the secretion of C-reactive protein (CRP) by an automated analyzer.
Results IL-17 and TNFα induced in synergy the hepatic IL-6 production (>8 fold, p<0.01) and CCL-20 expression (>100 fold, p<0.01). CRP secretion was induced by IL-17/TNFα and the anti-IL-6R inhibited this induction. CCL-20 expression was not increased by IL-6 stimulation or reduced by the addition of the anti-IL-6R to the IL-17/TNFα treatment. In HepaRG cell-PBMC cultures, IL-6 and IL-17 were produced only in PHA-activated conditions and the IL-6 and IL-17 levels were higher in co-cultures vs PBMC monocultures (>14 and >2 fold respectively, p<0.01). Transwell system that avoids direct cell-cell contact decreased the IL-6 and IL-17 secretion by 4- and 2-fold respectively in PHA-activated HepaRG cell-PBMC cultures. CRP production increased when PHH were cultured in presence of PHA-activated PBMC compared to PHH alone with PHA.
Conclusions The IL-17/TNFα synergistic effect on hepatocytes mediates systemic inflammation by inducing CRP secretion through the IL-6 pathway and mononuclear cell recruitment by acting on CCL-20 expression in an IL-6 independent manner. Direct and indirect hepatocyte-PBMC interactions contribute to the hepatic inflammatory response by increasing the IL-6 and IL-17 secretion. The induction of Th17 cell differentiation by IL-6 and the increase of CCL-20 mediated Th17 cell recruitment by IL-17 may lead to a vicious and chronic inflammatory cycle.
Disclosure of interest None declared

P078 A GENETIC VARIANT OF IL-32 IS ASSOCIATED WITH THE EX VIVO CYTOKINE PRODUCTION OF ANTI-TNF TREATED PBMCS ISOLATED FROM RHEUMATOID ARTHRITIS PATIENTS

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Introduction Since the introduction of biologics in the treatment of rheumatoid arthritis (RA) disease outcome improved. Still, about 40% of RA patients do not respond to therapy with TNFα blockers. Previously, a strong link between TNFα and interleukin (IL)−32 has been reported in RA.[1]
Objectives We hypothesise that a promoter single nucleotide polymorphism (SNP) in IL-32 can affect clinical responsiveness to anti-TNFα treatment in RA patients, functioning as a new biomarker in treatment of RA.[2]
Methods Peripheral mononuclear cells (PBMCs) from RA patients and healthy individuals were stimulated with RPMI or recombinant human (rh)TNFα to study the mRNA and protein expression of IL-32 and other pro-inflammatory cytokines. Moreover, disease activity scores (DAS28), '*in vitro response*' and clinical response to anti-TNFα therapy (etanercept, adalimumab), of RA patients were measured and all were stratified for the IL-32 SNP (C/T).
Results Stimulation of PBMCs from RA patients was followed by higher IL-32 protein production and a tendency towards higher IL-32β and IL-32γ mRNA expression compared to healthy individuals. When data was stratified for the IL-32 promoter SNP, patients bearing the CC genotype showed higher IL-32 protein expression. Of interest, these patients also produced more cytokines. Even though the DAS28 did not depend on the presence of the promoter SNP, the '*ex vivo*' cytokine response did have a different pattern in clinical responders depending on the genotype.
Conclusions IL-32 mRNA and protein production was higher in RA patients compared to healthy individuals, with a trend towards higher concentrations in patients bearing the CC genotype. Regardless of the fact that the promoter SNP was not associated with disease activity, IL-1 beta production in the CC-genotype might predict clinical response to either etanercept or adalimumab.

