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

[1]M Batignes, [1]F Santinon, [1,2]M-C Boissier, [1]N Bessis, [1]P Decker*. *[1]Inserm UMR 1125, University of Paris 13, Sorbonne Paris Cité; [2]Rheumatology 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 TGFb and provides to Tregs an available pool of TGFb. The function of this TGFb source for Tregs has not been investigated yet. Treg-derived TGFb 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 TGFb 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 TGFb 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 TGFb. In this regard basal phosphorylation of TGFb-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 TGFb availability and in a decreased TGFb signalling in CD4 Tregs. Diminished TGFb signalling leads in turn to an