Background and Objectives Our recent experiments have suggested that similarly to SLE, patients with primary Sjögren's syndrome (SS) manifest significantly increased phagocytosis of necrotic cell debris (secondary necrotic cell material, SNEC). This phenomenon has been attributed to serological aberrations of these patients, as indicated by the capacity of patients' sera to promote the uptake of SNEC by healthy phagocytes. In this study, we comparatively investigated the role of serum DNAse-I activity and IgG immunoglobulins from SS, SLE and RA patients in the promotion of SNEC-phagocytosis by healthy monocytes.
Materials and Methods The activity of DNase-I was assessed by single radial enzyme-diffusion assay (SRED) in the serum of patients with SS (n = 60), SLE (n = 22) and RA (n = 14) and healthy donors (HBD, n = 52). Total IgG immunoglobulins were isolated by negative selection from the serum of patients and controls using Melon Gel Resin columns. SNEC were prepared by heat-induced necrosis of normal lymphocytes and labelling with propidium iodide. The influence of serum components on SNEC-phagocytosis was assessed by flow cytometry in admixture experiments using normal phagocytes and SNEC pre-incubated with whole sera or purified serum IgG from patients or HBD.
Results Serum DNase-I activity in patients with SS and SLE was found significantly reduced compared to HBD and RA patients ($p < 0.0001$) and correlated inversely with the ability of these sera to promote SNEC-phagocytosis by healthy monocytes ($p = 0.0003$). The capacity of HBD sera to promote SNEC-phagocytosis by normal monocytes was significantly increased (by 90%) following the addition of the DNase-I-specific inhibitor G-actin (800 μg/ml), supporting the important physiological role of DNA degradation by serum DNase-I in the prevention of SNEC-phagocytosis. SNEC opsonised with IgG isolated from autoimmune patients or from HBD were found to be similarly ingested by normal monocytes. However, in the presence of normal serum, the opsonisation of SNEC with IgG isolated from SS or SLE sera was found to induce significantly increased SNEC-phagocytosis, compared to that observed with SNEC opsonised with IgG isolated from HBD sera ($p = 0.001$).
Conclusions Our results indicate that, in a manner similar to SLE, SS patients are characterised by deficient serum DNase-I activity. Such reduced serum capacity for degradation of nucleic acids, in conjunction with the opsonisation of SNEC by serum autoantibodies appears to lead to increased exposure of the immune system of these patients to necrotic cell debris, to enhanced SNEC-phagocytosis and consequently to the inflammatory responses that characterise the disorder.

A2.11 INVOLVEMENT OF THE NUCLEIC ACID RECOGNISING TOLL-LIKE RECEPTORS TLR7 AND TLR9 IN THE PATHOGENESIS OF EROSIVE ARTHRITIS

doi:10.1136/annrheumdis-2013-203215.11

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Background There is substantial evidence that abundant release and/or insufficient removal of endogenous nucleic acids can trigger autoimmune reactions via activation of nucleic acid recognising Toll-like receptors (TLR) such as TLR7 and TLR9, which may lead to the development of SLE and other systemic autoimmune diseases. However, in RA the involvement of these TLRs is less well understood. Interestingly, in rats with pristane-induced arthritis (PIA) disease can be transferred by T cells together with antigen-presenting-cells pre-activated with TLR7 or TLR9 agonists (Hoffmann *et al*, J Autoimmunity 2011; 36: 288–300).
Objective We aimed to study the role of TLR7 and TLR9 in the pathogenesis of inflammatory erosive arthritis by antagonising them in PIA and the KRN serum transfer model.
Methods Arthritis was induced in rats with the mineral oil pristane, and in C57Bl/6 mice by injection of KRN serum. Immunoregulatory oligodeoxynucleotide (ODN) sequences (IRS) antagonising TLR7 or TLR9 were applied either subcutaneously (PIA) or intra-peritoneally (KRN). A non-inhibitory ODN was used as control and PBS served as placebo. Arthritis was scored using established scoring systems, inflammation and bone erosion were quantitatively analysed by histology. Serum and cell culture cytokine levels were measured by ELISA.
Results While the TLR7 inhibitor and the control ODN showed no effect on arthritis development and severity, the TLR9 antagonist reduced arthritis severity significantly in PIA. Bone erosion was almost completely abolished, whereas it was moderately aggravated in animals treated with the TLR7 inhibitor. Furthermore, IL-6 serum levels were significantly reduced in animals treated with the TLR9 antagonist. However, these beneficial effects were only observed when the inhibitor was applied before disease onset. Moreover, neither inhibitor affected arthritis onset and severity in the serum transfer model, which is independent of the adaptive immune system.
Summary and Conclusions Inhibition of TLR9 significantly reduced inflammation and bone erosion in PIA but not in the KRN serum transfer model that reflects the late effector phase of erosive arthritis. Therefore, these results suggest important involvement of the DNA (CpG) recognising TLR9 in the initiation of autoimmune arthritis whereas nucleic acid binding TLRs do not seem to play a major role in the later phases of the disease. Antagonizing TLR9 in human RA may only act beneficial in the earliest phase of the disease.

A2.12 PHENOTYPE CHANGES OF BLOOD EOSINOPHILS REFLECT ACTIVITY AND SEVERITY IN SYSTEMIC SCLEROSIS

doi:10.1136/annrheumdis-2013-203215.12

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Background and Objectives We investigated whether surface marker expression analysis of blood eosinophils may reflect disease activity in systemic sclerosis (SSc), since a role for eosinophils in the pathogenesis of SSc may be suggested from the observation of increased counts in bronchoalveolar lavage.
Materials and Methods By flow cytometer analysis, eosinophils in whole blood were identified in 32 consecutive untreated patients using surface marker CD16 and CD9. Surface expression of markers CD11b, CD44, CD48, CD54, CD81 and HLA-DR was measured. Data were related to clinical measurements of the disease activity.
Results An increased blood eosinophil population with low surface expression of CD9 was identified in patients with early SSc, i.e. a disease duration of <2 years, compared to patients with longer disease duration ($P = 0.003$) and controls ($P = 0.029$). CD81 expression was lower in SSc patients compared to healthy individuals ($P = 0.003$). In patients with early SSc, CD81 levels correlated inversely to degree of skin involvement ($r = –0.67$; $P = 0.009$). CD48 levels were increased in early SSc and were associated with an increased concentration of alveolar nitric oxide in these patients ($r = 0.84$; $P < 0.001$). CD16 expression on blood eosinophils was also higher in patients with early disease and was associated with loss of nailfold capillaries ($r = –0.78$; $P < 0.001$).

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

doi:10.1136/annrheumdis-2013-203215.13

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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

doi:10.1136/annrheumdis-2013-203215.14

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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

doi:10.1136/annrheumdis-2013-203215.15

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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.