The presence of Dex significantly inhibited the expression of this DC marker in all cultures.

Conclusion This study demonstrates that synovial tissue macrophages have the capacity to differentiate into either functional osteoclasts or mature dendritic cells depending on the presence or absence of specific humoral factors.

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THU0039 LOCAL IL-17 GENE THERAPY ACCELERATES COLLAGEN ARTHRITIS WITH SEVERE BONE EROSION AND RANK LIGAND AND RANK EXPRESSION IN SYNOVIAL INFILTRATE AND AT BONE EROSION SITES

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Background

Objectives To examine the effects of local IL-17 application in the knee joint of type II collagen immunised mice on the induction of bone erosion.

Methods Collagen induced arthritis (CIA) was induced in male DBA-1 mice by immunising intradermally at the base of the tail with suboptimal dose of bovine type II collagen. On day 21, mice were given a booster injection (i.p.) of the same dose of type II dissolved in PBS. Just before expected onset, mice were intraarticularly (i.a.) injected into the right knee joint with 10^7 pfu of either an IL-17 expressing (AdIL-17) or control (AdControl) recombinant human type 5 adenovirus vector. Five days after the i.a. injection of the viral vector, arthritis was monitored visually and joint pathology was examined by histology. Formation of osteoclast-like cells was determined by tartrate-resistant acid phosphatase (TRAP) staining. In addition, RANKL and RANK protein expression was evaluated by specific immunohistochemistry.

Results Local IL-17 over-expression in the knee joint of type II collagen immunised mice promotes synovial inflammation. Five days after viral injection of AdIL-17, histologic analysis showed aggravation of bone erosion in the patella and femur/tibia region compared with the control vector group. Induction of bone destruction by IL-17 was accompanied with marked TRAP activity in the bone marrow and at bone erosion sites, indicating that IL-17 accelerates the formation of osteoclast-like cells. Interestingly, local IL-17 promotes local protein expression of RANKL and its receptor RANK in the synovial infiltrate and at bone erosion sites compared with the control vector group.

Conclusion These data shows that local IL-17 gene therapy during onset of collagen arthritis promotes osteoclastic bone erosion accompanied with accelerated expression of local RANKL and its receptor RANK. These findings suggest IL-17 to be a potent stimulator of osteoclastogenesis during arthritis.

THU0040 ANAEMIA OF CHRONIC DISEASE (ACD) IN A RODENT MODEL IS SIMILAR TO HUMAN ACD AND CAN BE ALLEVIATED BY ARANESP TREATMENT

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Background We previously reported that ARANESP™ alleviates ACD in a rodent model of peptidoglycan-polysaccharide polymer (PG-APS) mediated inflammation. We report here the further characterisation of this model and the effects of ARANESP™ treatment on ACD.

Objectives

Methods Immunisation of Lewis rats with PG-APS induces chronic systemic inflammation characterised by relapsing arthritis and hepatic granulomas. Associated with the inflammation is acute, severe anaemia followed by chronic, moderately severe anaemia. As previously established, a 30 µg/kg dose of ARANESP™ every 2 weeks starting day 36 normalised peripheral blood (PB) haemoglobin levels by day 64.

Results Acutely anaemic rats had greatly enhanced mean PB reticulocyte counts and greatly reduced mean RBC counts. Mean PB reticulocyte and RBC counts normalised during chronic anaemia, but RBC remained hypochromic and microcytic. Individual anaemic rats had transient increases in serum erythropoietin (EPO) concentrations. However, there was no significant difference in mean EPO concentrations compared to controls, which suggests EPO production was blunted. Histology of day 36 and day 112 anaemic rat spleen sections revealed greatly enhanced iron retention by splenic macrophages. In contrast, bone marrow (BM) macrophages were nearly devoid of iron. Serum iron concentrations were significantly reduced by day 7 and remained low throughout the study. Interestingly, ARANESP™ treated rats showed decreased iron retention in macrophages and increased serum iron starting day 98. To identify which cytokines may contribute to the chronic anaemia of this model, peritoneal exudate cells (PEC) were isolated and challenged with PG-APS *in vitro*. Unstimulated PEC produced little or no cytokines. PG-APS challenged PEC from anaemic rats produced IL-1α, TNF-α, and IFN-γ. PG-APS challenged PEC from ARANESP™ treated anaemic rats trended towards reduced levels of these cytokines relative to anaemic PEC cultures.

Conclusion In summary, we have shown this ACD model is similar to human ACD. Importantly, ARANESP™ treatment alleviates ACD in this model, thus indicating its potential therapeutic utility for human ACD.

THU0041 DISTINCT EFFECTS OF TNFALPHA BLOCKADE FROM TNFALPHA AND LTALPHA BLOCKADE IN A PRIMATE MODEL OF SUBCUTANEOUS ABSCESS FORMATION

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Background TNFα exerts both physiologic and pathologic effects in response to infection conferring the benefit of host defense against infection at the risk of eliciting severe pathology if the response is excessive or inappropriate.

Objectives To examine whether the currently used anti-TNF therapy would affect the innate response to infections in an animal model of subcutaneous abscess formation.

Methods Cynomolgus monkeys were randomly assigned to three treatment groups (saline, etanercept, and anti-TNF α Mab) by body weight and each group contained two male and two female monkeys. Abscess formation was induced with subcutaneous inoculation of *Staphylococcus aureus*.

