3rd European B Cell Forum 2021
From June 30 to July 2 2021

Book of Abstracts
Program - Abstracts
List of participants - List of sponsors

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3rd European B Cell Forum 2021

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### Opening of the 3rd EBCNet Forum

Thierry Defrance & Hans-Martin Jäck

### Session #1. B cell memory/B cell subsets

**Talk participants**

**Chairs: Michel Cogné & Marc Seifert**

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<td><strong>Session #1. B cell memory/B cell subsets</strong></td>
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<td>09:20-09:40</td>
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<td>09:40-10:00</td>
<td>Human memory B cell dynamics in blood and spleen change with age</td>
<td>Artur Kibler (Institute of Cell Biology, University of Duisburg-Essen, Essen, Germany)</td>
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<tr>
<td>10:00-10:20</td>
<td>Diversity of memory B cells repertoire cope with SARS-CoV2 variants after mRNA vaccination in naïve and recovered individuals</td>
<td>Matthieu Mahévas (Institut Necker Enfants Malades (INEM), INSERM U1151/CNRS UMS 8253, Université de Paris, Paris, France)</td>
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<td>10:20-10:40</td>
<td>Persistence of functional memory B cells recognizing SARS-CoV-2 variants despite loss of specific IgG</td>
<td>Stephan Winklmeier (Institute of Clinical Neuroimmunology, University Hospital, LMU Munich, Munich, Germany)</td>
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10:40-10:47 **Sponsor - Miltenyi Biotec**

- **SARS-CoV-2 specific B cells and antibodies Analysis by Miltenyi Biotec**
  Shahul Mouhamad - Miltenyi Biotec

10:47-11:00 **Break**

11:00-12:20 **Session #1 (continued). B cell memory/B cell subsets**

**Talk participants**

**Chairs: Pierre Bruhns & Deborah Dunn-Walters**

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<td>VAST: a novel and versatile vaccination platform that induces robust and durable antibody responses</td>
<td>Paraskevi Vlachou (D150, German Cancer Research Center (DKFZ), Heidelberg, Germany)</td>
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<td>11:20-11:40</td>
<td>Single-Cell Transcriptomic Analyses Define Distinct Peripheral B Cell Subsets and Discrete Development Pathways</td>
<td>Alexander Stewart (School of Biosciences and Medicine, University of Surrey, Guildford, UK)</td>
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<td>11:40-12:00</td>
<td>T-bet+ B cells infiltrating the human brain: from peripheral induction to local effector function</td>
<td>Marvin M. van Luijn (Department of Immunology, MS Center ErasMS, Erasmus MC, Rotterdam, The Netherlands)</td>
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<td>12:00-12:20</td>
<td>Phenotypic and functional division of human Bregs in CD45lo and CD45hi B cells and their dependence on Btk and mTOR</td>
<td>Vassilios Lougaris (Pediatrics Clinic and Institute of Molecular Medicine A. Nocivelli, Department of Clinical and Experimental Sciences, University of Brescia and ASST - Spedali Civili of Brescia, Brescia, Italy)</td>
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12:20-12:27  Sponsor - Stemcell

• Tools for B cell Research
  Graeme Milton - Senior European Market Development Manager, Immunology

12:27-14:00  Lunch

14:00-15:40  Session #2. B cell development/B cell neoplasia

Talk participants

Chairs: Marta Rizzi & Thierry Defrance

14:00-14:20  • Transcription factor networks coordinate transcriptional priming and cellular expansion in early B-cell development
  Mikael Sigvardsson (Division of Molecular Hematology, Lund University, Lund, Sweden)

14:20-14:40  • IL-7R signalling activates widespread V\textsubscript{H} and D\textsubscript{H} gene usage to drive antibody diversity in bone marrow B cells
  Anne Corcoran (Lymphocyte Signalling and Development Programme, Babraham Institute, Cambridge, UK)

14:40-15:00  • Tracking neonatal B cell function in the adult gut
  Joan Yuan (Division for Molecular Hematology, Lund Stem Cell Center, Department of Laboratory Medicine, Faculty of Medicine, Lund University, Lund, Sweden)

15:00-15:20  • Siglec-G controls the severity of Chronic Lymphocytic B-cell Leukemia
  Lars Nitschke (Division of Genetics, Department of Biology, University of Erlangen, Erlangen, Germany)

15:20-15:40  • Continuous MYD88 activation is associated with expansion and then transformation of IgM differentiating plasma cells
  Christelle Vincent-Fabert (UMR CNRS 7276/INSERM U1262 CRIBL. University of Limoges, and hematology Laboratory of Dupuytren Hospital University Center (CHU) of Limoges, France)

15:40-15:47  Sponsor - Cytek Biosciences

• How spectral flow could be the solution for your lab
  Juliette Desfrançois - Cytek Biosciences

15:47-16:00  Break

16:00-16:45  Keynote lecture

• Memory B cells differentiation
  Dr Charlotte Viant (Laboratory of Molecular Immunology, The Rockefeller University, New York, USA)

16:45-17:15  EBCnet General assembly
THURSDAY 1 JULY

09:00-10:20 Session #3. Plasma cells

**Talk participants**

**Chairs: Claude-Agnès Reynaud & Hans-Martin Jäck**

- **09:00-09:20**  
  **Dissecting antibody-secreting cells differentiation at single cell level**  
  Niels J.M. Verstegen (Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, Amsterdam, The Netherlands)

- **09:20-09:40**  
  **The Sec22b SNARE is indispensable for plasma cell maintenance and antibody secretion**  
  Marion Espéli (Université de Paris, Institut de Recherche Saint Louis, EMILy, Inserm U1160, Paris, France)

- **09:40-10:00**  
  **Single-cell analyses of autoreactive plasma cells from immune thrombocytopenia patients**  
  Pierre Bruhns (Unit of Antibodies in Therapy and Pathology, Institut Pasteur, UMR 1222 INSERM, Paris, France)

- **10:00-10:20**  
  **TFG controls ER stress, autophagy and plasma cell function**  
  Dirk Mielenz (Division of Molecular Immunology, Department of Internal Medicine 3, Nikolaus-Fiebiger-Zentrum, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany)

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10:20-10:27 **Sponsor - Immudex**

- **dCODE® Klickmer - Exploring B-cell immunity at the single-cell level**  
  Valérie Bodemeyer - Immudex

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10:27-10:40 **Break**

10:40-12:20 Session #3 (continued). Plasma cells

**Talk participants**

**Chairs: René E.M. Toes & Idit Shachar**

- **10:40-11:00**  
  **Metabolic flux analysis of individual antibody-secreting cells – the impact of space, time, and immunization**  
  Klaus Eyer (Laboratory for Functional Immune Repertoire Analysis, Institute for Pharmaceutical Sciences, D- CHAB, ETH Zürich, Switzerland)

- **11:00-11:20**  
  **Complementary but distinct protective roles of tumor-infiltrating IgG and IgA producing cells**  
  Yasmine Lounici (Centre de Recherche en Cancérologie de Lyon, Inserm U1052, CNRS 5286, Lyon, France)

- **11:20-11:40**  
  **The BCR acts as a switch between the canonical and non-canonical functions of plasma cells**  
  Thierry Defrance (Centre International de Recherche en Infectiologie/CIRI, INSERM U1111, Lyon, France)

- **11:40-12:00**  
  **The RNA m6A binding protein YTHDF2 promotes the B cell to plasma cell transition**  
  Martin Turner (Immunology Programme, The Babraham Institute, Cambridge, CB223AT, UK)

- **12:00-12:20**  
  **IgA production decrease is associated with B cell differentiation and proliferation defects in multiple sclerosis**  
  Jeremy Morille (Centre de Recherche en Transplantation et Immunologie, INSERM UMR1064, Nantes University, CHU de Nantes, Nantes, France)

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12:20-12:27 **Sponsor - Miltenyi Biotec**

- **SARS-CoV-2 specific B cells and antibodies Analysis by Miltenyi Biotec**  
  Shahul Mouhamad - Miltenyi Biotec

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12:27-14:00 **Lunch**  

.../...
14:00-15:20 **Session #4. B cells in disease**

**Talk participants**

*Chairs: Marion Espéli & Kai-Michael Toellner*

**14:00-14:20**
- **MHCII haplotype determines IgG autoantibody Fc N-glycosylation and development of murine autoimmune skin disease**
  Rudolf Armin Manz *(Institute for Systemic Inflammation Research, University of Lübeck, Lübeck, Germany)*

**14:20-14:40**
- **N-linked glycosylation of the immunoglobulin variable domain affects antigen binding and autoreactive B-cell activation**
  Theresa Kissel *(Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands)*

**14:40-15:00**
- **Autoantibodies deconvolution in Membranous Nephropathy**
  Sara Montagner *(Novartis Institutes for BioMedical Research, Basel, Switzerland)*

**15:00-15:20**
- **B cell defect in Prkcd G510S/G510S mice with autoimmunity and lymphoproliferation**
  Marion Moreews *(CIRI, Centre International de Recherche en Infectiologie, Univ. Lyon, Inserm, U1111, Université Claude Bernard, Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France)*

**15:20-15:27**
- **Tools for B cell Research**
  Graeme Milton - Senior European Market Development Manager, Immunology

**15:40-17:20** **Session #4 (continued). B cells in disease**

**Talk participants**

*Chairs: Yolanda R. Carrasco & Jean-Claude Weill*

**15:40-16:00**
- **Transcriptional dysregulation of CVID patients harboring the C104R TNFRSF13B mutation**
  Neftali Jose Ramirez *(Centre for Chronic Immunodeficiency (CCI), University Medical Centre Freiburg, University of Freiburg, Freiburg, Germany)*

**16:00-16:20**
- **Inverted direct allorecognition triggers early donor specific antibody response after transplantation**
  Xavier Charmetant *(CIRI, Centre International de Recherche en Infectiologie, Université de Lyon, INSERM U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France)*

**16:20-16:40**
- **Post-transcriptional control of neuroinflammation by HuR**
  Dunja Capitan-Sobrino *(Toulouse Institute for Infectious and Inflammatory Diseases, INSERM (U1291), Toulouse, France)*

**16:40-17:00**
- **Decreased Levels of T Follicular Helper (CD4+CXCR5+) Cells and CD27+CD38+ and CD27+CD38- B Cells in Ankylosing Spondylitis Patients Correlate with Marker of Inflammation**
  Kristina Lejon *(Department of Clinical Microbiology, Umeå University, Umeå, Sweden)*

**17:00-17:20**
- **Follicular helper-like T cells in the lung highlight a novel role of B cells in sarcoidosis**
  Laura Bauer *(Institute of Immunology, University Hospital Schleswig-Holstein, Kiel, Germany)*

**17:20-17:27** **Sponsor - Cytek Biosciences**

*How spectral flow could be the solution for your lab*

Juliette Desfrançois - Cytek Biosciences
**FRIDAY 2 JULY**

**Session #5. Ag recognition/BCR signaling**

**Talk participants**

*Chairs: Rudolf Manz & Marieke Van Ham*

- **09:00-09:20**  
  - BCR functions from within the endoplasmic reticulum  
    Marieta Caganova (Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany)

- **09:20-09:40**  
  - CD20 as a gatekeeper of the resting state of human B cells  
    Kathrin Kläsener (Biology III, faculty of biology, University Freiburg, Freiburg, Germany)

- **09:40-10:00**  
  - Tetraspanins: molecular organisers of the B-cell surface  
    Annemiek van Spriel (Dept. of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands)

- **10:00-10:20**  
  - Novel players in B cell antigen uptake and trafficking  
    Dessi Malinova (Wellcome-Wolfson Institute for Experimental Medicine, Queen’s University Belfast, Belfast, Ireland)

**10:20-10:27**  
**Sponsor - Immudex**

- dCODE® Klickmer - Exploring B-cell immunity at the single-cell level  
  Valérie Bodemeyer - Immudex

