A3.18 SYNOVIAL FIBROBLASTS INHIBIT INFLAMMATORY T CELL RESPONSES THROUGH TRYPTOPHAN METABOLISM

doi:10.1136/annrheumdis-2013-203216.18

[1]Lars-Oliver Tykocinski, [1]Anna-Marie Lauffer, [1]Antonia Bohnen, [1]Stefan Krienke, [2]Iris Oezen, [2]Ulrike Litzenburger, [2]Michael Platten, [1]Hanns-Martin Lorenz. *[1]Department of Medicine V, Division of Rheumatology, University of Heidelberg, Heidelberg, Germany; [2]Experimental Neuroimmunology, German Cancer Research Center (DKFZ), Heidelberg, Germany*

Background and Objectives It has been more and more accepted that fibroblasts participate in a dynamic interplay with other cells. Fibroblasts recruit immune cells and are able to influence their differentiation, proliferation, activation and survival. The development of rheumatoid arthritis (RA) is linked to functional changes of synovial fibroblasts (SFb) and a local infiltration of inflammatory cells, including B- and T cells. So far, little is known about the interaction of SFb and T cells. Here, we analysed the influence of normal SFb on the activation, proliferation and cytokine production of T cells in vitro.
Materials and Methods SFb were isolated from synovectomy tissue of patients with osteoarthritis. CD4+ T cells were stimulated with PHA and interleukin (IL)-2 in the presence or absence of fibroblasts, in direct contact or separated by a transwell chamber; their proliferation was measured by ^{3}H-thymidine incorporation or by PKH-26 staining. Cytokine secretion was quantified by ELISA. Surface marker expression was analysed by flow cytometry and mRNA levels of matrix metalloprotease (MMP) 1, MMP3 and Indoleamine 2,3-dioxygenase (IDO) were quantified by real-time PCR. IDO activity was measured by kynurenine levels detected by HPLC and blocked by 1-methyl-L-tryptophan.
Results SFb strongly reduced the proliferation of activated CD4+ T cells and the secretion of pro-inflammatory cytokines like interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha or IL-17. The suppression was consistent even when the T cells were separated by a transwell membrane. Interestingly, to produce suppressive mediators the SFb first needed to be stimulated by soluble factors released by activated T cells. The dynamic interplay between T cells and SFb was also shown by the fact that the SFb support the activation of T cells at early time points of coculture. The coculture induced the expression of MMP1 and MMP3, as well as IDO mRNA and the production of nitric oxide (NO) by the SFb. Blockade of IDO, but not of MMPs or iNOS, completely abrogated the suppression of T cells, indicating that the inhibitory effect of the fibroblasts is mediated by tryptophan metabolism.
Conclusions Secreted products of activated T cells induce IDO expression in SFb which in turn leads to decreased proliferation and cytokine production of the activated T cells. This intercellular mechanism may play an important role in preventing inappropriate T cell activation and in the termination of immune reactions. The insights gained from this study may help to define the malfunctions of pathogenic SFb of RA patients.

A3.19 THE miR-148a IS INDUCED BY TWIST1 AND TBET AND PROMOTES THE SURVIVAL OF EFFECTOR MEMORY T HELPER 1 LYMPHOCYTES BY REGULATING THE PROAPOPTOTIC GENE *BIM*

doi:10.1136/annrheumdis-2013-203216.19

[1]Claudia Haftmann, [1]Anna-Barbara Stittrich, [1]Kristyna Hradlikova, [1]Zhuo Fang, [1]Markus Bardua, [1]Kerstin Westendorf, [1,2]Gitta Anne Heinz, [3]Thomas Häupl, [3]Joachim Sieper, [1]Hyun-Dong Chang, [4]Nikolaus Rajewsky, [5]Hans-Martin Jäck, [1,*]Andreas Radbruch, [1,*]Mir-Farzin Mashreghi. *[1]Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin, Berlin, Germany; [2]Institute of Molecular Immunology, Helmholtz Zentrum München, Munich, Germany; [3]Charité Universitätsmedizin Berlin, Campus Charité Mitte, Berlin, Germany; [4]Max-Delbrueck-Center for Moleculare Medicine Berlin, Berlin, Germany; [5]Division of Molecular Immunology, Friedrich-Alexander University Erlangen Nuernberg, Erlangen, Germany*

