We also observed an increased expression of PDL2 on RA-B10⁺ cells compared to HC-B10⁺ cells that could explain this effect on Th1 differentiation.

Conclusions IL-10 secretion can be an interesting marker to define Breg subsets acting on regulatory T cell differentiation. Thus, increasing the number of B10⁺ cells, seems a promising therapeutic strategy especially in patients who lack the most of Treg cells. Additional markers are needed to define which subset of Breg cells can control inflammatory response. The role of PD1/PDL2 in B10⁺ cell function might also open new insights.

Disclosure of interest None declared

0003

CHARACTERISING THE ROLE OF NET-DERIVED IL-33 IN SLE PATHOGENESIS

^{1,2}S Georgakis*, ^{1,2}G Papadaki, ³I Gergianaki, ³C Adamichou, ³S Polia, ^{1,3}P Sidiropoulos, ⁴P Verginis, ^{1,2}K Gkirtzimanaki, ^{1,3}G Bertias. ¹Rheumatology, Autoimmunity and Inflammation Laboratory, Medical School, University of Crete; ²Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology – Hellas; ³Rheumatology Department, University General hospital, Heraklion; ⁴Biomedical Research Foundation, Academy of Athens, Athens, Greece

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Introduction Interleukin (IL)–33 is a cell necrosis-derived alarmin with immunostimulatory properties which depends on the context of immune cells and the inflammatory milieu.^{1,2} In Systemic Lupus Erythematosus (SLE), extracellular DNA (as in extracellular chromatin traps [NETs] or immune complexes [ICs]) combined with alarmins stimulate innate immunity receptors (such as Toll-like receptors [TLRs]) and the production of IFN α by plasmacytoid dendritic cells (pDCs).^{3,4}

Objectives We hypothesised that bioactive NET-derived isoforms of IL-33 might augment the interferogenic potential of extracellular DNA in SLE.

Methods Peripheral blood polymorphonuclear cells (PMNs) were isolated from healthy and SLE individuals and their ability to form IL33-decorated NETs was assessed by confocal microscopy. NET-supernatants from untreated and IC-treated SLE PMNs were administered to healthy pDCs and type I IFN production was monitored by qPCR and ELISA. The contribution of IL-33 in the interferogenic capacity of NETs was addressed by pre-treating pDCs with a specific mAb against IL33-receptor (anti-ST2L). Western blotting of SLE and healthy PMNs was used to detect different isoforms of IL-33.

Results Spontaneous-released NETs from peripheral blood PMNs of active SLE patients were decorated with IL-33 to a larger extent as compared to healthy PMN NETs. Treatment of SLE PMNs with ICs led to the release of NETs with extended IL-33 decoration and enhanced interferogenic capacity. IL33 receptor blockade significantly decreased the type I IFN response of NET-treated control pDCs. DNase treatment of NETs to disrupt their structure resulted in reduced interferogenic capacity and abrogated the pro-inflammatory effect of IL-33. Finally, western blotting on IC-mediated SLE NETs revealed the presence of cleaved isoforms of IL-33 which closely resemble the previously characterised highly bioactive isoforms of our protein of interest. Experiments are underway to explore the possible role of NET-bound proteases in IL-33 activation.

Conclusions NET-derived IL-33 is a novel mediator of the nucleic acid-driven aberrant type I IFN response which

exacerbates SLE disease. NET structure may be crucial in regulating the bioactivity of IL-33.

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0004

NIK-IKK COMPLEX CONTROLS NF-KB-DEPENDENT INFLAMMATORY ACTIVATION OF THE ENDOTHELIUM IN RESPONSE TO LTBR LIGATION

¹P Kucharzewska, ²KCM Jeucken*, ²CX Marade, ²JP van Hamburg, ¹H Olsson, ²SW Tas. ¹Department of Bioscience, RIA lmed, AstraZeneca, Gothenburg, Sweden; ²Experimental Immunology/Clinical Immunology and Rheumatology, AMC Amsterdam, Amsterdam, Netherlands

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Introduction Sites of chronic inflammation, such as rheumatoid arthritis (RA) synovial tissue, often contain tertiary lymphoid structures with high endothelial venules (HEV). Ligation of the lymphotoxin (LT)- β receptor (LT β R) results in activation of canonical and NF- κ B-Inducing Kinase (NIK)-dependent noncanonical NF- κ B signalling in endothelial cells (EC), leading to HEV development. However, the relative contribution of the individual NF- κ B pathways to inflammatory activation of EC is largely elusive.

