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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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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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-naive 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.