A4.2 ADIPOCYTES MODULATE T CELL FUNCTION THROUGH RELEASE OF LIPIDS

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Background and Objectives Obesity is characterised by the presence of inflammation in adipose tissue. Accumulation of several immune cell-types, including CD4+ T cells, has been previously reported in the increasing adipose tissue. This accumulation is also parallelled by changes in cytokine profiles and phenotype of the infiltrating cells. One of the possible mechanisms involved in these changes is the modulation of T cell function by tissue-resident adipocytes. Therefore, we investigated whether adipocytes derived from various adipose tissues can modulate CD4+ T cell cytokine production and proliferation and studied the mechanisms involved in this process.

Materials and Methods CD4+ T cells were purified from peripheral blood mononuclear cells using magnetic beads coated with anti-human CD4. Plate-bound anti-CD3 and soluble anti-CD28 antibodies were used to activate T cells. Adipocytes were isolated from IPF of OA patients by collagenase digestion and were either cultured with purified CD4+ T cells or were cultured in vitro for 24 hours in DMEM/F12 medium supplemented with 0.5% bovine serum albumin to generate adipocyte-conditioned medium (ACM). Cytokine/adipokine production was measured by intracellular cytokine staining (ICS), ELISA or cytokine multiplex. Lipids were isolated using hexane and lipid profiling was performed by liquid chromatography combined with mass spectrometry.

Results CD4+ T cells produced increased levels of IFNγ when activated in the presence of adipocytes. This effect is mediated by soluble mediators, as shown in transwell and adipocyte-conditioned medium (ACM) transfer experiments. Additionally, ACM induced increased proliferation of CD4+ T cells upon activation. Furthermore, adipose tissue contained more IFNγ-producing CD4+ T cells than peripheral blood of the same individuals, in 3 out of 3 cases tested, which indicates a possible in vivo relevance of our results.

To investigate the possible molecular mechanisms involved in this effect, we separated the protein and lipid fraction of ACM. Surprisingly, despite previous data indicating that several adipocyte-derived proteins can modulate T cell function, we have found that the increased proliferation of T cells is mainly due to the lipids isolated from ACM. Further separation of these lipids based on polarity revealed that the modulatory effect is mainly confined to fractions containing free fatty acids. All identified fatty acids were able to individually enhance T cell proliferation.

Conclusions These data indicate that adipocytes can modulate CD4+ T cell function through release of soluble mediators. Remarkably, within the soluble mediators identified, lipids and especially free fatty acids are the most prominent modulators of T cell proliferation.

A4.3 ADIPOCYTES MODULATE THE PHENOTYPE OF MACROPHAGES THROUGH SECRETED LIPIDS

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Conclusions These data indicate that adipocytes can modulate CD4+ T cell function through release of soluble mediators. Remarkably, within the soluble mediators identified, lipids and especially free fatty acids are the most prominent modulators of T cell proliferation.

A4.4 CIRCULATING METASTASIS PROMOTING PROTEIN S100A4 IN IDIOPATHIC INFLAMMATORY MYOPATHIES

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Background and Objectives Metastasis promoting protein S100A4 is involved in the pathogenesis of tumours and chronic autoimmune diseases such as rheumatoid arthritis (RA) and psoriasis. We recently described increased expression of S100A4 in inflamed muscle tissue in patients with idiopathic inflammatory myopathies (IIM). Since circulating levels of S100A4 correlate with disease severity in RA patients, we therefore evaluated the association between S100A4 protein and disease activity in patients with IIM and compared S100A4 serum levels in myositis patients and healthy controls in this study.

Methods Serum levels of S100A4 protein were determined in 43 dermatomyositis (DM), 39 polymyositis (PM) and 22 cancer associated myositis (CAM) patients and in 89 healthy controls. In 11 patients (4 DM, 3 PM, 4 CAM), S100A4 serum levels were measured before and after the start of treatment. The associations between S100A4 levels, inflammation, disease activity and muscle strength were examined. Disease activity was assessed using Disease Activity Core Set Measures developed by International Myositis Assessment & Clinical Studies Group (IMACS). Serum levels of
C-reactive protein (CRP) and muscle-associated enzymes creatine phosphokinase (CK) and lactate dehydrogenase (LD) were measured by routine laboratory methods.

