0001

LYMPHOID TISSUE MACROPHAGES EXPRESS PEPTIDYLARGININE DEIMINASE 2 AND 4: NEW IMPLICATIONS IN CITRULLINATION AND ANTI-CITRULLINE ANTIBODIES PRODUCTION IN AUTOIMMUNE ARTHRITIS

MEM El Shikh*, R El Sayed, L Fossati-Jimack, C Pitzalis. William Harvey Research Institute, Queen Mary University of London, London, UK

10.1136/annrheumdis-2018-EWRR2018.1

Introduction The role of citrullination in breach of tolerance in rheumatoid arthritis (RA) is well established, but the mechanisms linking protein citrullination and the generation of anti-citrullinated protein/p peptide antibodies (ACPA) are poorly understood. We have recently reported a critical role of peptidylarginine deiminase (PAD) 4 expression and extracellular trap formation by neutrophils in ACPA production in RA.¹ In lymphoid tissues, neutrophils are scarce, whereas macrophages are abundant antigen presenting cells where they regulate T and B cell responses and play fundamental roles in the chronicity of RA.

Objectives To test our hypothesis that antigen presenting cells (APCs) in lymphoid tissues express PADs and contribute to protein citrullination and ACPA production.

Methods We tested our hypothesis in collagen induced arthritis (CIA) mice and synovial biopsies from RA patients. PAD4 and 2 expression was assessed in the spleens and lymph nodes of CIA mice and their distribution was revealed by immunohistochemistry (IHC). The impact of macrophage depletion on lymphoid tissues' PAD activity, citrulline content, and serum ACPA levels was analysed; and the effect of macrophage stimulation on extracellular trap formation, PADs expression and release was investigated. Additionally, PAD4 expression in RA synovia was verified by IHC; and the correlation of PAD4 with macrophage-related genes was studied by RNA-Seq analysis.

Results Our results indicated that serum ACPA were detectable in CIA sera before clinical arthritis; and PAD4 and 2 were expressed in the draining lymph nodes and spleens as shown by qPCR and Western blotting. In addition, PAD4 and 2 labelling specifically co-localised with macrophages but not other APCs. Moreover, PAD4 and -2-expressing tingible body macrophages (TBM) were associated with citrulline-retaining follicular dendritic cells (FDCs) in the germinal centres (GC). *In vitro* TLR activation of macrophages enhanced PAD4 and 2 expression; and stimulation of TBMs with TNF- α induced Macrophage Extracellular Trap formation (METosis), release of apoptotic bodies, and DNA-associated citrullinated proteins. Whereas retention of citrullinated proteins on FDCs induced ACPA production in *in vitro* GC reactions, macrophage depletion in CIA mice significantly reduced citrullinated proteins in lymphoid tissues and inhibited serum ACPA levels. In RA synovia, PAD4 colocalized with CD68 macrophages and was expressed in TBMs associated with citrulline-retaining FDCs in ectopic lymphoid structures. RNA-Seq analysis of PAD4 expression in RA synovial biopsies identified significant positive correlation with 279 genes associated with macrophage activation and function.

Conclusions In conclusion, this work reports new mechanisms by which macrophages contribute to citrulline-induced autoimmunity. Therapeutic targeting of these mechanisms would inhibit citrullination, ACPA production and progression of autoimmune diseases.

REFERENCE

1. Corsiero E, Bombardieri M, Carloti E, Pratesi F, Robinson W, Migliorini P, Pitzalis C. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. *Ann. Rheum Dis* 2016;**75**:1866–1875.

