

determinants. An idiotype present on the majority of immunoglobulins derived from the V_H4–34 gene can be detected using the rat monoclonal antibody 9G4. 9G4 also allows the detection of B cells expressing B cell receptors derived from V_H4–34 gene. We therefore determined the effect of B cell depletion therapy with rituximab (RTX) on B cell subpopulations expressing the 9G4 idiotype in peripheral blood of patients with rheumatoid arthritis (RA) as a means of following the fate of a specific autoreactive B cell population.

Materials and Methods B-cell subpopulations were characterised by flow cytometry using combinations of IgD, CD5, CD27 and CD38 in healthy controls (HC) (n = 7), patients with RA before (n = 10) and at clinical relapse after RTX (n = 17). The frequency of each 9G4+ B cell subpopulation was calculated after gating on 9G4+ cells.

Results No significant differences were observed in the frequency of total 9G4+ B cells between both patient groups and HC, although the levels of 9G4+ B cells tended to be higher at relapse after RTX treatment. After RTX treatment, repopulating total B cells were predominantly transitional (IgD+CD38++) and naïve (IgD+CD38+), with lower frequencies of most memory B-cell subpopulations. At relapse, RA patients had significantly more 9G4+IgD–CD38– memory B cells compared to HC. This B cell subset was positively correlated with a 9G4+CD5+CD27+ subpopulation. The frequency of 9G4+IgD–CD38+ memory B cells also tended to be increased after RTX compared to HC and pre-RTX. Lower levels of 9G4+IgD+CD38+ naïve, 9G4+IgD+CD38– memory and 9G4+CD5–CD27+ memory B cells were found after RTX treatment compared with HC and pre-RTX. No significant differences were observed in 9G4+IgD+CD38++ transitional, or 9G4+IgD–CD38++ plasmablasts in RA patients before and after RTX treatment in comparison with HC. However, there was a positive correlation between the frequency of 9G4+IgD–CD38++ plasmablasts and time after RTX treatment.

Conclusions Alterations in the frequency of memory B cell subpopulations expressing 9G4 idiotype occur in clinically relapsing RA patients after RTX treatment, particularly in IgD–CD38– memory B cell subset. It is possible that this 9G4+ B cell subpopulation escapes B cell depletion therapy, being rescued in niches such as secondary lymphoid organs, or in inflammatory sites. Our results also suggest that the normal tolerance mechanisms preventing 9G4+ B cells from becoming antibody producing cells is defective in patients with RA.

A5.14 HOMOCITRULLINE-REACTIVE ANTIBODIES CAN BE GENERATED FROM SYNOVIAL B-CELLS FROM ACPA-NEGATIVE RA PATIENTS

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¹Fiona Murray, ¹Lena Israelsson, ¹Johanna Steen, ¹Khaled Amara, ²Jing Shi, ¹Lars Klareskog, ²Leendert Trouw, ¹Vivianne Malmström. ¹Rheumatology Unit, Department of Medicine at Karolinska University Hospital, Karolinska Institute, Solna, Stockholm, Sweden; ²Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

Background and Objectives Roughly two-thirds of rheumatoid arthritis (RA) patients carry antibodies, so-called ACPA, against peptides containing citrulline, a post-translationally modified version of the amino acid (AA) arginine in their sera and/or affected joints. A recent study reported the presence of antibodies against a different kind of post-translationally modified AA, homocitrulline, also known as carbamylated proteins (CarP), where lysine residues are altered by a non-enzymatic reaction involving cyanate. The authors reported IgG antibodies recognising homocitrullinated fibrinogen in the sera of >45% of RA-patients and that 16% of ACPA-negative patients carried such anti-CarP antibodies.

Furthermore, in vivo studies describe that immunisation of mice with homocitrulline-containing peptides induced erosive arthritis. Thus, homocitrulline represents an interesting immune target in the context of RA. Therefore, we aimed to assess the proportion of anti-CarP antibodies sourced from the joints of RA patients with active disease.

Materials and Methods Using a single B cell-based cloning technology to isolate the immunoglobulin (Ig) genes from joint-derived IgG+ CD19+ B-cells, we then co-transfected the paired heavy and light chains into a human cell line and produced recombinant monoclonal antibodies from each individual B cell. This approach has previously allowed for the generation of citrulline-reactive monoclonal antibodies. Here, we analysed for reactivity to carbamylated (homocitrullinated) fibrinogen and compared to its counterpart unmodified fibrinogen, by ELISA. So far, we have analysed 42 monoclonal antibodies from three ACPA-positive patients and one ACPA-negative patient, for homocitrulline reactivity.

Results Between six and eleven antibodies were tested for homocitrulline activity for the ACPA-positive patients and eleven were examined for the ACPA-negative patient. All clones screened for CarP-reactivity were negative for citrulline binding. Antibodies were made up to concentrations of 5 ug/ml down to 0.63 ug/ml in a 4-step dilution. So far, one antibody displayed reactivity to the carbamylated peptide and this antibody originated from the ACPA-negative patient.

