ABC7:
Autoimmunity, B cells and Complement

June 14-16, 2023
Tallinn, Estonia

European Journal of Immunology | EFIS European Federation of Immunological Societies

EIAS EESTI IMMUNOLOOGIDE JA ALLERGOLOOGIDE SELTS
ESTONIAN SOCIETY FOR IMMUNOLOGY AND ALLERGOLOGY

UNIVERSITY OF HELSINKI
FACULTY OF MEDICINE

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Nordic **C**omplement **C**ommittee **NCC / SSI**

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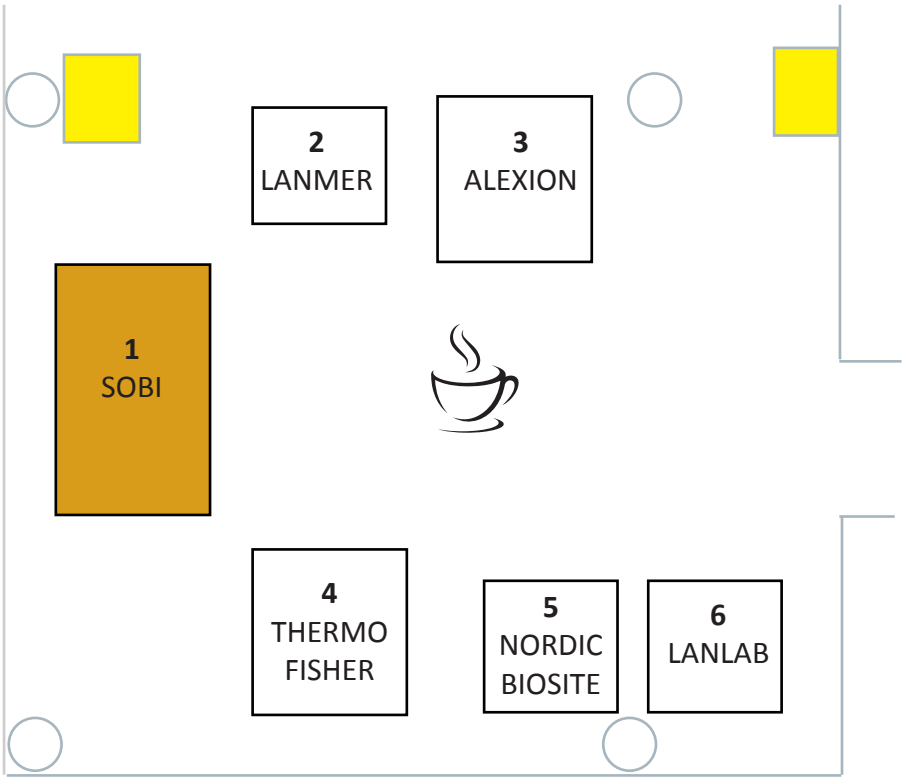
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PROGRAMME

Day I, 14.06

16:15	17:00	Meeting registration
17:00	17:15	Welcome note
17:15	18:15	Keynote lecture **Burkhard Becher** (University of Zurich, Switzerland) *IL-12 and IL-23: the paradox of simultaneous pro/anti inflammatory properties*
18:15	19:45	Arrival refreshments

Day II, 15.06

08:30	09:00	Registration
		Session 1
09:00	09:35	**Tobias Freitag** (University of Helsinki, Finland): *Antigen-specific immune tolerance induction in autoimmune diseases*
09:35	10:10	**Pablo Engel** (University of Barcelona, Spain): *Polyreactive autoimmune B-cell repertoire*
10:10	10:45	**Susan Wong** (University of Cardiff, UK): *B cells in Type 1 diabetes - the good and the bad - how do we balance these?*
10:45	11:05	Refreshment break (20 min)
11:05	12:05	**Session 2: Career development workshop by the Lithuanian Society for Immunology**
12:05	13:05	Lunch (60 min)
		Session 3: Complement
13:05	13:40	**Kristina Nilsson-Ekdahl** (Uppsala University, Sweden): *Contact activation of complement factor C3 on biomaterial and platelet surfaces*
13:40	14:15	**Peter Garred** (University of Copenhagen, Denmark): *The lectin pathway of complement*
14:15	14:25	Short stretch break

14.25	15:00	**Diana Karpman** (Lund University, Sweden): *Bridging the renin-angiotensin, kallikrein-kinin and complement systems in inflammatory diseases*
15:00	15:35	**Tom Eirik Mollnes** (University of Oslo, Norway): *Whole blood models for studying complement activation and inhibition*
15:35	15:55	Refreshment break (20 min)

Session 4: Complement

15:55	16:30	**Seppo Meri** (University of Helsinki, Finland): Regulatory disturbances of complement activation in human disease
16:30	17:05	**Kati Kaartinen** (University of Helsinki, Finland): *Thrombotic microangiopathies*
17:05	17:40	**Bo Nilsson** (Uppsala University, Sweden): *Thromboinflammation in COVID-19*
18:00		Leaving for the meeting dinner at the Kolu Inn of the Estonian Open Air Museum Bus leaves from Viru hotel at 18:00, there will be half an hour for exploring the territory before the dinner starts at 19:00. The bus will take everybody back at 22:00, expected arrival at Viru hotel approx. 22:30.

Day III, 16.06

08:45	09:00	Registration

Session 5

09:00	09:35	**Kai Kisand** (University of Tartu, Estonia): *Autoimmunity towards interferons*
09:35	10:10	**Eliisa Kekäläinen** (University of Helsinki, Finland): *Myasthenia gravis - antibody-mediated autoimmune disease of the thymus*
10:10	10:45	**Anna Šedivá** (Motol University Hospital, Czech Republic): *B cells in DiGeorge syndrome*
10:45	11:05	Refreshment break (20 min)

Session 6

11:05	11:40	**Jan Damoiseaux** (Maastricht University, Netherlands): *Immune-monitoring upon B-cell depletion*
11:40	12:15	**Pärt Peterson** (University of Tartu, Estonia): *Aire-deficient experimental models*
12:15	12:50	**Aili Tagoma** (University of Tartu, Estonia): *Immune responses to gut commensal in children*
12:50	13:50	Lunch (60 min)

13:50 15:50 **Session 7: Oral abstract presentation (PhD students)**

Asta Lučiūnaitė (Vilnius University, Lithuania)
Investigation of inflammatory response in macrophages activated by immune complexes