Objectives To identify the molecular pathways by which LT β R drives inflammatory activation of EC to promote interaction with leukocytes.

Methods Primary human EC were treated with LT β or LIGHT to activate LT β R, followed by analysis of downstream NF- κ B signalling pathways and expression of adhesion molecules and inflammatory cytokines. To repress canonical NF- κ B signalling pathway, a small molecule inhibitor of IKK β was used, and noncanonical NF- κ B signalling was repressed with siRNAs targeting NF κ B2. The role of NIK in LT β R signalling was investigated using small molecule inhibitors and siRNAs targeting NIK, as well as adenoviral overexpression of NIK. The role of NF- κ B signalling in RA was measured by stimulating EC with RA synovial fluid (RASf) followed by analysis of inflammatory mediators.

Results LT β R-triggering in EC resulted in activation of both canonical and noncanonical NF- κ B signalling, and induced inflammatory cytokine expression and immune cell adhesion. IKK β inhibition repressed LT β R-induced inflammatory activation of EC, indicating that this process was mediated through canonical NF- κ B signalling. Interestingly, inactivation of NIK also decreased LT β R-induced expression of inflammatory cytokines and leukocyte-adhesion, but silencing of NF κ B2 had no apparent effect. Further analyses, including silencing and overexpression of NIK, demonstrated a clear role for NIK in activation of the canonical NF- κ B pathway by amplifying IKK complex activity. RASf stimulation of EC resulted in

activation of canonical and noncanonical NF- κ B signalling, and increased the expression of inflammatory cytokines and adhesion molecules, which could be blocked by targeting NIK. **Conclusions** These findings suggest that in addition to regulating noncanonical signalling, NIK can serve as an amplifier of canonical NF- κ B signalling and associated inflammatory responses in EC, which may play a role in development and maintenance of chronic inflammation. Consequently, NIK may be an attractive therapeutic target.

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0005 SMOC2 HAS DIFFERENTIAL EFFECTS ON CARTILAGE AND BONE FORMATION

¹T Peeters*, ¹R Lories, ²F Cailotto. ¹Skeletal Biology and Engineering Research Centre – Dept. Development and Regeneration, KU LEUVEN, Leuven, Belgium; ²IMoPA, Université de Lorraine, Nancy, France

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Introduction SMOC2, a secreted calcium-binding protein of the BM-40/SPARC family was identified from a chondrogenic extract of articular cartilage and is increased in osteoarthritic cartilage. Alterations in bone and cartilage resulting from dysregulated signalling pathways are a hallmark of osteoarthritis.

Objectives To evaluate the role of SMOC2 in *in vitro* osteogenesis and chondrogenesis and its interaction with Wnt signalling.

Methods Wild-type Smoc2 (Smoc2+) or Smoc2 lacking the calcium binding domain (?CaBD) were stably overexpressed and wild-type Smoc2 was silenced (Smoc2-). Alkaline phosphatase (ALP) activity assay and Alizarin Red staining were performed in MC3T3 cells and human periosteal derived cells (hPDCs). Proteoglycan content and mineralization were assessed in ATDC5 micromasses by Alcian blue and Alizarin Red staining. Mice femoral head caps (FHC) were stimulated with recombinant IL-1b and SMOC2. Cartilage loss was evaluated by DMMB assay and Safranin O staining. TCF1 levels were analysed by immunohistochemistry. Human articular chondrocytes (hACs) were stimulated with recombinant SMOC2. Gene expression of osteoblast markers (*Opn*, *Osx*, *Col1a2*), chondrogenic markers (*Acan*, *Col2a1*, *Col10a1*, *Mmp13*) and canonical Wnt target genes (*Axin2*, *Lef1*) were analysed by qPCR and Wnt signalling by Western blot.