Conclusions Although the frequency of anti-CarP reactivity amongst the 42 antibodies examined does not match that previously reported for sera, the finding of one anti-CarP antibody from an ACPA negative patient, does support the earlier finding of significant frequencies of anti-CarP antibodies amongst ACPA-negative patients. It will be of great interest to expand the investigation for anti-CarP specificities, particularly among ACPA-negative patients and determine whether these antibodies could have pathological effects in RA patients.

A5.15 HOW MICRORNAS CONTROL PLASMA CELLS

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¹Julia Schmid, ¹Martina Porstner, ¹Andreas Brandl, ¹Edith Roth, ²Thomas Winkler, ³Michael Bösl, ⁴Andreas Radbruch, ¹Jürgen Wittmann, ¹Hans-Martin Jäck. ¹Division of Molecular Immunology, Nikolaus-Fiebiger-centre, Department of Internal Medicine III, FAU Erlangen-Nürnberg, Erlangen, Germany; ²Section of Hematopoiesis, Department of Biology, FAU Erlangen-Nürnberg, Erlangen, Germany; ³Max Planck Institute for Neurobiology, Martinsried, Germany; ⁴Deutsches Rheumaforschungszentrum (DRFZ), Berlin

Background and Objectives Our long-term goal is to understand how one class of interfering non-coding RNAs, the so-called microRNAs (miRNAs), regulates and fine-tunes the differentiation of mature B cells into effector cells, i.e., memory B cells and antibody-secreting plasma cells. miRNAs control the expression of specific target genes at the post-transcriptional level by binding to target sequences e.g., in the 3'-untranslated region of mRNAs, which, depending on the degree of the binding, results either in a block of translation or an accelerated degradation of the respective target mRNA. Lineage-specific deletion of the miRNA-processing DICER protein as well as of individual miRNAs revealed the importance of miRNA pathway in early steps of central B cell maturation. However, the mechanisms by which miRNA-dependent circuits control the antigen-induced phase of B cell activation of mature naïve B cells and their subsequent differentiation into effector cells remain largely elusive.

Methods To change this situation we established a transgenic knock-in mouse line with a floxed allele of DGCR8, an essential subunit of the nuclear miRNA processing complex. B cell-specific deletion of DGCR8 resulted in a complete maturation block at the pro-B stage, indicating that miRNA processing is essential for central

B cell maturation. We have also established a culture system that allows us to delete a floxed DGCR8 gene in freshly isolated B cells using retrovirally transduced Cre. Using this system, we found that the miRNA pathway is required for mitogen-induced in vitro proliferation of mature B cells.

Results We have also obtained genome-wide miRNA expression profiles of all major mouse B cell subsets, including long-lived plasma cells, and found a profound upregulation of one miRNA in plasma cells. Ectopic expression of this plasma cell signature-miRNA in primary mouse B cells accelerated the differentiation into antibody-secreting plasmablast, as indicated by upregulation of CD138 and enhanced IgM secretion. We also verified several targets of this miRNA, e.g., Bach2 and MiTF, that are part of the transcriptional circuit that controls germinal centre reactions and plasma cell differentiation.

Conclusions These studies will provide new molecular insights into regulatory circuits that control the production of antibodies and could potentially lead to new avenues for diagnosing or treating diseases associated with aberrant plasma cell development, e.g., primary antibody deficiencies, plasma cell malignancies, and autoimmune disorders.

A5.16 TNF- α RESPONSE OF SYNOVIAL FLUID MONOCYTE-MACROPHAGES TO ACPA IMMUNE COMPLEXES

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^{1,2}Cyril Clavel, ^{1,2}Florence Anquetil, ³Laurent Zabraniecki, ^{1,3}Évelyne Verrouil, ^{1,2}Guy Serre, ¹Mireille Sebbag. ¹Research Unit "Epidermis Differentiation and Rheumatoid Autoimmunity", UMR 5165 CNRS/1056 INSERM/Université de Toulouse; ²Laboratory of Cell Biology and Cytology, CHU de Toulouse; ³Rheumatology Centre, CHU de Toulouse; Toulouse, France

Background Autoantibodies to citrullinated proteins (ACPA) are specifically associated to rheumatoid arthritis (RA) and produced in the inflamed synovium where citrullinated fibrin, their main antigenic target, is abundant. Using a human in vitro model we showed that macrophages generated by differentiation of blood monocytes from healthy individuals or patients with RA secrete TNF- α in response to immune complexes formed by ACPA and citrullinated fibrinogen (ACPA-IC). Moreover while in both healthy individuals and RA patients the TNF- α response of macrophages was much higher than that of their monocyte precursors, the TNF- α production of blood monocytes and monocyte-derived macrophages from RA patients did not differ from that observed with the healthy controls.

Objectives To further assess the impact of ACPA-IC on joint inflammation, we evaluated the TNF- α response they prompt in monocyte-macrophages isolated from the synovial fluid (SF) of patients with RA and with other arthritides.

