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AB0161 ANTIPHOSPHOLIPID ANTIBODIES DIFFERENTIALLY REGULATE THE EXPRESSION & ACTIVITY OF THE LYSOSOMAL PROTEASES WITH EFFECTS UPON MONOCYTE AUTOPHAGY

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Background: Antiphosholipid antibodies (aPLs) are known to activate monocytes in the pathogenesis of antiphospholipid syndrome (APS), although the precise mechanisms by which this activation occurs are not fully understood. We have recently identified several novel protein targets using a comprehensive proteomic analysis of human monocytes treated with IgG from patients with APS. Amongst these novel targets lysosomal proteases, including cathepsin B and cathepsin D were identified. These proteases are important in protein degradation, clearance of autolysosomes, apoptosis and autophagy. Dysregulation of these homeostatic cellular functions may be important in the exposure of autoantigens and pathogenesis of the APS. Therefore, we have now studied the effects of APS IgG upon the expression/activity of different cathepsins and their effects upon autophagy.
Objectives: Determine the effect of pathogenic aPL antibodies on monocyte autophagy and its association with the regulation of lysosomal activity.
Methods: Healthy monocytes were treated with 200 μg/ml of IgG purified from (n=9) patients with APS or (n=9) healthy control (HC) IgG for 6 h. The expression of cathepsin B and cathepsin D were measured by western blotting. Activity assays for lysosomal proteases cathepsin D, cathepsin B and cathepsin L were performed using fluorescence based assays (RayBio®). Intracellular proteolytic activity of monocytes was determined using DQ-BSA (Molecular probes) and flow cytometry analysis. Autophagy was induced by treating monocytes with 50 μg/ml GM-CSF for 14 h.
Results: Consistent with our previous label free quantification mass spectrometry proteomic analysis, western blot analysis confirmed that levels of cathepsin B and cathepsin D were decreased in monocytes treated with APS IgG compared to HC IgG. Similarly, enzymatic assays revealed that cathepsin B and cathepsin D activities were significantly reduced in monocytes treated with IgG from patients with APS compared to HC (p=0.0188, 0.0323). In contrast, levels of enzymatic activity of cathepsin L were increased in monocytes treated with APS IgG compared to HC IgG (p=0.0106). To determine the effect of APS IgG on autophagy, we exposed healthy monocytes to IgG and induced autophagy by treating them with GM-CSF for 14 h. Subsequently we tested the intracellular proteolytic activity with DQ-BSA. Stimulation of monocytes with APS IgG reduced the lysosomal activity of GM-CSF-treated monocytes whereas HC IgG had no effect, indicating that APS IgG disrupts lysosomal degradation during monocyte autophagy.
Conclusions: We found that IgG from patients with APS regulate the expression and activity of lysosomal proteases cathepsins B/D and cathepsin L in opposite directions. Activity of cathepsin B and D was down-regulated by exposure to IgG from patients with APS whereas cathepsin L was up-regulated. Furthermore, we found APS IgG disrupts lysosomal degradation during monocyte autophagy. Additional experiments are now underway to increase our understanding of how modulation of cathepsin activity and autophagy may be important in the pathogenesis of APS and provide new therapeutic targets.
Disclosure of Interest: None declared
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AB0162 SERUM LEVEL OF PROINFLAMMATORY CYTOKINES IS NEGATIVELY ASSOCIATED WITH FATIGUE IN PRIMARY SJÖGREN'S SYNDROME