**10:27-10:40**  
**Break**

**Session #6. Genetics of immunoglobulins**

**Talk participants**

*Chairs: Ulf Klein & Lars Nitschke*

- **10:40-11:00**  
  - Immunoglobulin enhancers increase RNA polymerase 2 (Pol2) stalling at somatic hypermutation (SHM) target sequences  
    Jukka Alinikula (Unit of Infections and Immunity, Institute of Biomedicine, University of Turku, Finland)

- **11:00-11:20**  
  - Study of IgH Locus Suicide Recombination ‘LSR’ in human normal and pathological conditions  
    Israa Al Jamal (CBRS CRIBL UMR7276 CNRS INSERM1262, Limoges University, Limoges, France)

- **11:20-11:40**  
  - IgTreeZ: A toolkit for immunoglobulin gene lineage tree analysis  
    Ramit Mehr (The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel)

- **11:40-12:00**  
  - A dual effect upon deletion of Special A-T Rich Binding Protein 1 in B cells  
    Morgane Thomas (Contrôle de la réponse immune B et des Lymphoproliférations, CNRS UMR 7276 Inserm 1062, Limoges, France)

- **12:00-12:20**  
  - Inactivation of EXO1 nuclease activity on genome maintenance and tumor suppression in Exo1los173A mice in vivo  
    Richard Chahwan (Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland)

**Farewell/Closure of the 3rd EBCnet Forum**
SESSION #1. B CELL MEMORY/B CELL SUBSETS
Most isotype-switched memory B cells reside in bone marrow or spleen

Marta Ferreira-Gomes¹ (marta.fgomes@drfz.de), René Riedel¹, Richard Addo¹, Frederik Heinrich¹, Gitta Anne Heinz¹, Jannis Kummer¹, Victor Greiff², Daniel Schulz³, Cora Klaeden¹, Rebecca Cornelis¹, Gabriela M. Guerra¹, Anja E. Hauser¹,³, Sai T. Reddy⁴, Pawel Durek¹, Hyun-Dong Chang¹,⁴, Mir-Farzin Mashreghi¹,³, Andreas Radbruch¹,³

¹ Deutsches Rheuma-Forschungszentrum (DRFZ), an Institute of the Leibniz Association, Berlin, Germany; ² Department of Immunology, University of Oslo, Oslo, Norway; ³ Charité Universitätsmedizin Berlin, Berlin, Germany; ⁴ Department of Biosystems Science and Engineering, Eidgenössische Technische Hochschule (ETH Zürich), Basel, Switzerland; ⁵ Institute for Biotechnology, Technical University Berlin, Berlin, Germany

Introduction: Memory B cells are an essential component of the reactive immunological memory. However, it remains unclear how B cell memory is organized and maintained over time.

Objective: Determine if memory B cells persist mainly as circulating cells or as resident cells of particular tissues.

Methods: The heterogeneity of murine spleen and bone marrow (BM) switched memory B cells (Bsm) was investigated through the analysis of single cell transcriptomes and B cell receptor repertoires.

Results: We were able to reveal an unforeseen diversity by identifying six distinct Bsm subpopulations, among which only one qualified as migratory. Among the resident populations, one is located exclusively in the spleen, and a population of quiescent Bsm located exclusively in the BM. In the BM, Bsm are resting in terms of activation, proliferation and mobility, and are individually docked onto VCAM1-positive stromal cells.

Conclusion: Murine switched memory B cells are largely heterogeneous and mostly resident in the spleen and bone marrow, while the circulatory counterparts only account for about 20% of these cells [1]. The discrete and resident B cell memory of bone marrow may be key to rapid secondary humoral responses to systemic antigens.

References:

Human memory B cell dynamics in blood and spleen change with age

Artur Kibler (artur.kibler@uk-essen.de), Marc Seifert

Institute of Cell Biology, University of Duisburg-Essen, Essen, Germany

Introduction: Human splenic marginal zone (sMZ) B cells are important in the defense against blood-borne pathogens but their origin, spleen homing and retention, and relation to peripheral blood (PB) memory B cells (MBC) is largely unknown.

Objective: Analysis of the Ig-repertoire diversity, clonal composition and relation, and GC maturation history of splenic and PB MBCs.

Methods: MBC subsets were sort purified from paired PB and spleen samples from 17 donors (0-77 years) and Ig-repertoire deep sequencing was supported by flow cytometric characterization and functional assays of individual B cell subsets.

Results: Throughout life, virtually all MBCs are archived and expanded in the spleen, whereas PB clone members show hallmarks of ongoing diversification. sMZ B cells are stochastically induced by NOTCH2-dependent priming of archived MBCs. With age, class-switched MBCs accumulate, splenic Ig-repertoire diversity is compromised, and clonal outgrowth is uncoupled from class-switching.

Conclusion: Throughout life, the spleen serves as a systematic MBC archive and the dynamic relation of circulating, archived and primed memory changes with age, thereby contributing to immune aging. [1]

Keywords: Human memory B cells - Marginal zone - BCR repertoire dynamics

References:
Diversity of memory B cells repertoire cope with SARS-CoV2 variants after mRNA vaccination in naïve and recovered individuals

Pascal Chappert\textsuperscript{1,2,*}, Aurélien Sokal\textsuperscript{1,3,*}, Giovanna Barba-Spaeth\textsuperscript{4,*}, Ignacio Fernandez\textsuperscript{4,*}, Matteo Broketta\textsuperscript{5,*}, Imane Azzaoui\textsuperscript{7,8,**}, Andrea de la Selle\textsuperscript{**1,}, Alexis Vandenberghe\textsuperscript{**1,3,6,*}, Slim Fourati\textsuperscript{7,8,**}, Annalisa Meola\textsuperscript{1,}, Magali Bouvier-Alias\textsuperscript{7,8,*}, Etienne Crickx\textsuperscript{3,*}, Laetitia Languille\textsuperscript{1,*}, Marc Michel\textsuperscript{1,*}, Bertrand Godeau\textsuperscript{3,*}, Florence Canoui-Poitrine\textsuperscript{9,*}, France Noizat-Pirenne\textsuperscript{6,10,**}, Jérôme Megret\textsuperscript{11,*}, Jean-Michel Pawlowski\textsuperscript{7,8,*}, Pierre Bruhns\textsuperscript{5,*}, Felix A Rey\textsuperscript{4,*}, Jean-Claude Weiβ\textsuperscript{11,*}, Claude-Agnès Reynaud\textsuperscript{11,*}, Matthieu Mahévas\textsuperscript{1,3,6,*} (matthieu.mahevas@aphp.fr)

* these authors contributed equally. ** these authors contributed equally. †shared senior authorship

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Introduction: The emergence of SARS-CoV-2 variants bearing mutations in key epitopes has raised concerns that viral evolution will erode natural immunity.

Objective: We aim to characterize the dynamic and functionality of anti-SARS-CoV-2 memory B cells (MBC) after mRNA vaccination in naïve and COVID-19 recovered individuals.

Methods: We analyzed 33 COVID-19 recovered, and 23 naïve individuals before and after mRNA vaccination and performed a longitudinal functional screening (VH sequencing, affinity and neutralization assays) of over 1587 naturally expressed antibodies from single-cell cultured RBD-specific MBCs.

Results: Anti-SARS-Cov-2 Memory B cells appear stable in most patients up to 1 year post COVID-19 and vaccinal boost mobilizes the MBC pool without causing any reduction in its overall diversity. In recovered patients, RBD-specific MBCs show clear evidence of affinity selection, a process still ongoing in naïve individuals two months after their second dose. Only a small proportion of RBD-specific MBC clones failed to recognize the B.1351 variant RBD in both cohorts. This, however, preferentially affects recurrent and convergent VH families with high neutralizing potential. Nonetheless, potent B.1351-neutralizer MBCs could still be detected in all naïve and COVID-19 recovered individuals.

Conclusion: Owing to its diversity and affinity, the MBC repertoire selected against WT RBD protein upon infection or vaccination contains clones that can cope with viral evolution.

Keywords: Memory B cells - Repertoire - SARS-CoV2
Persistence of functional memory B cells recognizing SARS-CoV-2 variants despite loss of specific IgG

Stephan Winklmeier\textsuperscript{1,2} (stephan.winklmeier@med.uni-muenchen.de), Katharina Eisenhut\textsuperscript{1,2}, Damla Taskin\textsuperscript{1,2}, Heike Rübsamen\textsuperscript{1,2}, Celine Schneider\textsuperscript{1,2}, Peter Eichhorn\textsuperscript{3}, Oliver T. Keppler\textsuperscript{4}, Matthias Klein\textsuperscript{5}, Simone Mader\textsuperscript{1,2}, Tania Kümpfel\textsuperscript{1,2}, Edgar Meinl\textsuperscript{1,2}

\textsuperscript{1} Institute of Clinical Neuroimmunology, University Hospital, LMU Munich, Munich, Germany; \textsuperscript{2} Biomedical Center (BMC), Faculty of Medicine, LMU Munich, Martinsried, Germany; \textsuperscript{3} Institute of Laboratory Medicine, University Hospital, LMU Munich, Munich, Germany; \textsuperscript{4} Max von Pettenkofer Institute, Virology, LMU Munich, Munich, Germany; \textsuperscript{5} Department of Neurology, University Hospital, LMU Munich, Munich, Germany

**Introduction:** In most individuals, anti-SARS-CoV-2 serum antibodies (Abs) persist for more than 6 months after primary infection, but some patients rapidly lose their specific Abs, especially those that experienced a mild disease course.

**Objective:** We aimed to assess the persistence of SARS-CoV-2-specific B cells in patients who have lost specific IgGs and analyzed the reactivity of the immunoglobulins produced by these B cells.

**Methods:** PBMCs from each donor were stimulated to differentiate into Ab-secreting plasmablasts [1]. Reactivity to SARS-CoV-2 was determined by ELISA. The frequency of SARS-CoV-2-specific B cells was calculated according to the Poisson distribution [2]. Cross-reactivity to receptor-binding domains (RBDs) of emerging variants was tested and the ability of in vitro produced Abs to block the binding of RBD to its receptor angiotensin-converting enzyme 2 (ACE-2) was determined [3].

**Results:** Circulating IgG memory B cells specific for SARS-CoV-2 were detected in all 16 patients 1–8 months post-infection. Four patients lost specific serum IgG after 5–8 months but had SARS-CoV-2-specific-B-cell levels comparable to those of seropositive donors. Abs produced after in vitro differentiation blocked RBD binding to ACE-2, indicating neutralizing activity. Memory-B-cell-derived IgGs recognized the RBD of B.1.1.7 similarly to the wild-type, while reactivity to B.1.351 and P.1. decreased by 30% and 50%, respectively.

**Conclusion:** Memory-B-cell differentiation into Ab-producing cells is a more sensitive method for detecting previous infection than measuring serum Abs. Circulating SARS-CoV-2 IgG memory B cells persist, even in the absence of specific serum IgG; produce neutralizing Abs; and show differential cross-reactivity to emerging variants.

**Keywords:** SARS-CoV-2 - Variant of concern - Memory B cell

**References:**
VAST: a novel and versatile vaccination platform that induces robust and durable antibody responses

Paraskevi Vlachou1 (p.vlachou-efstathiou@dkfz.de), Gianna Triller1, Johan Zeelen2, Monique Van Straaten2, Joseph Verdi2, Erec Stebbins2, Nina Papavasiliou1

1 D150, German Cancer Research Center (DKFZ), Heidelberg, Germany; 2 D160, German Cancer Research Center (DKFZ), Heidelberg, Germany

Introduction: Vaccination strategies intend to imitate the endogenous ability of the immune system to remember the antigens it has been in contact with, leading to faster and optimized immune response. An optimal immune response results in high, neutralizing antibody titers that persist in the body and long-lasting functional memory.

Objectives: Substantial strides have been made in our understanding of the antibody repertoires produced under natural infection conditions, that lead to pathogen clearance. However, identifying vaccination platforms that can yield optimal antibody repertoires matching those raised during natural infection and clearance is a goal that has yet to be met.