Julia Roquigny (INSERM, UMR, France; Sorbonne Université, France; Georges Pompidou European Hospital, France)
A C3-targeting nanobody inhibits the C3NeF activity from patients with C3G by promoting the dissociation of the C3bBb convertase

Alexandra Kapogianni (Sofia University, Bulgaria)
Correlation between secretory phospholipase A2 activity and autoantibodies to complement C3 and factor H in SLE patients

Eva Krzyżewska-Dudek (University of Helsinki, Finland)
Nanoparticles containing Influenza A (H1N1) viral peptides induce antigen-specific immune tolerance in a mouse model of narcolepsy type 1

Martti Vanker (University of Tartu, Estonia)
Autoantibodies neutralizing type III interferons are uncommon in patients with severe Covid-19 pneumonia

Kristi Alnek (University of Tartu, Estonia)
Immunological and immunogenetic markers in recent-onset type 1 diabetes among children and adults

Anu Bärenson (University of Tartu, Estonia; Children's Clinic of Tartu University Hospital, Estonia)
Maternal characteristics of women at risk for gestational diabetes influencing the development of asthma and atopic dermatitis in their offspring

Lehte Türk (University of Tartu, Estonia)
Quantifying CD8+ temra cells using statistical model

15:50 16:35 Poster Session (with coffee and snacks)

16:35 17:00 Concluding remarks

ABSTRACTS / ORAL PRESENTATIONS

IMMUNOLOGICAL AND IMMUNOGENETIC MARKERS IN RECENT-ONSET TYPE 1 DIABETES AMONG CHILDREN AND ADULTS

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Type 1 diabetes (T1D) is a chronic disease where the destruction of pancreatic β-cells leads to gradually diminishing insulin production. T1D is not only a childhood disease, but can develop at any age. We compared the immunological and HLA class II profiles over a spectrum of childhood- and adulthood-onset T1D. The cross-sectional study involved participants with recently diagnosed T1D (n=168) aged 2.9–68.2 years. Blood samples were taken up to 7 days after diagnosis. HLA, thyroid and coeliac diseases associated autoantibodies and anti-enterovirus (EV) antibodies were measured and analysed in relation to diabetes associated autoantibodies (DAA – autoantibodies against zinc transporter 8 (ZnT8A), islet antigen-2

(IA2A) and glutamic acid decarboxylase 65 (GADA)) status or age group at diagnosis, using logistic regression modelling. The most significant protective factor for multiple DAA positive participants compared with single DAA positive participants was older age (adOR 0.94, p=0.001), whereas anti-EV IgG acted as risk factor (adOR 4.01, p=0.013). When comparing childhood- and adulthood-onset T1D, the HLA-DR3/x genotype increased the odds for multiple DAA positivity (adOR 11.23, p=0.009). In conclusion, we showed that a more aggressive autoimmune attack on pancreatic beta cells is characteristic of childhood-onset T1D, whereas adulthood-onset T1D is characterised by subtler progression of T1D. Anti-EV IgG increases the odds for multiple DAA positivity. These results confirm immunological variability in recent-onset T1D cases at all ages and stress the importance of further studies to define the comprehensive immunological profile of the disease subgroups.

MATERNAL CHARACTERISTICS OF WOMEN AT RISK FOR GESTATIONAL DIABETES INFLUENCING DEVELOPMENT OF ASTHMA AND ATOPIC DERMATITIS IN THEIR OFFSPRING

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There is controversial evidence that links maternal gestational diabetes (GDM) to the development of asthma and atopic dermatitis in their offspring. Several other factors during pregnancy may contribute to the risk of developing these diseases as well. The aim of this longitudinal study was to investigate maternal characteristics during pregnancy in women at risk of GDM (based on current guidelines) that might influence development of asthma and/or atopic dermatitis in their offspring at 1, 2, and 5 years of age. The study cohort comprised of 223 children (52% male). Mothers of 86 (39%) children had GDM. Information about offspring´s diseases of interest (allergic and non- allergic asthma, allergic rhinitis, atopic dermatitis) was obtained from Electronic Health Records. The overall prevalence of at least one diagnosis in the GDM group at 1, 2, and 5 years of age was 6.3%, 9.4% and 6% respectively. In the non- GDM group the prevalence was respectively 12.5%, 13.5% and 15.4%. Adjusted logistic regression analysis was used to evaluate associations between several maternal characteristics (e.g., parity, age at delivery, mode of delivery, pre-pregnancy BMI, gestational weight gain, GDM diagnosis etc.) and offspring´s asthma and/or atopic dermatitis diagnosis. Maternal pre-pregnancy BMI > 30 kg/m^2 raised the odds of a respiratory tract related diagnosis at 2 years of age (OR 1.12, p=0.04). In conclusion, our results show that maternal pre-pregnancy BMI has an important adjunctive effect on the development of asthma in childhood.
The study was supported by EU HEDIMED grant https://www.hedimed.eu/ and Estonian Science Foundation grant no. 712.

INVESTIGATION OF INFLAMMATORY RESPONSE IN MACROPHAGES ACTIVATED BY IMMUNE COMPLEXES

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As demonstrated in the COVID-19 pandemic, viral infection can cause severe inflammation, both organ-specific and systemic. Also, vaccination can induce very strong activation of the immune system leading to serious adverse effects. The role of innate immune cells in promoting the inflammatory processes mediated by the immune complexes (IC) formed by viral antigens and their specific antibodies is insufficiently explored, in particular their potential impact on the development of autoimmune inflammation. To avoid over-activation of the immune system, a better understanding of IC-mediated innate immune responses is essential. Recently, we demonstrated a strong inflammatory response of macrophages activated by viral proteins of human polyomaviruses (PyV). The inflammation was driven by the intracellular innate immune component – the NLRP3 inflammasome. To expand this study, we investigated macrophage response to IC compared to viral antigens alone. We used mouse primary macrophages as a cell model and a collection of murine monoclonal antibodies specific to viral proteins. Virus-like particles (VLPs) formed by recombinant capsid protein of PyV were used as antigens. Secretion of inflammatory cytokines was measured by ELISA, activated cellular components were identified by the immunofluorescence assay, and cell viability was assessed by the LDH assay. IC-induced activation of the inflammasome in macrophages was evaluated by IL-1β release, caspase-1 activation, and formation of apoptosis-associated speck-like protein containing a CARD (ASC). We detected higher secretion of inflammatory cytokines and inflammasome activation after macrophage treatment with IC compared to VLPs alone. IC formed by IgG2a antibody showed the highest activation profile compared to IC formed by IgG1 and IgG2b. Formation of IC also increased the phagocytosis of viral antigens. In conclusion, our results demonstrated that IC enhanced the inflammatory response induced by VLPs. This data could be relevant to other studies that investigate IC-mediated inflammation as a consequence of viral infections or vaccine adverse effects.

