead to the rare auto-inflammatory disease STING-associated vasculopathy with onset in infancy (SAVI). Previously, only heterozygous and mostly de novo *STING1* variants have been reported as causative for SAVI. Interestingly, one variant that leads to SAVI only in homozygosity, namely c.841C>T p.(Arg281Trp), has recently been described.

Methods: Whole-exome sequencing and Whole-genome sequencing was performed to identify causative variant in *STING1*. Quantitative real-time PCR was performed to detect relative mRNA expression of IFN-stimulated genes. The routine immunological and clinical work up as well as chest X-ray and ultra-low dose CT were performed.

Results: Here, we report on four patients of four unrelated families harboring the homozygous pathogenic variant c.841C>T p.(Arg281Trp) in *STING1* (NM_198282.3). While all four patients showed SAVI features including ILD, tachypnea and hypoxia, failure to thrive, and increased IFN activation, only one of them displayed skin vasculitis – a hallmark phenotypic feature of SAVI. Steroid and baricitinib treatment had a mitigating effect on the disease phenotype in two cases, but failed to halt disease progression. In contrast to homozygous patients, heterozygous carriers of these families were healthy and showed normal interferon activation.

Conclusions: Until recently, SAVI has been listed as an exclusively autosomal dominant inherited trait in relevant databases. We aim to raise awareness for autosomal recessive inheritance in this rare, severe disease, which may aid in early diagnosis and development of optimized treatment strategies.

Optical Genome Mapping – a promising method for the detection of disease-causing structural variants in Inborn Errors of Immunity

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Background Nowadays, more than 450 causative genes are known in Inborn Errors of Immunity (IEI). However, in 70-90% of affected IEI patients a genetic diagnosis cannot be established conventionally by Next Generation Sequencing. The additional use of Optical Genome Mapping (OGM) may improve the diagnostic yield by the detection of pathogenic Structural Variants (SVs) and the identification of new disease-causing genes.

Methods OGM is a new cytogenomic method allowing the detection of all different kinds of structural aberrations including deletions, duplications, insertions, and inversions. It is based on labelling of ultrahigh molecular weight DNA that is linearised in nanochannels and used for de novo assembly. Subsequently, the resulting maps are compared to reference genomes and filtered for different criteria. OGM allows a sensitive and accurate detection even in very complex regions (e.g. repetitive regions).

Results We analysed 37 undiagnosed patients with IEI by OGM. Exemplarily and in concordance with WES a de novo heterozygous likely pathogenic deletion of 834kb on chromosome 3 (3p21.31) was detected in a 2.5yr old boy presenting with recurrent febrile episodes with pharyngitis and mild lymphadenopathy. It affects around 33 genes, including ARIH2 (ariadne RBR E3 ubiquitin protein ligase 2). ARIH2 acts as negative regulator of NLRP3 inflammasome and haploinsufficiency might explain the autoinflammatory phenotype. Further experiments on patient-derived cell lines are being performed to investigate this hypothesis.

Conclusions The use of OGM can improve the detection of disease-causing variants in undiagnosed patients, which allows further investigations for a better understanding of new pathomechanisms in IEI.

Saturday, May 6th

11:00 – 12:30	**Session 8: Innate immunity and inflammation** *Chairs: Almut Meyer-Bahlburg (Greifswald), Sujal Ghosh (Düsseldorf)*
11:00 – 11:40	Macrophage differentiation in chronic inflammatory diseases *Antigoni Triantafyllopoulou (Berlin)*
11:40 – 11:55 (10'+5')	Extended clinical phenotype and treatment modalities in thirty JAGN1 deficient patients - an ESID/EBMT IEWP multi-center study *Julia Fekadu (Frankfurt)*
11:55 – 12:10 (10'+5')	CGD patient cohort analysis: an update *Myriam Lorenz (Ulm)*

Extended clinical phenotype and treatment modalities in thirty JAGN1 deficient patients - an ESID/EBMT IEWP multi-center study

Julia Fekadu[1], Jan Robert Heusel[1], Andre Willasch[1], Fabian Hauck[2], Luis Ignacio Gonzalez-Granado[3], Zahra Chavoshzadeh[4], Samin Sharafian[4], Franziska Cuntz[5], Cornelia Zeidler[6], Blandine Beaupain[7], Christine Bellanné-Chantelot[8], Gerhard Kindle[9], Michael Albert[2], Jean Donadieu[7], Peter Bader[1], Benedicte Neven[10], Shahrzad Bakhtiar[1]

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Background and aims: Autosomal recessive mutations in *JAGN1* (Jagunal-homolog1) lead to congenital neutropenia (CN) with early onset aphthosis, skin abscesses, and invasive bacterial infections due to aberrant differentiation and maturation of neutrophilic granulocytes in the bone marrow. In addition, an associated syndromic phenotype including facial features, short stature and neurodevelopmental delay has been reported. In a retrospective multicenter study supported by the Inborn Errors Working Party (IEWP) data were collected on patients with JAGN1-deficiency. We aimed to perform a phenotype-genotype analysis and evaluate the treatment modalities.

Methods: Patient data were gathered via case report forms with added data from the published literature if the patient was previously reported. Data on clinical manifestations and allogeneic hematopoietic stem cell transplantation (HSCT) were collected and analyzed.

