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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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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