CORRELATION BETWEEN SECRETORY PHOSPHOLIPASE A2 ACTIVITY AND AUTOANTIBODIES TO COMPLEMENT C3 AND FACTOR H IN SLE PATIENTS

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Topic: Development of an inflammatory response is typical for Systemic Lupus Erythematosus (SLE) and its most common manifestation Lupus Nephritis (LN) due to the tissue deposition of immune complexes between autoantibodies and their target antigens. Diagnostic markers for SLE and LN are the increased serological titers of autoantibodies to dsDNA, other nuclear antigens and complement proteins. An increase of the secretory phospholipase A2 (sPLA2) in serum is another diagnostic marker of SLE development. The higher enzyme activity of sPLA2 generates free fatty acids and lysophospholipids that play a crucial role in the maintenance of inflammatory process. We studied the correlation between the sPLA2 activity and the titers of autoantibodies against the complement proteins C3 and Factor H. Methods: Sera from 18 patients in acute phase of SLE were analyzed for the sPLA2 activity with spectrophotometric assay using the chromogenic lipid substrate 4-nitro-3-octanoyloxy-benzoic acid (NOBA). Detection of autoantibodies to complement C3 and Factor H was performed with homemade ELISA in 96-well microtiter plates, coated with purified C3 and Factor H proteins. Pooled serum from healthy donors (n=26) was used as a control. Results: Among the 18 analyzed patients, 5 showed increased sPLA2 activity and anti-C3 and/or anti- Factor H antibodies. The patients that were negative for increased sPLA2 activity could be divided in three groups: positive for anti-C3 antibodies (n=3), positive for anti-Factor H antibodies (n=3) and positive for both anti-C3 and anti-Factor H antibodies (n=4) Conclusion: Increased serum level of sPLA2 was found to correlate with the presence of autoantibodies to either C3 or Factor H or both.

NANOPARTICLES CONTAINING INFLUENZA A (H1N1) VIRAL PEPTIDES INDUCE ANTIGEN-SPECIFIC IMMUNE TOLERANCE IN A MOUSE MODEL OF NARCOLEPSY TYPE 1

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Narcolepsy type 1 (NT1) is a chronic central nervous system disease. Almost all NT1 patients carry the HLA-DQB1*06:02 allele, thereby suggesting an immunopathological origin of the disease. An association of NT1 with influenza A (H1N1) infection or pandemic influenza A H1N1 vaccination (Pandemrix®) has been observed. T-cell immunity against specific influenza virus epitopes is enhanced in Pandemrix-associated NT1 patients. Poly(lactide-co-glycolide) nanoparticles encapsulating peptide or protein antigens can induce tolerance in animal models of autoimmune diseases. Here, we investigated the immunomodulatory efficacy of nanoparticles encapsulating one of three different influenza A virus peptides in a mouse model of NT1. 2.5mg of nanoparticles were administrated intravenously on days -11 and -3 before Pandemrix vaccination (days 0 and 14; 50ul i.m.) to three groups of HLA-DQ6 NOD-C57BL/6 F1 hybrid mice. Additionally, a mix of all three nanoparticles was administrated to another group. A control group received only immunization (n=11-13). On day 28, spleen cells were harvested and re-stimulated for 6 days with viral peptides. Secreted inflammatory cytokines were measured in cell culture supernatants (multiplex ELISA). Additionally, the expression of CD69 on CD4, CD8 or CD19 positive cells was evaluated by FACS. The results

showed that pre-treatment with nanoparticles decreased the secretion of inflammatory cytokines in a peptide-specific fashion. For all three viral peptides, pre-treatment with mixed nanoparticles was equally effective than single nanoparticles. Additionally, lymphocyte activation was reduced by pre-treatment with nanoparticles. This strategy might be developed for the immunomodulatory treatment of patients of NT1.

A C3-TARGETING NANOBODY INHIBITS THE C3NEF ACTIVITY FROM PATIENTS WITH C3G BY PROMOTING THE DISSOCIATION OF THE C3BBB CONVERTASE

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C3 glomerulopathy (C3G) is a rare kidney disease secondary to dysregulation of the complement alternative pathway (AP) in plasma and the glomerular microenvironment. This unrestrained complement activation is frequently associated with C3 Nephritic

factors (C3NeFs), a group of antibodies binding an unknown neoepitope generated in the AP C3 convertase (C3bBb), and that stabilize it. We explored whether C3-nanobodies (hC3Nb1, hC3Nb2, hC3Nb3) being potent inhibitors of the AP, may influence C3NeF activity and promote the dissociation of the C3bBb convertase. Using Luminex, we set up a new functional C3NeF detection assay, in which C3bBb convertases are generated on the surface of magnetic beads. Preformed-C3bBb were allowed to decay with purified IgG, and the residual bound-C3bBb was estimated by measuring Bb fragments to the bead surface. Sixteen patients with biopsy-proven C3G and hypocomplementemia, were screened for C3NeF and IgG binding to C3bBb using Luminex and ELISA. In both models, each nanobody was incubated with patients IgG on the preformed-C3bBb. We identified 12/16 patients with anti-C3bBb, for which the IgG binding to C3bBb was inhibited with hC3Nb3 but not with hC3Nb1 and hC3Nb2. By LuminexTM, 9/16 patients IgG increased the residual bound-Bb compared with healthy donors IgG (HDs). In all cases the IgG-mediated C3bBb stabilization was specifically inhibited by hC3Nb3. In addition, we observed that hC3Nb3 exclusively induced the decay of stable C3bBb in absence of C3NeF-positive IgG. We demonstrated that hC3Nb3 interfere with the binding and functional effect of patients IgG on the C3bBb convertase. Our results showed that its capacity to dissociate stable C3bBb strongly participate to inhibit C3NeF function, and suggest that C3NeF and hC3Nb3 share a common binding site on C3bBb. It also implies that hC3Nb3 may specifically prevent the C3 cleavage mediated by C3NeF, thus providing a new potential therapeutic application in GC3.