Results: We identified thirty patients with JAGN1-deficiency from fifteen centers. Patients showed nine distinct homozygous mutations in *JAGN1*. All patients showed CN with early onset aphthosis and infectious complications including pneumonia, otitis, mastoiditis and skin abscesses. Moreover, twelve patients presented a syndromic phenotype with short stature and facial features such as forehead enlargement and a flat nose. In four patients of three families neurodevelopmental delay was found. Four patients received allogeneic HSCT due to G-SCF-refractory CN and severe infections and one because of secondary acute myeloid leukemia

(AML). Two patients had to undergo a second HSCT because of autologous reconstitution. One untransplanted patient died at the age of five years due to pancolitis and septicemia. All other patients were reported alive and well at last follow-up. All patients receive G-CSF treatment except four who did not respond to the therapy. The oldest patient was 66 years of age.

Conclusion: In this retrospective study, we present the extended phenotype of a cohort of 30 patients with JAGN1-deficiency. All patients had CN, but 4 did not responded to G-CSF and one developed secondary AML. Carefully monitoring for the occurrence of invasive infections and hematopoietic malignancies is required. Allogeneic HSCT was curative in five patients due to severity of the disease and malignancy. Extrahematopoietic features occur, but are not fully penetrant.

CGD patient cohort analysis: an update

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Background: In about 70 % of 99 families referred to our center between 2010 -2022 for CGD genetic testing the disease-causing variation was detected. We now focus on the 30% in which the genetic cause of CGD is not identified so far.

Methods: We designed primers for exon amplification of all of the presently known genes causing CGD including novel genes. We sequenced by a Big Dye Terminator v.1.1 Cycle Sequencing Kit on an Applied Biosystems Prism 3100 Genetic Analyzer using Sanger Sequencing.

Results: Reanalysis of our patient cohort revealed genetic variations in the *NCF4* gene in 3 families. Sanger sequencing resulted in a homozygous missense variation p.R57H in *NCF4* in one patient unknown in the literature. This variation is of uncertain significance in the ClinVar database and is listed in the GnomAD-database with a frequency of 0,0018%. It is predicted as probably damaging in PolyPhen-2 and as deleterious in SIFT. In 2 further patients we detected the homozygous splice site variation c.118-1G>A and the pathogenic variation p.R58C in *NCF4,* both described in the literature.

Conclusion: Expanding standard routine analysis to rarely affected genes in CGD might be important for genetic diagnosis. The location of p.R57H in *NCF4* in the center of the PX domain and the proximity to the known pathogenic mutation R58C as well as the fact that p.R57 is highly conserved among species implies that this variation resides in an important functional domain. Further functional analyses may contribute to the evaluation of the pathogenicity of this variation.

Lack of Efficacy of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) in Patients with JAGN1 Deficiency

Susan Farmand[1], Katharina Wustrau[2], Susanne Aydin[1], Francis Ayuk[3], Udo zur Stadt[4], Gabriele Escherich[4], Julia Skokowa[5], Ingo Müller[1], Kai Lehmberg[1]

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Background: JAGN1 deficiency due to homozygous or compound heterozygous mutations causes severe congenital neutropenia. In addition, several extra-hematopoietic manifestations have been observed. JAGN1-deficient patients present with severe early onset bacterial infections and have been described as low-responders to recombinant granulocytecolony stimulating factor (G-CSF) therapy. Interestingly, in a murine JAGN1 knockout model, treatment with GM-CSF was able to restore the functional defect.

Patients: We present two unrelated patients with biallelic JAGN1 mutations, who were both treated with subcutaneous GM-CSF (Sargramostim/Leukine®) after treatment failure to GCSF. The first patient was an 18-year-old pregnant woman who received GM-CSF at 12 weeks of gestation up to a dose of 10 µg/kg/d for 7 days. The second patient was a 5-month-old girl who received GM-CSF for a total of 9 days at a dose of up to 20 µg/kg/d.

Results: GM-CSF did not increase neutrophil counts in our patients. Treatment was stopped when neutrophil numbers declined further, no beneficial effect was noticed, and patients presented with infections. No side effects were observed in both patients and the fetus. In contrast to the data from the mouse model, GM-CSF therapy did not ameliorate the phenotype. Despite severe immunological compromise, no additional extra-hematopoietic manifestations were evident in our patients. Ultimately, both patients underwent HSCT with successful outcome.

Conclusion: In two unrelated patients, GM-CSF did not have any beneficial effect. JAGN1deficient patients with reduced G-CSF responsiveness and elevated infection rate should be evaluated early for stem cell transplantation.

A forward rescue mutation in *ELANE* can revert the phenotype of severe congenital neutropenia

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Background: Severe congenital neutropenia (SCN) in patients with neutrophil elastase *(ELANE)* mutations is an autosomal dominant immune deficiency, usually presenting with severe bacterial infections. Genetic reversions have been described for several immunodeficiencies.

Case: In an infant girl with SCN phenotype, Sanger sequencing identified the heterozygous *ELANE* mutation c.158A>T/ p.His53Leu, which has been described previously. On segregation analysis of the non-consanguineous asymptomatic parents with normal neutrophil count, both the wildtype allele and the mutated allele c.158A>T were surprisingly detected in paternal DNA obtained from peripheral blood. In addition, the father was found to carry a third allele with a second mutation at the same site (c.158A>C, p.His53Pro). This mutation has not yet been described for patients with SCN nor is it found in the general population.

