Methods PBMCs were isolated and we first determined monocytes subpopulations and looked at expression of membrane TNF (mTNF). After negative selection of monocytes, Macrophages were Derived from Monocytes(MDM) by 7 days of culture in the presence of M-CSF (M2 differentiation) or GM-CSF (M1 differentiation). Expression of total macrophages markers (CD11b and CD71) and the M2 macrophage polarisation markers (CD163 and CD206) were evaluated.

Results We have confirmed that the CD14⁺CD16⁺ monocytes subset was expanded in RA patients. For the first time, we have demonstrated that mTNF expression was significantly increased only in monocytes in RA patients (CD14⁺: Mean 5.6% HD versus 10.6% RA, CD14⁺CD16⁺: Mean 3.3% HD versus 11.1% RA, CD16⁺: Mean 2.4% HD versus 6.3% RA). Moreover, mTNF expression on monocytes correlated with the activity of the disease assessed by DAS28 CRP (Spearman r 0.548 and $p=0.0021^*$). We have observed a significant decrease of macrophages induction by M-CSF in RA patients as shown by a decreased expression of CD11b-CD71 (Mean 87.2% HD versus 57.1% RA, P value ***). Among MDM, we have found a specific decreased level of M2 markers (CD206 Mean 78.1% HD versus 27% RA and CD163 56.2% HD versus 37.2% RA) suggesting an impaired maturation to M2 stage in RA patients.

Conclusions Monocytes from RA patients have an increased expression of mTNF linked to activity of the diseases. RA patients have an impaired maturation of monocytes to M2 macrophages. This might suggest that RA monocytes have a propensity for preferential maturation towards a pro-inflammatory M1 phenotype thus contributing to synovial inflammation. Deeper characterisation of M1/M2 and effect of the different types of anti-TNF on this differentiation process are on-going and will be presented.

Disclosure of interest None declared

P022 CHECKPOINT INHIBITORS ACTIVATE STORE-OPERATED CA²⁺ ENTRY AND ERK1/2 SIGNALLING AND PROMOTE TH17 DIFFERENTIATION

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

10.1136/annrheumdis-2018-EWRR2018.47

Introduction Anti-tumour therapy with immune checkpoint inhibitors for programmed-death 1 receptor (PD1), such as nivolumab is often accompanied by immune-related adverse events (irAE). The cellular and molecular mechanisms underlying this phenomenon are not defined yet. Interaction of PD-1 with its ligand (PD-L1) mediates potent inhibitory signals to hinder proliferation and effector function of T cells. Interruption of PD1:PD-L1 interaction by nivolumab during anti-tumour therapy might therefore amplify T cell receptor (TCR) signalling and facilitate the development of a pro-inflammatory autoimmune response.

Objectives To investigate the impact of PD1 inhibition on intracellular signalling mechanisms down-stream of the TCR such as calcium (Ca²⁺) influx and activation of mitogen-activated protein kinases (MAPK) pathway and to assess the effect of PD1 inhibition on T cell effector function by evaluating the cytokine profile of the nivolumab-treated T cells.

Methods CD4 memory T cells were stimulated with anti-CD3 and anti-CD28 in the presence of nivolumab for 24 hour.

Afterwards intracellular Ca²⁺ influx in response to ionomycin was assessed by flow cytometry following loading the cells with Fluo-8. Expression of proteins involved in store-operated Ca²⁺ entry (SOCE), stromal activation molecule (STIM) 1, an activator of Ca²⁺ release-activated Ca²⁺ (CRAC) channels, and Orai1, a component of CRAC channels was determined by TaqMan real-time PCR. Phosphorylation of extracellular signal-regulated kinase 1 and 2 (Erk1/2) in response to anti-CD3/28 was determined by intracellular flow cytometry. The cytokine profile of nivolumab-treated cells was assessed after four-day culture by intracellular flow cytometry.

Results Treatment of CD4 T cells with nivolumab led to a pronounced increase of the ionomycin-mediated Ca²⁺ influx. At the same time expression of SOCE proteins, STIM1 and Orai1, was significantly up regulated in the nivolumab-treated cells. Phosphorylation of Erk1/2 in response to short anti-CD3/CD28 restimulation was almost twice as high in CD4 T cells cultured with as without nivolumab. Finally nivolumab-treated cells contained higher frequencies of IL-17-producing T cells (Th17 cells).

Conclusions Interruption of PD1:PD-L1 interaction by nivolumab activate SOCE and promotes Erk1/2 activation. Both T cell signalling pathways are essential for a proper mounting an immune response. Their deregulation might therefore precede an abnormal T cell response as shown for example by increased Th17 cell frequency and facilitate the onset of autoimmune phenomena such as irAE.

Acknowledgements This work was supported by DFG grants SK59/09-1 and Schu1683/10-1, and by BMBF Project Arthromark 01EC1401B.

Disclosure of interest None declared

P023 SMOKING IS ASSOCIATED WITH LOW SERUM LEVELS OF SOLUBLE PD-L1 IN RHEUMATOID ARTHRITIS

¹C Wasén*, ¹M Erlandsson, ^{2,3,4}A Bossios, ²L Ekerljung, ²C Malmhäll, ¹S Töyrä Silfverswärd, ¹R Pullerits, ²B Lundbäck, ¹M Bokarewa. ¹Department of Rheumatology and Inflammation Research; ²The Krefling Research Centre, Department of Internal Medicine and Clinical Nutrition, University of Gothenburg, Gothenburg; ³Department of Respiratory Medicine and Allergy, Karolinska University Hospital; ⁴Department of Medicine, Karolinska Institutet, Huddinge, Sweden

10.1136/annrheumdis-2018-EWRR2018.48

Introduction Smoking is a risk factor for the development of rheumatoid arthritis (RA) and associates with positivity for disease specific anti cyclic citrullinated peptide (aCCP) antibodies. It is not known exactly how smoking promotes autoimmunity but we have previously demonstrated that smoking limits the expression of the T cell co-inhibitory receptor programmed death-1 (PD-1).¹

Objectives To investigate if smoking can interfere with the interaction between PD-1 and its ligand by influencing the serum levels of soluble PD-1 ligand 1 (sPD-L1).

Methods Serum samples were collected from 254 RA patients and 168 healthy controls with known smoking status and analysed with ELISA for levels of sPD-L1 and inflammatory cytokines IL-6 and IL-1b. Fc-receptor mRNA expression analysis of peripheral blood monocytes (PBMC) from a group of 10 healthy controls and 15 RA patients was done by qPCR.

Results In RA patients current smokers had 5 times lower median serum levels of sPD-L1 compared to never smokers ($p=0.027$). This difference persisted in former smokers that quit smoking <25 years ago ($p=0.0086$). Expectedly, sPD-L1