AB0052  
TREATMENT WITH BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN REDUCES CRISTAL INFLAMMATION AND COLLAGEN-INDUCED ARTHRITIS IN MICE  
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Background: Bacterial/permeability-increasing protein (BPI) is an antibacterial glycoprotein produced by polymorphonuclear cells (PMN). Although BPI has been detected in synovial fluid of patients with different type of arthrits, its effects in these diseases remain unexplored.  

Objectives: To investigate the effects of BPI in mouse models of crystal-induced inflammation and collagen-induced arthritis (CIA).  

Methods: Arthritis models were raised on the backs of CD1 mice (n=14 per condition). 2 mg of calcium pyrophosphate (CPP) crystals in 1 ml of PBS were injected into the pouch in the presence or absence of 0.1 mg of BPI for 3 hours. Controls received 1 ml of PBS. After the sacrifice, pouch fluids were recovered by washing with 2 ml of PBS. Leukocyte count in lavage fluids was obtained using a hemocytometer and the PMN percentage was determined by May-Grünwald-Giemsa staining.  

Results: The injection of CPP crystals into the pouches induced leukocyte infiltration (26.27±4.14 × 10^6 cells/ml) comprising 73.5%±12.2% of PMN. IL-1β (80.99±3.65 pg/ml), IL-6 (892.90±28.14 pg/ml), CXCL1 (762.82±50.08 pg/ml) and TNFα (52.70±4.99 pg/ml) were measured in lavage fluids. The co-injection of crystals with BPI decreased histological scores for pannus formation and inflammation by 56%, cartilage and bone destruction in knee and ankle joints (score 0–5). IL-1β, IL-6, CXCL1 and TNFα levels were measured by ELISA in pouch fluids and serum from collected blood.  

Conclusions: This study shows inhibitory effects of BPI on crystal-induced inflammation and CIA, supporting a therapeutic potential of this protein for arthritis by down-regulating inflammatory process.  

Disclosure of Interest: None declared  

AB0054  
PDE4 INHIBITOR ATTENUATION OF IL-23 SECRETION FROM MONONUCLEAR CELLS  
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Background: IL-23 is a cytokine heavily implicated in the immunopathology of both psoriasis and psoriatic arthritis. Emerging evidence suggest IL-36g, a novel inflammatory cytokine which has been heavily implicated in psoriatic immunopathology, is able to stimulate the release of IL-23.1 However, the ability to translate this knowledge into human subjects is presently unknown. Additionally, any potential relationship between IL-36g and IL-23 is not well understood.  

Objectives: To determine the bioactivity of TGFβ in SSC serum  

Methods: Blood mononuclear cells from healthy patients, (n=5) were pre-treated with either bacterial or fungal toll like receptor adjuvants and subsequently treated with either anti-TGFβ1/2/3 for 1 hour at RT before use.  

Results: Control sera significantly induced reporter activity by 3.5-fold. However, SSc sera only induced a 2.5-fold increase in luciferase activity, indicating significantly lower bioactivity of TGFβ (p<0.0001). This difference was not due to a difference in total TGFβ levels; after activation of all TGFβ both HC and SSc sera induced a similar 8-fold increase in signal strength. These data show that in HC sera approximately 75% of all TGFβ is bioactive compared to only 42% in SSc sera. The anti-TGFβ1/2/3 induced a decrease in bioactivity (p<0.0001) of both HC and SSc serum, and of both acidified and not acidified sera (p<0.0001), showing that our bioassay is indeed TGFβ-dependent. To investigate if reduced bioactivity is a more general phenomenon we measured BMP activity. BMP proteins are structurally closely related to TGFβ and also circulate in inactive form. Both HC and SSc sera induced a similar 8-fold increase in BRE-1 activity, and this activity was increased to a 16-fold induction after acidification for both sera. BMPs in SSc sera are thus not less bioactive. This illustrates the uniqueness of our observation on TGFβ bioactivity.  

Conclusions: TGFβ in SSc serum is less bioactive than in control serum whereas BMPs are not less bioactive.  

Disclosure of Interest: None declared  

AB0053  
A BIOASSAY TO MEASURE TGFβ ACTIVITY REVEALS DECREASED TGFβ ACTIVITY IN SSC SERUM  
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Background: Systemic sclerosis (SSc) is a severe disease characterised by auto-immunity, vasculopathy and excessive fibrosis of connective tissues. The pathophysiology of SSc is still poorly understood, but its symptoms imply a role for auto-immunity, vasculopathy and excessive fibrosis of connective tissues. The dysregulated transforming growth factor (TGFβ) signalling: e.g. this cytokine is known to regulate vascular and connective tissue biology. TGFβ circulates in both inactive and active forms bound to latency-associated peptide and latent TGFβ1/2/3 binding proteins. This latent TGFβ1/2/3 first has to be activated before it can become bioactive. With the use of a bioassay, TGFβ’s bioactivity can be measured in a complex mixture like serum, unlike with an ELISA which cannot take cellular activation processes into account.  

Objectives: To determine the bioactivity of TGFβ in SSC serum compared to that of healthy controls  

Methods: Serum was collected of 10 SSc patients and 10 age and sex-matched healthy controls. Primary human fibroblasts of 3 donors were transduced with CAGAα-luc—which produces luciferase in response to TGFβ/Smad3 or BRE-luc which produces luciferase in response to BMP/Smad1/5. These cells were treated with 10% serum for 16 hour and luciferase activity was measured. To activate all TGFβ, sera were treated with 4M HCl for 1 hour at RT, after which pH was normalised with 4M NaOH. Controls were treated with HCl and NaOH simultaneously. To verify that TGFβ signalling was measured in this reporter assay, sera were treated with anti-TGFβ1/2/3 for 1 hour at RT before use.  

Results: Control sera significantly induced reporter activity by 4.5-fold. However, SSc sera only induced a 2.5-fold increase in luciferase activity, indicating significantly lower bioactivity of TGFβ (p<0.0001). This difference was not due to a difference in total TGFβ levels; after activation of all TGFβ both HC and SSc sera induced a similar 8-fold increase in signal strength. These data show that in HC sera approximately 75% of all TGFβ is bioactive compared to only 42% in SSc sera. The anti-TGFβ1/2/3 induced a decrease in bioactivity (p<0.0001) of both HC and SSc serum, and of both acidified and not acidified sera (p<0.0001), showing that our bioassay is indeed TGFβ-dependent. To investigate if reduced bioactivity is a more general phenomenon we measured BMP activity. BMP proteins are structurally closely related to TGFβ and also circulate in inactive form. Both HC and SSc sera induced a similar 8-fold increase in BRE-1 activity, and this activity was increased to a 16-fold induction after acidification for both sera. BMPs in SSc sera are thus not less bioactive. This illustrates the uniqueness of our observation on TGFβ bioactivity.  

Conclusions: TGFβ in SSc serum is less bioactive than in control serum whereas BMPs are not less bioactive.  

Disclosure of Interest: None declared  