The three alleles (wildtype c.158A>T; c.158A>C) are detected in the father at the same ratio on Sanger sequencing in whole blood, T cells, and buccal mucosa. In isolated peripheral neutrophils in contrast, the c.158A>C allele is enriched while the frequency of c.158A>T is decreased. First cloning experiments confirm the allele ratios in the paternal neutrophils. Currently, *ELANE* sequencing of paternal whole blood, buccal mucosa, and neutrophils with a next generation sequencing technique is underway to confirm the shift of allele frequency in paternal neutrophils as compared to the other samples.

Conclusions: We assume the father has developed a somatic forward mutation (c.158A>C, p.His53Pro) on the background of a heterozygous germline mutation (c.158A>T, p.His53Leu) that rescues granulopoietic cells from maturation arrest at promyelocyte level, leading to numerically normal neutrophil count and absence of severe infections.

Thursday, May 4th

15:00 – 15:30

Poster Session 1

Chair: Kai Lehmberg (Hamburg)

Unraveling the molecular causes of polyautoimmunity including alopecia areata as a common feature

Buket Basmanav (Bonn)

IKAROS-deficiency in father and son with progressive B-cell lymphopenia and hypogammaglobulinemia

Renate Krüger (Berlin)

IKAROS associated disease as rare differential diagnosis of small vessel vasculitis and hypogammaglobulinaemia

Gregor Dückers (Krefeld)

Rapidly evolving anti-drug antibodies in a ADA2-deficient patient

Leif Hanitsch (Berlin)

EBV-positive mucocutaneous ulcers in a patient with combined immunodeficiency

Geraldine Engels (Würzburg)

Unravelling the molecular causes of polyautoimmunity including alopecia areata as a common feature

F. Buket Basmanav[1] and Regina C. Betz[1]

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Alopecia areata (AA) is an autoimmune disorder and a common cause of hair loss affecting both children and adults with a lifetime risk of 2%. AA is regarded as a multifactorial disease with both environmental and genetic factors playing a role in its etiology with the latter being mostly attributed to the joint contribution of common risk variants of modest effect sizes. In the field of AA genetics, it has however not received attention that AA can also manifest as an associated clinical feature of a number of monogenic syndromes. These syndromes caused by mutations in central immune genes such as AIRE, CTLA4 and FOXP3, severely impact the immune system and lead to immune deficiency and/or polyautoimmunity.

We have one of the largest AA cohorts worldwide composed of ~2800 individuals; and ~1/6th of our patients are affected by additional autoimmune diseases. We refer to these individuals as the AAPAD (Alopecia Areata Plus other Autoimmune Disease(s)) sub-cohort and hypothesize that AAPAD cases are due to a distinct pathophysiology and are positioned between the very rare autoimmune syndromes and the multifactorial AA cases, representing a continuous spectrum of autoimmunity with AA as the common feature. The aim of the current study is to elucidate the genetic basis of AAPAD by performing whole genome-sequencing in 400 AAPAD cases. Identification of genetic variants in known and unknown immune genes in AAPAD may have a profound impact on the clinical diagnostic scheme of AA, such that individuals with severe autoimmunity may benefit from molecular diagnostics.

IKAROS-deficiency in father and son with progressive B-cell lymphopenia and hypogammaglobulinemia

Renate Krüger[1], Volker Wahn[1], Anna Stittrich[2,3], Nadine Unterwalder[4], Uwe Kölsch[4], Christian Meisel[2, 4,5], Britta Siegmund[6], Leif Gunnar Hanitsch[2,5], Horst von Bernuth[1,2,4,7]

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Introduction: IKAROS-deficiency due to variations in *IKZF1* leading to either haploinsufficiency, disturbed dimerization or a dominant-negative effect is a rare genetic cause of inborn errors of immunity (IEI) with broad phenotypic variations. Recurrent infections due to B-cell lymphopenia and hypogammaglobulinemia as well as autoimmunity and malignancy in affected patients have been described (1-3). In addition to loss-of-function (LOF) mutations gain-of-function (GOF) mutations have been described but are not discussed here.

Case report: A male patient was diagnosed with agammaglobulinemia at the age of 30 years following recurrent viral and bacterial airway infections since childhood. B cells were absent, NK cells decreased and $CD3^+CD8^+$ T cells increased with an inverted CD4/CD8 ratio. He had also suffered from recurrent diarrhea and profound impairment of swallowing solid food due to esophageal strictures resembling eosinophil esophagitis. Whether epilepsy, liver steatosis, a kidney cyst and gallbladder polyps are related to his IKAROS-deficiency is unclear.

His son suffered from recurrent minor viral airway infections since the first year of live. Levels of IgG were normal up to the age of 2.5 years, of IgA not detectable, of IgG2 and IgG4 (determined at 2.5 years) decreased. There were no sustained IgG responses to tetanus, pneumococcal and measles vaccinations. B-cell numbers progressively declined with age and showed impaired B-cell maturation. NK cells and $CD3^+CD8^+$ T cells were within normal ranges up to the current age of 9 years. *Genetic analyses:* Whole Exome Sequencing (WES) including the analysis of Copy Number Variations (CNV) revealed a >13kb deletion spanning exons 4 and 5 in *IKZF1*. *Therapy:* Both patients receive s.c. IgG substitution (SCIG) and require high IgG dosages to obtain levels within normal ranges; despite SCIG they both suffer from persistent upper airway infections.

