development and bone homeostasis. It has recently been demonstrated that RANK-activated osteoclasts release CCL20 and attract T cells to the central nervous system in a model of Multiple Sclerosis and that transgenic RANK expression in the skin promotes aberrant epithelial cell proliferation and is sufficient to induce ectopic formation of tertiary lymphoid structures (TLS). Ductal epithelial cells (SGEs) have been implicated in Sjögren’s Syndrome (SS) pathogenesis where they mediate immune recruitment by expression of pro-inflammatory chemokines and support the formation of pre-malignant myoepithelial lesions.

Objectives To address the role of RANK-RANKL interaction in primary (p) SS.

Methods A combination of human and mouse studies were used to explore the RANK-RANKL interaction in pSS. Salivary glands (SGs) and saliva samples from patients recruited in the OASIS cohort (University of Birmingham) were studied to evaluate this pathway in human disease. Consecutive stimulated saliva samples (n=69) were analysed using Proseek Multiplex INF96 × 96, covering 92 unique inflammation-related protein biomarkers. Taking advantage of a viral induced model of pSS we studied the effect of this pathway with a RANKL blocking antibody and by inducing gain of function with direct cannulation in the salivary glands of recombinant RANKL. Murine SGs were studied by immunofluorescence, flow cytometry and qPCR on total tissue and sorted cells.

Results Fourteen proteins in saliva were significantly separated between pSS and sicca controls, and elevated levels of just two proteins, RANKL and TNFβ, could classify pSS or sicca with 75% accuracy. Levels of salivary RANKL and CCL20 were strongly correlated (r=0.6; p<0.01). We demonstrated that both human and murine inflamed SGs upregulate both RANK and CCL20, a chemokine known to recruit pathogenic T cells. Upregulation of RANKL was found in human Th2 cells, classically associated with humoral responses and germinal centre (GC) formation. SGEs from mice treated with anti-RANKL antibody showed decreased epithelial proliferation, reduced T cell infiltration and defective TLS establishment. On the contrary, viral infected SGs treated with recombinant RANKL showed increased T cell infiltration, CCL20 expression and enhanced differentiation of GC B cells.

Conclusions In vivo RANK-RANKL interaction mediates recruitment of activated T cells that are skewed toward a Th2 phenotype. These, in turn, will favour the establishment of TLS in the SG. Those data were confirmed in human pSS, where expression of RANK is found in inflamed epithelium and RANKL detection in saliva is able to differentiate patients with pSS from sicca controls, thus candidates this pathway both for drug targeting and patient stratification.

Disclosure of interest None declared.
TOLL-LIKE RECEPTOR 9 INFLUENCES INFLAMMATORY ARTHRITIS AND OSTEOCLASTOGENESIS

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Introduction Release and insufficient removal of endogenous nucleic acids may be involved in triggering autoimmune reactions important in the initiation of systemic autoimmune diseases including rheumatoid arthritis (RA). Nucleic acid sensing molecules, such as the endosomal Toll-like receptors (TLRs) 7 and 9, have been linked to pathogenic autoimmune processes, but their role in RA is less clear.

Objectives To gain more insight into the role of TLR9 in autoimmune arthritis, TLR9 inhibition was investigated in rats with pristane-induced arthritis (PIA). To further investigate TLR9 involvement, streptococcal cell wall (SCW) arthritis was induced in TLR9-/- mice.

Methods Arthritis was induced in mice with SCW lysates and in rats with the mineral oil pristane. Rats were treated with a TLR9 antagonist, starting before disease induction. Arthritis was scored using established scoring systems, inflammation and bone erosion was quantified by histological analysis of the paws. Levels of α-1-acid-glycoprotein (AGP), rheumatoid factor (RF) and IL-6 in sera were analysed. The role of TLR9 in osteoclast differentiation was investigated in vitro.

Results In PIA, which is a T cell-dependent, the TLR9 antagonist reduced arthritis severity by ~50%. This was accompanied by a reduction of AGP, IL-6 and RF in the sera of these animals. In addition, TLR9 inhibition led to reduced inflammation, bone erosion and cartilage degradation in the paws. Moreover, the T cell-dependent chronic phase of SCW arthritis was significantly suppressed in TLR9-/- mice. Remarkably, TLR7 and TLR9 mRNA levels strongly differed in the course of in vitro osteoclastogenesis. Whereas TLR7 expression did not change throughout osteoclastogenesis, expression of TLR9 was higher in precursor cells than in mature osteoclasts and stimulation with a TLR9 agonist (CpG) completely inhibited osteoclast differentiation. The results suggest a crucial role for TLR9 in the T cell-dependent phases of PIA and SCW arthritis and thus an important involvement of the DNA (CpG) recognising TLR9 in the induction of arthritogenic autoimmune reactions. In addition, TLR9 also seems to play a role in the initiation of osteoclast differentiation which needs to be further elucidated in future experiments.

Disclosure of interest None declared