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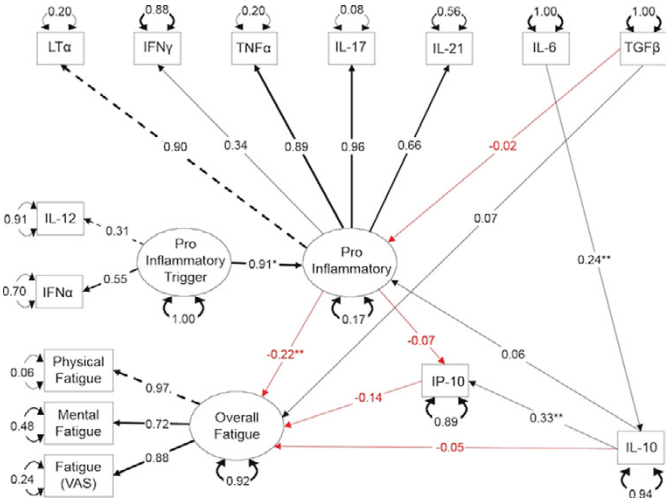
Background: We have previously described a model of fatigue in patients with primary Sjogren's syndrome (PSS) based on the levels of serum cytokines, pain and depression scores. Importantly, removal of cytokines from this model substantially reduced the accuracy suggesting that cytokines may have a key role in the biological basis of fatigue [1]. However, interpreting the model is complicated by the complexity of the immune system and the likely multiple interactions between numerous cytokines and other variables [2]. Structural equation modelling (SEM) is a statistical technique that allows for analysis of one or multiple independent variables with one or multiple dependent variables. SEM consists of two components – the structural model, which represents the relationships between the theoretical variables, and the measurement model, which are the relationships between the latent variables and their measures [3].
Objectives: To use SEM to test our hypothesis that the balance between pro-inflammatory and anti-inflammatory cytokines play an important role in determining severity of fatigue in patients with PSS.
Methods: We used Canonical Correpondence Analysis (CCA) to investigate the variation in cytokine expression across our spectrum of fatigue patietns to explore interactions and dependencies between cytokines. We then built a conceptual model based on the literature representing the likely relationships between fatigue and various proinflammatory and anti-inflammatory cytokines and other soluble molecules in the serum. This conceptual model was then challenged using serum data and fatigue scores of 161 PSS patients from the UK primary Sjogren's syndrome registry. Model fit was assessed using the Confirmatory Factor Index, the Root Mean Square Error of Association and the Standardised Root Mean Square Residual. We also analysed changes in fatigue scores over a period between 1–4 years.
Results: CCA revealed the first axis of ordination (CCA1) broadly correlates with fatigue, consists of many pro-inflammatory cytokines including TNFα, LTα and IFNγ, IL17, which were negatively correlated with fatigue while IL-6 and MCP1, which were positively associated with increased fatigue severity. The second axis (CCA2) reflects a trend in cytokines which appear to relate to patients' age. Fatigue scores were largely stable over time and therefore data were not included in the SEM analysis. The main pro-inflammatory SEM model showed fatigue was negatively associated with pro-inflammatory cytokine activity (p=0.019); IL-10 drove IP-10 (p=0.000); and IL-10 was driven by IL-6 (p=0.006) (Fig. 1)



Conclusions: Chronic fatigue in PSS is negatively associated with many pro-inflammatory cytokines. We hypothesize that it reflects adaptive biological processes, which occurs after chronic exposure to inflammation in conditions such as PSS.
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AB0163 MESENCHYMAL STEM CELLS INDUCE CD1C+ TOLEROGENIC DENDRITIC CELLS IN HUMAN SYSTEMIC LUPUS ERYTHEMATOSUS VIA UP-REGULATING FLT-3 LIGAND

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Background: $CD1c^+$ tolerogenic dendritic cells (DCs) play important roles in the induction of peripheral tolerance and control of adaptive immune response. Umbilical cord (UC)-derived mesenchymal stem cells (MSCs) exhibit immunoregulation effects in systemic lupus erythematosus (SLE). However, the underlying immunosuppression mechanism of MSCs via tolerogenic DCs in SLE remains largely unknown.
Objectives: The aim of this study was to examine tolerogenic DCs levels in SLE patients, and to further investigate the mechanism of MSCs in the regulation of tolerogenic DCs.
Methods: Tolerogenic DCs were isolated as Lin $(CD3/19/56/14)^-$ HLA $DR^+CD11c^+CD1c^+$ from peripheral blood mononuclear cells (PBMCs). Levels of tolerogenic DCs were determined by flow cytometry, and serum concentration of Flt-3 ligand (FLT3L) were determined by ELISA from 17 healthy controls and 25 SLE patients. Eight SLE patients were given UC MSCs infusions. We compared the levels of tolerogenic DCs and serum FLT3L before and 24 hours after UC

MSCs transplantation. PBMCs from 8 patients were collected and co-cultured with UC MSCs at ratios of 1:1, 10:1 and 50:1, for 24 hours, 48 hours and 72 hours, respectively, to detect the levels of tolerogenic DCs. The FLT3L in the supernatant solution were determined. FLT3L siRNA was added to the co-culture system, and the level of tolerogenic DCs were detected.
Results: The levels of peripheral $CD1c^+$ DCs and serum FLT3L were significantly decreased in SLE patients compared to healthy controls. After UC MSCs transplantation, the levels of $CD1c^+$ DCs increased, along with an increase in serum FLT3L. In vitro studies showed that UC MSCs time-dependently up-regulated peripheral $CD1c^+$ DCs, but not dose-dependently. The supernatant FLT3L level significantly increased after co-cultured with MSCs. However, the addition of FLT3L siRNA significantly abrogated the up-regulation of $CD1c^+$ DCs by MSCs.
Conclusions: UC MSCs induce $CD1c^+$ tolerogenic DCs through up-regulating FLT3L in lupus patients.
Disclosure of Interest: None declared
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Scleroderma, myositis and related syndromes - etiology, pathogenesis and animal models