Methods: Here we have co-opted the inherited immunogenicity of the dense surface coat of the African trypanosome, to engineer a novel vaccine carrier that elicits excellent neutralizing antibody responses as well as immune memory. Using fentanyl as an example, we prime mice. We then employ single-cell RNA sequencing and B cell receptor analysis in combination with FACs index sorting, to identify recurrent memory B cell clones with high affinity to fentanyl.

Results: We demonstrate the generation and maintenance of serological memory, and full protection from near-lethal fentanyl challenge. Via cloning of the recurring paired antibody genes, and reconstitution in HEK293 cells, we validate that our approach can yield superb anti-small molecule reactivities. Follow-up structural characterizations of the antibodies together with their fentanyl hapten reveal unique structural features that explain the high affinities of the cloned antibodies.

Conclusions: Overall, we have developed an integrated approach capable of generating exquisitely specific antibodies to a range of different epitopes that are traditionally thought of as “hard to target”.

Keywords: Vaccine platform - Memory B cells - Antigen-specific antibodies.

References:
Single-Cell Transcriptomic Analyses Define Distinct Peripheral B Cell Subsets and Discrete Development Pathways

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Separation of B cells into different subsets has been useful to understand their different functions in various immune scenarios. In some instances, the subsets defined by phenotypic FACS separation are relatively homogeneous and so establishing the functions associated with them is straightforward. Other subsets, such as the “Double negative” (DN, CD19+CD27-IgD-) population, are more complex with reports of differing functionality which could indicate a heterogeneous population. Recent advances in single-cell techniques enable an alternative route to characterize cells based on their transcriptome. To maximize immunological insight, we need to match prior data from phenotype-based studies with the finer granularity of the single-cell transcriptomic signatures. We also need to be able to define meaningful B cell subsets from single cell analyses performed on PBMCs, where the relative paucity of a B cell signature means that defining B cell subsets within the whole is challenging. Here we provide a reference single-cell dataset based on phenotypically sorted B cells and an unbiased procedure to better classify functional B cell subsets in the peripheral blood, particularly useful in establishing a baseline cellular landscape and in extracting significant changes with respect to this baseline from single-cell datasets. We find 10 different clusters of B cells and applied a novel, geometry-inspired, method to RNA velocity estimates in order to evaluate the dynamic transitions between B cell clusters. This indicated the presence of two main developmental branches of memory B cells. A T-independent branch that involves IgM memory cells and two DN subpopulations, culminating in a population thought to be associated with Age related B cells and the extrafollicular response. The other, T-dependent, branch involves a third DN cluster which appears to be a precursor of classical memory cells. In addition, we identify a novel DN4 population, which is IgE rich and closely linked to the classical/precursor memory branch suggesting an IgE specific T-dependent cell population.
T-bet+ B cells infiltrating the human brain: from peripheral induction to local effector function

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Introduction: In multiple sclerosis (MS), B cells escape from peripheral tolerance, enter the brain and associate with lesions [1]. The exact B-cell subsets and mechanisms involved in these events remain elusive.

Objective: We aim to delineate the functional program of brain-infiltrating B cells to better understand the cause and course of MS.

Methods: Functional B-cell subsets were characterized both ex vivo (blood, brain tissues) and in vitro (Tfh cell-related differentiation, crossing of brain endothelial monolayers) using different MS cohorts [2, 3].

Results: Naive B cells from untreated MS patients were prone to develop into CXCR3(T-bet)high class-switched populations under IFNGR- and TLR9-inducing conditions. MS risk allele IFNGR2 and EBV load augmented this type of B-cell development. CXCR3high B cells showed brain-infiltrating capacity in vitro and accumulated in the blood of natalizumab (α-VLA-4) responders. CXCR3 was upregulated on CD19+ cells from distinct postmortem MS brain tissues. ASC/B-cell ratios were elevated and corresponded to the presence of CD4+ T cells in lesions versus control brain tissue. Accordingly, CXCR3high B cells of MS patients preferentially matured into ASC in vitro.

Conclusion: CXCR3(T-bet)high class-switched B cells are poised to infiltrate and mature into ASC in the MS brain.

Keywords: Brain-infiltrating B cells - Multiple sclerosis - CXCR3

References:
Phenotypic and functional division of human Bregs in CD45\textsuperscript{lo} and CD45\textsuperscript{hi} B cells and their dependence on Btk and mTOR

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Background: B regulatory cells (Bregs) represent a recently identified B cell subset with particular immunoregulatory properties, able to produce Interleukin-10 (IL-10). Bregs have been extensively studied in various autoimmune and immunomediated disorders. One of the major experimental approaches applied in human Breg induction involves Toll-like receptor 9 (TRL9) activation via CpG stimulation of human B cells. On the other hand, the AKT/mTOR axis plays important role in TLR-dependent B cell activation. To date, limited data on the potential role of BTK and/or mTOR in the induction and function of Bregs in humans are available.

Methods: Flow cytometry, in vitro IL-10 production, in vitro BTK and mTOR inhibition.

Results: Our data show for the first time that human Bregs upon CpG stimulation can be divided in CD45\textsuperscript{lo} and CD45\textsuperscript{hi} IL-10 producing B cells. Both CD45\textsuperscript{lo} and CD45\textsuperscript{hi}, the latter to a higher extent, depend on BTK, but not mTOR for cell survival. Regarding IL-10 production, both subsets, ie CD45\textsuperscript{lo} and CD45\textsuperscript{hi}, depend on BTK and mTOR for IL-10 production, with the CD45\textsuperscript{hi} population showing a higher sensibility to the inhibition of both pathways.

Conclusions: These data provide the first evidence for a phenotypic and functional division of human Bregs in CD45\textsuperscript{lo} and CD45\textsuperscript{hi} B cells, with both subsets resulting dependent on Btk for survival and on BTK and mTOR for IL-10 production, with the CD45\textsuperscript{hi} subset showing increased dependence on both pathways.
SESSION #2. B CELL DEVELOPMENT/B CELL NEOPLASIA
Transcription factor networks coordinate transcriptional priming and cellular expansion in early B-cell development

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B-lymphocyte development is dependent on the interplay between the chromatin landscape and lineage specific transcription factors. We have used chromosome conformation capture in combination with ATAC-seq analysis to enable highly efficient annotation of both proximal and distal transcriptional control elements to genes active in B-lineage progenitors. A large majority of these genes were annotated to at least one regulatory element with an accessible chromatin configuration in multipotent progenitors. Our data unravel an extensive epigenetic priming at regulatory elements annotated to lineage restricted genes and provide insight into the interplay between the epigenetic landscape and transcription factors in cell specification. One of these transcription factors were Early B cell Factor 1 (EBF1), a protein of critical importance for lineage specification and survival of B-lymphoid progenitors. Functional analysis revealed that impaired EBF1 function in mouse B-cell progenitors results in reduced expression of Myc. Ectopic expression of MYC partially rescued B-cell expansion in the absence of EBF1 both in vivo and in vitro. Using chromosome conformation analysis in combination with ATAC-seq, ChIP-seq and reporter gene assays, we identified six EBF responsive enhancer elements within the Myc locus. CRISPR-Cas9 mediated targeting of EBF1 binding sites identified one element of key importance for Myc expression and pro-B cell expansion. These data provide evidence that Myc is a direct target of EBF1. Furthermore, ChIP-seq analysis revealed that several regulatory elements in the Myc locus are targets of PAX5. However, ectopic expression of PAX5 in EBF1 deficient cells inhibits the cell cycle and reduces Myc expression, suggesting that EBF1 and PAX5 act in an opposing manner to regulate Myc levels. This hypothesis is further substantiated by the finding that Pax5 inactivation reduces requirements for EBF1 in pro-B cell expansion. This work highlight the importance of balanced transcription factor networks for the differentiation and expansion of early B-lymphoid cells.
IL-7R signalling activates widespread V<sub>H</sub> and D<sub>H</sub> gene usage to drive antibody diversity in bone marrow B cells

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Introduction: Generation of the primary antibody repertoire requires V(D)J recombination of hundreds of gene segments in the immunoglobulin heavy chain (I<sub>gh</sub>) locus. The role of interleukin-7 receptor (IL-7R) signalling in I<sub>gh</sub> recombination has been difficult to partition from its role in B cell survival and proliferation.

Objective: To elucidate the role of the IL7R in V(DJ) recombination.

Methods: We used VDJ-seq to gain a detailed description of the I<sub>gh</sub> repertoire in murine IL-7Ra<sup>-/-</sup> bone marrow, and fetal liver B cells, and RNA and ATACseq to study underlying mechanisms.

Results: We demonstrate that IL-7R signalling profoundly influences V<sub>H</sub> gene selection during V<sub>H</sub>-to-DJ<sub>H</sub> recombination. We find skewing towards 3' V<sub>H</sub> genes during <i>de novo</i> V<sub>H</sub>-to-DJ<sub>H</sub> recombination, more severe than the fetal liver (FL) repertoire, and uncover a role for IL-7R signalling in D<sub>H</sub>-to-J<sub>H</sub> recombination. Transcriptome and accessibility analyses suggest reduced expression of B lineage transcription factors (TFs) and targets, and loss of D<sub>H</sub> and V<sub>H</sub> antisense transcription in IL-7Rα<sup>-/-</sup> B cells.

Conclusion: In addition to its roles in survival and proliferation, IL-7R signalling shapes the I<sub>gh</sub> repertoire by activating underpinning mechanisms.

Keywords: Interleukin-7 receptor - Immunoglobulin recombination - Antisense transcription.
Tracking neonatal B cell function in the adult gut

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Introduction: The adult B cell pool consists of naïve and antigen experienced subsets of vastly different life spans and developmental timing. Using a lineage tracing model, we have time stamped neonatal B cells to track their function in the adult. Our results reveal a large contribution of neonatal B cells to IgA plasma cells (PCs) in the gut mucosa of unimmunized mice.

Objective: Elucidate the functional division of labor between neonate and adult derived B cells in mucosal immunity.

Methods: Rotavirus (RV) infection of time stamped mice was used to interrogate the role of neonatal B cells in the adult gut. GFP bound RV-like particles were used to isolate antigen specific IgA PCs.

Results: We demonstrate that neonatal B cells, despite their abundance in the adult gut, do not significantly contribute to anti-RV responses in the adult. Instead, antigen specific responders predominantly derive from adult origin. Neonatal infection on the other hand produces long-lived RV specific PCs which harbor unique clonotypes not found in adult infected mice.

Conclusion: Our work reveals a layer of neonatal B cells that is actively maintained in the adult gut and carries the memory of early life exposure. Importantly, the same enteric antigen elicits distinct IgA responses in neonatal life. This finding highlights a window of opportunity for the recruitment of non-redundant clonotypes into the long-lived memory repertoire and has implications in immune imprinting.
Siglec-G controls the severity of Chronic Lymphocytic B-cell Leukemia

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Siglec-G is a negative regulator of B-cell receptor (BCR) signalling. Siglec-G deficient mice show an expansion of the population of CD5+ B1a cells and increased Ca2+ signalling in these cells. Human chronic lymphocytic B-cell leukemia (B-CLL) is characterized by B cell leukemic cells developing from CD5+ B cells. BCR signalling is crucial for the maintenance of the B cell leukemic populations, as BCR inhibitors such as Ibrutimib are successful treatments of B-CLL. We used the mouse B-CLL model of Tcl1 transgenic mice to determine how Siglec-G influences the severity of B-CLL. Siglec-G-deficient (KO) x Tcl1 transgenic mice showed a significantly faster and higher expansion of the leukemic CD5+ B cell population in the blood than Tcl1 transgenic mice. While the disease develops in 40-week old Tcl1 transgenic mice, this occurred already in about 12-week old Siglec-G KO x Tcl1 transgenic mice. In Siglec-G KO transgenic mice spleen, lymph nodes and liver were affected by infiltration of the leukemic cells at much earlier time points. The Ig repertoire of Siglec-G KO x Tcl1 transgenic mice was changed in comparison to Tcl1 transgenic controls, as typical PC- or PtC binding Igs were reduced in number, despite the earlier leukemia development. Mechanistically, we could show that increased Ca2+ signalling of Siglec-G KO leukemic B cells is responsible for the more severe leukemic disease. Furthermore, we crossed Siglec-G knockin mice, with about 5-fold overexpression of Siglec-G on B cells, to Tcl1 transgenic mice. Overexpressed Siglec-G leads to a higher BCR inhibition. These Siglec-G overexpressing mice show a strong suppression of the disease, compared to normal Tcl1 transgenic mice. In those mice, there was hardly any development of leukemic cells in the blood and a normal spleen, lymph node and liver composition. Interestingly, human Siglec-10 (the homolog to Siglec-G) was downregulated from the B cell surface of human B-CLL patients, suggesting also a protective role of Siglec-10 in humans. In conclusion, the inhibitory receptor Siglec-G/ Siglec-10 has a crucial role in the pathogenesis of B-CLL. This will allow new therapeutic targeting strategies for Siglec-G/-10.
Continuous MYD88 activation is associated with expansion and then transformation of IgM differentiating plasma cells

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Introduction: Activating mutations of MYD88 (MYD88L265P being the far most frequent) are found in most cases of Waldenström macroglobulinemia (WM) as well as in various aggressive B-cell lymphoma entities with features of plasma cell (PC) differentiation, such as activated B-cell type diffuse large B-cell lymphoma (DLBCL).