QUANTIFYING CD8+ TEMRA CELLS USING STATISTICAL MODEL

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Introduction. Accumulation of terminally differentiated effector memory T (Temra) cells with age has been associated with many chronic inflammatory conditions. So far, Temra cells have been counted by flow cytometry (FC). Recently, statistical model using site-specific DNA methylation levels was developed to quantify CD8+ Temra cells from whole blood, which is less costly and time-consuming compared to FC. The aim of this study was to test CD8+ Temra model on a new dataset and compare the results with FC data. Methods. Whole blood from 593 individuals was collected into EDTA vacutainers. For bisulfite sequencing, DNA was treated with sodium bisulfite and then sequenced with Illumina MiSeq. Additionally, 123 samples were analysed with flow cytometry (LSR Fortessa) using whole-blood staining. Statistical analysis and graphs were made with R. This project is part of a COVID-19 Longitudinal study. Results. Model-based (predicted) Temra values and FC data were in good correlation (R=0.72). There was an increase in predicted and FC measured Temra cells amongst >50-year-olds compared to <50-year-olds. FC values had a weak correlation with age (R=0.35), but the relationship was lost with the predicted values. Even though there was an increase in FC measured Temra cells in individuals with hypertensive diseases and inflammatory polyarthropathies, there was no significant differences in predicted Temra numbers between disease and control groups. Conclusions. Although predicted Temra values didn't correlate with age, they did correlate with FC values. As correlation between predicted and FC values was not perfect and correlation between FC values and age weak, it is understandable, that the correlation with age was lost with the predicted values. Thus, the model needs to be tested on other datasets or improved using new Temra specific methylation sites.

AUTOANTIBODIES NEUTRALIZING TYPE III INTERFERONS ARE UNCOMMON IN PATIENTS WITH SEVERE COVID-19 PNEUMONIA

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Autoantibodies neutralizing type I interferons (IFN) underlie about 15% of cases of critical COVID-19 pneumonia. The impact of autoimmunity towards type III IFNs remains unexplored. We included samples from 1002 patients with COVID-19 (50% with severe disease) and 1,489 SARS-CoV-2-naïve individuals. We studied the prevalence and neutralizing capacity of autoantibodies towards IFNλ and IFNα. Luciferase based immunoprecipitation method was applied using pooled IFNα (subtypes 1, 2, 8 and 21) or pooled IFNλ1-3 as antigens, followed by reporter cell-based neutralization assay. In the SARS-CoV-2-naïve cohort IFNλ autoantibodies were more common (8.5%) than those targeting IFNα2 (2.9%) and were less associated with older age. In the COVID-19 cohort the presence of autoreactivity

to IFNλ did not associate with severe disease (OR 0.84; 95% CI 0.40-1.73), unlike to IFNα (OR 4.88; 95% CI 2.40-11.06; p<0.001). Most IFNλ autoantibody positive COVID-19 samples (67%) did not neutralize any of the three IFNλ subtypes. Pan-IFNλ neutralization occurred in five patients (0.50%), who all suffered from severe COVID-19 pneumonia, and four of them neutralized IFNα2 in addition to IFNλ. Overall, autoantibodies to type III IFNs are rarely neutralizing, and do not seem to predispose to severe COVID-19 pneumonia on their own.

ABSTRACTS / POSTER PRESENTATIONS

COMPARISON OF STOOL BACTERIOME IN WOMEN AT RISK OF GESTATIONAL DIABETES AND THEIR OFFSPRINGS

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Gestational diabetes mellitus (GDM) is a metabolic disease defined as any degree of glucose intolerance with the first onset or recognition during pregnancy. It is associated with several adverse maternal and offspring outcomes. Maternal gut microbiome status differ significantly between the GDM and non-GDM mothers. We hypothesize that stool bacteriome of children born to GDM versus non-GDM mothers, may also differ. In addition, we hypothesized that allergic and non-allergic children´s stool bacteriome differs from each other. Fifty-four mothers (GDM n=18 and non-GDM n=36) and their children (1–2 years old) from Woman's Clinic of Tartu University Hospital (September 2020 to June 2021) were included in this study. Stools samples were collected using IsohelixTM Stoolfix collection tubes. Stool bacteriome was profiled by 16S rDNA sequencing. Sequencing was performed on a MiSeq instrument using a 2x250 cycles sequencing Reagent Kit v2 (both Illumina, San Diego, CA). The measures of alpha diversity of samples from GDM mothers did not differ from non-GDM mothers or their children (both p-values >0.05). No significant difference in alpha diversity was found between children with and without allergies (p-value >0.05). Beta diversity measures did not reveal clustering by any case-control status. In conclusion, stool bacteriome was not associated with mothers GDM and the same applies for their children. However, bacteriome composition may differ between studied groups and this needs further analysis.

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THE DYNAMICS OF TYPE I INTERFERON AUTOANTIBODIES AND SALIVARY GLAND INFLAMMATION IN AIRE-DEFICIENT RATS

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Thymic epithelial cells express autoimmune regulator (AIRE), which has a key role in the development of central tolerance. Lack of functional AIRE protein causes a rare autosomal- recessive disorder in humans called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). Although APECED has a highly variable phenotype, almost all cases are characterized by the development of type I interferon (IFN) autoantibodies, whose functions are not well understood. To further study the disease, mouse and rat models have
been constructed with the latter being the more promising but least studied of the two.

The aim of our research was to systematically study the dynamics of anti-IFN-α autoantibodies and of the salivary gland inflammation while also analyzing their relations to each other in Aire-deficient rats. To this end, we compared Aire-KO rats with controls, conducting flow cytometry, immunofluorescence microscopy, hematoxylin and eosin staining on salivary gland samples, luciferase immunoprecipitation system on serum and RT-qPCR on poly(I:C) stimulated, serum incubated splenocytes.

