NOVEL SUBCLASS OF NON-CLASSICAL MONOCYTES ARE CRITICAL FOR INFLAMMATION

AB Montgomery*, 1PJ Homan, 1D Winter, 1HR Perlman. 1Northwestern University; 2Rheumatology, Chicago, USA

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

Objectives We aim to contrast Ly6c<sup>lo</sup> monocytes from the circulating, lining vessels, and tissue to determine their involvement in inflammation.

Methods Female 8–10 week old NR4A<sup>-/-</sup> mice and C57Bl/6 mice were used in all studies. CX3CR1<sup>ERCre.zsGFP</sup> and C57Bl/6 mice were utilized for cell tracking studies and joint shielded bone marrow chimeras 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<sup>+</sup> CD11b<sup>+</sup> Ly6G<sup>-</sup> TIM4<sup>-</sup> and subdivided into intravascular (labeled, CD43<sup>+</sup>), trans-vascular (labeled CD43) and extravascular (no label).

Results NR4A<sup>-/-</sup> mice retain only 5% of circulating Ly6c<sup>lo</sup> monocytes but all joint Ly6c<sup>lo</sup> cells. STIA was comparable in NR4A<sup>-/-</sup> and C57Bl/6 mice suggesting circulating Ly6c<sup>lo</sup> are redundant. Transcriptional profiling of Ly6c<sup>lo</sup> cells identified distinct pathways enriched in upregulated genes between Ly6c<sup>lo</sup> from joint and blood. In the joint we identified three populations of Ly6c<sup>lo</sup> monocytes: extravascular unlabeled cells, labeled trans-vascular cells, and labeled intravascular cells adherent to endothelium. Mice given tam D8 of gestation had GFP<sup>+</sup> microglia only, whereas D15 tam induced GFP<sup>+</sup> synovial macrophages and unlabeled Ly6c<sup>lo</sup> monocytes. Both labeled Ly6c<sup>lo</sup> populations were GFP, indicating unlabeled and unlabeled Ly6c<sup>lo</sup> arise from different progenitors. This was confirmed by bone marrow chimera studies showing labeled Ly6c<sup>lo</sup> cells are replenished from blood monocytes. Clodronate loaded liposomes depleted labeled CD43<sup>+</sup> cells but did not affect CD43<sup>-</sup> cells or unlabeled cells. With our previous finding that colo-lip prevents STIA, these suggest adherent CD43<sup>+</sup> Ly6c<sup>lo</sup> cells are essential. This is supported by the finding that labeled Ly6c<sup>lo</sup> monocytes expand rapidly during the first 1 hour of STIA. Adherent CD43<sup>+</sup> cells expand especially rapidly, increasing in population size by 30x.

Conclusions We have identified and described two previously uncharacterized populations of Ly6c<sup>lo</sup> cells in the joint- intravascular adherent and trans-vascular which have distinct origins and phenotype from both extravascular and circulating Ly6c<sup>lo</sup>. The findings presented here strongly suggest adherent Ly6c<sup>lo</sup> monocytes are a key effector cell in inflammatory arthritis.

Disclosure of Interest None declared.
Career situation of first and presenting author Student for a master or a PhD.

Introduction Endothelial cells (EC) are important contributors to inflammation via expression of inflammatory mediators, including chemokines and adhesion molecules. Production of these inflammatory mediators can be induced via canonical and NF-κB-inducing kinase (NIK)-dependent noncanonical NF-κB signalling. The ligands activating these pathways are well studied, but less is known about the cells producing ligands that can activate NF-κB signalling in EC.

Objectives To study the effects of soluble factors produced by activated memory T cells on NF-κB dependent inflammatory activation of EC.

Methods CD4+CD45RO+ Memory T cells were isolated from healthy PBMC using MACS sorting and cultured in medium containing anti-CD3 and anti-CD28 for 72 hour, after which supernatant was harvested. Human umbilical cord EC (HUVEC) were treated with 50% Tm supernatant (Tm sup). After 72 hour of Tm sup stimulation HUVEC protein and RNA was harvested and NF-κB signalling and downstream expression of inflammatory mediators was analysed using qPCR and Western Blot. Culture supernatant was analysed by ELISA to detect presence of inflammatory mediators. To repress canonical NF-κB signalling an inhibitor of IKKβ (iIKKβ) was used and to repress NIK-dependent NF-κB signalling an inhibitor of NIK (iNIK) was used.

Results Stimulation with Tm sup led to activation of both canonical NF-κB signalling, indicated by increased levels of phosphorylated (p)-IkBα, and noncanonical NF-κB signalling, indicated by increased p100 to p52 processing. HUVEC stimulated with Tm sup had increased mRNA levels of all tested inflammatory mediators compared to non-treated cells. Gene expression of chemokines (CXCL1, CXCL5, IL6, ILβ and GM-CSF) after Tm sup stimulation was significantly reduced after treatment with iIKKβ and to a lesser, but still significant, extent after treatment with iNIK. Interestingly, treatment with iIKKβ also led to a reduction in mRNA levels of the adhesion molecules VCAM-1 and ICAM-1, while this effect was minimal after iNIK treatment. In addition, treatment with either iKKβ or iNIK led to a significant reduction in CXCL5 in the culture supernatant of HUVEC stimulated with Tm sup.

Conclusions This study provides new insights into the cellular interactions leading to production of inflammatory mediators by EC. Our findings demonstrate that treatment with Tm cells factors produce factors that can cause NF-κB-dependent inflammatory activation of EC. Targeting canonical NF-κB signalling and, although to a lesser extent, NIK-dependent NF-κB signalling reduces inflammatory activation of the endothelium.

Disclosure of Interest None declared.