Objective: No animal model for WM exists for instance. Considering MYD88 mutation as the first oncogenic event, our aim is to understand how MYD88 activation exerts its transformation potential.

Methods: We developed a new mouse model in which the MYD88L252P protein, the murine ortholog of human MYD88L265P, is continuously expressed in CD19 positive B-cells (Myd88L252P mice) [1].

Results: Myd88L252P mice develop B-cell lymphomagenesis characterized by a splenomegaly with lymphoplasmacytic cells and IgM plasma cell differentiation. With age, those mice exhibited an Ig peak in blood associated with increase in IgM secretion. Comparison with MYD88L265P WM showed that Myd88L252P tumors also shared the typical lymphoplasmacytic transcriptomic signature of WM bone marrow purified tumor B-cells.

Conclusion: Altogether, these results demonstrate for the first time that continuous MYD88 activation is specifically associated with clonal transformation of differentiating IgM B-cells. Since MYD88 L252P targets the IgM PC differentiation continuum, it provides an interesting preclinical model for development of new therapeutic approaches to both WM and aggressive MYD88 associated DLBCLs.

Keywords: Waldenström macroglobulinemia - Myd88 - IgM plasma cell differentiation.

References:
SESSION #3. PLASMA CELLS
Dissecting antibody-secreting cells differentiation at single cell level

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Introduction: Differentiation of B cells into antibody secreting cells (ASCs) is a key process during the course of an immune response. Dysregulation of this process can cause severe pathological conditions, such as alloimmunization during blood transfusions or self-reactivity in auto-immune disease.

Objective: Detailed understanding of the cues that control antibody-secreting cell differentiation is important to devise strategies to modulate antibodies formation in both health and disease.

Methods: We profiled the transcriptomes of single cells derived from an in vitro culture system that differentiate human naive B cells into ASCs [1]. We analyzed the B-cell receptors (BCR) and applied RNA velocity to get more insights into, respectively, the receptor editing processes and the different naive to ASCs cellular transitional stages in the culture system. Publicly available single cell transcriptomics data from bone marrow, peripheral blood and tonsils derived ASCs were further analyzed for comparative purposes.

Results: Five transcriptional distinct subsets of cells were detected in our in vitro system. Two subsets showed a clear ASCs gene signature, highly comparable to ex vivo derived ASCs. Class-switched but unmutated BCRs were detected. RNA velocity identified a pre-ASCs cellular stage and a possible second route of naive to memory B cell differentiation, including a germinal-center like intermediate phenotype.

Conclusion: Taken together, these data demonstrates that our in vitro naive B cell differentiation system highly reflects the in vivo situation and can therefore be used to identify previously unknown key regulators of ASCs formation.

Keywords: B cell - Antibody-secreting cell - Single cell RNA sequencing

References:
The Sec22b SNARE is indispensable for plasma cell maintenance and antibody secretion

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Introduction: Plasma cells (PC) are key actors of the immune response through secretion of large quantities of antibodies. To accommodate this important protein load, PC display unique cellular features including expansion of the endoplasmic reticulum (ER) and adaptation of the unfolded protein response. Despite the essential role of PC in health and disease the cellular mechanisms controlling their development and their secretory function are still poorly understood.

Objective: The goal of this project is to unravel the role of SNARE molecules in PC generation, maturation, survival and function.

Methods: We identified SNAREs particularly highly expressed by PC. We used ex vivo and in vivo models to unravel the crucial role of these molecules in PC biology.

Results: Using a B cell specific deficient mouse model, we showed that the loss of Sec22b caused an almost complete loss of PC and a dramatic reduction of serum antibody titers. Accordingly, these mice fail to mount a protective immune response. Our ex vivo results demonstrate that Sec22b is indispensable for PC maturation, maintenance and efficient antibody secretion through the control of ER expansion and mitochondria biogenesis.

Conclusion: Altogether, our results demonstrate a unique and critical role for SNAREs in PC biology.

Keywords: Plasma cell - Endoplasmic reticulum - SNARE
Single-cell analyses of autoreactive plasma cells from immune thrombocytopenia patients

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Introduction: 80% immune thrombocytopenia (ITP) patients possess anti-integrin αIIbβ3 (GPIIbIIIa) IgG autoantibodies causing platelet opsonization and phagocytosis. Spleen is considered the main site of autoantibody production by autoreactive B cells¹ and platelet destruction, but 40% patients fail platelet number recovery after splenectomy.

Objective: Determine the affinity for GPIIbIIIa of IgG-secreting cells (IgG-SC) from chronic ITP patients from spleen, blood and bone marrow.

Methods: We used a droplet microfluidic approach² to compartmentalize individual IgG-SC and determine their secretion rate and affinity for GPIIbIIIa, and analyzed >3,300 single IgG-SC from 28 ITP patients.

Results: Analyses revealed high inter-individual variability in affinity of IgG-SC for GPIIbIIIa with variations over 3 logs. IgG-SC dissemination and range of affinities was however similar per patient between spleen, bone marrow and/or blood. Longitudinal analysis of autoreactive IgG-SC during experimental immunotherapy using anti-CD38 daratumumab demonstrated variable outcomes, from complete remission to failure with persistence of high-affinity anti-GPIIbIIIa IgG-SC in the bone marrow.

Conclusion: These studies demonstrate the existence and dissemination of high-affinity autoreactive plasma cells in multiple anatomical compartments of ITP patients that may be the cause of failure of current therapies.

Keywords: Plasma cell - Autoimmunity - Antibody affinity.

References:
TFG controls ER stress, autophagy and plasma cell function

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Introduction: Activated B cells and plasma cells need to handle endoplasmic reticulum (ER) stress. TFG (Trk-fused gene) is a 50 kDa protein involved in organization of the ER-Golgi intermediate compartment and COPII vesicle transport. We have identified TFG in B cells as part of the CARD11-BCL10-MALT1 signaling complex (1). Within the B cell lineage, Tfg expression is highest in plasma cells and TFG becomes up-regulated during plasmablast differentiation in vitro (2). H. M.<author>Mielenz, D.</author></authors></contributors><auth-address>Division of Molecular Immunology, Department of Internal Medicine 3, Nikolaus-Fiebiger-Zentrum, Friedrich-Alexander-Universitat (FAU.

Objective: To determine the function of TFG in B cell activation and plasma cell differentiation.

Results: TFG prevents ER stress as well as cell death while supporting autophagy flux in CH12 B cells (2). H. M.<author>Mielenz, D.</author></authors></contributors><auth-address>Division of Molecular Immunology, Department of Internal Medicine 3, Nikolaus-Fiebiger-Zentrum, Friedrich-Alexander-Universitat (FAU.

Proteomic analyses to identify autophagy substrates in CH12 tfg KO B cells revealed that TFG controls, amongst others, the abundance of BCL10 and the Ig JCHAIN. Bioinformatic processing of the proteomic data and subsequent lipidomics uncovered furthermore a role for TFG in lipid metabolism. To address the function of TFG in vivo we generated tfgΔ/Δ mice that were crossed to mb1-Cre mice. Ongoing analyses support the idea that TFG is important for plasma cell generation and function.

Conclusion: TFG is a novel player in plasma cell biology by regulating autophagy.

Keywords: Plasma cell - Autophagy - ER stress

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Metabolic flux analysis of individual antibody-secreting cells – the impact of space, time, and immunization

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Introduction: Cellular state and functionality of immune cells are highly intertwined with cellular metabolism. While functionality can be assessed on the single-cell level and shows notable heterogeneity, metabolic fluxes are currently only accessible in bulk. Therefore, the metabolic consequences of high activity and functionality in individual, rare immune cells is currently masked.

Objective: We aimed to develop a technology linking antibody secretion to metabolic flux measurements with single-cell resolution.

Methods: We combined our recently published ‘DropMap’ technology¹,² with bioassays that allow measuring various metabolic fluxes, such as oxygen consumption rates (OCR) as a measure of oxidative phosphorylation (OxPhos) and lactate secretion as a measure of anaerobic glycolysis (AG).

Results: We could confirm that antibody-secreting cells in the bone marrow mostly used oxidative phosphorylation for IgG production after immunization in mice³. The OCR capped the maximal observed secretion rate in this compartment. However, splenic ASC shifted from OxPhos to AG (and back) during immunization, strongly depending on immunization conditions.

Conclusion: The integration of these bioassays allowed to link metabolic flux and functionality in individual ASCs, and suggests a Warburg effect in splenic ASCs.

Keywords: Single-Cell Analysis – Metabolism - Antibody-secreting cells

References:
Complementary but distinct protective roles of tumor-infiltrating IgG and IgA producing cells

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Antibody-secreting cells (ASC) emerge as critical actors of anti-tumor immunity, but their diversity and clinical impact remain controversial.

We aim to study the heterogeneity of ASCs and their consequences on patient prognosis in breast cancer. Flow cytometry, in situ IF imaging, antibody specificity profiling and transcriptome analysis were performed with fresh and FFPE breast tumor samples.

ASC account for ≈ 8% of B cells in invasive tumors, localize mainly in the stroma, mostly consists of plasmablasts and produced IgG, but also monomeric and dimeric IgA. Strikingly, all in situ carcinomas display an over-dominance of IgA producers among ASC - while this occurs only in about 15% of advanced invasive tumors - and serum and tumor IgG and IgA mostly target different antigens. In addition, high IgA-ASC infiltration is associated with increased survival even in patients low for IgG ASC. Interestingly, IgA ASC rich only tumors (G-Lo/A-Hi) show increased expression of mast cell and neutrophil genes, while genes of activated adaptive immune cells are overexpressed in the G-Hi/A-Lo group.

Therefore, IgA and IgG ASC infiltrate breast tumors, target different antigens and play complementary yet distinct, positive roles on patient survival.
The BCR acts as a switch between the canonical and non-canonical functions of plasma cells

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Introduction: It has been demonstrated that IgM and IgA-secreting PCs, but not IgG-producing PCs, express a functional BCR leading to the notion that the PC pool can be subdivided into conventional PCs, lacking a BCR (IgG\(^+\)) and unconventional BCR-expressing PCs (IgM\(^+\) or IgA\(^+\)). Moreover, previous work from our team has demonstrated that IgM\(^+\) PCs initiate a biological response biased towards cytokine production, characterized in particular by upregulation of the CCL5/RANTES and IL10 transcripts upon in vivo antigenic challenge (1).

Objective: To characterize the functional output of BCR ligation on unconventional PCs and the contribution of these cells to the humoral immune response.