Discussion: The clinical course of the patients further adds to the broad phenotypic spectrum in IKAROS-deficiency. This case report underscores the importance of CNV analyses in WES in patients with IEI to detect deletions or duplications in genes related to IEI.

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IKAROS associated disease as rare differential diagnosis of small vessel vasculitis and hypogammaglobulinaemia

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The index patient is a 11year old Caucasian girl of a non-consanguineous family, presenting with recurrent episodes of ANCA negative, small vessel vasculitis, edematous swelling of limbs, physical dyspnea and muscular weakness. At the age of 5 years she was diagnosed to have a unilateral cholesteatoma with repeated ENT surgery and necessity to wear a deaf-aid.

Her father had presented at 37 years of age, with splenomegaly, ANCA negative leucocytic-clastic vasculitis and chronic and relapsing bipolar aphthosis. Chronic recurrent episodes of pulmonary infection and weight loss led to pulmonary CT scan, bronchoscopy with biopsy of lungs. Histology revealed: granulomatous lung disease with bronchiolitis obliterans, chronic interstitial lymphocytic pneumonia with signs of alveolar hemorrhage and multiple central granulocytic, perivascular granuloma. The brother of the index patient is asymptomatic, grandfather had metastatic colon CA, DM type II, liver cirrhosis.

Genetic analyses showed a heterozygous mutation in IKFZ1 (c.1480_c.1481delAT; p.(Met494Valfs*86)) in the index patient, the brother and her father.

Lab findings in the index patient and other mutation carriers were low B (Index: 3% (56/µl); brother 2% (47/µl); father: 11% (58/µl) and low NK (Index: 3% (64/µl), father 5,8% (29/µl), leukocytopenia, microcytic anemia, hypogammaglobulinaemia (IgG, IgA) and significantly decreased or absent antibodies against pneumococcal polysaccharides.

IKAROS associated disease may presents with a wide phenotypical variation in members of the same family. IKAROS associated disease is a differential diagnosis in rare cases of treatment refractory small vessel vasculitis in patients with a suspicious family history and hypogammaglobulinaemia.

Rapidly evolving anti-drug antibodies in a ADA2-deficient patient

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Background: Homozygous mutations in ADA2 result in decreased ADA2 activity. Patients with DADA2 present with a broad spectrum of auto-inflammatory manifestations, including immunodeficiency, vasculopathy and hematologic disease. TNF alpha blockade has proven to be highly effective in treating inflammatory manifestations and preventing CNS vasculitis and stroke.

Methods: We report clinical and immunological parameter of a 23-year old patient with DADA2 before and during TNF alpha blockade.

Results: Our patient is affected by early childhood autism and suffered a stroke at the age of 7 years. Recurrent aphtous lesions of the oral cavity and episodes of fever started during adolescence. At 18 years he developed an abscess requiring proctologic surgery. With 21 years he developed watery diarrhea and massive weight loss of 25kg. The same year patient required ICU treatment due to an intra-abdominal abscess and septic peritonitis.

Immunological work-up revealed a severe neutropenia (<0,1/nl), mild thrombocytopenia and mild hypergammaglobulinemia. Genetic testing showed a homozygous mutation in ADA2 (c.753G>A). Enzymatic activity was reduced.

Patient was started on intravenous infliximab (5mg/kg) and switched to s.c. treatment after two infusions. Due to severe neutropenia no additional MTX was offered. Prompt clinical improvement. 10 weeks after initiation of TNF alpha treatment we detected mildly elevated anti-infliximab auto-antibodies (19,6 AU/ml; Norm <10) without affecting therapeutic drug levels (5,1µg/ml; range: 3-7). 14 weeks after beginning treatment patient reported again aphtous lesions, fever bouts and diarrhea. Anti-infliximab auto-antibodies increased (124,3 AU/ml; Norm <10), resulting in reduced drug levels (0,6µg/ml). Patient was switched to adalimumab 40mg biweekly with good clinical response.

Conclusions: Patients with DADA2 might present an increased risk of developing anti-drug antibodies and early monitoring should be encouraged.

EBV-positive mucocutaneous ulcers in a patient with combined immunodeficiency

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Background: Epstein-Barr virus (EBV) positive B-cell lymphoproliferative disorders may arise in states of acquired or inherited immunodeficiency and depict a spectrum of disease that range from self-limiting, localized conditions to aggressive lymphomas.

Case report: The male patient has been diagnosed with combined immunodeficiency at age 4 years. He initially presented with Evans syndrome, lymphoproliferation, recurrent respiratory tract infections hypogammaglobulinemia and decreased number of T cells and absent switched memory B cells. Whole-genome sequencing could not reveal a genetic cause for his disease yet. He was treated with immunoglobulin substitution and received mycophenolate mofetile which controlled autoimmune thrombocytopenia and autoimmune hemolytic anemia. Sirolimus has not been tolerated well by the patient and did not reduce lymphoproliferation. At age 18 years the patient has been splenectomized due to massive splenomegaly. At that time he also suffered from diarrhea and weight loss. Colonoscopy revealed inflammation of the terminal ileum and entire colon with aphtous and ulcerating lesions. Histological work-up showed an EBER- as well as CD20/CD79a-psoitive cell population within the ulcerative lesions compatible with an EBV-positive B-cell lymphoproliferative disorder. PET-CT showed hypermetabolic activity in the colon as well as multiple hypermetabolic and enlarged cervikal, thoracal and abdominal lymph nodes. Only slight traces of EBV could be detected in the peripheral blood. In synopsis of these findings the diagnosis of intestinal EBV-positive mucocutaneous ulcers was made. The patient was treated with rituximab which resulted in complete resolution of the inflammatory lesions and clinical symptoms.

