

P084 PROINFLAMMATORY ENVIRONMENT IN RA AND OA STRONGLY INFLUENCES ADIPOSE TISSUE ACTIVITY

M Plebanczyk*, A Radzikowska, U Musiałowicz, I Janicka, A Kornatka, W Maslinski, E Kontny. *National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland*

10.1136/annrheumdis-2018-EWRR2018.101

Introduction Rheumatoid arthritis (RA) and osteoarthritis (OA) are inflammatory diseases leading to joint destruction and disability. Apart from joint inflammation they are characterised by high-grade (RA) or low-grade (OA) systemic inflammation. Thus, it is possible that proinflammatory environment affect not only articular (AAT), but also subcutaneous adipose tissue (ScAT) activity.

Objectives The aim of present work was to compare reactivity of AAT and ScAT from RA and OA patients to proinflammatory stimulus.

Methods AAT and ScAT explants, obtained from OA (n=44) and RA (n=43) patients during knee joint replacement surgery, were cultured (100 mg/ml) for 24 hours in medium (DMEM) alone or in the presence of proinflammatory IL-1 β (1 ng/ml), known to be overproduced in inflamed joint. Concentrations of cytokines (proinflammatory IL-6 and TNF, anti-inflammatory IL-1Ra, IL-10, TGF β), chemokines (CCL2, CCL5) and metalloproteinase MMP-3 were measured in culture supernatants by ELISA. OA patients were treated with non-steroidal anti-inflammatory drugs while RA patients were given disease modifying drugs and/or glucocorticosteroids. Data were analysed as stimulation to control ratio (IS, index of stimulation) using Mann-Whitney U test and are expressed as median.

Results In OA the levels of secreted factors were usually significantly higher in AAT, but ScAT was characterised by significantly higher increase of IL-6 (IS=16.4), TGF β (IS=2.0), CCL2 (IS=1.8), and MMP-3 (IS=9.9) production than AAT (IS=70.3; 1.3; 1.3; 2.4 respectively). The up-regulation of other cytokines was similar in both tissues. By contrast, in AAT and ScAT from RA patients IL-1 β -triggered increase of all tested factors secretion was similar. Moreover, upon IL-1 β treatment the up-regulation of IL-6 (IS=13188) and MMP-3 (IS=9,1) in AAT of RA patients as well as of CCL2 from rheumatoid AAT (IS=3.2) and ScAT (IS=3.7) was significantly higher than in OA tissues (IS=70.3; 2.4; 1.3; 1.8 respectively).

References We report that in OA ScAT, exposed *in vivo* to low-grade inflammation, retains higher reactivity to proinflammatory stimulus than AAT located in chronic high-grade inflammation. In RA, however, reactivity of both adipose tissue is rather similar but in the case of secretion of some factors (CCL2 and/or IL-6, MMP-3) higher than in OA, reflecting more destructive character of this disease.

Acknowledgements This work was funded by the National Science Centre (grant number 2012/N/NZ5/00074).

Disclosure of interest None declared

P085 ABSTRACT WITHDRAWN

P086 ADIPOCYTOKINES LINKING OBESITY AND OSTEOARTHRITIS

¹M-L Hülser*, ¹C Schreijaeck, ²Y Luo, ²A Bozec, ²G Schett, ¹E Neumann, ¹U Müller-Ladner. ¹Department of Internal Medicine and Rheumatology, Kerckhoff-Klinik, Justus-Liebig-University Giessen, Bad Nauheim; ²Department Clinic of Medicine 3 – Immunology and Rheumatology, University of Erlangen-Nürnberg, Erlangen, Germany

10.1136/annrheumdis-2018-EWRR2018.102

Introduction In the Western society, obesity and the resulting metabolic changes are an increasing field of medical research. Both conditions alter the expression of adipokines such as adiponectin, visfatin or leptin, which can modulate key pathways of the immune system or the progress of osteoarthritis (OA).

Objectives To evaluate cross-linking influences of the above-mentioned comorbidities, we combined a high-fat diet (HFD) with the DMM osteoarthritis mouse model (Destabilisation of the Medial Meniscus). The serologic as well as local tissue effects of HFD at different stages of OA development were correlated to the local and systemic adipokine expression.