Disclosure of interest None declared

0002

TARGETING IL-10 PRODUCING B CELLS IN RHEUMATOID ARTHRITIS AND PRIMARY SJÖGREN SYNDROME IS PROMISING TO INCREASE REGULATORY T CELLS BUT NOT TO DECREASE PRO-INFLAMMATORY T CELLS

J Mielle*, R Audo, A Nutz, M Hahne, L Macia, B Combe, P Guilpain, J Morel, C Daien. Montpellier University, Montpellier, France

10.1136/annrheumdis-2018-EWRR2018.2

Introduction The role of regulatory B cells in tolerance has been established in both preclinical and clinical studies. There is no specific marker for human regulatory B cells. They have been mainly ascribed to CD24^{hi}CD38^{hi} and C24^{hi}CD27⁺ B cells which can increase regulatory T cells and decrease pro-inflammatory Th1 and TNF α -producing T cells. However, in patients with auto-immune diseases, such as Rheumatoid Arthritis (RA) or Sjögren Syndrome (pSS), CD24^{hi}CD38^{hi} and C24^{hi}CD27⁺ B cells lose their regulatory functions. It is thus needed to find a better marker of Breg cells that correlate with regulatory functions in both healthy controls and patients. As Breg cell functions are mainly mediated by IL-10, some authors use IL-10 production to define Breg cells, which are then called B10⁺ cells. We previously showed that B10⁺ cells are decreased in RA and negatively correlated with the disease activity, suggesting a key role of regulatory B10⁺ cells in autoimmune diseases. Before using B10⁺ cells as a therapeutic strategy it is necessary to precisely determine their functions. However, it remains unknown if any B cell secreting IL-10 has regulatory functions and which ones.

Objectives To assess the functions of B10⁺ cells, compared to non-IL-10 producing B cells (B10^{neg} cells) on T cell differentiation in healthy controls (HC) and in patients with autoimmune diseases, namely RA and pSS.

Methods B10⁺ cells were induced by a 24 hour stimulation with CpG and sorted using isolation kits and flow cytometry. We evaluated their effect on naïve T cell differentiation into Th1, TNF α -producing T cells, Treg cells and IL-10 producing T cells (Tr1). We characterised the interaction between B10⁺ cells and T cells, using blocking antibodies against IL-10, CD80, CD86, PD1 and transwell experiments.

Results B10⁺ cells of HC converted more naïve T cells into Treg cells and Tr1 than B10^{neg} cells (p=0.03 and p=0.02). Blocking either IL-10 secretion or cellular contacts between B10⁺ cells and T cells abolished the induction of Treg cells. Surprisingly, conversely to CD24^{hi}CD27⁺ B cells, B10⁺ cells failed to decrease Th1 and TNF α -producing T cell conversion, compared to B10^{neg} cells. This might be due to a different expression of PDL1 and PDL2 between B10⁺ cells and CD24^{hi}CD27⁺ B cells. Similarly to HC, B10⁺ cells from RA and pSS patients induced more Treg cells compared to B10^{neg} cells (p=0.02 in RA; p=0.03 in pSS). However, conversely to HC and pSS-B10⁺ cells, RA-B10⁺ increased the conversion of naïve T cells into Th1, compared to B10^{neg} cells (p=0.033).

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

10.1136/annrheumdis-2018-EWRR2018.3

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.

REFERENCES

1. Foo Yew Liew, et al. Interleukin-33 in health and disease. *Nature Reviews* 2016.
2. Lefrancais E. IL-33 is processed in mature bioactive isoforms by neutrophil elastase and cathepsin G. *PNA's* 2012
3. Garcia Romo G. Netting neutrophils are major inducers of type I IFN production in paediatric systemic lupus erythematosus. *Science Translational Medicine* 2011.
4. Lande R. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 2007.

Acknowledgements We thank Dr. Ronnblom and Dr Eloranta for providing us with immune complexes material for our scientific project.

Disclosure of interest None declared

0004

NIK-IKK COMPLEX CONTROLS NF- κ B-DEPENDENT INFLAMMATORY ACTIVATION OF THE ENDOTHELIUM IN RESPONSE TO LT β R 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

10.1136/annrheumdis-2018-EWRR2018.4

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