**Results** In patients with FM, serum levels of S100A4 protein were significantly higher than those observed in healthy controls or DM patients (148.6 ± 351.5 versus 80.75 ± 285.1, p < 0.01 and 43.55 ± 53.03, p < 0.05, respectively). No significant differences in S100A4 levels were found between CAM patients (119.9 ± 414.0) and healthy controls or other myositis patients. In the whole group of IIM patients, serum S100A4 levels correlated with MYOACT score (r = 0.39; p < 0.001) and its components Constitutional Disease Activity (DA) (r = 0.34; p < 0.001) and Pulmonary DA (r = 0.44; p < 0.001). Serum S100A4 correlated also with Muscular DA (r = 0.25; p < 0.05), CK (r = 0.33; p < 0.01) and LD (r = 0.40; p < 0.001). S100A4 levels correlated with Cutaneous DA (r = 0.46; p < 0.01) in DM patients and with Extramuscular Global Assessment only in PM patients (r = 0.55; p < 0.001). No significant correlations of S100A4 serum levels in patients with CAM were found. In the 11 longitudinal IIM samples there was no significant decrease of S100A4 serum levels observed. Multiple regression of the whole IIM patients group showed significant association of S100A4 serum levels with Pulmonary DA (β = 0.369; p < 0.01), LD (β = 0.545; p < 0.01) and severity of dysphagia (β = -0.250, p < 0.05). In PM patients, S100A4 levels were associated with Extramuscular Global Assessment (β = 0.552; p < 0.01) and in DM patients with MYOACT (β = 0.557; p < 0.01) and CRP (β = 0.391; p < 0.05).

**Conclusions** This is the first study showing that circulating levels of S100A4 are associated with several features of IIM disease activity, particularly with extramuscular components. We did not find any association of S100A4 levels and cancer associated myositis. Further studies analysing bioactive form of S100A4 and the role of S100A4 in cancer associated myositis are needed.

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**A4.6 EFFECTS OF CHOLIC ACID AND ITS DERIVATIVES IN EXPERIMENTAL ARTHRITIS**

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**Introduction** Bile acids play an important role in cholesterol metabolism and act as intestinal detergents for digestion and absorption of fats and fat-soluble vitamins. Disruption of bile flow causes cholestatic liver diseases. Derivatives of cholic acid (CA) such as nor-ursodeoxycholic acid (norUDCA) are promising therapeutic agents in the treatment of cholangiopathies. Previous studies also demonstrated anti-inflammatory and anti-fibrotic properties of norUDCA in experimental sclerosing cholangitis.

**Objective** To investigate the anti-inflammatory potential of CA and its derivatives ursodeoxycholic acid (UDCA) and norUDCA in in Collagen-induced arthritis (CIA), an animal model for inflammatory, erosive arthritis.

**Methods** Mice were prophylactically treated with CA, UDCA or nor-UDCA enriched diet pellets (5 mg/kg diet) or standard diet pellets (Placebo) ad libitum starting 1 week before the first immunisation with collagen. Animals were weekly assessed for clinical signs of arthritis, body weight and food consumption during the experimental period. After 10 weeks of treatment hind paws, liver, sera and lymph nodes were isolated for further analysis. Sera were investigated for anti-collagen antibodies, cytokine responses and liver parameters such as alkaline phosphatase (AP) and alanine transaminase (ALT). Paraffin-sections of hind paws were examined for histopathological changes in synovial inflammation, subchondral bone erosion, cartilage damage and osteocyte formation. Cell populations within synovial pannus were identified by immunohistochernical stainings and were determined using HistoQuest software (from TissueGnostics).

**Results** Uptake of CA, UDCA and norUDCA was confirmed by serum analysis. Prophylactic treatment of CIA mice with UDCA and norUDCA could not significantly prevent disease incidence. In contrast, treatment with CA led to a marked increase in disease incidence and severity compared to Placebo treated animals. Whereas UDCA and norUDCA showed a similar course of clinical signs of progression were determined using a multivariate normal regression model (EAC cohort) or by generalised estimated equations (GARP cohort). Adjustments were made for age, gender, treatment strategy and Body Mass Index (BMI).

**Results** In RA patients totAPN associated positively with radiographic progression (Sharp van der Heijde scores) (association estimate 3.65, p = 0.002), whereas in patients with hand OA, totAPN associated negatively with radiographic progression (joint space narrowing (JSN)) (Odds 0.24/Odds 0.21, p = 0.002/p = 0.002 two highest tertiles compared to the lowest tertile). HmwAPN on the other hand, did not associate significantly with radiographic progression in patients with hand OA or RA, although in patients with RA we did observe a trend towards a positive association (association estimate 1.55 p = 0.07) upon correcting for age, gender and treatment strategy. This trend was lost after further adjustment for BMI. Similar results were obtained when joint space narrowing (JSN) was used as outcome measurement.

**Conclusions** Our data further substantiate the connexion between APN-levels and radiographic progression in rheumatic disease and indicate that the differential effects associated between totAPN and radiographic progression in either in RA and hand OA is not mediated by (a selective effect of) hmwAPN.