Methods Arthritis was induced by collagen-induced arthritis (CIA) and passive collagen antibody induced arthritis (CAIA) in respectively C57BL/6 and RAG2-/- (T- and B-cell deficient) mice. Animals were subjected to different regimens of mechanical strain. Increased strain occurred in voluntary running mice whereas tail suspension (unloading) abolished mechanical strain; both were compared to control housing conditions. The impact of different loading conditions was measured on clinical disease score, histology, micro-CT images and erosion quantification, gene induction in tendon and synovial tissue, immune cell recruitment *in situ*, development of anti-collagen antibodies and their pattern of siaylation and galactosylation.
Results Voluntary running of CIA in C57BL/6 mice markedly induced an early onset and increased progression whereas no disease onset could be observed in the hind paws from animals in unloaded conditions. CAIA in running RAG2-/- mice also induced early arthritic symptoms and severe progression. Intriguingly, running conditions were sufficient to induce arthritis without the need of LPS as an inflammatory trigger. Mechanical strain did not alter however IgG autoantibody levels nor their levels of galactosylation and sialylation. Furthermore, we demonstrate that mechanical strain on stromal cells results in recruitment of classical monocytes into specialised mechano-sensitive regions characterised by a unique microanatomy. This promotes local inflammation and differentiation into local osteoclasts which induce regional erosions. A striking similarity was observed in the pattern of joint erosions in human patients with RA and SpA which were also confined to these mechanosensitive regions.
Conclusions This study provides the first evidence that mechanical strain controls the transition from systemic autoimmunity into site-specific joint inflammation. Homing of inflammation and development of erosions was confined to mechano-sensitive regions, characterised by a high number of attachment- and contact points for tendons.This represents a novel paradigm and explains why arthritis in mice and humans is characterised by a regional and patchy distribution. Curiously, this pathway does not rely on adaptive immunity but rather on stromal cells. Mechano-stimulation of mesenchymal cells induced CXCL1 and CCL2 permitting recruitment of classical monocytes which can differentiate into bone-resorbing osteoclasts. Thus, mechanical strain controls the site-specific direction of inflammation and tissue damage in arthritis.
Disclosure of interest None declared

P001 MECHANISMS OF BONE EROSION AND PAIN TRIGGERED BY ANTIBODIES TARGETING POST-TRANSLATIONAL PROTEIN MODIFICATIONS IN RHEUMATOID ARTHRITIS

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Introduction Pain and bone loss are cardinal features of rheumatoid arthritis (RA), which can be triggered by anti-citrullinated protein antibodies (ACPAs).
Objectives We aimed to investigate whether ACPA fine specificities could influence osteoclast (OC) induction and we studied the contribution of ACPA Fc and Fab regions in the regulation of OCs. We also investigated whether *in vivo* targeting of OCs by bis-phosponate could influenceACPA-induced pain in mice.
Methods Polyclonal ACPAs and control IgGs were purified from the peripheral blood of RA patients using protein G and anti-cyclic citrullinated peptide (CCP)−2 affinity chromatography. Monoclonal ACPA and rheumatoid factor (RF) IgGs were generated from single synovial plasma cells or antigen tetramer-sorted peripheral blood memory B-cells. OCs were generated from CD14 +monocytes of healthy individuals or bone marrow cells of FcgRIII or FcRg chain knockout mice in the presence of the various antibodies. OC differentiation was monitored by counting TRAP-positive multinucleated cells or in bone erosion assays. Mechanical hypersensitivity was assessed over time by Von Frey filaments and the up-down method in female adult wild type or FcRg chain knockout mice injected i.v. with polyclonal or monoclonal ACPA or control antibodies. Bone density was measured by micro-CT. Zoledronate (100 ug/kg) was injected i.p every third day to examine if blocking osteoclast activation alter ACPA-induced pain.
Results Polyclonal and two out of the nine tested monoclonal ACPAs increased osteoclastogenesis. Addition of a monoclonal RF antibody to OC cultures could not influence osteoclastogenesis in itself, but it significantly increased the effect of ACPAs. Dimeric Fab fragments prepared from polyclonal ACPAs could increase OC numbers similarly as the intact antibodies, suggesting a crucial role of the cell surface antigens triggered by ACPA binding in mediating the effects of these antibodies. On the other hand, whereas ACPAs increased osteoclastogenesis from bone marrow precursors of wild type mice, no stimulatory effects could be observed when bone marrow cells of FcgRIII or FcRg chain knockout mice were used, suggesting that Fc receptors might also be important for ACPA-mediated OC stimulation. Polyclonal and monoclonal ACPA induced pronounced mechanical hypersensitivity lasing for at least 3 weeks and injection of polyclonal and certain combinations of monoclonal ACPA lead to bone erosion detectable by micro-CT- Bisphosphonate treatment and FcRg chain depletion prevented development of pain-like behaviour in those groups.
Conclusions We demonstrated that ACPAs with certain specificities have the capacity to increase osteoclastogenesis whereas most of the tested clones showed no effect on OCs. The mechanism triggered by ACPA binding to developing OCs was mediated through both Fc-dependent and independent signals. ACPA-mediated pain hypersensitivity was dependent both on osteoclast activity and Fcg receptors.
Disclosure of interest None declared

P002 HOW DO GLYCANS AFFECT IMMUNE CELLS IN RHEUMATOID ARTHRITIS?