Methods ND (normal diet) and HFD were fed to C57Bl/6 mice over a period of 3 months followed by surgical OA induction (time point 0). Tissues and sera were collected 4, 6, and 8 weeks after surgery. Serum levels of adipokines were measured by ELISA. Liver fat was scored to evaluate metabolic changes. Arthritis progression was scored and quantified based on histologic stainings of the joints (H/E, safranin O, Pappenheim and Masson-Goldner's trichrome). Immunohistochemical stainings of the joints were performed to evaluate local distribution of adipokine positive cells. Metabolic parameters were correlated to the progression of OA.

Results At all time points, OA could be induced in all settings, especially when combined with HFD (e.g.: OA score at 6 weeks HFD 3.7 vs. ND 1.4). Systemically, leptin levels were significantly higher in HFD compared to ND, suggesting a leptin and established insulin resistance. However, DMM led to decreased leptin levels at all time points, independent from diet (e.g. 4 weeks: HFD healthy 18.4 ng/ml vs. HFD DMM 3.7 ng/ml). Systemic leptin levels and fatty-liver score were positively correlated. Interestingly, the systemic increase of adiponectin mediated by DMM was only visible at week 8 (HFD healthy 5176 ng/ml vs. HFD DMM 6149 ng/ml). HFD, DMM or the combination of both did not show significant effects on serum levels of adiponectin, visfatin or IL-6. The local adipokine distribution in the joints was independent from systemic parameters.

Conclusions Our data show that similar to observations in humans, OA is deteriorated by HFD. This is reflected by increased numbers of adipokine positive cells in areas of destruction. This phenomenon appears to be based on local

mechanisms as systemic adipokine levels did not reflect the intraarticular adipokine distribution.

Disclosure of interest None declared

P087

RECIPROCAL CONTROL OF REGULATORY T LYMPHOCYTES AND NEUTROPHILS IN BOTH PHYSIOLOGICAL AND PATHOLOGICAL ENVIRONMENTS

¹M Batignes, ¹F Santinon, ^{1,2}M-C Boissier, ¹N Bessis, ¹P Decker*. ¹Inserm UMR 1125, University of Paris 13, Sorbonne Paris Cité; ²Rheumatology Department, Avicenne Hospital, AP-HP, Bobigny, France

10.1136/annrheumdis-2018-EWRR2018.103

Introduction Regulatory T lymphocytes (Treg) play a key role in the control of autoimmunity. However, under inflammatory conditions like rheumatoid arthritis (RA), Treg become less suppressive and may shift toward a Th17 profile. On the contrary, polymorphonuclear neutrophils (PMN) are typical inflammatory cells activated in RA, although regulatory PMN sub-populations have been described. Treg and PMN are thus supposed to have opposite functions, but in both cases these functions can be reverted. Very few data are available on Treg-PMN communication in normal conditions, and even less during inflammation, especially in RA.

Objectives The aim was to analyse the interplay between Treg and PMN and their reciprocal modulation, both in physiological and pathological inflammatory environments.

Methods Splenic Treg and bone marrow PMN (C57BL/6 mice) were purified by magnetic sorting. Treg and PMN of healthy donors were freshly isolated by dextran sedimentation and magnetic sorting from peripheral blood. Co-cultures (1:1 ratio) were unstimulated or exposed to anti-CD3/anti-CD28 antibodies and/or LPS to stimulate Treg and/or PMN. CD4⁺FoxP3⁺ Treg (mouse and human), Ly6G⁺ (mouse) and CD66b⁺ (human) PMN were identified by flow cytometry. Cell activation was studied using antibodies against CD39, CD25, CTLA-4 (Treg) and CD11b (PMN). Treg maintenance was evaluated as the frequency of FoxP3 expression among CD4⁺ cells, and their proliferation by CFSE staining followed by flow cytometry analysis. Co-cultures were performed using Transwell (0.4 µm) to determine the respective involvement of soluble mediators/cell-contacts. Cytokine levels were quantified in culture supernatants by ELISA. Collagen-induced arthritis (CIA) was induced in C57BL/6 mice by immunisation with chicken type II collagen in complete Freund's adjuvant at days 0 and 21.