Materials and Methods SF samples were obtained from 7 patients with RA (4 ACPA-positive, 3 ACPA-negative) and 8 ACPA-negative control patients with various arthritides. Polymorphonuclear cells were eliminated by centrifugation over Ficoll or capture with CD15-conjugated magnetic beads then monocyte-macrophages further purified using CD14-beads (median purity: 95%). The purified cells were stimulated with IC generated by capture of ACPA from IgG fractions prepared from RA sera, on immobilised citrullinated fibrinogen, as described (Clavel, Arthritis Rheum, 2008).

Results With SF monocyte-macrophages from both RA and control patients, secretion of TNF- α sometimes occurred in the absence of any stimulation. However in RA patients, irrespective of their ACPA status, TNF- α secretion increased when the cells were cultured on ACPA-IC (median (range) = 16 (2–110) pg/ml). Such activation was also observed with the SF monocyte-macrophages from control patients (184 (33–638) pg/ml). In the whole series of SF samples, the increase in TNF- α secretion was found to be highly significant ($p < 0.001$).

Conclusions In contrast with our previous observations on CD14-positive blood monocytes and on derived macrophages, it appears that CD14-positive monocyte-macrophages can be pre-activated in the SF and secrete TNF- α spontaneously, but that they can nevertheless be further activated by ACPA-IC. These properties are not restricted to RA patients and seem to be characteristic for the SF CD14-positive monocyte-macrophages.

Since citrullinated fibrin and ACPA have been described in the SF of RA patients, it is highly probable that ACPA-IC play a direct pro-inflammatory role in the SF by inducing or enhancing TNF- α secretion by the SF monocyte-macrophages.

A5.17 IGG4(+) B-CELL CLONES DOMINATE THE PERIPHERAL BLOOD IN IGG4-ASSOCIATED CHOLANGITIS

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^{1,*}Marieke Doorenspleet, ^{3,*}Lucas Maillette de Buy Wenniger, ¹Paul Klarenbeek, ²Frank Baas, ¹Paul-Peter Tak, ^{3,*}Ulrich Beuers, ^{1,*}Niek de Vries. ¹Department of Clinical Immunology and Rheumatology; ²Department of Genome Analysis; ³Department of Gastroenterology and Hepatology, all at Academic Medical Center/University of Amsterdam, The Netherlands;

*.*These authors contributed equally to this work

Background IgG4-related disease (IgG4-RD) is a novel disease entity characterised by elevated serum IgG4 levels, IgG4(+) plasmacytic infiltration and fibrosis in various organs. We hypothesised that, if elevated IgG4 titers would be caused by antigen-driven immune responses, clonally expanded, IgG4(+) B cells and plasma cells might be present in affected tissue and peripheral blood.

Objectives To analyse the presence of IgG4(+) expanded B-cell clones in peripheral blood (PB) before and after successful therapy of patients with IgG4-associated cholangitis (IAC), and to compare this to healthy and disease controls using a newly developed high throughput sequencing (HTS) protocol for analysis of the B-cell receptor (BCR) repertoire.

Methods In 6 IAC patients the BCR repertoire was assessed in PB before and after 4 and 8 weeks of prednisolone (median 40mg, range 20–40 mg) treatment. As controls 6 healthy controls (HC) and 6 treatment-naïve disease controls with PSC and pancreaticobiliary cancer were analysed (DC). In two patients a paired duodenal papilla biopsy was obtained at baseline. After isolation of mRNA the BCR_{heavy}-chain was amplified, including the CDR3 region, thus fingerprinting unique clones. In our HTS protocol the number of clonal reads can be used as a measure for 'dominance'; clones with a frequency of >0.5% were considered dominant.

Results At baseline, a mean of 15.1% of all clones was IgG(+) in IAC (similar in HC/DC). Among the IAC IgG(+) clones, the most dominant ones were IgG4(+) (mean rank: 1st in IAC versus 63th in HC ($p < 0.005$) and 55th in DC ($p < 0.005$)). Across all isotypes in every IAC patient IgG4(+) BCR clones were present among the 10 most dominant BCR clones which was not observed in any of the controls. The papilla contained the same dominant IgG4(+) clones that were detected in the paired blood samples. After 4 and 8 weeks of therapy, the contribution of IgG4(+) clones specifically to the BCR-repertoire was negligible (median 0.19%, IQR 0.16–0.21%), mirroring a sharp decline in serum IgG4 in conjunction with regression of clinical symptoms.

Conclusions Our findings indicate that IgG4(+) clones are abundantly present within the repertoire of IAC patients, in contrast to healthy or disease controls. The inflamed tissue was shown to contain the same expanded IgG4(+) clones, suggesting an antigen-driven immune response in IgG4-RD. A possible central role for IgG4(+) B cells is furthermore supported by the finding that IgG4(+) clones in peripheral blood specifically disappear upon successful corticosteroid therapy.