Methods: We have crossed Blimp-1Cre mice with mice expressing two floxed alleles of Syk (3) to target invalidation of the BCR signaling pathway in PCs. Different parameters of the humoral response were compared in Blimp-1\(^{Cre/+}\) Syk\(^{+/+}\) (PC Syk WT) and Blimp-1\(^{Cre/+}\) Syk\(^{lox/lox}\) (PC Syk KO) mice at the steady state and in the context of polymicrobial sepsis induced by cecal ligation and puncture (CLP).

Results: At the steady state, PC Syk KO mice were characterized by a 4-5 fold increase in serum IgA while titers of polyclonal IgM and IgGs were not affected. Comparable frequencies and numbers of IgA PCs were found in the gut-associated lymphoid tissues of PC Syk KO and PC Syk WT mice but the cytoplasmic IgA density was increased in IgA PCs of PC Syk KO as compared to their WT counterparts. Both the sepsis severity score and serum titers of the proinflammatory cytokine IL6 were increased in PC Syk KO as compared to PC Syk WT animals, suggesting that impairment of BCR signaling in PCs aggravates the systemic inflammatory response induced by CLP. Finally, we showed that BCR ligation is compulsory for induction of IL-10 production by sorted PCs in vitro.

Conclusion: We propose that the BCR signaling pathway in PCs dampens their canonical Ig secretion function but maximizes their non-canonical function of cytokine secretion suggesting that development of the PC regulatory function may be driven by Ag signals.

Keywords: Plasma cell - BCR signaling - B cell memory

References:
The RNA m6A binding protein YTHDF2 promotes the B cell to plasma cell transition

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Introduction: Humoral immunity requires the differentiation of B cells into antibody-secreting plasma cells. B cell terminal differentiation involves the coordinated remodelling of the gene expression program. The role of post-transcriptional regulation during differentiation is not well understood.

Objective: We aimed to identify RNA binding proteins (RBP) that regulate post-transcriptional gene expression during the B cell to plasma cell transition.

Methods: We generated a custom sgRNA library targeting RBPs and used this in a CRISPR/Cas9 knockout screen of B cell terminal differentiation.

Results: In our genetic screen, the m6A binding protein YTHDF2 was identified as promoting B cell terminal differentiation, whereas other CCR4-NOT trans-acting factors enriched for roles inhibiting differentiation. In mixed bone marrow chimeras, in the absence of YTHDF2 the germinal centre reaction is broadly intact, however, plasma cells fail to accumulate in the bone marrow.

Conclusion: Our data support a role for YTHDF2 in promoting B cell differentiation or promoting the survival of differentiated plasma cells in vivo. Furthermore, our in vitro genetic experiments support a role for the CCR4-NOT complex in coordinating competing regulatory pathways of B cell differentiation at the post-transcriptional level.
IgA production decrease is associated with B cell differentiation and proliferation defects in multiple sclerosis

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Introduction: The efficacy of B-cell depleting therapies in multiple sclerosis (MS) has proven the B cell contribution to pathogenesis. However, the failure of TACI-Ig, a treatment that blocks B cell differentiation into plasma cells has pointed out a potential protective function of plasma cells in MS.

Objective: The aim was to explore IgA production and plasma cell differentiation in MS patients.

Methods: IgA levels were measured by ELISA in MS patients (n=33) and healthy controls (n=15). B cell differentiation was assessed in vitro. IgA-memory B cells were sorted by flow cytometry. Libraries preparation and sequencing were performed by Next Generation Sequencing Platform of Curie Institute.

Results: We report a lower concentration of IgA in MS patient serum compared to controls. A lower proliferation and differentiation of B cells from MS patients was observed and associated with reduced IgA secretion. RNA sequencing analysis of IgA memory B cells showed a specific signature in MS. Two blockers of proliferation were found upregulated and 1 gene of cell survival was downregulated.

Conclusion: our results suggest that IgA decreased production is linked to a defect of proliferation and B cell differentiation in MS.

Keywords: IgA - Plasma cells - Multiple sclerosis
SESSION #4. B CELLS IN DISEASE
MHCII haplotype determines IgG autoantibody Fc N-glycosylation and development of murine autoimmune skin disease

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Introduction: Epidermolysis bullosa acquisita (EBA) is an autoimmune skin-blistering disease driven by IgG-autoantibodies to type VII collagen (Col7), in mice strictly associated with expression of the MHCII H2s haplotype. But the mechanisms by which MHC-associated genetic susceptibility translates into autoimmune skin disease are poorly understood [1].

Objective: To compare the development of murine EBA skin disease, Col7-specific plasma cells, Col7-specific CD4+ T cells, autoantibodies and IgG Fc glycosylation in congenic mouse strains with the disease-permitting H2s or -non-permitting H2b MHC haplotypes.

Methods: Intracellular binding of fluorochrome labelled Col7 was used to detect autoreactive Col7-specific plasma cells, Col7-specific CD4+ T cells were analyzed by the rapid antigen-induced upregulation of CD154 [2]. Autoantibodies were quantified by ELISA. IgG Fc glycan composition was determined by liquid chromatography–mass spectrometry/mass spectrometry.

Results: Both, susceptible B6.s and non-susceptible B6.s mice developed autoantibodies. However, susceptible B6.s (H2s) mice developed a higher frequency of IgG autoantibodies with an agalactosylated, proinflammatory N-glycan moiety, which induced strong ROS release from neutrophils, the main drivers of autoimmune skin inflammation in this model. Disease development correlated with increased IL-21- and IFN-γ-production by Col7-specific CD4+ T cells. These cytokines are known to promote the IgGs with agalactosylated N-glycan moieties.

Conclusion: Together, these results indicate that MHCII-associated susceptibility to autoimmune diseases acuminates in an altered cytokine profile of T helper cells eventually resulting in the production of autoantibodies with increased inflammatory potential [3].

Keywords: Autoreactive B cells - Autoreactive T cells - MHC - Autoimmunity.

References:
N-linked glycosylation of the immunoglobulin variable domain affects antigen binding and autoreactive B-cell activation

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Introduction: All IgG contain N-linked glycans in the Fc-region of the molecule. These glycans can profoundly impact IgG effector functions by affecting Fc-receptor and complement activation. 10-15% of human IgG molecules also contain N-linked glycans in the Variable domain (V-domain). The impact of these glycans on the function of B cells or the antibodies they produce is largely unknown. Anti-citrullinated protein antibodies, the hallmarking autoantibodies in Rheumatoid Arthritis (RA), are characterized by the high prevalence (>90%) of N-linked glycans in the V-domain. Their occurrence results from the selective introduction of N-glycosylation sites by somatic hypermutation and their presence is predictive for the transition from pre-disease autoimmunity towards RA.

Objective: to elucidate the biological implication of V-domain glycans on antigen binding and B-cell activation.

Results: Autoantibody crystal structures show that V-domain glycans are positioned in the vicinity of the binding pocket and dynamic modelling shows their potential to interfere with antigen binding, which is confirmed by binding assays. Noteworthy, human Ramos B cells carrying V-domain glycosylated B-cell receptors undergo increased signalling after stimulation compared to their non-glycosylated counterparts.

Conclusion: We show that V-domain glycans increase B-cell activation, providing a rationale on how their acquisition by autoreactive B cells contributes to a breach of tolerance in a prominent autoimmune disease.

Keywords: V-domain glycans - Autoimmunity - Rheumatoid arthritis
Autoantibodies deconvolution in Membranous Nephropathy

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Introduction: idiopathic Membranous Nephropathy (iMN) is an autoimmune disease caused by the deposition of immune complexes composed by autoantibodies with their target phospholipase A2 receptor (PLA2R1) on the surface of podocytes, which results in the disruption of the glomerular barrier in the kidneys.

Objective: We aim at characterizing both the serological and the memory cellular component in iMN patients.

Methods: primary B cells isolated from the peripheral blood of the patients have been characterized by flow cytometry and expanded to clone IgG PLA2R1-specific antibodies. The analysis of the serum reactivity has been performed by ELISA and GyroLab technology.

Results: circulating IgG PLA2R1-specific antibodies have high affinity for their target (KD in pM range). iMN patients showed an expansion of the B cell memory compartment, with an increased frequency of CD27neg IgG4 B cells. We observed relatively more IgG4⁺CD27highCD20⁻CD19⁺ cells in the patients than in healthy controls. Moreover, we isolated and characterized one anti-PLA2R1 IgG antibody.

Conclusion: we performed a comprehensive characterization of the autoreactive B cell memory compartment in iMN, shedding lights on the complexity of the autoantibody repertoire.

Keywords: PLA2R1 - Autoantibody - B cell memory
B cell defect in Prkcd G510S/G510S mice with autoimmunity and lymphoproliferation

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Introduction: Systemic lupus (SL) is an autoimmune disease that can affect various organs. Environmental and genetic factors have been linked to the disease in early adult SL while early pediatric SL can be caused by a single gene mutation [1].

Objective: We aimed to identify and study of genetic forms of this disease to help to better understand the mechanisms of tolerance.

Methods: A large-scale genetic analysis was performed on a cohort of pediatric SL patients in which bioinformatic analyses identified potentially causal mutations in several patients. Due to the scarcity of pediatric samples, we have generated mouse models with the same mutations found in patients.

Results: Our results showed that mice with a Prkcd variant developed a severe and early autoimmune phenotype. We demonstrated that the mutation induces overactivation of B cells with excessive proliferation and high expression of activations markers. Our data shows also an alteration of the cellular signaling of the B cell in our model especially on the signaling pathway of the B cell Receptor «BCR».

Conclusion: The Pkc-delta protein encoded by Prkcd would therefore play a key role in the control of homeostasis and tolerance of B cells.

Keywords: Monogenic lupus - Tolerance - Autoimmunity.

References:
Transcriptional dysregulation of CVID patients harboring the C104R \textit{TNFRSF13B} mutation

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Introduction: Common variable immunodeficiency (CVID) is the most common primary immunodeficiency in humans. Up to 30% of CVID cases can be explained by monogenetic alterations. For the majority of patients the genetic causes is unknown.

Objective: To determine additional factors contributing to the development of CVID, we investigated the perturbations of transcription factor binding and transcriptome profiles in CVID patients harboring the C104R mutation in \textit{TNFRSF13B}.

Methods: ATAC-seq was performed on naïve and class switched memory B cells of six CVID patients heterozygous for the C104R mutation, six of their healthy relatives carrying the same heterozygous mutation, and eight healthy wild-type donors. RNA-sequencing was performed on three C104R-heterozygous CVID patients, their unaffected relatives, and three healthy donors. For functional validation, intra-cellular staining was performed by flow cytometry.

Results: Our analysis revealed 25 \% less accessible chromatin in class-switched memory B cells from \textit{TNFRSF13B}-mutant carriers compared to healthy donors. 1.356 and 1.069 differential accessible regions were detected in naïve and class-switched memory B cells from mutation carriers compared to healthy donors, respectively. The NF-κB binding motif was most enriched in both B cell subsets. IκBa expression (downstream of NF-κB) was increased in patients. RNA-seq analysis revealed 687 dysregulated genes in naïve B cells and 617 in class-switched B cells from CVID patients. Gene ontology analysis highlighted the NF-κB signaling and systemic lupus erythematosus pathway in naïve- and class-switched memory B cells, respectively.

Conclusion: Here we showed the transcriptional dysregulation of naïve- and class-switched memory B cells of individuals with the C104R mutation in \textit{TNFRSF13B}. The most enriched transcription factor binding motif was for NF-κB, confirmed by transcriptome analysis, and the increase of IκBa expression. NF-κB is essential for regulating immune response. Thus, its changes may lead to variety of disease-causing differences.

Keywords: CVID - TACI - Epigenetics
Inverted direct allorecognition triggers early donor specific antibody response after transplantation

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Introduction: Transplantation is a unique situation in which the immune system is exposed to foreign MHC. The direct allorecognition is specific to this context. It consists in the recognition, by recipient’s T cells, of the intact donor’s MHC molecules on the surface of the donor’s APC. The indirect allorecognition has all the features of a thymo-dependent response: recipient’s T cells recognise alloantigen-derived peptides within self-MHC. This leads to donor specific antibody (DSA) production.

