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Disclosure of Interest None declared.

P008

INFLUENCE OF M-CSF POLARISATION OF MONOCYTES AND ACTIVATION OF THE M-CSF MACROPHAGES BY PRO-INFLAMMATORY OR OTHER POLARISING CYTOKINES, ON EXPRESSION OF PEPTIDYLARGININE DEIMINASES 2 AND 4

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Autoantibodies to citrullinated proteins (ACPA) are specifically associated to rheumatoid arthritis (RA) and likely involved in its pathophysiology. ACPA are produced in the inflamed synovial tissue (ST) where peptidylarginine deiminase (PAD) 2 and 4, responsible for fibrin citrullination, generate the ACPA-targeted epitopes. PAD2 and 4 are expressed in the intima and the subintimal inflammatory infiltrates by CD68⁺ mononuclear cells.¹ That made macrophages (Mφ) of the ST suspect to synthesize PADs. Moreover, we demonstrated that among various polarised subsets, the M-CSF-Mφ present the highest pro-inflammatory response to ACPA-containing immune complexes.² Recently, we confirmed that PAD2 and 4 are expressed in monocytes and showed that PAD2 is expressed at various degrees in monocyte-derived polarised Mφs generated in the presence of IFN-γ, IL-4, IL-10 or M-CSF, while PAD4 is only detected in the IFN-γ Mφ.

Objectives To evaluate expression of PAD2 and 4 in Mφ polarised by M-CSF, activated by various polarising or pro-inflammatory cytokines, expressed in the ST.

Methods CD14⁺ monocytes from healthy donors were differentiated in Mφ in the presence of M-CSF and were subsequently exposed for 18 hour either to polarising cytokines or to pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 or IL-17. *PADI2* and 4 gene expression was measured by RT-qPCR and expression of the related proteins evaluated by immunoblotting on total cell extracts.

Results *PADI2* mRNAs are less detected in M-CSF-Mφ than in the monocytes while *PADI4* gene expression is totally suppressed. All the polarised M-CSF-Mφ subsets retain *PADI2* gene expression, and interestingly, after IFN-γ stimulation *PADI2* mRNAs are detected at a higher relative rate. However IFN-γ, IL-4 or IL-10 does not induce *PADI4* gene re-expression. Whereas PAD4 protein remains undetectable, PAD2 is detected in all the polarised M-CSF-Mφ subsets but not more after IFN-γ stimulation.

M-CSF-Mφ activation by TNF-α, IL-8 or IL-17 does not modulate expression of the *PADI2* and 4 genes. IL-6 induces a significant decrease of *PADI2* gene expression and has no effects on the *PADI4* gene. Finally, the protein expression of PAD2 is not modulated in the various activated M-CSF-Mφs, and PAD4 is never induced.

Conclusions This study shows that in M-CSF-Mφ *PADI2* gene expression can be modulated by pro-inflammatory cytokines while *PADI4* gene expression cannot be re-induced. Our results reinforce the hypothesis of monocytes and Mφ involvement in generation of the ACPA epitopes in the ST of RA patients.

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P009/O22

TRANSCRIPTOMICS UNVEILS UNIQUE BIOLOGICAL PROFILE OF TERTIARY LYMPHOID STRUCTURES GERMINAL CENTERS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction The development of the B cell repertoire is regulated by the process of affinity maturation that occurs within the inner part of the B cell follicles within secondary lymphoid organs (SLOs). In autoimmunity process might occur in ectopic lymphoid structures (ELS), aggregates of lymphocytes that form in target organs of disease (i.e. the salivary glands of patients with Sjogren's Syndrome (SS)). The phenotypical and functional features supporting ELS pathogenic properties have not been identified. Moreover, the functional proof that ELS independently from SLOs contribute to the autoimmune response has not been provided.

Objectives To characterise the transcriptome profile of human ELS isolated from SS salivary glands in comparison with SLO and to dissect, in an animal model of ELS development, the ability of ELS to contribute to the autoreactive response.

Methods Frozen salivary gland biopsies were obtained from SS patients and selected for presence of germinal center +ELS. Samples were stained and microdissected, RNA isolated and transcribed and used for RNAseq using ClonTech SMARTseq v4 kit.

Salivary gland ELS and autoimmunity was established as previously described¹ in FAP-DTR mice. Animals were locally treated (day 2 and 6) with DTX to induce selective ELS disaggregation; glands and serum were harvested for analysis at day 15.

Results Transcript analysis provide evidence that the ectopic ELS are characterised by upregulation of TNF, INFγ, BAFF, APRIL, CXCL12 and CXCL13, FAS and FASL as compared to SLOs. Sequencing unveiled an altered cell-proliferation profile with downregulation of BCL6 and AID, the enzymes responsible for B cell affinity maturation. Selective depletion of FAP +cells induced loss of anatomical segregation of ELS and profoundly compromised their anatomy with significant impact on the local autoimmune response.

Conclusions Our study provides the first evidence of a significant molecular difference between ELS and SLOs. We

demonstrated that, although characterised by similar anatomical organization, ELS pathogenic cytokine signature is associated with low levels of bcl6 and AID and aberrant apoptosis, unveiling impaired regulation of the B cell cycle, responsible for the survival of autoreactive, poorly selected B cell clones. Accordingly, selective depletion of ELS *in vivo* profoundly impact local and systemic autoimmunity.