*****Equal contribution*

Background Repeatedly activated effector memory (EM) T helper 1 (Th1) cells in the context of autoimmunity and chronic inflammation exhibit a varity of differences in their cellular programme compared to regular EM Th1 cells. They function independently of activation signals and therefore escape physiological and therapeutical regulation. The acquirement of these properties is probably mediated by reduced expression of the pro-apoptotic protein *Bim*. Recent results suggest that microRNA (miRNA) mediated regulation of *Bim* may play an important role for the persistence of EM Th1 cells in autoimmune disease. Therefore, we aimed to identify miRNAs with the ability to suppress the expression of *Bim* in EM Th1 cells.
Material and Methods Assuming that Th1 cells involved in autoimmune inflammation have a history of repeated restimulation by persistent (auto-) antigens, we use in vitro generated acutely (once) and chronically (four times) activated murine EM Th1 cells. By using high-throughput sequencing of miRNA expression libraries, we have identified miRNAs being differentially expressed between once and repeatedly reactivated Th1 cells. By performing gain or loss of function experiments we examined the functional impact of miR-148a in chronically activated EM Th1 cells.
Results We found that among Th subsets chronically activated EM Th1 cells uniquely express microRNA-148a. MiR-148a regulates expression of the proapoptotic gene *Bim* leading to a decreased *Bim/Bcl2* ratio. When inhibiting miR-148a using antagomirs in Th1 cells Bim expression increases, leading to enhanced apoptosis and reduced expansion of repeatedly reactivated EM Th1 cells. Knockdown of *Bim* expression by siRNA in miR-148a antagomir treated cells restored viability of the Th1 cells. This clearly proofs that miR-148a controls viability exclusively by regulating Bim expression. T cells isolated from the synovium of arthritic patients exhibit elevated miR-148a expression. Interestingly, Tbet (Th1 master transcription factor) and Twist1 (marker for chronically activated EM Th1 cells) induce expression of miR-148a.
Conclusions Taken together the data imply that Tbet and Twist1, besides controlling pathogenicity of Th1 cells, also regulate the longevity in chronic inflammation via the miR-148a-Bim-axis. MiR-148 plays an important role for the survival of EM Th1 cells in autoimmunity and chronic inflammation, thus, represents a highly potent molecular target for therapeutical treatment.

A3.20 TNF REGULATES CD3ζ EXPRESSION OF T LYMPHOCYTES VIA SRC-LIKE ADAPTOR PROTEIN-DEPENDENT PROTEASOMAL DEGRADATION

doi:10.1136/annrheumdis-2013-203216.20

[1]Barbara Érsek, [1]Viktor Molnár, [2]Andrea Balogh, [2]János Matkó, [3]Andrew P Cope, [1]Edit I Buzás, [1]András Falus, [1,4]György Nagy. *[1]Department of Genetics, Cell, and Immunobiology, Faculty of Medicine, Semmelweis University, 1089 Budapest, Hungary; [2]Department of Immunology, Eötvös Loránd University, 1117 Budapest, Hungary; [3]Division of Immunology, Infection and Inflammatory Disease, Academic Department of Rheumatology, Center for Molecular and Cellular Biology of Inflammation, King's College School of Medicine, King's College London, London WC2R 2LS, UK; [4]Department of Rheumatology, Semmelweis University, Medical School, 1023 Budapest, Hungary*

Background and Objectives although ζ-chain downregulation of human T lymhocytes is common at sites of chronic inflammation, the precise mechanism of ζ-chain regulation is not known. Src-like adaptor protein (SLAP) is a regulator of T cell activation; earlier data have reported that SLAP regulates immunoreceptor signalling. We studied the mechanism of CD3 ζ-chain downregulation.
Materials and Methods CD3ζ and SLAP protein levels of T lymphocytes were measured by Western blot. Jurkat cells were transiently transfected with siRNAs to silence SLAP, knockdown efficiency of the siRNAs was measured by real-time RT-PCR and by Western blot. For confocal microscopy experiments cells were transfected with eGFP-SLAP cDNA vector or control eGFP vector.