Methods and results: In this work, we show that despite being devoid of T cells, CD3εKO (H2b) mice develop a rapid but transient wave of switched DSA after transplantation with a CBA (H2k) heart. CD4+ T cells can be isolated from the heart of CBA mice and are efficiently depleted by an anti-CD3 monoclonal antibody. T cell depletion in the donor abrogates DSA generation in CD3εKO recipient mice. Interaction between H2k T cells and H2b B cells were evidenced in vitro, allowing demonstrating that donor CD4+ T cells recognise intact recipient’s MHC-II molecules expressed by BCR-activated allospecific B cells.

Conclusion: Our work demonstrates that donor CD4+ T cells transplanted with the graft can provide help to allospecific B cells through inverted direct recognition, and lead to the production of DSA.

Keywords: Transplantation - Allorecognition
Post-transcriptional control of neuroinflammation by HuR

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Introduction: B cells play dual roles in autoimmunity depending on their ability to produce cytokines that either promote or repress neuroinflammation (Fillatreau et al., 2002)(Matsushita et al., 2008). We have shown that EAE disease initiation and progression are differentially influenced by the depletion of B cells from mice with otherwise intact immune systems. CD20 antibody-mediated B cell depletion before EAE induction substantially exacerbated disease symptoms and increased encephalitogenic T cell influx into the CNS. Increased symptom severity resulted from the depletion of a rare IL-10-producing CD1dhiCD5+ regulatory B cell subset (B10 cells). Emerging evidence have shown that timely and balance production of cytokines is regulated at the post-transcriptional level by RNA binding proteins (RBPs) (Díaz-Muñoz & Turner, 2018). Location, stability, and translation are the other key steps for final gene expression, and they are all controlled by RNA-binding proteins (RBPs).

Objective: Our preliminary data has uncovered HuR as an essential modulator of B-cell mediated immunity. Thus, we hypothesized that HuR modulates neuroinflammation by post-transcriptionally regulating B cell function and cytokine production.

Methods: To address this, we have used conditional KO mice to assess the intrinsic role of HuR in B cells in a model of EAE. Clinical and phenotypical characterization of B and T cell subsets revealed an important role for HuR in B1 B cells that has been further examined in-vivo and in-vitro.

Results: Conditional deletion of HuR in B cells resulted in a non-remitting EAE phenotype associated to decreased B cell infiltration and IL10 cytokine production. Molecular characterization of HuR function using iCLIP and RNAseq revealed distinctive gene programs in B2 and B1 cells that modulate their function.

Conclusion: In summary, our data show that HuR-post transcriptional regulation is a key instrument for B cell function and cytokine production during neuroinflammation.

Keywords: Neuroinflammation - RNA binding proteins - B1 cells

References:
Decreased Levels of T Follicular Helper (CD4+CXCR5+) Cells and CD27+CD38+ and CD27+CD38- B Cells in Ankylosing Spondylitis Patients Correlate with Marker of Inflammation

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The role of different lymphocyte subsets in ankylosing spondylitis (AS) is still to be elucidated. In addition, the connection to disease related parameters is still to be fully revealed.

Levels of CD4+TFH cells and CD27+CD38+/CD38- B cells in AS patients and controls was investigated by flow cytometry analysis of peripheral blood mononuclear cells from a cohort of 50 patients with AS and 50 pair wise matched blood donors. In addition, associations between these cell subsets and determined clinical markers was analyzed.

When comparing AS patients and controls pair wise, we observed on average a 50% reduction of TFH (CD3+CD4+CXCR5+) cells among CD45+ lymphocytes in PBMCs from patients (p=0.000008). Furthermore, a 20-30% reduction among memory/plasma cells (CD19+CD27+CD38+ and CD19+CD27+CD38low) among CD45+ lymphocytes in PBMCs from patients (p=0.002 and p=0.007 respectively). For female patients a correlation between TFH and ESR (Rs=-0.551 p=0.022) was observed. Moreover, negative correlations between the two B cell subsets (CD19+CD27+CD38+ and CD19+CD27+CD38low) and ESR were observed for female patients (Rs =–0.476 p=0.053 and Rs =–0.522 p=0.032 respectively). Our observations indicate a role of the humoral immune response in AS.
Follicular helper-like T cells in the lung highlight a novel role of B cells in sarcoidosis

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Introduction: Pulmonary sarcoidosis is thought to be a Th-1- and macrophage-driven disease. However, mouse models recently revealed that chronically inflamed lung tissue also harbors Tfh-like cells and represents a site of active T cell / B cell cooperation.

Objective: We assessed the role of pulmonary Tfh- and germinal center (GC)-like lymphocytes in sarcoidosis.

Methods: Bronchoalveolar lavage (BAL) fluid, lung tissue, and blood samples from sarcoidosis patients were analyzed by flow cytometry, histology, RNA-seq and in vitro T/B cooperation assays for phenotypical and functional characterization of GC-like reactions in the inflamed tissue.

Results: We identified a novel population of Tfh-like cells marked by high expression of CD40L and IL-21 in sarcoidosis BAL, which provided potent B cell help for plasmablast differentiation. In lung tissue, we observed peribronchial infiltrates with T and B cells in close contact, with most clusters being non-ectopic (i.e., FDC-negative) and a large number of IgA+ plasmablasts.

Conclusion: Active T/B cooperation and local production of pathogenic antibodies in the inflamed lung marks a new pathomechanism in sarcoidosis and should be considered from both diagnostic and therapeutic aspects.

Keywords: Sarcoidosis - T cell / B cell cooperation - Tfh-like cells
SESSION #5. AG RECOGNITION/BCR SIGNALING
BCR functions from within the endoplasmic reticulum

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Resting B cell dependence on the B cell receptor (BCR) has been attributed solely to its signalling competence (1,2). We have recently provided evidence that the presence of BCR is sensed in the endoplasmic reticulum (ER), and maintains ER homeostasis and mitochondrial function both in primary B cells and Burkitt lymphoma cell lines. Unexpectedly, in Ramos cells this role of the BCR has been shown to be independent of BCR signals from the cell membrane (3).

To address this latter function of the BCR in primary resting B cells, we used i) ex vivo CRISPR-Cas9-mediated mutagenesis of BCR components and ii) an in vivo mouse model which enabled us to distinguish between the cell membrane-bound and the ER-resident function of the BCR.

We show that the defects observed in B cells lacking the BCR in the ER were rescued by the expression of immunoglobulin heavy chain unable to be exported to the cell membrane.

We provide evidence that in mouse resting B cells the BCR exerts a dual function, controlling cell survival and fitness through signals from the cell surface and from within the ER.

References:
CD20 as a gatekeeper of the resting state of human B cells

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Introduction: CD20 is an atypical tetraspanin expressed only on mature B cells. Due to its B cell-specific expression, CD20 is an ideal target of B cell-depleting monoclonal antibodies, most prominently rituximab (RTX).

Objective: Despite widespread usage of anti-CD20 antibodies for B cell depletion therapies, the biological function of their target remains unclear.

Methods and Results: CRISPR/Cas9-mediated ablation of CD20 in resting B cells resulted in re-localization and interaction of the IgM-class B cell antigen receptor with the co-receptor CD19. This receptor rearrangement led to a transient activation of B cells, accompanied by the internalization of many B cell surface marker proteins. Re-expression of CD20 restored the expression of the B cell surface proteins and the resting state of Ramos B cells. Similarly, treatment of Ramos- or naïve human B cells with the anti-CD20 antibody rituximab induced nanoscale receptor rearrangements and transient B cell activation in vitro and in vivo. A departure from the resting B cell state followed by the loss of B cell identity of CD20 deficient Ramos B cells was accompanied by a PAX5 to BLIMP-1 transcriptional switch, metabolic reprogramming towards oxidative phosphorylation and a shift towards plasma cell (PC) development. Our finding of plasma cell characteristics and adaptations in CD20 negative cancer B cells opens new possibilities for patients that have relapsed after RTX medication.

Conclusion: CD20 is an organizer of the IgD-class nanocluster on the B cell membrane. The loss of CD20 on human B cells results in a dissolution of the IgD-class nanocluster and a transient B cell activation inducing a B cell to PC differentiation. Thus, CD20 is an essential gatekeeper a membrane nanodomain and the resting state of naive B cells.

Keywords: B cell activation - Membrane nanoscale organization - Therapeutic antibodies

References:
Tetraspanins: molecular organisers of the B-cell surface

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Introduction: The plasma membrane of B cells contains thousands of different proteins that need tight spatial organization. Tetraspanins are evolutionary conserved four-transmembrane proteins that control membrane organization. We discovered that tetraspanin proteins (CD37, CD53) organise membrane receptors and signaling molecules into specialized nanoscaled domains at the B-cell surface.

Objective/Methods: We investigated the humoral immune response in tetraspanin-deficient mice and studied B-cell signaling in tetraspanin-knockout human B-cell lines using CRISPR/Cas9 technology.

Results: Cd37-deficient mice have defective antibody responses that are B-cell intrinsic. Moreover, deficiency of CD37 induces spontaneous development of B-cell lymphoma in mice and is directly correlated with worse clinical outcome in patients with aggressive lymphoma. CD37 interacts with suppressor of cytokine signaling 3 (SOCS3), and when absent drives tumor development through constitutive activation of the IL-6 signaling pathway. B cells lacking CD53 show impaired PKC recruitment to the membrane upon B cell receptor signaling, and Cd53-deficient mice have impaired humoral immune responses compared to wild-type controls.

Conclusion: These studies demonstrate that tetraspanin domains act as novel membrane signaling hotspots in B cells, and show the importance of cell surface organization during humoral immunity.

Keywords: B-cell membrane - Signaling - Humoral immunity

References:

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Novel players in B cell antigen uptake and trafficking

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Introduction: Antigen binding to the B cell receptor (BCR) triggers rapid endocytosis, intracellular trafficking into processing compartments and loading of peptides onto MHCII molecules. This regulates the number and repertoire of peptide-MHC complexes presented to T cells and impacts germinal centre formation, affinity maturation and antibody production.

Objective: The current model of BCR uptake involves clathrin-mediated endocytosis though evidence exists for additional mechanisms, which we aim to uncover.

Method: Whole-genome CRISPR/Cas9 screens for genes affecting antigen uptake and subsequent validation identified over 70 regulators, most previously not associated with B cell endocytosis.

Results and conclusion: Candidates include signalling proteins, ubiquitin ligases, and intracellular trafficking proteins. Many have been validated in primary cells highlighting the potential for novel discoveries. We detected a role for Endophilin A2, which regulates clathrin-independent endocytosis of ligand-bound receptors. Endophilin was recruited to signalling BCR, and its depletion reduced antigen uptake in primary mouse B cells. Loss of Endophilin resulted in reduced germinal centre responses and serum antibody titres in vivo, suggesting a critical role for Endophilin in T-dependent B cell responses.
SESSION #6. GENETICS OF IMMUNOGLOBULINS
Immunoglobulin enhancers increase RNA polymerase 2 (Pol2) stalling at somatic hypermutation (SHM) target sequences

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SHM is targeted to Ig genes by their enhancers (DIVACs; diversification activators), but how they mediate this activity is unknown.
We investigate the locus-specific targeting of SHM by characterizing the effects of DIVACs on constitutively expressed transcription units.
We use ChIP-seq, GRO-seq and in situ bisulfite assay of genome-integrated GFP-based reporters in DT40 and Ramos cell lines.
We show that DIVACs that strongly stimulate SHM increase the occupancy and phosphorylation of Pol2 in the mutating gene with little or no increase in elongation-competent Pol2 or production of full-length transcripts, indicating the accumulation of stalled Pol2. DIVAC-induced stalling is weakly associated with increased single-stranded DNA in the mutating target gene. We found no evidence for anti-sense transcription in the areas targeted for SHM, indicating that convergent transcription is not necessary for efficient SHM targeting.
Our findings argue for a connection between Pol2 stalling and cis-acting targeting elements in the context of SHM and thus define a mechanistic basis for targeting of SHM and suggest that DIVACs make the target gene a suitable platform for AID-mediated mutation without the need for increasing transcriptional output.