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P079 INTERLEUKIN-27 REGULATES THE MAGNITUDE OF THE ECTOPIC GERMINAL CENTRE RESPONSE IN A VIRAL-INDUCIBLE MODEL OF SIALADENITIS

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Introduction Ectopic lymphoid structures (ELS) are leukocytes aggregates that form in tissues affected by chronic inflammation. In autoimmunity, ELS play an active role in disease progression and are typically associated with aggressive disease. Thus, understanding the mechanisms that trigger ELS formation is of paramount importance for therapeutic targeting of this process. Interleukin-27 (IL-27) is prominently associated with the negative control of adaptive immunity, and in particular in the suppression of Th17-type responses.
Objectives To elucidate the role of IL-27 in the control of lymphoid neogenesis and its functional relationship with aberrant IL-17 production in a murine model of inducible ELS, where the local administration of a replication-deficient adenovirus (AdV) triggers the formation of ectopic germinal centres in salivary glands (SG).
Methods A single administration of AdV was delivered by cannulation directly into the SG of wild-type (WT) and IL-27R-deficient (*Il27ra*$^{-/-}$) mice. For IL-17A blockade, an anti-mouse IL-17A antibody or IgG control was administered systemically. ELS development and peripheral immune responses were temporally tracked by immuno-histopathology, flow cytometry, and real-time qPCR.
Results AdV cannulation induced an early upregulation of IL-27 and IL-27R in WT mice SG, which was mirrored by an increase in the infiltration of IL-27-producing T, B and NK cells. AdV-challenged *Il27ra*$^{-/-}$ mice developed exacerbated salivary gland inflammation, and by day-19 post AdV challenge developed larger and more abundant ELS as compared to WT mice. Moreover, *Il27ra*$^{-/-}$ mice displayed a heightened expression of homeostatic cytokines, chemokines and their corresponding receptors that are required for lymphoid neogenesis (e.g., *Cxcl13*, *Ccl19* and *Ltb*). IL-27R-deficient mice also displayed elevated markers of functional germinal centre responses (e.g., activation-induced deaminase, AID). Underpinning the exaggerated development of ELS in *Il27ra*$^{-/-}$ mice was the preferential expansion of IL-17-producing T helper (Th)17 cells, which was linked to a reduction in the Th1 cell

population. This was confirmed using a neutralising antibody to IL-17A, which resulted in a reduction in the size of ELS as determined by immunofluorescence detection of T and B-cell involvement. The inhibition of ELS development by anti-IL-17A treatment was also reflected by the reduced expression of lymphoid chemokines and AID. Notably, the infiltration of IL-22-producing CD4 +cells, a key effector population involved in ELS formation, was also reduced in anti-IL-17-treated *Il27ra*$^{-/-}$ but not WT mice.

Conclusions Here we show that IL-27 has a non-redundant inhibitory role in the regulation of the magnitude of ectopic germinal centre responses in inflamed SG. In the absence of a regulatory IL-27 signal, an exaggerated Th17 cell response was linked to dysregulated ELS size and activity. These findings provide new insights into the mechanisms governing ELS formation and highlight the role of IL-27 as an endogenous inhibitor of lymphoid neogenesis which could be exploited for therapeutic purposes in autoimmune diseases.

Disclosure of interest None declared

P080 C-REACTIVE PROTEIN: NOT ONLY A MARKER, BUT ALSO A CAUSE OF INFLAMMATION THROUGH METABOLIC REPROGRAMMING OF HUMAN MACROPHAGES

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Introduction C-reactive protein (CRP) is an acute-phase protein produced in high quantities by the liver in response to infection and during chronic inflammatory disorders such as rheumatoid arthritis (RA). As a consequence, CRP is in widespread clinical use as a general marker of inflammation. Although CRP is known to facilitate clearance of cell debris by phagocytic cells by binding to its ligand phosphocholine on dead cells, it is still unclear whether CRP displays additional immunological functions.

Objectives Here, we set out to investigate whether CRP, which is present in high concentrations in synovial fluid of active RA patients, also plays a role in the orchestration of inflammation in the inflamed joint.