We found that type I IFN autoantibodies develop primarily in three to five month-old Aire-KO rats. This happens prior to the onset of other symptoms induced by Aire-deficiency. The production of autoantibodies was found to increase continuously and reach its peak in nine to twelve-month-old rats, after which it remained stable. The most severe inflammation was observed in three to five-month-old Aire-KO rats. After the peak inflammation decreased and salivary glands began to resemble those of healthy heterozygous rats. Additionally, we observed significant downregulation of inflammatory gene expression in poly(I:C) stimulated splenocytes that were incubated with old AIRE-KO rat serums.

In conclusion, our data suggests a negative correlation between salivary gland inflammation dynamics and anti-IFN-α autoantibody dynamics, implying anti-IFN autoantibodies may protect Aire-KO rats against the development of autoimmune inflammations.

THERAPEUTIC EFFECT OF DUPILUMAB IN CHRONIC RHINOSINUSITIS

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Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose that is associated with severe sex and population-specific symptoms and affecting over 10% of adults in Europe. Due to its heterogeneous symptomatic and inflammatory nature, treatment of this incurable disease requires a better understanding of the disease mechanisms as well as personalized therapy. In our study, we seek to understand the longitudinal cell type specific response to an innovative anti-inflammatory medication named dupilumab on both the local environment of the nose and peripheral system. Dupilumab is a monoclonal antibody targeting type 2 inflammation, an underlying immune reaction of CRS disease. Here, we sequenced single-cell transcriptomes from nasal specimens of CRS patients and healthy individuals and applied flow cytometry and inflammatory marker analyses on matched blood samples. The nasal scrapings analysis revealed various types of epithelial cells including basal, apical, goblet, ciliated, ionocytes and immune cells such as T cells, mast cells and neutrophils with varying proportions between conditions and across treatment. Inflammatory marker analysis suggested a decreased inflammatory response to dupilumab treatment and its correlation with the clinical outcome pinpointed the specific immune processes of pa-

tients who did not respond to treatment. We will use this information to study the further differences on a cellular and gene expression level between local and systemic responses to treatment. Our results have the potential to significantly advance our understanding about the cellular basis of CRS and pave the way towards new therapeutic approaches.

HARNESSING EPIGENETIC CHANGES TO ESTIMATE IMMUNE CELL LEVELS

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Epigenetic modifications refer to changes in the chemical structure of DNA that can affect gene expression without altering the underlying DNA sequence. DNA methylation is one of the most well-studied epigenetic modifications, which consists of adding a methyl group to a cytosine base in a CpG dinucleotide. Accumulation of DNA methylation is an age-dependent factor in the human body, with certain genomic regions being particularly susceptible to methylation changes with increasing age. Interestingly, DNA methylation patterns can act as biomarkers for ageing and immune system status, with specific regions being more informative for one or the other. Each cell type in the body has its characteristic epigenetic profile, and DNA methylation changes in specific regions can be used to estimate the levels of particular cell types in whole blood samples. In our work, we used whole blood samples from donors of various ages (ranging from 20 to 99 years old) to sequence CpGs of bisulfite-treated DNA and, in parallel, PBMCs from the same individuals to determine the levels of selected cell populations. By combining this data with the previously existing one, we built a model for predicting the proportions of specific cell types. This work demonstrates the possibility of using DNA methylation to determine the levels of T effector memory cells re-expressing CD45RA (TEMRA), a type of memory T cell that plays a crucial role in the immune response to viral infections. Furthermore, this study suggests that epigenetic models could be used as alternatives to flow cytometry. Overall, we have shown the potential of using epigenetic modifications, particularly DNA methylation, as a valuable tool for estimating cell populations and understanding age-related changes in the body.
Keywords: Ageing, epigenetics, DNA methylation, TEMRA cells

TRANSCRIPTOME ANALYSIS TO STRATIFY SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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Introduction. Systemic Lupus Erythematosus (SLE) is a very heterogenous autoimmune disease which can affect almost every major organ system, making the treatment of this disease very challenging. One of the most common characteristics of SLE is upregulation of type I interferons (IFN), which causes interferon-stimulated gene signature (ISG), thus making them of interest in the diagnosis and treatment of SLE. There is a need for more personalized treatment and for that it is important to know the endotype of the disease, which could be based on many different factors (e.g. ISG signature, IFNα levels, transcription patterns) to provide more precise treatment to every single patient. The aim of our study is to describe the molecular profile of SLE patients. Material and Methods. We analyzed 70 Estonian SLE patients and 20 controls (age- and sex-matched). IFNα levels were measured by Single Molecule Array. RNA was isolated from whole blood using Tempus Spin Isolation kit and cDNA was synthesized. We performed qPCR (Quantitative Polymerase Chain Reaction) using ISGs and RNA-sequencing. Results. When comparing patients to controls, we discovered 404 differentially expressed genes which were upregulated in patients. Most of the top upregulated genes were ISGs. We also compared patients based on IFNα concentration (high vs low) and discovered 118 upregulated genes, but interestingly very few of them were interferon-stimulated. We tested 9 ISGs with qPCR and calculated IFN score based on the results. We compared IFN score to IFNα levels and discovered that they were highly correlated. Conclusions. Based on our results we suggest that there is a subset of patients who harbor interferon-stimulated gene signature. However, there is also another subset of patients, non-dependent of ISGs, whose pathogenicity may be affected by other immune pathways. Our results from qPCR suggest that IFN score could be used to predict IFNα concentration in SLE patients.

THE PROFILE OF MATERNAL CYTOKINE LEVELS DURING PREGNANCY IN WOMEN AT RISK OF GESTATIONAL DIABETES AND THE DEVELOPMENT OF ATOPIC DERMATITIS IN THEIR OFFSPRING

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BACKGROUND. During gestational diabetes (GDM) carbohydrate metabolism is disturbed in pregnant women due to insulin resistance. GDM usually manifests in the 2nd or 3rd trimester of pregnancy, and although it resolves after pregnancy, it can affect the health of both mother and fetus. GDM is associated with disorders of the mother's immune system that can have an additional effect on the long-term health outcomes of the child. Cytokines are immune system's modulators, which could play an important role in controlling several of these processes.