Keywords: Somatic hypermutation - AID - Pol2 stalling
Study of IgH Locus Suicide Recombination ‘LSR’ in human normal and pathological conditions

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Introduction: Locus Suicide Recombination ‘LSR’ and Class Switch Recombination ‘CSR’ are genetic rearrangements of the IgH locus in activated B-cells. LSR deletes constant IgH genes and results in loss of BCR and B-cell death [1]. LSR is a physiological event but its function in B-lymphocyte homeostasis is not yet been elucidated.

Objective: We aim to study of the LSR mechanism in human normal and pathological B-cells.

Methods: We used High Throughput Sequencing (NGS) and chromatin immunoprecipitation (ChIP) technics.

Results: We obtained results implicating for the first time the association of abnormal LSR in a pathological condition. In chronic lymphocytic leukemia (CLL), we subdivided CLL samples on the basis of the LSR junction count compared to normal B-cells into two subsets with specific IgH locus features (VH mutational status, LSR junction count and diversity and LSR repair profile) associated with differential prognosis (TFS, Binet stages and VH mutational status). These data implicated LSR alteration in tumoral transformation as a cause, result or passenger IgH recombination.

Conclusion: Our results provide new insights on the LSR molecular process and function.

Keywords: LSR - Normal B cell - Tumor cells.

References:

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IgTreeZ: A toolkit for immunoglobulin gene lineage tree analysis

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Introduction: Somatic hypermutation (SHM) is an important diversification mechanism that plays a part in the creation of immune memory. Immunoglobulin (Ig) variable region gene lineage trees were used over the years to model SHM and the selection mechanisms operating on B cell clones. [1-3]

Objective: We have developed IgTreeZ (Immunoglobulin Tree analyZer), a python-based software tool that analyses many aspects of Ig gene lineage trees and their repertoires.

Methods: Using simulations, we show that IgTreeZ can truthfully detect and quantify population transitions in trees, reveal differences in tree topology characteristics, and can be used for mutation and selection analyses.

Results: We used IgTreeZ on empirical data, found evidence for different mutation patterns in different B cell subpopulations, and gained insights into antigen-driven selection in COVID-19 patients.

Conclusion: Overall, we present a comprehensive lineage tree analysis tool that can reveal new biological insights into B cell repertoire dynamics.

Keywords: Immunoglobulin variable region gene - Somatic hypermutation - Software tool

References:
A dual effect upon deletion of Special A-T Rich Binding Protein 1 in B cells

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Introduction: Mature B cells undergo genetic remodeling of their Immunoglobulin (Ig) genes, such as Class Switch Recombination (CSR) and Somatic Hypermutation (SHM), upon antigen stimulation. For a meticulous control of such events, the IgH locus contains different cis-regulatory regions such as the Core-Eμ enhancer flanked by two Matrix Attachment Regions (MARsEμ) a structure strikingly conserved between species. We suspect some MARs binding protein (MARs BP) as SATB1 (Special A-T rich Binding protein 1) as "container-title": “Journal of Biological Chemistry”, "page":"11463-11470", "volume":"272", "issue":"17", "source": "www.jbc.org", “abstract”: "SATB1 is a cell type-specific nuclear matrix attachment region (MAR, which is described as a nuclear factor involved in T-cell development, to be involved as well in B cell specific mechanisms.

Objective: Since this MAR BP are involved in multiple tumor development clinicopathological parameters, Ki67 expression and MGMT promoter methylation status was evaluated, and the prognostic value of SATB1 expression in patients with gliomas was analyzed. SATB1-specific shRNA sequences were synthesized, and U251 cells were transfected with SATB1 RNAi plasmids. Expression of SATB1 mRNA and protein was investigated by RT-PCR and immunofluorescence staining and western blotting. The expression of c-Met, SLC22A18, caspase-3 and bcl-2 protein was determined by western blotting. The apoptosis of U251 cells was examined with a flow cytometer. The adherence, invasion, and in vitro angiogenesis assays of U251 cells were done. The growth and angiogenesis of SATB1 low expressing U251 cells was measured in an in vivo xenograft model.

Results: Of 70 tumors, 44 (62.9% and is crucial for T cell maturation, it should be interesting to study it effect on genetic remodeling mechanisms in B-lineage cells.

Methods: This study is performed on conditional knock out mice that permit to delete Satb1 in B-lineage cells upon expression of cre recombinase. B cell development, Somatic Hypermutation and Class switch recombination were analyzed in Satb1 conditional–knock out context.

Results: Peripheral B cells devoid of Special A-T rich Binding protein 1 display a changing Ig (immunoglobulin), expression pattern depending of their subset, through a mechanism involving transcription. This MAR BP acts like an activator of IgH locus transcription in inactivated B cell, and becomes a repressor when B cell are activated. Its deletion induces an increase of SHM frequency at Ig gene loci.

Conclusion: SATB1 seems to be involved in Ig gene regulation and consequently participate to B cell remodeling mechanisms by modulating transcription. Understanding all SATB1 gene targets in B cell will help to decipher mechanisms potentially underlying involvement of this nuclear factor in tumor development.

Keywords: SATB1 - B cell genetic remodeling mechanisms.

References:
Inactivation of EXO1 nuclease activity on genome maintenance and tumor suppression in Exo1<sup>Δ173A</sup> mice in vivo

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DNA damage response pathways rely extensively on nuclease activity to process DNA intermediates. Exonuclease 1 (EXO1) is a pleiotropic evolutionary conserved DNA exonuclease. It is involved in various DNA repair pathways, replication, antibody diversification, and meiosis. But, whether EXO1 is performing its activity through its enzymatic or scaffolding functions in mammalian cells remains unclear. Here we dissect the contribution of EXO1 enzymatic versus scaffolding activity by comparing mice expressing proven nuclease-dead Exo1<sup>ΔA/DA</sup> to nuclease-deficient Exo1<sup>-/-</sup> and nuclease-proficient Exo1<sup>+/+</sup> mice. We show that Exo1<sup>ΔA/DA</sup> and Exo1<sup>-/-</sup> mice are compromised in canonical DNA repair processing, suggesting that the EXO1 enzymatic role is important for error-free DNA mismatch and double strand break repair pathways. However, in non-canonical repair pathways EXO1 seems to have a more nuanced function. Next-generation sequencing of heavy chain V region in B cells showed the mutation spectra of Exo1<sup>ΔA/DA</sup> mice to be intermediate between Exo1<sup>+/+</sup> and Exo1<sup>-/-</sup> mice, suggesting that both catalytic and scaffolding roles of EXO1 are important for somatic hypermutation. Similarly, while overall class switch recombination in Exo1<sup>ΔA/DA</sup> and Exo1<sup>+/+</sup> mice was comparably defective, switch-switch junction analysis suggests that Exo1 might fulfill an additional scaffolding functions downstream of class switching. Although Exo1<sup>+/+</sup> mice are infertile, meiosis progressed normally in Exo1<sup>ΔA/DA</sup> and Exo1<sup>-/-</sup> cohorts, indicating that the structural but not the nuclease function of Exo1 is critical for meiosis. However, both Exo1<sup>ΔA/DA</sup> and Exo1<sup>-/-</sup> displayed similar mortality and cancer predisposition profiles. Taken together, these data demonstrate that EXO1 has both scaffolding and enzymatic functions in distinct DNA repair processes and suggest a more composite and intricate role for EXO1 in DNA metabolic processes and disease.

Keywords: Mismatch repair - Double strand break repair - Exo1 - Exonuclease - Meiosis - Resection
mRNA vaccine-induced immunity is less protective than post-infectious immunity in kidney transplant patients

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Introduction: Kidney transplant recipients (KTR), who are more likely to develop severe SARS-Cov2 infection, have been prioritized for vaccination. However, because KTR receive therapeutic immunosuppression they are known to have reduced response to vaccines.

Methods and results: In the present study, we demonstrate that KTR with previous history of COVID never experience reinfection with SARS-Cov2, in contrast with vaccinated KTR. In order to understand why infection provides a more robust protection than mRNA vaccine we monitored anti-SARS-Cov2 humoral and cellular immune responses in KTR after infection (n=22, COVID KTR) or mRNA-1273 vaccination (n=29, Vacc KTR). In contrast with Spike specific CD8+ T cells and anti-Spike IgA responses, which were similar in the two groups, almost all (91%) COVID KTR developed anti-RBD IgG, whereas only 45% of the Vacc KTR did so (p<0.001). The low numbers of Spike-specific Tfh17 cells in non-responders Vacc KTR point to defective B-cell help, which could be explained by higher doses of anti-metabolite drugs and a lack of immunogenicity of the vaccine.

Conclusion: These data suggest that a better vaccine protection of KTR could be obtained by transiently adapting immunosuppression and/or increasing the vaccine’s immunogenicity (e.g. by administering a 3rd dose).

Keywords: SARS-Cov2 - Transplantation - Humoral response
Maintenance of longlived memory plasma cells by contact to stromal cells through activation of PI3K/AKT and inactivation of FoxO1/3 prevents activation of caspase 3

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Longlived plasma cells (PC) secreting antibodies are maintained for decades in the bone marrow, in close contact to mesenchymal stromal cells 1,2.

The molecular signalling network enabling their long survival has been elusive.

We have established ex vivo cell culture conditions mimicking the bone marrow environment of PC, providing them with cell-contact to a stromal cell line and the cytokine APRIL, which are necessary and sufficient to prevent caspase-mediated death of PC. Persistence of PC depends on cell contact-induced PI3K signalling and inactivation of FoxO1/3 and prevents activation of caspases 3 and 7. APRIL signalling, via the NF-κB pathway, prevents activation of the ER stress-associated caspase 12 3.

Thus, stromal cells and APRIL ensure persistence of memory PC by complementing each other in providing resilience of memory PC to lethal mitochondrial and ER stress. Single cell transcriptomes of stromal cells and memory PC reveal an unforeseen diversity of both, and point to distinct differences in the synapses of different memory PC populations, differences which might impact on their longevity, and which might allow to target them specifically in vaccination or in the therapy of antibody-mediated diseases.

Keywords: Immunological memory - Signalling – Plasma cell.

References:

Post-transcriptional control by the RNA binding protein HuR is essential for germinal centre responses

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Introduction: Germinal centres (GC) are essential for the generation of high affinity antibodies and immunological memory. Nowadays we have a good understanding of how transcription factors modulate the GC reaction. However, little is known about the post-transcriptional mechanisms that mediate B cell activation, proliferation, selection and differentiation in GCs.

Objective: We hypothesize that HuR is part of an integrative post-transcriptional regulatory network that shapes the transcriptome of GC B cells to enable high-affinity antigen-specific immune responses.

Methods: We have combined conditional knock out mice, model antigens, transcriptomics and protein: RNA interactome analyses to uncover the essential functions of HuR in GCs.

Results: Our data show that HuR plays an essential role in the sustenance of GC responses. In its absence, the GC reaction and production of high-affinity antibody is severely impaired. Mechanistically, HuR modulates mRNA splicing and stability shaping, qualitatively and quantitatively, the transcriptome of GC B cells. HuR enables the expression of Myc and a Myc-dependent transcriptional program that is essential for GC B cell proliferation and Ig somatic hypermutation. HuR also controls the expression of mRNAs required for entry into and transition through the S phase of the cell cycle and it modulates a gene signature associated with DNA deamination, protecting GC B cells from DNA damage and cell death.

Conclusion: In summary, HuR is a key component for the post-transcriptional control of the GC reaction allowing expansion and selection of high-affinity, antigen-specific B cells.