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Disclosure of Interest None declared.

P010

CHARACTERIZATION OF DISTINCT DENDRITIC CELL SUBSETS IN THE CONTEXT OF CHRONIC INFLAMMATION

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Career situation of first and presenting author Assistant.

Introduction Dendritic cells (DCs) are central regulators of the balance between immunity and tolerance, and alteration of the specialized DCs system is a common feature in chronic inflammatory diseases, such as rheumatoid arthritis (RA). In RA, synovial tissues are characterized by aberrant distribution of DCs populations, with altered phenotype. However, an in-depth characterization of the altered phenotype and functions of DCs in RA has been hampered by a lack of specific DCs markers and relevant *in vitro* culture model.

Objectives To perform an in-depth phenotypic characterization of DCs found in RA joints with an extensive panel of monoclonal antibodies specific of DCs, and to establish a relevant *in vitro* culture model to generate DCs resembling to those found in joints.

Methods Flow cytometry was used for analysis of cell surface molecules expressed on DCs found in RA synovial fluid (SF). Staining was performed with a panel of 100 mouse monoclonal antibodies specific of DCs, generated by immunization with different subsets of human DCs. Phenotype of DCs from synovial fluid was then compared to the phenotype of monocytes co-cultured *in vitro* with synoviocytes from RA patients, in presence or not of synovial fluid, or cytokines.

Results In RA SF patients, two main DCs subsets were identified, with distinct surface protein phenotypes. One subset was characterized by markers of plasmacytoid DCs (CD303, CD123), whereas the other subset expressed markers of myeloid/conventional DCs (CD11c, DCIR). This last subset of DCs expressed some markers found on inflammatory DCs, the main inducers of Th17 cells in RA joints. Surprisingly, the two subsets of DCs expressed also surface proteins shared by synoviocytes. Culture of human monocytes with synoviocytes and/or combination of pro-inflammatory cytokines (TNF, M-CSF, IL-4) yielded DCs with a phenotype close to the phenotype of conventional DCs found in RA synovial fluid.

Conclusions We uncovered previously unreported phenotypic heterogeneity of DCs in a chronic inflammatory context, and characterized two main subsets of DCs. The next steps will be

to understand the consequences of this heterogeneity on the pathogenicity of the disease. This will be facilitated by our relevant *in vitro* culture model that allows the generation of DCs, closely resembling those found in joints of patients with RA.

Disclosure of Interest None declared.

P011

THE ROLE OF CELLULAR METABOLISM IN RHEUMATOID AND PSORIATIC ARTHRITIS

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Career situation of first and presenting author Post-doctoral fellow.

Introduction Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) share common features such as synovial hyperplasia, cartilage degradation and subchondral bone remodelling, however, there are distinct pathologic differences, which aid their diagnosis, and may account for the differential responses to specific treatments. Increased proliferation and angiogenesis transforms the synovium into an aggressive, tumour-like 'pannus' which contributes to disease pathogenesis. Hypoxia, resulting from dysregulated angiogenesis, can cause cells to switch from mitochondrial respiration to anaerobic glycolysis in order to meet the cellular energy demand. The RA synovium is more hyperplastic and invasive than that of PsA, while a more abnormal vasculature is observed in the PsA synovium and correlates with a reduction in tissue pO₂ levels.

Objectives The aim of this study was to compare the metabolic profiles of RA and PsA synovial fibroblast cells (SFC) and to determine whether there is a correlation between dysregulated metabolism, cell function and disease pathogenesis.

Methods The metabolic profiles of RA and PsA SFC were analysed using the XF96 Extracellular Flux Analyzer. Gene expression was determined by quantitative-PCR. SFC migration and invasion were observed by microscopy following wound-scratch and transwell invasion assays.

Results RA SFC displayed increased migratory capacity and invasiveness compared to PsA SFC. Expression of IL-6, IL-8 and the glycolytic markers, GLUT1/3, HK2, PKM1/2, PDK2 and LDHA is higher in RA SFC compared to PsA SFC and is upregulated following TNF- α treatment. PsA SFC displayed higher baseline oxygen consumption rate (OCR), baseline extracellular acidification rate (ECAR) and ECAR:OCR ratio than RA SFC. Treatment with TNF- α increases the ECAR:OCR ratio in both RA and PsA SFC.

Conclusions Consistent with clinical observations, RA SFC have a greater migratory and invasive capacity than PsA SFC. Despite the lower expression of glycolytic markers, PsA SFC display a more glycolytic phenotype than RA SFC, as indicated by a higher ECAR:OCR ratio. Interestingly, however, RA SFC display a higher glycolytic reserve, possibly due to the higher expression of glycolytic markers, which can be called upon in response to increases in cellular ATP demand. Preliminary data suggest a positive correlation between the ECAR:OCR ratio and DAS28 in RA but a negative correlation in PsA. Further investigation is required to determine the precise role of metabolism in specific pathogenic processes.

Disclosure of Interest None declared.