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Introduction Anti-Citrullinated Protein Antibodies (ACPAs) are specific for Rheumatoid Arthritis (RA) and have been implicated in disease pathogenesis. The fragment antigen-binding domain (Fab) of ACPA was recently shown to be extensively glycosylated.[1] It is known that glycans play a key role in controlling innate and adaptive immunity,[2] however to date there

is limited understanding on the mode of action of glycans in RA. We hypothesise that the glycans on ACPA interact with glycan binding receptors and thus modulate immune responses in RA. Therefore, our aim is to elucidate the glycan effect of ACPA and other glycans on immune cells of RA patients to increase our understanding of RA pathogenesis.

Methods A whole blood flow assay was used to study glycan interactions with leukocytes. Leukocytes were isolated from blood and cells were incubated for 2 hours at 4°C with 15 µg/ml highly glycosylated ACPA and immunomodulatory glycoconjugates, such as sialic acid, Lewis-x, Lewis-y, mannose and as a negative control GlcNac. Glycan binding and identification of immune cell subsets was assessed with flow cytometry using a whole blood flow antibody panel.

Results This study examined the glycan-binding capacity of leukocytes in healthy donors via the whole blood flow assay. B cells appear to be superior in their interaction capacity with a variety of glycans, including the Fab glycan on APCA. This is an important finding because B cells play a key role in the pathogenesis of RA, through their antigen presenting capacity as well as their production of ACPA. In future studies RA patients material will be used to assess the glycan binding capacity to immune cell and to elucidate their role in the pathogenesis of RA.

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P003 IGF1R SIGNALLING IS ESSENTIAL FOR NEUROLOGICAL SYMPTOMS IN RA

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Introduction In addition to inflammation of the joints, rheumatoid arthritis (RA) has a neurological part consisting of pain, fatigue, depression and cognitive deterioration. These symptoms are critical for the patients' ability to cope with daily life, but are not alleviated completely with modern anti-rheumatic drugs. Sufficient IGF1R signalling is important for neurogenesis in the hippocampus. Its misbalance correlates with depression and anxiety.

Objectives The aim of this study was to evaluate the pathological changes in the brain during experimental RA and investigate their connexion to IGF1R.

Methods Characteristics of pain, depression and anxiety were collected among 214 RA patients and analysed in relation to IGF1R expression in WBC. Experimental RA was induced by immunisation with collagen II. Inhibition of IGF1R was achieved by injection of shRNA-producing lentiviral construct. The behavioural pattern of each mouse was recorded by filming. The hippocampus was analysed morphometrically, and gene and protein expression were analysed by qPCR and immunohistochemistry, respectively.

Results The RA patients' perception of depression and anxiety was associated with high IGF1R expression in WBC. This group of patients was also less physically active. In experimental RA, an enrichment of IBA1 +microglia and high expression of CD68 and IL-1b was found in the hippocampus. This was followed by an increased density of IGF1R+cells in cornu ammoni, and a decreased neurogenesis by limited expression DCX in the subgranular layer of the dentate gyrus. This results in a significant reduction of the hippocampus area. These changes in the brain were associated with immobility in RA mice. Treatment with shRNA targeting IGF1R improved arthritis, but led to increased immobility.

Conclusions RA induces remote inflammation in the hippocampus reducing neurogenesis and physical activity. The neurological symptoms in patients and in experimental RA are connected to IGF1R expression and signalling, and further expands our knowledge of neurological processes in RA.

Disclosure of interest None declared

P004 ABSTRACT WITHDRAWN

P005 INFLUENCE OF MACROPHAGE POLARISATION ON EXPRESSION OF PEPTIDYLARGININE DEIMINASES 2 AND 4 THAT CATALYSE CITRULLINATION OF THE PROTEINS TARGETED BY ANTI-CITRULLINATED PROTEIN/PEPTIDE AUTOANTIBODIES (ACPA)

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Introduction Autoantibodies to citrullinated proteins (ACPA) are specifically associated to rheumatoid arthritis (RA) and probably involved in its pathophysiology. ACPA are produced in the inflamed synovial tissue (ST). We demonstrated that peptidylarginine deiminases (PAD) 2 and 4 are present in the tissue and probably responsible for fibrin citrullination, and consequently for genesis of the epitopes targeted by ACPA. PAD2 and 4 are expressed in the intima and the subintimal inflammatory infiltrates, essentially by CD68 +mononuclear cells.[1] We suspected macrophages (MΦ) of the ST to be responsible for synthesis and release of PADs in the interstitium, inducing citrullination of the local fibrin deposits. We generated *in vitro* various subsets of MΦ in the presence of IFN-γ, IL-4, IL-10 or M-CSF, and observed a major influence of the phenotype of polarised MΦ on cytokine response to ACPA-containing immune complexes.[2]

Objectives The aim of the current study was to evaluate expression of PAD2 and 4 by various subsets of polarised MΦ.