Conclusions: EBV-positive mucocutaneous ulcers is a rare, indolent condition within the spectrum of B-cell lymphoproliferative disorders, which is localized to skin and mucosal surfaces.

Friday, May 5h

10:30 – 11:00

Poster Session 2

Chair: Carsten Speckmann (Freiburg)

Heterozygous pathogenic *FOXN1* variant causes nail dystrophy and low CD8+ T cells
Olga Staudacher (Berlin)

HSCT in a two-year-old with IL2RB-Defect
Ommo Mauss (Ulm)

Compound heterozygous *DOCK8* mutation - time for transplantation?
Ursula Holzer (Tübingen)

A novel *SIK3* mutation presenting with combined immune deficiency (CID) in the context of severe skeletal dysplasia
Andrea Meinhardt (London)

Identification of a New Variant in *RFXAP* in a Patient with Hypomorphic Bare Lymphocytes Syndrome and Short Stature
Sybille Landwehr-Kanzel (Hannover)

Heterozygous pathogenic *FOXN1* variant causes nail dystrophy and low CD8⁺ T cells

Olga Staudacher[1,2], Sandra von Hardenberg[3], Uwe Kölsch[2], Anna Stittrich[4], Mirjam Völler[1], Sarah Dinges[1], Oliver Blankenstein[5], Christian Meisel[2,6], Horst von Bernuth[1,2,7,8]

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Introduction: Forkhead box protein N1 (FOXN1) regulates the development of epithelial cells in both skin and thymus. Homozygous pathogenic loss of function variants in *FOXN1* cause complete athymia, alopecia and nail dystrophy. Patients with homozygous as well as heterozygous pathogenic variants in *FOXN1* are identified through newborn screening for severe combined immunodeficiency due to reduced numbers of T receptor excision circles. Although a majority of *FOXN1*$^{+/-}$ infants have low CD3⁺, CD4⁺, and CD8⁺ T cells their risk of severe infections and autoimmunity seems to be low.[1]

Case report: A newborn with as heterozygous, pathogenic *FOXN1* variant was identified through screening for severe combined immunodeficiency. His nails were dystrophic. After establishing the genetic diagnosis, the (*a priori* healthy) father was identified as a heterozygous carrier of the same variant (c.1337delA p.(His446Profs*104)). The mother does not carry a pathogenic variant in *FOXN1.* During follow-up of 9 months CD3⁺ T cells rose from 30/µl to 320/µl, CD4⁺ T cells from 30/µl to 280/µl, and CD8⁺ T cells from 0/µl to 40/µl. The percentage of naïve CD4⁺ T cells rose from 6% to 25%. No infections occurred on immunoglobulin substitution, fungal and antibiotic prophylaxis. The infant keeps thriving.

Conclusion: Nail dystrophy and low CD8⁺ T cells are the hallmark in patients carrying heterozygous pathogenic variants in *FOXN1*. In *FOXN1*$^{+/-}$ infants there is most likely no need for thymus transplantation.

[1] Bosticardo et al. Am J Hum Genet 2019 Sep 5;105(3):549-561

HSCT in a two-year-old with IL2RB-Defect

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We report on a two-year-old girl of consanguineous parents of Pakistani origin. First hospitalization at 6 months with suspected MIS-C. Afterwards repeated hospitalizations for FUO, failure to thrive, aggravating skin eczema and reduced general condition. In addition, she showed pronounced generalized lymphadenopathy and EBV in serum and lymph-node biopsies. Laboratory testing showed Hypergammaglobulinemia (IgG+IgE), increased B-cells, normal NK-cells and low CD8+-T-cells with increased CD4/CD8-ratio, low naïve T-cells but a polyclonal TCR-panel. FOXP3+/CD25+ T-cells were drastically reduced, T-cell-proliferation to IL-2 stimulation was absent (tested post steroids). Genetic testing revealed a homozygous mutation in IL2Rß-gene Exon 4, c.230T>C, p.(Leu77Pro), a mutation previously described to increase intracellular turnover and reduced surface expression of IL2Rß.

We initiated immunosuppressive therapy with prednisolone (systemically and topical) and Sirolimus. Being EBV positive, she received four doses of Rituximab. With partial response of the symptoms (lymphadenopathy and eczema) we added Dupilumab, an anti-IL-4/IL-13 antibody, to her treatment-protocol resulting in further improvement in overall condition and reduction of eczema to <5% of body surface. HSCT was performed from MUD 9/10 after conditioning with Busulfan/Fludarabine/Alemtuzumab/Rituximab.

Lymph-nodes and skin further improved under conditioning. She engrafted timely and was discharged 2,5 months post-HSCT in good general condition. Except for aGvHD (°I, skin) and mild engraftment-syndrome no complications occurred during transplantation. Currently, no eczema or lymph-node-bulks are detectable.