PURPOSE. To analyze and find associations between blood plasma cytokine levels during 2nd and 3rd trimester of pregnancy in women at risk of GDM and the development of atopic dermatitis in their children. MATERIAL AND METHODS. The study group consisted of 88 pregnant women who were at risk of GDM development. Of them 39 (44%) were diagnosed with GDM. Blood plasma was obtained during gestational week 23-30 and the level of CCL2, CXCL8, GM-CSF, IFNγ, IL-1β, IL-5, IL-6, IL-10, IL-12p70, IL-17A, and TNFα was measured with a multiplex assay on the Luminex 200TM. Information on offspring's diagnosis of atopic dermatitis was obtained from Electronic Health Record. IgE sensitization was measured with Phadiatop Infant on ImmunoCap 100 (Thermo Fisher). RESULTS. The odds of developing atopic dermatitis in children (age 1-6 years) were increased if their mothers had higher levels of: CCL2 (OR=1.09; P=0.040), IFNγ (OR=1.24; P=0.016), IL-1β (OR=1.28; P=0.020), IL-10 (OR=3.44; P=0.025), IL-12p70 (OR=1.43; P=0.039), IL-17A (OR=1.87; P=0.045), and TNFα (OR=1.62; P=0.035), irrespective of maternal GDM diagnosis or IgE sensitization.

CONCLUSIONS. Maternal blood plasma cytokine levels during pregnancy were associated with an increased likelihood of atopic dermatitis in their children while no association with GDM was found.

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THE COMPLEMENT SYSTEM AS THERAPEUTIC TARGET IN HIGH-GRADE SEROUS OVARIAN CANCER

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Epithelial ovarian cancer is the sixth leading malignancy amongst women. High-grade serous ovarian cancer (HGSOC) is the most common and fatal subtype. Most patients develop ascites, which promotes metastasis through the peritoneal cavity by increased pressure and contains cytokines that stimulate immune escape. However, ascites may also contain components of an active immune system, such as complement factors and tumor-directed antibodies. The tumor cells protect themselves from complement killing through for instance overexpression of the complement inhibitors CD59 on membranes and Factor H in the fluid phase. Our goal is to study whether local inhibition of these complement inhibitors could allow antibodies and complement in ascites to kill tumor cells.

We obtained ascites fluid samples (n=101) from ovarian cancer patients treated at Helsinki University Hospital Women's Clinic. The ascites samples were screened for the presence of tumor-directed IgG antibodies by immunoblotting. The presence of classical pathway (CP) complement factors in ascites was examined by in-house sandwich ELISA. We observed a wide diversity between patients in both levels and types of antibodies and CP activity in ascites samples. Preliminary results of hemolysis assays showed that monoclonal rat IgG2 anti-human CD59 (YTH53.1) allowed about 25% of ascites samples to kill red blood cells at a comparable level as normal human serum. We will use these ascites samples together with YTH53.1, anti-Factor H and/or classical carboplatin drugs, to improve killing of HGSOC cell lines and patient's own tumor cells. Preliminary results suggest that some ascites samples, rich in tumor-directed IgG and classical complement pathway activity, are able to kill the HGSOC cell line OVCAR8 in the presence of YTH53.1.

In summary, tumor-directed IgG and classical complement activity are present in ascites fluids of HGSOC patients and seem to be able to kill RBCs and HGSOC cell lines, when CD59 is inhibited.

SERUM IgA AND IgG ANTI-GLIADIN ANTIBODIES IN THE OFFSPRING OF MOTHERS WITH GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) is defined as an abnormal glucose tolerance with onset during pregnancy. GDM is associated with several offspring's long-term complications, such as allergies, obesity, metabolic syndrome, cardiovascular disease, non-alcoholic fatty liver disease, and autism, all of which are associated with increased intestinal permeability. It has been proposed that wheat protein gliadin disrupts the intestinal barrier function and anti-gliadin antibodies (AGA) might therefore be reflective of intestinal permeability. In this study, IgA and IgG AGA were measured from sera of offspring of mothers with GDM (n=34) and without GDM diagnosis (n=40). Sera were collected at two visits one year apart. Anti-gliadin IgG and IgA antibodies were measured using an in-house ELISA. Offspring's allergy diagnoses were obtained from Electronic Health Records system. Our results showed that there were no statistically significant differences in IgA or IgG AGA levels between offspring of mothers with and without GDM. IgA AGA levels were found to be lower in older children at the second visit ($p=0.005$), but there was no difference in IgG AGA levels ($p > 0.05$ for all comparisons). Offspring with atopic dermatitis had higher IgA and IgG AGA levels compared to offspring without this diagnosis at both visits ($p<0.05$ for all comparisons). No such correlations were seen with asthma diagnosis. AGA levels correlated neither with mothers' pre-pregnancy BMI nor with the way of delivery. Offspring breastfed until the second visit had lower IgA AGA levels compared to offspring who were weaned from breastmilk ($p=0.007$). No correlation was found between AGA levels and duration of breastfeeding. Although GDM and non-GDM mothers' children did not differ in AGA levels, we noted that intestinal permeability increases in atopic dermatitis despite the general decreases when a child matures.
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ANTIBODIES AGAINST STREPTOCOCCUS PNEUMONIAE PNEUMOLYSIN CORRELATE WITH MODIFIED HDL LEVELS AND COMPLEMENT ACTIVATION MARKERS IN ATHEROSCLEROSIS

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Streptococcus pneumoniae is an opportunistic pathogen that causes pneumonia and systemic infections such as sepsis. Pneumolysin (PLY) is a cholesterol dependent pore-forming cytolysin that is an important virulence factor for S. pneumoniae. Earlier we found that PLY changes the structure and function of HDL leading to reduction in atheroprotective properties of HDL. Antibodies against PLY can be detected from healthy donors and patients with pneumonia and pneumococcal infections may cause acute complications in atherosclerosis patients. Therefore, we next investigated the role of anti-PLY antibodies in peripheral arterial disease patients in in vitro functional assays and in ex vivo atherosclerotic plaques of femoral arteries. We found a positive correlation between plasma anti-PLY IgG and malondialdehyde (MDA) modified HDL levels. Interestingly, isolated IgG from the patients with high anti-PLY levels significantly inhibited PLY induced hemolysis. Plasma levels of complement activation markers correlated positively with anti-PLY. Atherosclerotic sections revealed complement activation and inhibition in different locations of femoral artery plaques. These results indicate that anti-PLY IgG might have both protective and inflammatory roles in atherosclerotic processes and therefore, detailed mechanistic function of this antibody needs to be further explored.