Results Without stimulation of both Treg and PMN from normal mice, no effect on any cell type was observed in co-culture. In contrast, co-culture of activated Treg with activated PMN resulted in increased maintenance of Treg with higher CTLA4 but lower CD39 expression, sustained PMN activation evidenced by CD11b up-regulation, and higher secretion of MIP-2, IL-6 and IL-17 but not IFN-γ in normal mice. All these effects were lost in transwell experiments. Nevertheless, transfer of supernatants from LPS-activated PMN also partly increases Treg maintenance. In addition, co-cultures led to Treg proliferation. Similar results were observed in co-cultures of activated Treg/PMN isolated from CIA mice. Likewise, human Treg-PMN co-cultures led to enhanced Treg maintenance with higher expression of CTLA4/CD25 in a cell contact-dependent manner. Secretion of both IL-8 and IL-10 was enhanced in co-cultures.

Conclusions Our results show that cross-talk between Treg and PMN mainly leads to an activation of both cell types in normal or inflammatory conditions. Although cell contacts are clearly required, soluble mediators are also involved. Whether these synergistic interactions lead to a global suppressive or inflammatory milieu with functional modulation of either partner needs to be determined.

Disclosure of interest None declared

P088

TGFB BOUND TO GARP PROMOTES ACETYLATION-MEDIATED FOXP3 PROTEIN STABILISATION

P Lehmkuhl*, B Zapp, H Schulze-Koops, A Skapenko. *Sektion Rheumatologie und Klinische Immunologie, Medizinische Klinik und Poliklinik IV, Universität München, Munich, Germany*

10.1136/annrheumdis-2018-EWRR2018.104

Introduction Regulatory T cells (Tregs) play a critical role in maintaining homeostasis and limiting autoimmunity. The transcription factor Forkhead box P3 (Foxp3) has been identified as the key regulator of Treg function and development. Its activity and stability are tightly regulated by acetylation dependent on the interplay between histone deacetylases (HDAC) and histone acetyltransferases (HAT). Tregs express distinctively on their cell surface glycoprotein A repetitions predominant (GARP). GARP binds latent TGFβ and provides to Tregs an available pool of TGFβ. The function of this TGFβ source for Tregs has not been investigated yet. Treg-derived TGFβ might on the one hand be involved in mediating suppressive functions of Tregs and on the other hand be important for maintaining Treg homeostasis.

Objectives To analyse the function of surface TGFβ bound to GARP utilising GARP-deficient Tregs from conditional CD4 specific GARP knockout mice.

Methods CD4 positive CD25⁺ or CD25⁻ T cell from GARP-deficient or wild type mice were purified using MACS separation and stimulated with anti-CD3 and anti-CD28 in the presence or absence of TGFβ for different times. mRNA expression profile was evaluated in non-stimulated and stimulated cells by Affymetrix gene array analysis and confirmed by real-time PCR. Acetylation, Foxp3 protein expression and Smad2/3 phosphorylation were assessed by intracellular flow cytometry. Half-life of Foxp3 was evaluated in cell cultures using cycloheximid. Acetylation of Foxp3 was determined by fluorescence resonance energy transfer (FRET) utilising a FRET antibody pair for Foxp3 and acetylated lysine. The regulatory capacity of Treg was assessed *in vivo* in reconstitution experiments of Rag-deficient mice with CD25-CD4 T cells or bone marrow cells.

Results GARP-deficient Tregs exhibited a markedly diminished intracellular protein acetylation. Acetylation of Foxp3 was also significantly lower. Consistently, expression of a Treg specific HDAC, HDAC9, was up regulated in Tregs lacking GARP. Both acetylation and HDAC9 expression levels could be restored by addition of exogenous TGFβ. In this regard basal phosphorylation of TGFβ-dependent transcription factors Smad2/3 was diminished in Tregs lacking GARP. Further analysis revealed an unstable phenotype of GARP-deficient Tregs characterised by a shorter half-life of Foxp3, a faster loss of Foxp3 in response to anti-CD3/28 stimulation and restricted regulatory capacity.

Conclusions Lack of GARP on the cell surface results in a reduced TGFβ availability and in a decreased TGFβ signalling in CD4 Tregs. Diminished TGFβ signalling leads in turn to an