With little data available about the disease and therapeutic options, we successfully performed HSCT and eradicated initial disease symptoms. However, it appears vital that diagnosis and treatment occur at early stages of the disease.

Compound heterozygous DOCK8 mutation - time for transplantation?

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Background: Dedicator of cytokinesis 8 (DOCK8) deficiency is an autosomal recessive combined immunodeficiency. Laboratory findings include high IgE plus hypereosinophilia, low IgM and T- and NK-cell lymphopenia. In addition to initial presentation with severe allergic inflammation, the patients suffer from a broad spectrum of infections, including chronic cutaneous viral infections, recurrent respiratory infections, and mucocutaneous candidiasis. Furthermore, malignancies such as lymphoma and squamous cell carcinoma are reported. Here we present a patient with compound heterozygous likely pathogenic variants in the DOCK8 gene.

Case presentation: The male patient is the third child of non-consanguineous, healthy parents. At the age of 5 years he presented with a history of recurrent eczema, diarrhea and short stature. Except for pyelonephritis at the age of 3 months, he did not suffer from increased or severe infections.

Results: Clinical findings showed eczema and dental anomalies with enamel defects. IgE was elevated (6550 kU/l) with mild hypereosinophilia (660/µl), and there was no measurable IgM but normal IgG. Number and proliferation of CD4+ and CD8+ cells were normal as well as expression of IL17 and IFNγ. MRI did not reveal any abnormalities of the lung. Genetically bi-allelic changes in the DOCK8 gene could be detected (c.1730T>G, p.Leu577Arg and c.(3530+1_3531-1)_(5817+1_5818-1)del, p.? resulting in reduced, but not absent DOCK8-expression.

Conclusions: Our patient with compound heterozygous variants in the DOCK8 gene exhibits a mild form of the disease without severe infections. However, the question remains, if a stem cell transplantation might be an option to avoid potential life-threatening infections and reduce the risk of malignancies.

A novel SIK3 mutation presenting with combined immune deficiency (CID) in the context of severe skeletal dysplasia

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Background: SIK3 is an essential positive regulator of mTOR signalling that functions by triggering DEPTOR degradation in response to PTH/PTHRP during skeletogenesis. In 2018, Csukasi et al. reported two siblings with a SIK3 homozygous mutation associated with Krakow-type spondyloepimetaphyseal dysplasia and CD4+ lymphopenia.

Methods: We report a 4-year-old girl of consanguineous parents with a novel homozygous mutation in the catalytic region of SIK3.

Results: Antenatal scans at 16 weeks demonstrated skeletal dysplasia. Antenatal CGH was negative as were genetic investigations for skeletal dysplasia. She was born at 26+3 weeks with severely under mineralised bones, metaphyseal ossification defects, impaired spine ossification and absent PTH. She was admitted to the hospital for 6 months with respiratory distress requiring assisted ventilation and evolving into chronic lung disease. At the age of 10 months, she developed RSV and influenza A bronchiolitis requiring non-invasive ventilation. Panlymphopenia (particularly low B-cells) was noted at 12 months with CD3+ 698/µl, CD4+ 386/µl, CD8+ 316/µl, CD19+ 38/µl, CD16+ 115/µl. IgM was low and vaccine response absent even after booster vaccination. The clinical course was complicated by intermittent pancytopenia with hypocellular marrow requiring repeated red cell transfusions, renal dysplasia, recurrent bacterial and viral infections as well as BCGosis. Trio-WES confirmed a de novo homozygous mutation in SIK3; c.746T>G,p.Leu249arg. At 4 years old, she remains well on home oxygen, immunoglobulin replacement and antibacterial prophylaxis.

Conclusion: We describe a novel SIK3 mutation associated with combined immunodeficiency. Our findings of B-cell lymphopenia and intermittent pancytopenia extend the previously described phenotype of this defect.

Identification of a New Variant in *RFXAP* in a Patient with Hypomorphic Bare Lymphocytes Syndrome and Short Stature

Sandra v. Hardenberg [1], Sybille Landwehr-Kenzel [2,3], Agnes Bonifacius [2], Anna Raab [3], Bernd Auber [1], Britta-Eiz-Vesper [2], Rita Beier [3], Michaela Nathrath [4], Ulrich Baumann

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Background: Bare lymphocyte syndrome (BLS) is a form of SCID caused by MHC deficiency. While BLS-I, also known as MHC-I deficiency, is due to defects in one of the TAP proteins, MHC-II deficiency is classified as BLS-II, caused by mutations of transacting transcription factors *RFX5, RFXAP, RFXANK and CIITA*. MHC class-I and class-II promotors, however, share similar conserved motifs, including the X-box which is bound by the RFX complex.

Patient and Methods: We report on a 2-yrs old boy of consanguineous parents, born at term but SGA. The patient presented with the clinical picture of CID, lymphoproliferation, anemia, and short stature. Diagnostic work-up was performed to characterize the clinical phenotype on a molecular and functional level.