THE ROLE OF THE STAUFEN PARALOGS IN THE GERMINAL CENTER B-CELLS

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To become an antibody-producing cell, activated B-cells undergo a differentiation process in disctinct secondary lymphoid structures termed germinal center (GC). Although the transcriptional networks guiding GC B-cells (GCBs) differentiation are well studied, the regulation at the level of translation is yet poorly understood.

Post-transcriptional regulation by RNA binding proteins (RBPs) influences mRNA stability, localization and translation. Proteomic analysis focused on up-regulated RBPs in GCBs showed Staufen 2 (Stau2) but not its paralog Staufen 1 (Stau1) is up-regulated in this cell population. To dissect the role of both Staufen (Stau) paralogs in the GC response, we immunized mice lacking Stau1 in the whole body, mice lacking Stau2 only in B-cells and mice deficient in both Stau paralogs in B-cells. While B-cells deficient either in Stau1 or Stau2 shown almost no differences with the controls in the GC formation, B-cells conditional Stau double knockout (DKO) are affected both in the total GC formation and the specific response.

Using the murine GCB-like cell line A20, we performed irCLIP to decipher the RNAs being targeted by the Stau paralogs. As expected, between the Stau there is an overlapping in their targets. However, only the Stau2 deletion in A20 cells dramatically change the proteome, affecting the expression of Stau-targets relevants for the DNA repairment, lipid metabolism, regulation of the transcription and cell cycle.

According to co-IP associated to mass spectrometry results, Stau paralogs might conform distinct RNPs since they have different RBPs interactors. While Stau1 interact with classical nucleolar RBPs, Stau2 seems to have a spread cytoplasmic activity where interact even with translational initiator. Thus, we speculate that although both Stau paralogs share the majority of their targets, there is a dynamic functional compartmentalization when totally absent in the StauDKO mice is detrimental for the GC formation.

DETECTION OF BIFIDOBACTERIUM BREVE ANTIBODIES BY FLOW CYTOMETRY IN BLOOD SERUM OF CHILDREN OF MOTHERS AT RISK OF GESTATIONAL DIABETES

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Gestational diabetes (GDM) is a disorder of carbohydrate metabolism occurring during pregnancy and the intestinal microbiota dysbiosis is considered to promote the disease. Specific bacterial taxa associated with GDM may be transmitted to the offspring and the dysbiosis may be related to allergies that occur in early childhood. Bifidobacterium breve has been found in the intestines of breastfed children and is thought to be involved in activating the immune system and preventing allergies. The aim of this longitudinal study was to determine IgA, IgG and IgG2 type antibodies that react with B. breve in the blood serum of children, whose mothers were at risk of GDM during pregnancy. The study group consisted of 88 children aged 1 – 6 years. Blood samples were taken at two time-points one year apart during the subjects paediatrician's visit. B. breve DSM20213 was diluted to a concentration of 5x10˘6 cells/µl and antibody reactivity was detected on a LSRFortessa flow cytometer using the adapted method of Moor et al., Nat Protoc. 2016. The obtained results were compared with the clinical data of children (gender, age, breastfeeding, way of delivery and maternal GDM diagnosis). IgE sensitization was measured with Phadiatop Infant on ImmunoCap 100 (Thermo Fisher). Reactivity to B. breve did not differ between children based on their mother's GDM diagnosis. We detected mainly IgA type antibodies that reacted with B. breve, espe-

cially in younger children who were breastfed, delivered by vaginal birth and who were IgE-negative. With increasing age, IgA type reactivity decreased and IgG type reactivity increased. We could not detect IgG2 type antibodies towards B. breve. The study was supported by EU Human Exposomic Determinants of Immune Mediated Diseases (Hedimed) Consortium grant https://www.hedimed.eu/ and Estonian Science Foundation grant no. 712.

AUTOANTIBODIES AGAINST INTERLEUKIN 1-ALPHA: FREQUENCY, POTENTIAL AND BIOLOGICAL ROLE

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Interleukin 1-alpha (IL-1α) is a pro-inflammatory cytokine. Autoantibodies that bind and neutralize this cytokine can therefore influence immune response. Our work investigated the presence of IL1-α autoantibodies in 849 plasma samples from three different cohorts (healthy controls (n=225, average age=46, SD=24.4), elderly patients with multiple diseases from Tartu University Clinics (n=510, average age=75.7, SD=6.9) and recovered COVID-19 patients (n=114, average age=56.7, SD=13.09) and their ability to neutralize IL1-α.

IL1-α autoantibody levels were determined by luciferase-based immunoprecipitation system (LIPS). Cut-off values were calculated according to the control group values: average + 3SD. Samples over the cut-off were assessed for their neutralizing ability. For this, IL-1α was pre-incubated with patient plasmas and added to the reporter cell line NHDF. After the overnight incubation supernatants were collected and IL-1α induced chemokine CXCL8 release was measured with commercial ELISA set.

IL1-α autoantibody prevalence differed between the study groups: 7% in the control population, 14% in samples collected from Tartu University Clinics and 19% of the COVID-19 convalescents were autoantibody positive. No correlation between age and antibody frequency was found. It was found that plasmas with high levels of IL1-α autoantibodies are also stronger neutralizers. Recovered COVID-19 patients were divided into four groups based on WHO disease severity. It was found that IL1-α autoantibody frequency was the highest in the critically-ill patients (WHO4). However, no difference in neutralization ability was found when comparing the groups (WHO4 to WHO1-3).

Our work revealed that IL1-α autoantibody prevalence has no correlation with age instead the increase is affected by various diseases.

CENTRAL TOLERANCE MECHANISMS IN AN APECED RAT MODEL

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The Thymus is an essential organ involved in thymocyte development and plays an important role in establishing central tolerance. Autoimmune regulator (Aire), which is expressed in thymic epithelial cells (TECs), is a critical molecule in inducing central tolerance and Aire deficiency causes multi-organ autoimmune disease APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy). The aim of the current study was to describe the Aire knock-out (KO) rat model in respect to its peripheral autoimmune phenotype and in respect to the intrathymic alterations mechanisms by which Aire deficiency may result in autoimmunity. We confirmed with immunofluorescent microscopy (IF), real-time PCR and flow cytometry (FC) that the autoimmune phenotype of Aire KO rats resembled the one of Aire KO mice and was characterized by autoimmune infiltrations in peripheral organs. Regarding the thymus, we found no disturbances neither in thymic morphology nor thymic epithelial differentiation as opposed to the previously characterized mouse model. However, the whole genome expression analysis (GeneTitan) of TECs revealed that Aire regulates tissue restricted antigen (TRA) and interferon stimulated gene (ISG) expression whereas chemokine expression was not affected. Also, similarly to the mice, Aire KO rats were characterized by reduced thymic regulatory T-cell numbers. In conclusion, the study suggests that the breakdown of central tolerance in Aire KO rats is likely to be caused by impaired TRA expression and regulatory T-cells induction and may be modified by impaired thymic interferon signalling.

