Introduction Macrophage activation syndrome (MAS) is a severe condition, which can appear as a complication of inflammatory rheumatic diseases such as systemic juvenile idiopathic arthritis (sJIA) and adult onset Still's disease (AOSD), a viral infection, or a malignancy. Interleukin (IL)-18 is a pro-inflammatory cytokine of the IL-1 family, known as a strong interferon (IFN)- γ inducer, that is naturally inhibited by IL-18 binding protein (IL-18BP). High levels of unbound biologically active IL-18 have been described in patients with sJIA, AOSD, and MAS, suggesting that IL-18 is involved in the pathogenesis of these diseases.

Objectives To examine the effect of excessive IL-18 signalling in a mouse model of MAS induced by repetitive toll like receptor (TLR)9 stimulation, and explore the consequences of IL-18 or IFN- γ blockade on MAS manifestations.

Methods MAS was induced by repeated intraperitoneal CpG injections in IL-18BP deficient (*IL-18BP*^{-/-}) mice and in wild type (WT) littermates. Anti-IL-18 receptor (IL-18R) or anti-IFN- γ monoclonal antibodies were administered prior to CpG injections. Clinical and biological manifestations of MAS were studied. Plasma levels of free IL-18 were measured by ELISA. Expression levels of IFN- γ and downstream IFN- γ -induced effectors (CXCL9, CIIA) were explored by ELISA or Luminescence in plasma, and by RT-qPCR in spleen and liver.

Results Naïve *IL-18BP*^{-/-} mice had no spontaneous phenotype. After repeated CpG injections, *IL-18BP*^{-/-} mice displayed significantly more severe MAS phenotype than their WT littermates, including more pronounced weight loss, splenomegaly, anaemia, thrombocytopenia, hyperferritinemia and hepatitis. This phenotype was associated with elevated plasma levels of unbound IL-18 and the presence of bone marrow hemophagocytes in *IL-18BP*^{-/-} mice only. In addition, *IL-18BP*^{-/-} mice displayed higher plasma levels of IFN- γ and CXCL9, as well as increased *Ifn γ* , *Cxcl9* and *Ciita* mRNA expression in the spleen and liver. IL-18 blockade using an anti-IL-18R antibody attenuated MAS manifestations in *IL-18BP*^{-/-} mice and abrogated IFN- γ production and downstream signalling. IFN- γ blockade using an anti-IFN- γ antibody also attenuated the MAS phenotype.

Conclusions By using *IL-18BP*^{-/-} mice, we showed that unopposed IL-18 signalling was detrimental in the TLR9-induced MAS model. Importantly, blocking IL-18, as well as IFN- γ , improved disease in *IL-18BP*^{-/-} mice. Altogether, our results suggest that IL-18 exerts a pathogenic role in this model of MAS, acting upstream of IFN- γ .

Disclosure of interest None declared

0026

WHICH PLACE FOR THE NLRP3 INFLAMMASOME IN THE MSU CRYSTAL-INDUCED INFLAMMATORY RESPONSE IN VIVO?

¹A Mariotte*, ²Z Zhang, ¹A de Cauwer, ³B Frisch, ¹S Bahram, ²R Ricci, ¹P Georgel. ¹Laboratoire d'Immunologie-Rhumatologie Moléculaire – INSERM S1109, Institut d'Hématologie et d'Immunologie; ²UMR 7104 CNRS/U 964 INSERM, IGBMC, Strasbourg; ³Laboratoire de Bio-véctorologie, Faculté de pharmacie, Illkirch, France

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Introduction The NLRP3 (Nucleotide-binding and oligomerisation domain, Leucine-Rich repeat and Pyrin containing 3) inflammasome is a multiprotein complex made of the NLRP3 sensor, the caspase 1 enzyme and an adaptor, ASC. Upon exposure to sterile or infectious stimuli, the NLRP3 inflammasome assembles and cleaves pro-IL-1 β and pro-IL-18 into their

bioactive forms. Gout is a common microcrystalline arthritis induced by MSU (Mono-Sodium Urate) crystals accumulation which triggers an inflammatory response depending on the NLRP3-IL-1-IL-18 axis *in vitro*¹; however, this relationship is far less understood *in vivo*. PKD (Protein Kinase D) were recently shown as important regulators of the NLRP3 inflammasome.²

Objectives To study the *in vivo* relevance of the PKDs-NLRP3 axis in two mouse models of gout.