Results Intravenous administration of anti-TNF α Mab on days -7, -3, 1, and 4 delayed the onset and reduced the incidence and the severity of *S. aureus*-induced abscess formation as compared with the saline-treated group at both 10⁹ and 10¹⁰ CFU/ml of bacterial inocula given on day 1. In contrast, no improvement in subcutaneous abscess formation as measured by swelling scores was observed in the receptor construct-treated animals as compared with the saline-treated animals when delivered with the same regimen at the same doses as anti-TNF α Mab. Rather, treatment with receptor construct resulted in a modest increase in the incidence and severity of abscess formation with inocula of 10⁹ and 10¹⁰ CFU/ml.

Conclusion Our results provide the initial evidence that a specific anti-TNF α monoclonal antibody therapy administered at 3 mg/kg provides a beneficial effect against *S. aureus*-induced infection as shown in this non-human primate model of abscess formation.

THU0042 **IN VIVO PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS**

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Background Pro-inflammatory and anti-inflammatory cytokines play important roles in the rheumatoid activity process.

Objectives This study aims to demonstrate the cytokine profiles in rheumatoid arthritis (RA) patients with active and inactive disease process.

Methods Fifty-three Chinese rheumatoid arthritis patients (8M:45F) and 38 healthy individuals (19M:19F) were enrolled into the study. RA patients were classified as having active disease or in remission according to clinical and serological criteria (morning stiffness, joint pain, joint tenderness, joint swelling, ESR, CRP). At venepuncture, the patients and healthy controls did not have any active infective process. All except 3 patients (in remission) were on SAARDs. Sera obtained in the morning were assayed for the following cytokines: TNF α , IFN γ , TGF β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 and for rheumatoid factor (RF) by the ELISA method. HLA-DRB1*04 typing was performed using PCR-RFLP.

Results Forty-two patients had active disease and 11 patients were in remission. Rheumatoid factor was detected in 28 patients and HLA-DR*0405 allele was significantly elevated in RA patients ($p < 0.001$). TNF α , IL-6 and IL-8 were significantly elevated in RA patients when compared to controls ($p < 0.01$, $p < 0.001$, $p < 0.018$, respectively). Patients with RF positivity were noted to have much higher levels of these 3 pro-inflammatory cytokines. There was however no association with HLA-DR*0405. The anti-inflammatory TGF β was noted to be significantly lower in active patients when compared to inactive patients or controls ($p < 0.05$).

Conclusion In conclusion, the serum pro-inflammatory cytokines were significantly elevated in RA patients irrespective of their clinical disease activity while serum TGF β was significantly

depressed in active RA patients and normalises when in remission. The exact role that TGF β plays in RA is yet to be fully elucidated.

THU0043 **THE EXPRESSION OF CYCLOOXYGENASE, LIPOXYGENASE AND NEUROPEPTIDES IN MUSCLE TISSUE OF PATIENTS WITH POLYMYOSITIS AND DERMATOMYOSITIS**

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Background

Objectives To investigate pathogenesis of inflammation in muscles of patients with idiopathic inflammatory myopathies (IIM) – dermatomyositis (DM), polymyositis (PM) by studying expression of cox, lox and neuropeptides, using *in situ* hybridization and immunohistochemistry.

Methods Muscle tissue samples were obtained by needle biopsy from affected and non-affected sites distinguished by MRI-STIR technique. The second biopsy was performed after treatment. For studying cox, lox we used *in situ* hybridization with mRNA probes – antisense cox1, 2 and lox 5. Neuropeptides, such as substance P (SP), calcitonin gene related peptide (CGRP), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY), were evaluated by using immunohistochemistry.

Results 11 patients with DM or PM (9 female, 2 male) were examined. The expression of all investigated molecules (cox1, cox2, lox5, CGRP, SP, VIP, NPY) was detected on all muscle tissue. mRNA expression of cox and lox was increased in affected tissue (detectable expression was on 47 sections of 53–89%) in comparison with non-affected (40 sections of 64 $\pm$ 63%) and tissue after treatment. Expressing cells were muscle cells, inflammatory cells and vessels. Cox1 expression was found mainly on inflammatory cells and vessels, cox2 on muscle cells, inflammatory cells and vessels, lox5 mainly on muscle cells. Expression of neuropeptides was detected mainly on vessels, not on nerve cells. SP and CGRP were expressed in all tissues, VIP and NPY mainly in affected tissue. CGRP was expressed on smaller and thicker vessels in non-affected tissue and on bigger and thinner vessels in affected tissue, SP on smaller and thicker vessels in affected tissue. VIP and NPY staining was weak.

Conclusion Expression of all investigated molecules was observed in all muscle tissue samples from IIM patients. This expression was increased in affected tissues compared with non-affected and tissue after treatment, which indicates the role of cox, lox and neuropeptides in pathogenesis of IIM. Expression of neuropeptides was detected on vessels, not on nerve cells that suggests activation of vascular system in inflamed tissue.

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THU0044 **METHOTREXATE INHIBITS EX-VIVO IFN-GAMMA PRODUCTION IN WHOLE BLOOD OF RHEUMATOID ARTHRITIS PATIENTS**

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