Keywords: RNA binding proteins - Post-transcriptional gene regulation - B cell development - DNA damage
Improved cell signalling analysis by biofunctionalized nanospheres and imaging flow cytometry

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Introduction: The analysis of immune cell signalling is critical for the understanding of the biology and pathology of the immune system, and thus a mandatory step for the development of efficient biomarkers and targeted therapies. Phosflow, which has progressively replaced the traditional western blot approach, relies on flow cytometry to analyse various signalling pathways at a single-cell level. This technique however suffers a lack of sensitivity largely due to the low signal/noise ratio that characterises cell signalling analysis.

Methods and results: In this study, we describe a new technique, which combines the use of biofunctionalized nanospheres (i.e. synthetic particulate antigens, SPAg) to stimulate the immune cells in suspension and imaging flow cytometry to identify homogenously-stimulated cells and quantify the activity of the chosen signalling pathway in selected subcellular regions of interest.

Conclusion: Using BCR signalling as model, we demonstrate that SIBERIAN (SPAg-assisted suB-cEllulaR sIgnaling ANalysis) allows assessing immune cell signalling with unprecedented sensitivity and specificity.
Missing self-induced activation of NK cells synergises with low DSA titles to accelerate graft loss in complement-independent antibody-mediated rejection

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Introduction: During antibody-mediated rejection (AMR), the binding of donor-specific antibodies (DSAs) to graft endothelial cells recruits innate effectors and sometimes triggers complement activation. Innate effectors, in particular NK cells, damage graft vasculature by antibody-dependent cell-mediated cytotoxicity (ADCC). Recently, we demonstrated that recipients’ NK cells also trigger antibody-independent microvascular inflammation by sensing the absence of expression of self HLA class I molecules (“missing self” (MS)) on graft endothelial cells. In this study, we evaluated whether MS-induced NK activation could synergise with DSA-dependent NK activation to worsen AMR outcome.

Methods and results: Among the 1682 renal transplant patients that underwent a graft biopsy in our centre between 2004 and 2017, the 135 that fulfilled the diagnostic criteria for AMR were enrolled. Patients with complement-fixing DSAs (73.54%) had a higher risk of graft failure (p=0.002). Among the remaining patients (62.46%), with low titre DSAs, those with a MS exhibited a worse allograft survival (p=0.02). Cocultures of human NK cells and endothelial cells confirmed that addition of MS to ADCC increased endothelial damage.

Conclusion: We conclude that assessment of MS at time of the diagnosis of complement-independent AMR identifies patients at high risk for allograft failure.
Deep immune phenotyping of SARS-CoV-2 specific B cell profiles after primary infection

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Introduction: T cell-driven B cell responses are needed to induce class switched antibodies upon infection or vaccination, thereby establishing long-term protection against subsequent infections. Although SARS-CoV-2 infections and SARS-CoV-2 vaccines indeed induce protective antibodies against the virus, (Objective) it remains to be elucidated which unique SARS-CoV-2 specific B cell subsets become activated upon primary infection, if the various B cell differentiation pathways induced differ between primary disease severities and how they relate to formation of long-lasting antibodies against SARS-CoV-2.

Methods: Using multiparameter high-dimensional spectral flow cytometry, we elucidated deep immune profiles of the SARS-CoV-2-specific B cell responses in patients who recovered from COVID-19 with varying degrees of disease severity.

Results: We identified distinct populations of antigen-specific B cells targeting Nucleocapsid, Spike and RBD sites on SARS-CoV-2 in these patients when compared to other pathogens. Currently, we are investigating how the SARS-CoV-2 specific B cell subsets observed after three months after recovery of COVID-19 relate to the longevity of induced SARS-CoV-2 antibodies in persons who recovered from COVID-19 with high persistence or quick decay of antibody titers.

Conclusion: This study aims to elucidate the ongoing and established B cell responses against SARS-CoV-2 and to establish their role in COVID-19 disease for future therapies and vaccinations.

Keywords: Adaptive immunity - B lymphocytes - Infectious disease
Characterization of different immune escape mechanisms in Waldenström’s Macroglobulinemia

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Introduction: Waldenstrom’s macroglobulinemia (WM) is a rare subtype of indolent non-Hodgkin B cell lymphoma. This disease is characterized by the presence of MYD88\textsuperscript{L265P} mutation, which leads to constitutive activation of NFκB signaling and B-cell clonal expansion. However, WM is currently incurable, and the role of the microenvironment on its development remains unclear.

Objective: Identify different immunomodulation mechanisms involved in WM to characterize a protumoral microenvironment which can explain WM development; and propose novel targeted therapies which may improve the treatment arsenal of WM.

Methods: Through an original in vivo mouse model of WM which constitutively expresses MYD88\textsuperscript{L252P} in the B cell lineage (Ouk et al., Front. Immunol., 2021), we are studying expression of immune checkpoint molecules (PD-L1, CTLA-4, MHC-II) on tumor and immune cells. We are also studying different populations such as Tumor Infiltrated T lymphocytes (TILs), regulatory T-cells and tumor associated macrophages (TAMs).

Results and conclusion: Preliminary results show a deregulation of PD-1/PD-L1 axis with an impact on T-cells infiltration and exhaustion. We now focus our work on other escape mechanisms to perform an exhaustive study of the tumor microenvironment and its role on WM development.

Keywords: Waldenstrom’s macroglobulinemia - Immunomodulation - Immune checkpoint molecules - TILs - TAMs

References:
Termination of CD40L signaling drives human naïve B cell differentiation into antibody-secreting cell formation

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Introduction: Human naïve B cells are notoriously difficult to differentiate into antibody-secreting cells (ASCs) in vitro while maintaining enough cell numbers to evaluate the differentiation process. Insights in factors controlling the T cell-dependent differentiation of B cells into ASCs are much needed in order to understand the generation of effective humoral immunity against invading pathogens or to prevent undesired antibody formation in autoimmunity and blood transfusion. B cells require T follicular helper (Tfh) cell derived signals like CD40L and IL-21 during the germinal center (GC) response in order to undergo ASC differentiation. However, the cognate interaction between B and Tfh cells are short; after Tfh contact, B cells migrate away for renewed proliferation to GC dark zones where Tfh cells and thus stimulation, is absent.

Objective: Here we elucidated that enforced termination of CD40L-CD40 stimulation strongly promotes naïve B cell-to-ASC differentiation in vitro. Our data show that efficacy of naïve B cell differentiation is dependent on release of CD40 stimulation and is dramatically induced in the appropriate cytokine environment.

Methods: Using multiparameter phospho-flow and transcription factor (TF)-flow cytometry.

Results: We show that CD40L blocking, after initial CD40L and IL-21 stimulation, regulates the kinetics of the NF-κB and STAT3 pathways yielding downregulation of the B cell signature TF PAX5 and promoting ASC TFs BLIMP1 and XBP-1s.

Conclusion: Our data are the first steps to provide further insights in the regulation of human naïve B cell differentiation to ASCs. This is crucial in improving vaccination strategies and will also aid in the prevention and treatment of autoimmunity.
In severe COVID-19, SARS-CoV-2 induces a chronic, TGF-β-driven adaptive immune response that does not target itself

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Introduction: Immune responses elicited by SARS-CoV-2 are heterogeneous - ranging from asymptomatic to lethal. The molecular basis for this heterogeneity is unclear.

Objective: Here we investigated the adaptive immune response against SARS-CoV2 for the identification of mechanisms driving severe COVID-19.

Methods: Plasmablasts (PB) from severe COVID-19 patients admitted to the ICU for up to 60 days were sorted and examined by single cell RNA-Seq and BCR repertoire analysis.

Results: A continuous egress of PBs into the blood of severe COVID-19 patients was detected, which is a reflection of a chronic immune reaction. Early after ICU admission, this immune response is directed against SARS-CoV-2, with all patients acquiring virus specific IgG antibodies. Later, IgA-expressing PBs are formed, mirroring instruction by TGF-β. Only 30% of the patients expressed Spike-specific IgA, with one expressing IgA2. BCR repertoire of the PBs were rather oligoclonal and somatically mutated but not specific for SARS-CoV-2 S or N protein.

Conclusion: TGF-β is a key cytokine regulating a chronic immune response in severe COVID-19 that is no longer directed against SARS-CoV-2. Targeting of TGF-β may be a way to ameliorate severe COVID-19 (1).

Keywords: COVID-19 - Plasmablasts - TGF-β driven isotype switch.

References:
Modulation of the anti-tumor NK response by EBV latency III B cells: an escape route from immune surveillance?

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Introduction: B cell immortalization is related to many viruses and associated with lymphomas. Epstein Barr Virus (EBV) has the ability to immortalize B cells (EBV latency III B cells). They can inhibit the anti-tumor T cell response by overexpressing PD-L1. T and NK cells are major players in B lymphoma immunosurveillance, as the result of an activating/inhibiting balance.

Objective: We aim to study CMH and emergent inhibitor checkpoints for NK cells in the case of EBV latency III B cells.

Methods: We characterized three newly established EBV latency III B cell Lines (LCL), by comparison with peripheral blood B cells (flow cytometry).

Results: High expression for CMH could protect cells from NK lysis. Overexpression on cells of CD80, CD86, PD-L1 and PD-L2 could inhibit NK response by interaction with CTLA4 and PD-1. PD-L1, PD-L2, galectins 3 and 9, highly expressed at the intracellular level, could be secreted and inhibit NK cells by interaction with PD-1, LAG3 and TIM3. CD112, CD155 and Ceacam 1 (ligands for PVRIG, TIGIT and TACTILE) are not expressed.

Conclusion: EBV latency III B cells may be strong inhibitors for the anti-tumor NK response and contribute to the emergence of EBV associated B lymphomas.

Keywords: B lymphomas - NK cells - Immunosurveillance

References:
1. Auclair et al., 2019 Journal of Immunology 203 (6) 1665-1674.
Chronic lymphocytic leukemia evolves from a reservoir of Ig-diversified, persisting tumor precursor cells

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Introduction: The early pathogenesis of chronic lymphocytic leukemia (CLL) is poorly understood. Previous studies revealed mature CD5⁺ B cells to represent the cellular origin of CLL, in particular a rare subpopulation of CD5⁺ memory B cells in healthy adult peripheral blood (PB) to show high molecular similarity to CLL cells. However, the existence and functional role of such cells in CLL patients has not been investigated.

Objective: Assuming that the early pathogenesis of CLL is often connected to germinal center reactions, we aimed to characterize persisting CD5⁺ memory B cells in the PB of CLL patients to explore Ig diversity and tumor plasticity in early CLL pathogenesis.

Methods: We purified normal B cells and tumor cells from CLL patients and generated single-cell-RNA and -Ig gene repertoire-sequencing (scRNAseq/VDJseq) profiles. Moreover, we performed Ig repertoire profiling from longitudinal cryopreserved patient samples and used Ig-mutation phylogenetics to investigate GC-diversification and subclonal evolution in CLL pathogenesis. Tumor precursor cells were functionally analyzed in vitro.

Results: In Ig-mutated and Ig-unmutated CLL a minor tumor subset exists that is in phenotype and intraclonal IGHV diversity similar to normal CD5⁺ memory B cells. The distinct tumor subset efficiently responds to stimulation in vitro, which further contrasts with the main CLL population. Longitudinal analysis shows that the small CLL subset is generated pathogenetically early and persists, maintaining a-priori CLL subclone diversity. During CLL progress, competitive outgrowth of selected subclones can be observed. CLL diversity is reduced by efficient treatment, but entirely reconstructed upon relapse.

Conclusion: We observed a reservoir of a-priori existing CLL subclones which carry a conserved IGHV gene mutation pattern, along which the main CLL population is distributed either more upstream (uCLL) or downstream (mCLL) to the root of the Ig-dendrogram, and which can be used to monitor CLL evolution dynamics. Our study identified early generated and persisting “CLL precursor cells”.

Keywords: CLL pathogenesis - Persisting tumor precursor cells - Clonal evolution
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