Methods Human macrophages were differentiated from blood monocytes of healthy volunteers, or sorted from synovial fluid of RA patients. Cells were stimulated with complexed CRP (c-CRP) and/or ligands for Toll-like receptors (TLRs) and NOD-like receptors (NLRs), mimicking the stimuli in the inflamed joint. Responsible signalling pathways were identified using small molecule inhibitors and RNA interference. Metabolic pathways were identified using specific inhibitors and the Seahorse metabolic analyzer.

Results Strikingly, we here provide evidence that CRP is not only a marker, but also a cause of inflammation by strongly amplifying the production of RA-associated pro-inflammatory cytokines. We show that complex formation of CRP as a result of binding to its ligand phosphocholine selectively enhanced TNFα, IL-1β, and IL-23 production by human macrophages. While c-CRP did not induce cytokine production individually, c-CRP synergized with TLRs and NLRs to amplify cytokine gene translation. We identified Fc gamma receptor I and IIa (FcγRI and FcγRIIa) as the main receptors responsible. Moreover, we unravelled the responsible molecular mechanism of c-CRP-induced inflammation, which crucially depends on signalling through kinases Syk and PI3K, resulting in enhanced gene translation of pro-inflammatory cytokines through metabolic reprogramming, particularly through amplified glycolysis and fatty acid synthesis.

Conclusions These data indicate that CRP is not only a marker, but also a cause of inflammation in RA patients by selectively promoting RA-associated pro-inflammatory cytokine production by human macrophages, thereby exacerbating pathology. From a therapeutic point of view, inhibition of c-CRP-induced immune activation, e.g. by targeting the identified molecular mechanisms, may be a valuable tool to suppress inflammation.

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P081 SCLEROSTIN AFFECTS RANKL-MEDIATED OSTEOCLAST DIFFERENTIATION

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Introduction Sclerostin is a Wnt inhibitor and has anti-anabolic effects on bone formation by negatively regulating osteoblast differentiation. The lack of sclerostin in humans and mice leads to a higher bone mass and bone strength known as sclerosteosis. Therefore, inhibition of sclerostin is currently investigated as a treatment against osteoporosis. Surprisingly, the genetic deficiency or pharmacological inhibition of sclerostin causes a deterioration of disease severity in a TNFα-dependent arthritis mouse model (hTNFtg). hTNFtg mice lacking sclerostin displayed enhanced joint inflammation, cartilage loss and bone erosion associated with an elevated number of osteoclasts within the joint.

Objectives We want to investigate the impact of sclerostin on osteoclast differentiation and bone erosion in arthritis.

Methods Sclerostin knockout (sost$^{-/-}$) mice were crossbred with hTNFtg mice to obtain sost$^{-/-}$/hTNFtg synovial fibroblasts. Co-cultures of synovial fibroblasts and wildtype bone marrow macrophages were analysed by TRAP staining. RANKL expression was measured by ELISA and cytokine expression by array analysis and Western Blot. Moreover, the influence of sclerostin on osteoclastogenesis was additionally analysed in mono-cultures.

Results In our co-culture system of synovial fibroblasts and bone marrow derived macrophages, fibroblasts from sost$^{-/-}$/hTNFtg mice strongly promote osteoclastogenesis in comparison to hTNFtg synovial fibroblasts. Notably, no increased expression of receptor activator of NF-kB ligand (RANKL) was detectable in sost$^{-/-}$/hTNFtg fibroblasts even after stimulation with inflammatory cytokines. Interestingly, basal secretion of IL-1α, which is known to stimulate osteoclastogenesis, was higher in sost$^{-/-}$/hTNFtg compared to hTNFtg fibroblasts. Accordingly, sclerostin inhibited osteoclastogenesis when administered in the pre-differentiation phase, whereas no effect was observed in the differentiation phase, indicating an inhibitory effect of sclerostin on osteoclast precursors.

Conclusions Sclerostin deficiency in hTNFtg synovial fibroblasts promotes RANKL-mediated osteoclastogenesis, which is most likely dependent on IL-1α.

Disclosure of interest None declared