Results: Clinically the boy suffered from recurrent bacterial major infections, severe iron deficiency, hypergammaglobulinemia, chronically replicative CMV infection, eosinophilia, and splenomegaly. The immunological phenotype was characterized by a reduced expression of MHC-I (T-, B- and NK-cells) and MHC-II (monocytes and $\gamma\delta$-T-cells). The composition of the T-cell compartment showed reduced $CD45RA^+$-T-cells, the CD4/CD8-ratio was inverted. Within the B-cell compartment mature IgM^+ memory B-cells were markedly reduced. Antibody testing showed IgA-deficiency but chronic hypergammaglobulinemia with a normal response to MMR vaccination. In contrast, no antibody response to pneumococcal polysaccharides, hepatitis-B and varicella zoster virus was detectable. Genome sequencing, identified a homozygous likely pathogenic variant of *RFXAP* (ENST00000255476.2):c.28_34dup_p.(Pro12Argfs*63).

Conclusions: We identified a new variant in *RFXAP* causative for hypomorphic BLS. As infectious complications constitute a high risk in non-transplanted patients with BLS and increase the TRM, early HSCT is currently discussed.

Friday, May 5h

15:00 – 15:30

Poster Session 3

Chair: Helmut Wittkowski (Münster)

A Toolkit for Monitoring Immunoglobulin G Levels from Dried Blood Spots of Patients with Primary Immunodeficiencies

Bodo Grimbacher (Freiburg)

The ABACHAI clinical trial protocol: Safety and Efficacy of abatacept (s.c.) in patients with CTLA-4 insufficiency or LRBA deficiency – establishment of a disease-specific scoring system

Máté Krausz (Freiburg)

A 10-year old girl with X-linked CGD presenting with polyarthritis and cheilitis granulomatosa

Nina-Christine Knopf (Dresden)

Gastric Adenocarcinoma due to immunodysregulation and chronic gastritis

Marcus Jakob (Regensburg)

Severe autoinflammatory syndrome/ CINCA with pathogenic de novo mutation in the NLRP3 gene with prenatal anemia with intrauterine blood transfusion, Ascites, generalized perinatal edema and massive splenomegaly

Anna Raab (Hannover)

A Toolkit for Monitoring Immunoglobulin G Levels from Dried Blood Spots of Patients with Primary Immunodeficiencies

Hanna Haberstroh[1,2], Aleksandra Hirsch[3,4], Sigune Goldacker[3,4], Norbert Zessack[5], Klaus Warnatz[3,4], Bodo Grimbacher[1,2,3,4,6,7,*], Ulrich Salzer[3,4,*]

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Baxalta GmbH, a Takeda company, provided funding for this collaborative research in terms of study development, conduct, and analysis.

Purpose. This study assessed whether measuring immunoglobulin G (IgG) from dried blood spots (DBSs) using nephelometry is a suitable remote monitoring method for patients with primary immunodeficiencies (PID).

Methods. Patients receiving immunoglobulin replacement therapy for PID were included in this non-interventional single-arm study (DRKS-ID: DRKS00020522) conducted in Germany from December 4, 2019 to December 22, 2020. Three blood samples, two capillary DBSs (one mail-transferred and the other direct-transferred to the laboratory) and one intravenous, were collected from each patient. IgG levels were determined using nephelometry. IgG levels were summarized descriptively and significant differences assessed using Wilcoxon matched pairs signed-rank tests. Correlation and agreement between IgG levels were assessed using Spearman correlation and Bland–Altman analyses, respectively.

Results. Among 135 included patients, IgG levels measured from DBS samples were lower than those measured in serum (p<0.0001). There was no significant difference between IgG levels in direct- and mail-transferred DBS samples. There was a high degree of correlation between IgG levels in serum samples and DBS samples (r=0.94–0.95). Although there was a bias for higher levels of IgG in serum than DBS samples, most samples were within the 95% interval of agreement. There was a high degree of correlation between IgG levels measured in direct- and mail-transferred DBS samples (r=0.96) with no bias based on shipment process and most samples within the 95% interval of agreement.

Conclusion. Monitoring IgG levels from DBS samples is a suitable alternative to the standard method and results are not substantially affected by mailing DBS cards.
Keywords: Dried blood spot, Immunoglobulin replacement therapy, Nephelometry, Primary immunodeficiency, Remote Monitoring, Subcutaneous immunoglobulin

The ABACHAI clinical trial protocol: Safety and Efficacy of abatacept (s.c.) in patients with CTLA-4 insufficiency or LRBA deficiency – establishment of a disease-specific scoring system

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Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) insufficiency and lipopolysaccharide-responsive and beige-like anchor protein (LRBA) deficiency are both complex immune dysregulation syndromes with an underlying regulatory T cell dysfunction due to the lack of CTLA-4 protein. As anticipated, the clinical phenotypes of CTLA-4 insufficiency and LRBA deficiency are similar. Main manifestations include hypogammaglobulinemia, lymphoproliferation, autoimmune cytopenia, immune-mediated respiratory, gastrointestinal, neurological, and skin involvement, which can be severe and disabling. The rationale of this clinical trial is to improve clinical outcomes of affected patients by substituting the deficient CTLA-4 by administration of CTLA4-Ig (abatacept) as a causative personalized treatment.

Our objective is to assess the safety and efficacy of abatacept for patients with CTLA-4 insufficiency or LRBA deficiency. The study will also establish a CTLA4- and LRBA-specific disease-severity scoring system and investigate how treatment with abatacept affects the patients' quality of life.