Results Alizarin red staining and ALP activity were reduced in Smoc2 +MC3 T3 cells. Osteoblast gene expression levels were altered in Smoc2 +cells. ?CaBD cells and calcium supplementation to Smoc2 +showed a partial rescue of the effects of Smoc2 +cells. We validated these results in hPDCs. We could not observe an effect on osteogenesis when silencing Smoc2. Smoc2 +ATDC5 micromasses showed reduced chondrogenic gene expression and Alcian blue and Alizarin Red staining intensity. We observed a reverse pattern with an increased chondrogenic gene expression in Smoc2- ATDC5 micromasses. Smoc2 +and Smoc2- ATDC5 cells altered canonical Wnt signalling in opposite directions. SMOC2 also alters protein levels of CamKII. IL-1b triggered cartilage loss was increased and TCF1 levels were reduced in mice FHC stimulated with recombinant SMOC2. In hACs, we observed a negative effect of SMOC2 on chondrocyte markers and canonical Wnt target genes.

Conclusions Our results suggest an inhibitory role of Smoc2 on mineralization. The inhibitory effects on mineralization are at least partially regulated through interaction with calcium. Moreover, Smoc2 is a regulator of chondrogenic differentiation and cartilage homeostasis by regulating canonical and non-canonical Wnt signalling.

Disclosure of interest None declared

0006 LUMICAN: A NOVEL GLYCOPROTEIN MEDIATING INFLAMMATION IN OSTEOARTHRITIS

¹G Barreto*, ²B Senturk, ¹L Colombo, ³P Neidenbach, ³G Salzmann, ²M Rottmar, ¹M Zenobi-Wong. ¹ETH Zurich, Zurich; ²Swiss Federal Laboratories for Materials Science and Technology, Dübendorf; ³Schulthess Klinik, Zurich, Switzerland

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Introduction Lumican (LUM) is major extracellular matrix protein which is present in glycoprotein format in adult articular cartilage. Recently, LUM was shown to augment pathogen-associated molecular patterns (PAMPs) activation of the TLR4 signalling cascade. Given that TLR4 is highly associated with inflammation in rheumatic disease, we aim to decipher the LUM role in Osteoarthritis and TLR4 associated inflammation.

Objectives To measure LUM in synovial fluid from patients with arthritic conditions and to study the role of LUM on TLR4 activation in osteoarthritis.

Methods Synovial fluid (SF) was obtained from knee meniscus tear (n=11), first carpometacarpal (CMC-I) OA (n=11) and knee OA (n=40) patients. LUM glycoprotein levels were analysed by enzyme-linked immunosorbent assay (ELISA). Human monocytes were isolated from healthy individuals and differentiated into M1-like and M2-like macrophages by lipopolysaccharide (LPS) and interleukin-4, respectively. LUM (1 ug/ml) was added either together with macrophage polarisation inducers or alone. Primary chondrocytes and OA cartilage explants were stimulated for 24 hour with LUM (1 µg/ml), LPS (10 ng/ml), or a combination of both. Conditioned media was analysed for selected secreted molecules by xMAP technology. Macrophages surface expression markers CD197 and CD206 were analysed by Fluorescence-Activated Cell Sorting (FACS). Cartilage explants were immunofluorescently double stained for collagen type II and X.

Results LUM glycoprotein levels were significantly upregulated in knee OA SF vs. controls. In normal articular chondrocytes, LUM combined with LPS caused an upregulated secretion of catabolic markers characteristic of OA such as IL-6, MMP-1, and MMP-13 in comparison to LPS stimulation. LUM alone had no observable effects. A similar response occurred in cartilage explant cultures, with increased MMP-1 secretion levels and marked cartilage degradation with the LPS/LUM combination. Interestingly, LUM stimulation with the polarisation inducer, LPS for M1, and IL-4 for M2 macrophages, upregulated M1 macrophage secretion of TNF-alpha and downregulated IL-10 secretion in M2 macrophages relative to control and LUM alone. FACS results followed the same trend, with increased CD197 expression in LUM/LPS combination vs. LUM alone or control, and downregulation of CD206 expression when LUM/IL-4 combination vs. LUM alone or control.

Conclusions The results presented here show that LUM is highly upregulated in SF of OA and RA patients. In addition, we observed that TLR-expressing chondrocytes do not respond to LUM, however LUM augmented the LPS-induced TLR4 signalling cascade and consequently the catabolic response.