NOVEL SUBCLASS OF NON-CLASSICAL MONOCYTES ARE CRITICAL FOR INFLAMMATION

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

Introduction Monocytes in mice are distinguishable by expression of Ly6c. Ly6c\textsuperscript{hi} (classical) monocytes are associated with pro-inflammatory responses, while Ly6c\textsuperscript{lo} (non-classical) are involved in patrolling endothelial membranes. We have previously shown that deletion of monocytes prevents serum transfer induced arthritis (STIA) in mice, and that Ly6c\textsuperscript{lo} monocytes are the critical population.

Objectives We aim to contrast Ly6c\textsuperscript{lo} monocytes from the circulation, lining vessels, and tissue to determine their involvement in inflammation.

Methods Female 8–10 week old NR4A1\textsuperscript{-/-}, CX3CR1\textsuperscript{ERCre.zsGFP} and C57Bl/6 mice were used in all studies. CX3CR1\textsuperscript{ERCre.zsGFP} were utilized for cell tracking studies and joint shielded bone marrow chimera via administration of tamoxifen (tam). Intravascular monocytes were identified by I.V. anti-CD45 labeling before perfusion. STIA was induced via I.V. KBxN sera. Cell populations were quantified by flow cytometry and FACS sorted for RNA-seq. Monocytes were identified CD45\textsuperscript{+} CD11b\textsuperscript{+} Ly6G\textsuperscript{-} TIM4\textsuperscript{-} (no label). Results NR4A1\textsuperscript{-/-} mice retain only 5% of circulating Ly6c\textsuperscript{lo} monocytes but all joint Ly6c\textsuperscript{lo} cells. STIA was comparable in NR4A1\textsuperscript{-/-} and C57Bl/6 mice suggesting circulating Ly6c\textsuperscript{lo} are redundant. Transcriptional profiling of Ly6c\textsuperscript{lo} cells identified distinct pathways enriched in upregulated genes between Ly6c\textsuperscript{lo} from joint and blood. In the joint we identified three populations of Ly6c\textsuperscript{lo} monocytes: extravascular unlabeled cells, labeled trans-vascular cells, and labeled intravascular cells adherent to endothelium. Mice given tam D8 of gestation had GFP\textsuperscript{+} microglia only, whereas D15 tam induced GFP\textsuperscript{+} synovial macrophages did not affect CD43\textsuperscript{-} cells or unlabeled cells. With vascular adherent and trans-vascular which have distinct origins and phenotype from both extravascular and circulating Ly6c\textsuperscript{lo}. The findings presented here strongly suggest adherent Ly6c\textsuperscript{lo} monocytes are a key effector cell in inflammatory arthritis.

Disclosure of Interest None declared.

SALIVARY GLAND EPITHELIAL CELLS FROM SJÖGREN’S PATIENTS INCREASE B LYMPHOCYTES SURVIVAL AND ACTIVATION

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Introduction Primary Sjögren’s syndrome (pSS) is a chronic autoimmune disorder characterized by lymphocytic infiltrates and destruction of the salivary glands (SG). Several lines of evidence support the hypothesis that SG epithelial cells (SGECs) are not only the target of autoimmunity in pSS but may also play a role for its initiation and maintenance.

Objectives To study the survival and the activation of B lymphocytes cocultured with SGECs from pSS patients compared to controls.

Methods Primary cultures of SGECs were established from minor SG biopsies. Patients had pSS according to 2016 EULAR/ACR criteria and controls had sicca symptoms without any antibodies and with normal SG biopsies. The coculture involved B lymphocytes isolated by CD19 magnetic bead positive selection from healthy donors: blood (purity >80%). Several conditions of stimulation were tested: IFNa 2400 U/mL, IFNg 5 ng/mL, Poly(IC) 10 µg/mL or 30 µg/mL. After 5 days, the viability, the activation (CD38) and the differentiation (CD27) of B lymphocytes were assessed by flow cytometry. Mann-Whitney (unpaired data) and Wilcoxon (paired data) were used for statistical analysis.

Results A significant increase of B lymphocytes survival was observed when cocultured with SGECs compared to B lymphocytes cultured alone, in all conditions of stimulation (p<0.05). The survival of B lymphocytes (percentage of alive cocultured B lymphocytes percentage of alive cultured alone B lymphocytes) was increased when the cocultures were performed with SGECs from pSS patients (n=5) compared to SGECs from controls (n=5), in all conditions of stimulation (p<0.05), except IFNg. Moreover, there was a trend for an increase of B lymphocytes activation, assessed by higher percentages of CD38\textsuperscript{+} B lymphocytes when the cocultures were performed with SGECs from pSS patients compared to SGECs from controls. This difference was statistically significant (p<0.05) in the condition stimulated with TLR3 agonist (Poly(IC) 10 µg/mL). The percentage of CD27\textsuperscript{+} B lymphocytes was not affected by the cocultures and no difference between pSS and controls SGECs was observed.

Conclusions This coculture model showed a differential effect of SGECs from pSS compared to controls on B lymphocytes survival. Interestingly, there was also a trend for a higher activation level of B lymphocytes when cocultured with SGECs from pSS compared to controls. This results suggest that SGECs could play a major role in pSS pathophysiology through B lymphocytes support and activation.

Disclosure of Interest None declared.

ACTIVATED MEMORY T CELLS PRODUCE LIGANDS THAT CAUSE NF-KB-DEPENDENT INFLAMMATORY ACTIVATION OF THE ENDOTHELIUM

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Introduction That cause NF-KB-dependent inflammatory activation of the endothelium.

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