SURFACE EXPRESSION OF CD28 AND TIGIT CORRELATES WITH C-PEPTIDE LEVEL IN RECENTLY DIAGNOSED ADULTHOOD-ONSET TYPE 1 DIABETES

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Co-stimulatory and co-inhibitory immune cell surface molecules, also called immune checkpoints, along with their ligands play the fundamental role in shaping the immune response. Abnormalities in their expression and function correlate with different immunological diseases, including type 1 diabetes (T1D). T1D is a chronic autoimmune disorder resulting from destruction of pancreatic beta cells by cytotoxic CD8+ lymphocytes. In this study, we analysed surface expression profiles of checkpoint molecules in 19 adults with recent-onset T1D (mean age 29.0 ± 6.0 years) and 68 age- and sex-matched healthy donors before and after cell stimulation using flow cytometry. In particular, we measured the median fluorescence intensity (MFI) of co-stimulatory CD226 and CD28 molecules and co-inhibitory TIGIT (T-cell immunoreceptor with Ig and ITIM domain) as well as the percentage of marker-positive cells. The samples of diabetic patients were collected in the course of three weeks after diagnosis; also, the blood C-peptide level representing insulin production was measured. Within our study population, we observed no significant differences between the surface expression of CD226, TIGIT, and CD28 on unstimulated or stimulated cells in major T-cell subsets. However, patients with C-peptide level higher than 0.2 nmol/L showed lower CD28 MFI on CD8+ effector memory (EM) T cells and a higher percentage of TIGIT+ cells within the CD28-CD8+ EM subset. Lower CD28 expression and a higher proportion of TIGIT+ cells within the CD8+ EM population may indicate better regulatory control over autoimmune processes in these donors, which in turn provides higher residual insulin production and a milder disease course. This work shows that TIGIT has a potential for implementation as a treatment target not only in cancer immunotherapy, but also in the prevention of T1D. This study was supported by Estonian Science Foundation grant no. 712.

CLINICAL CHARACTERISTICS OF PATIENTS WITH AUTOIMMUNE RELATED ACQUIRED FVIII DEFICIENCY (ACQUIRED HAEMOPHILIA A) IN A SINGLE CENTRE COHORT

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Introduction: An autoimmune-mediated process with autoantibodies against coagulation factor VIII causes severe bleeding and is defined as acquired haemophilia A (AHA). The incidence is approximately 1,5 to 3 per million people in a year. The antibodies develop in individuals with no previous history of bleeding. The cause remains idiopathic in 50% of cases and is associated with pregnancy, autoimmune diseases, medications, infections, malignancies. The occurrence is common in elderly patients. The basic strategies of the management of AHA patients include maintaining haemostasis, eradication of antibodies and diagnosis of underlying pathology Patients and methods: A retrospective chart review of patients diagnosed and treated in the Haemophilia Treatment Centre of Berlin in 2016-2019 Results: 12 patients were diagnosed with AHA (median age 78 years, range 38 to 92) and experienced 14 bleeding episodes with a need for transfusion in 8 bleedings (Hemoglobin ranged 3.1-10.2g/dl (N:12.0-15.6g/dl) with 3 doses (median, range 2-14 doses). The bleeding episodes were treated in all patients with recombinant porcine FVIII, in 3 episodes later with rFVII and aPCC and in all patients concomitantly with tranexamic acid. All patients received immunosuppressive treatment with prednisolone, one case additional cyclophosphamide and four cases additional anti-CD20 treatment with rituximab. There were two deaths in this cohort, one related to haemorrhage and one to septic shock. One relapse occurred in one patient 7 months after first diagnosis. Two patients got thrombotic complications during follow-up. Conclusion: AHA is a rare coagulation disorder and challenging in diagnosis and treatment. There are good treatment options and in case of a response to the treatment, a good life expectancy. The patients need repeated follow-up to manage relapses early.

CLONING AND ANALYSIS OF THE ANTIGEN-BINDING REGION OF HYBRIDOMA-DERIVED MONOCLONAL ANTIBODY SPECIFIC TO HUMAN CARBONIC ANHYDRASE

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Biopharmaceutical antibodies are often derived from murine hybridomas producing monoclonal antibodies (MAbs) of desired specificity. In order to generate recombinant antibody, the constant sequences of murine immunoglobulins (Ig) are replaced with the respective regions of human Ig by genetic engineering leading to reduced immunogenicity. The variable fragments of heavy (VH) and light (VL) chains must remain unchanged to maintain the same antigen-binding properties as the maternal antibody. Identification of Ig variable region sequences is challenging but necessary step to generate recombinant antibodies. Here we describe the determination of the variable region sequences of hybridoma-derived MAb 14D6 directed against cancer-associated human carbonic anhydrase XII (CA XII). The VH and VL fragments were cloned from total RNA isolated from a stable hybridoma clone 14D6 by PCR using a set of degenerative primers specific for the framework and constant regions of mouse IgG heavy and light chains. DNA sequencing was applied to identify the nucleotide sequence of Ig variable regions, following the analysis with various sequence alignment software, data bases, and tools (IMGT/V-QUEST, IgBLAST, Geneious Biologics). The sequences with accidental frameshifts, stop codons, deletions or atypical amino acids were eliminated and the plausible VL and VH sequences were obtained. Sequences of the complementarity determining regions (CDRs) of VL and VH were also defined, thus providing valuable information for the subsequent generation of recombinant antibodies. The identified VL and VH sequences were further verified by expressing the single-chain fragment variable (scFv) of 14D6 antibody in E. coli, which was shown to recognize CA XII by Western blot and ELISA. In conclusion, our study provides new data on the anti-CA XII antibody by identifying its antigen-binding region, which is a first step in developing recombinant antibodies of potential therapeutic relevance.

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