Methods Experiments were conducted in mice deficient for NLRP3, for PKD1 and 3 or mice in which PKD1, 2 and 3 were blocked by a pan-PKD inhibitor. Peritoneal macrophages were stimulated by MSU crystals to assess IL-1 β production. Gouty arthritis was triggered by subcutaneous injections of MSU crystals in the hind-paws and MSU-induced peritonitis by crystals injection in the peritoneal cavity.

Results We confirmed the absence of IL1 β release by MSU-challenged peritoneal macrophages isolated from NLRP3KO animals. Pharmacological pan-PKD inhibition gave similar results, whereas genetic loss of PKD1 and 3 did not. The peritoneal gout model showed a reduction in neutrophils recruitment in NLRP3KO animals, and an increased neutrophils influx in PKD1-3dKO mice. Finally, we did not observe any alteration in gout severity in NLRP3-deficient and pan-PKD inhibitor-treated mice following MSU crystal subcutaneous injections; PKD1-3dKO animals even displayed more severe symptoms in this assay.

Conclusions Our data seem to confirm that the PKD-NLRP3 axis exerts a major role in sensing MSU crystals *in vitro* but the relevance of this pathway in our *in vivo* gouty arthritis models is not so clear. We speculate that an alternative pathway could compensate for the loss of IL-1 β in NLRP3 inflammasome-deficient mice. Unravelling this possibility might be of major fundamental interest and could even lead to new therapeutics in the field of inflammation.

REFERENCES

1. Martinon F, et al. *Nature* 2006.
2. Zhang Z, et al. *J. Exp. Med* 2017.

Disclosure of interest None declared

0027

MECHANICAL STRAIN DETERMINES THE SITE-SPECIFIC DIRECTION OF INFLAMMATION AND TISSUE DAMAGE IN ARTHRITIS

¹I Cambré*, ¹D Gaublonne, ¹A Burssens, ¹P Jacques, ¹D Elewaut, ²R Lories, ³G Schett, ⁴G Kollias, ⁵A de Muynck, ⁶L Meuris, ⁶N Callewaert, ⁷S Lambrecht, ²S Carter. ¹Rheumatology, Ugent/VIB, Ghent; ²Skeletal biology and engineering research centre, KULeuven, Leuven, Belgium; ³Rheumatology and immunology, Friedrich Alexander University of Erlangen-Nuremberg and Universitätsklinikum, Erlangen, Germany; ⁴Division of Immunology, Alexander Fleming, Attica, Greece; ⁵UGCT-Department of Physics and Astronomy, Ugent; ⁶Unit of medical biotechnology, Ugent/VIB; ⁷Rheumatology, Ugent, Ghent, Belgium

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Introduction Many pro-inflammatory pathways leading to arthritis act systemically on the immune system rather than locally in the joint. However, the reason behind the regional and patchy distribution of arthritis represents a longstanding paradox.

Objectives Explore the relation between mechanical strain and joint inflammation and understand the underlying basis of joint pattern involvement in inflammatory rheumatic diseases.

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 sialylation 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

¹A Krishnamurthy*, ²K Sandor, ²A Jurczak, ³MJ Andrade, ¹J Steen, ¹C Grönwall, ¹G Wigerblad, ¹P Titcombe, ¹H Wähämaa, ¹B Rethi, ²C Svennson, ¹A Catrina. ¹Medicine, Rheumatology Unit; ²Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; ³Department of Unidad Academica Multidisciplinaria Reynosa Aztlan, Universidad Autónoma de Tamaulipas, Reynosa, Tamaulipas, Mexico

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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-phosphonate could influence ACPA-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 FcγRIII or FcγR 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 FcγR chain knockout mice injected i.v. with polyclonal or monoclonal ACPA or control antibodies. Bone density was measured by micro-CT. Zoledronate (100 µg/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 FcγRIII or FcγR 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 lasting 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 FcγR 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 Fcγ receptors.

Disclosure of interest None declared

P002

HOW DO GLYCANS AFFECT IMMUNE CELLS IN RHEUMATOID ARTHRITIS?

¹A Molhoek*, ²L Hafkenscheid, ²R Toes, ¹S van Vliet, ¹Y van Kooyk. ¹MCB, VUmc, Amsterdam; ²LUMC, Leiden, Netherlands

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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