ABACHAI is a phase IIa prospective, non-randomized, open-label, single arm multi-center trial. Altogether 20 adult patients will be treated with abatacept 125 mg s.c. on a weekly basis for 12 months, including (1) patients already pretreated with abatacept, and (2) patients not pretreated, starting with abatacept therapy at the baseline study visit. For the evaluation of drug safety infection control during the trial, for efficacy, a newly developed CHAI-Morbidity Score will be used. The trial was fully recruited in March 2022; altogether 18 CTLA-4 and 2 LRBA patients were included. The trial is registered in the German Clinical Trials Register (Deutsches Register Klinischer Studien, DRKS) with the identity number DRKS00017736.

Key words: CTLA-4 insufficiency, LRBA deficiency, abatacept, clinical trial protocol, disease severity score, immunodeficiency

The study ist supported German Federal Ministry for Education and Research (BMBF) through a grant to the German Auto-Immunity Network (GAIN) [grant number 01GM1910B]. The trial medication is provided by Bristol-Myers Squibb (BMS, New York, USA) [grant number IM101-774].

A 10-year old girl with X-linked CGD presenting with polyarthritis and cheleitis granulomatosa

Nina-Christine Knopf[1,2], Lissy Tille[1], Sarah Koss[1], Aschoff Roland[2], Min Ae Lee-Kirsch[1,2], Normi Brück[1], und Catharina Schütz[1,2]

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Background X-linked chronic granulomatous disease (CGD) is a rare inherited disorder of the innate immune system. Uncontrolled inflammation accompanied by granuloma formation are hallmarks of the disease.

Patient characteristics A 10-year old girl presented at the age of 3 years with polyarticular juvenile idiopathic arthritis (ANA, RF, CCP positive). Initially she was treated with methotrexate, later in combination with etanercept, which lead to stable remission. At the age of 9 years she developed recurrent episodes of swelling of her upper lip. There was no personal or family history of severe or chronic infections nor of inflammatory bowel disease.

Immunological and genetic findings Flow cytometry-based DHR (Dihydrorhodamine 123) oxidation assay revealed two granulocyte populations. The larger population (84%) showed an impaired respiratory burst typical for X-linked CGD carriers with random X-chromosome inactivation. Genetic testing revealed the variant (c.1151-21151+2delAAGT) in the CYBB gene.

Conclusion Autoimmune disorders like discoid or systemic lupus erythematodes or juvenile idiopathic arthritis are not uncommon in X-linked CGD carriers. Individualized treatment of these patients is challenging because immunosuppressive drugs may increase susceptibility to infection. Allogeneic HSCT is reserved for symptomatic carriers with severe manifestation as described by Tsiflis et al. 2023 (submitted).

Gastric carcinoma due to immunodysregulation and chronic gastritis

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Background: Chronic gastric inflammation is a well-known predisposition for gastric cancer. However an atrophic gastritis - gastric cancer - sequence presumably rare in children.

Case-report: We report a 15-year-old girl who presented with anemia due to defeciency of iron, folic acid and vitamine B 12. Gastroscopy showed a severe Type-A-gastritis with multiple polyps and histologic confirmation of a mixed-type gastric carcinoma.

Results: Immunological workup revealed hypogammaglobulinemia with lowered IgA and IgG (IgG1 and 4). In Trio-Exome-Sequencing we found an interstitial deletion on chromosome 14q13.1q13.3 with involvement of NFKBIA and SRP54.

Conclusion: Immunodeficiency therefore might be a possible cause for atrophic gastritis leading to gastric cancer. Immune-pathway-mechanisms in this patient remain unclear but might deliver a potential target for therapeutic immune-modulation.

Severe autoinflammatory syndrome/ CINCA with pathogenic de novo mutation in the NLRP3 gene with prenatal anemia with intrauterine blood transfusion, Ascites, generalized perinatal edema and massive splenomegaly

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Background: Heterozygous pathological changes in the NLRP3 gene are associated with the (OMIM #606416) chronic infantile neurological cutaneous articular (CINCA) syndrome, or neonatal onset multisystem inflammatory disease (NOMID).

Patient and Methods: Trio exome analyses was done in child's DNA from umbilical cord.

Results: Pregnancy with polyhydramnion and profound anemia requiring two 2 intrauterine blood transfusions. Prenatal diagnostics include trio whole exome analysis and detected a sequence change: c.1706A>G p (Glu569Gly) in the NLRP3-gene (NM_004895.4) in 25% of the reads, probably pathogenic (class 4) (UKM).

Born at 32 weeks of gestational age. At birth the patient presented with maculopapular exanthema and systemic inflammatory response syndrome (SIRS) with highly elevated CRP- and I-l6, thrombocytopenia, leukocytosis and anemias. She required 3 more blood transfusions. She had failure to thrive (age corrected Z- score of weight -2.4). By the age of 6 weeks, she received human interleukin- 1- receptor antagonist (anakinra) upon suspected autoinflammatory syndrome. Within 1 day, exanthema and inflammation subsided. However for full control of inflammation including encephalitic changes, she required high and frequent anakinra treatment up to 4 mg/kg b.d.

Conclusions: This as yet unpublished variant c.1706A>G p (Glu569Gly) of NLRP3 is related to an unusually active phenotype requiring higher and more frequent doses as previously published. We hypothesize that the pre- and postnatal anemia was a sequela of the high level of inflammation. Anemia has not been reported in CINCA syndrome. For anakinra has better effects in suppression of encephalitis it was preferred to canakinumab (Rodriguez-Smith J. et. al., 2017).

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