Conclusions Blood eosinophils in patients with SSc display a diverse phenotype depending on disease duration. In early disease, surface marker expression on eosinophils is associated with disease activity and severity.

A2.13 PHENOTYPIC AND MOLECULAR PROFILE OF INNATE LYMPHOID CELLS IN CHRONIC SYNOVIAL INFLAMMATION

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Background and Objective Innate lymphoid cells (ILCs) represent a novel family of effector and regulatory cells in innate immunity and tissue remodelling. The family comprises several phenotypically and functionally distinct subsets that produce various cytokines such as IL-22, IL-17, IFNγ, TNF, IL-13 and IL-5, of which many have been shown to be important in arthritis pathogenesis.

The IL-17 and IL-22 producing ILCs are of major interest as they are implicated in chronic gut inflammation. Based on the broad clinical overlap between inflammatory bowel disease and spondyloarthritis (SpA) and the clinical importance of IL-17 in SpA we hypothesise that IL-17 and IL-22 producing ILCs contribute to inflammation and remodelling in SpA synovitis. As these cells have never been described in the joint our first aim was to characterise ILC in chronic inflammatory arthritis.

Material and Methods ILCs (lineage negative, $CD45^+CD127^+$) were analysed and sorted by flow cytometry from synovial tissue and fluid from rheumatoid arthritis (RA) and SpA patients as well as in blood from SpA patients and healthy donors. mRNA expression of sorted and expanded cells was analysed by qPCR.

Results ILCs were identified in blood as well as in synovial tissue and fluid from both RA and SpA patients. The frequency of ILCs was higher in the inflamed joint (0.5–3.3% of the lymphocyte population) than in the peripheral blood compartment (0.1%). In blood, there was no marked difference in the frequency of the different ILC subset between healthy controls (n = 10) and SpA patients (n = 5). In the inflamed joint, the ILC3 (CRTH2-NKp44+ckit+) and ILC1 (CRTH2-NKp44-ckit–) populations, previously shown to express IL-22 and IFNγ respectively in other tissues, were present in all samples whereas the Th2 cytokine expressing ILC2s (CRTH2+) were found in very low frequencies. Frequencies of ILC subpopulations varied considerably between patients and no differences could be detected between RA and SpA patients. qPCR analysis of expanded cells revealed that ILC1 expressed TBX21 whereas ILC3 expressed RORC. Accordingly, stimulated ILC3 expressed transcripts for both IL-23R and IL-22 but not IL-17.

Conclusions ILC1 and ILC3 are present in the chronically inflamed joint and express the key transcription factors associated with specific cytokine profiles. These data indicate that ILC could contribute to local cytokine-driven immune alterations in SpA and RA.

A2.14 POTENTIAL IN VITRO IMMUNOMODULATORY EFFECTS OF THE RECOMBINANT HUMAN ALPHA-ENOLASE ON PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) FROM HEALTHY DONORS

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Background Identification of autoantibodies associated with rheumatoid arthritis has been of major interest. In this context, we have previously identified for the first time α-enolase (ENO) as a new auto-antigen in early RA. ENO is an evolutionary conserved protein involved both in glycolysis pathway and as a plasminogen receptor which confer it a role in anti-infectious inflammatory response. In vivo, preliminary studies showed that ENO had immunomodulatory effect in the collagen induced arthritis mouse model. [1] To better understand the immunological mechanisms of ENO, the aim of this in vitro study was to determine the effects of ENO on PBMCs from healthy donors.

Methods In one hand, PBMCs or different cell types (monocytes, B and T cells, and immature dendritic cells [iDC]) (n = 3) were cultured with ENO (20 µg/mL) or Bovine Serum Albumin (20 µg/mL). TNFα and IL-10 production was measured in the supernatants by ELISA at different times. On the other hand, TNFα and IL-10 production were evaluated in PBMCs, monocytes or B and T cells after LPS stimulation and pre-incubation with ENO for 24 h (n = 3).

Cytometric analyses have evaluated the ability of ENO to inhibit the differentiation of monocytes into iDC. Before differentiation into iDC (GM-CSF and IL-4), monocytes (1.10^6 cells/mL) were incubated with ENO (20 or 50 µg/mL) for 24 h.

Results In cultures of PBMCs, monocytes or iDC, ENO induces, dose dependently, an early production of TNFα followed by extended secretion of IL-10. PBMCs or individual cells (monocytes, B and T cells) stimulated by LPS secreted successively TNFα and IL-10, while PBMCs or individual cells, stimulated by LPS but previously incubated with ENO for 24 h did not secrete these cytokines.

In contrast to LPS, ENO did not induce differentiation of immature dendritic cells into mature cells. But ENO has not the capacity to inhibit differentiation from monocytes to iDC.

Conclusions This study suggests that ENO has no pro-inflammatory effect unlike LPS. Indeed, ENO might have immunomodulatory properties via IL-10 production. Others studies focused on an extended cytokine panel and different signalling pathways are underway to better understand the immunological mechanisms induced by ENO.

Reference

1. C Guillou *et al*, *Arthritis and Rheumatism* 2011; 63:S815.

A2.15 RELATIVE OVEREXPRESSION OF TRANSMEMBRANE VERSUS SOLUBLE TNF IN HUMAN AND EXPERIMENTAL SPONDYLOARTHRITIS

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Background Macrophages and their pro-inflammatory cytokines, including TNF, are pivotal mediators of chronic synovitis in rheumatoid arthritis (RA) as well as spondyloarthritis (SpA). Despite similar levels of synovial macrophage infiltration and similar clinical responses to TNF blockade in both diseases, SpA is characterised by a more pronounced infiltration with alternatively activated CD163+ macrophages and ongoing osteoproliferation. This study aimed to investigate whether these differences were related to a differential expression and/or function of TNF between both diseases.

Methods Expression of transmembrane TNF (tmTNF) and soluble TNF (sTNF) was measured in IFN-γ, IL-4 or IL-10 polarised macrophages obtained from healthy donors. Expression of TNF and its receptors was measured in synovial fluid (SF) and synovial tissue biopsies (ST) of actively inflamed knee joints of SpA and RA patients. Mice transgenically overexpressing tmTNF (TgA86) were evaluated for spondylitis and arthritis.