AB0164 AMYLOIDOSIS IN PROGRESSIVE SYSTEMIC SCLEROSIS – A POSTMORTEM CLINICOPATHOLOGIC STUDY OF 12 PATIENTS

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Background: Systemic sclerosis (SSc), like all chronic autoimmune disorders, may be complicated by AA amyloidosis (AAa). An associated B-cell lymphoma my cause amyloid λ or κ light-chain deposition and AL amyloidosis (ALa) in SSc as well.
Objectives: The aim of this study was to determine the prevalence and type of amyloidosis in SSc patients, appraise the extent of amyloid deposits in various organs.
Methods: 12 patients (females 11, age: 54.82 years, range 66–32, onset of SSc: 48.86, disease duration: 6.43 years; one male, age: 65.0 years at death, onset of SSc and disease duration not known) were studied. All patients were autopsied. SSc was diagnosed clinically according to the criteria of the ACR [1].
Amyloid deposits on different tissue structures [arteriole, small artery, medium size artery, venule, small vein, medium size vein, interstitial collagen fiber, reticulin fiber (collagen IV), basal lamina, nerve, renal glomerulus] of 6 organs [heart, lungs, kidney, gastrointestinal tract, skin and brain] were determined histologically. The extent of amyloid A deposits was evaluated by semi-quantitative, visual estimation on a 0 to 3 plus scale, based on the number of involved tissue structures per light microscopic field [2] ("0": no amyloid deposits, "1": Sporadic, minimal amyloid deposits on different tissue structures, "2": less than five, "3": five or more involved tissue structures per microscopic field at objective magnification of x20)
The prevalence and extent of amyloid deposits in various organs were compared by Student (Welch) t-probe.
Results: Systemic AL-l light-chain amyloidosis was diagnosed in 1 (8.0%) 67 year old female patient (onset of SSc: 66 years, disease duration 1 year), and systemic AAa in 1 (8.0%) 53 year old female patient (onset of SSc: 41 years, disease duration 12 years).
The prevalence (in %) and the average extent of AL-l light-chain and amyloid A deposits (absolute value) in various organs of SSc patients are summarized in Table 1.

Tab;e 1

Organs	SSc-λ Prevalence in %	SSc-AAa Prevalence in %	p<	SSc-λ Average extent	SSc-AAa Average extent	p<
Skin	80.00	0.00	**0.0001**	1.45	0.00	**0.0002**
Heart	72.73	63.64	0.3329	1.45	0.91	0.117
Kidney	63.64	63.64	0.1228	1.09	0.73	0.185
G-I Tract	41.67	66.67	0.1185	1.08	1.00	0.433
Lung	40.00	40.00	0.5000	1.00	0.50	0.077
Brain	0.00	0.00		0.00	0.00	–
Average/Organ	50.79	41.27	**0.052**	1.040	0.556	**0.0036**
Average/Patient	45.56	35.96	0.144	0.965	0.494	**0.0006**

Conclusions: AL-λ deposits were present earlier and were more prominent in the skin, heart and kidney, than in the, G-I tract and the lung. Amyloid A deposits were present earlier and were more prominent in the G-I tract, heart and kidney, than in the, lung and the skin. In the brain AL-λ and amyloid A deposits were absent in both diseases.
Higher prevalence (80.0% versus 0.0%; $p<0.0001$) and massive AL-λ deposition in the skin (1.45 versus 0.0; $p<0.0002$) may be explained by qualitative differences of AL-λ and amyloid A and by a diverse affinity of circulating amyloid precursors to the qualitative changed interstitial collagen and reticulin fibers. In systemic sclerosis patients qualitative change of collagens has been demonstrated [3–5].

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AB0165 CXCL4 MAY PLAY A KEY ROLE IN SYSTEMIC SCLEROSIS BY DRIVING CD4 T CELLS TO PRODUCE IL-17

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Background: CXCL4 regulates multiple facets of immune response and its level is highly increased in various Th17-associated rheumatic diseases, including systemic sclerosis (SSc)[1–4]. Recently, CXCL4 was shown to induce type I interferon production as well as endothelial activation in SSc patients[1]. Th17 skewing has been demonstrated in SSc[5–6], however, whether CXCL4 plays a role in the induction of IL-17 production by human CD4 T cells is currently unclear.
Objectives: To investigate the effect of CXCL4 on human CD4 T cell phenotype in particular IL-17 production in the absence or presence of antigen presenting cells.
Methods: Blood was obtained from healthy donors and CD4 T cells, monocytes, dendritic cells, were isolated using magnetic-based sorting (n=20). In addition, CD4 T cells from SSc patients were isolated (n=10). CD4 T cells were activated using anti-CD3/CD28, or in co-cultures with antigen presenting cells, stimulated using superantigen Staphylococcal enterotoxin B. Exogenous recombinant human CXCL4 was added during (co-)culture in different concentrations. Cytokine production and proliferation were analyzed using Luminex immunoassays, intracellular cytokine staining, and flow cytometry.
Results: CXCL4 directly induced CD4 T cells secreting both IL-17 and IFN-γ, as well as IL-22, when costimulated with anti-CD3/CD28 ($p<0.05$). In many SSc patients, similar IL-17 increase upon CXCL4 treatment was observed, although this did not reach statistical significance. This might be due to the fact that CD4 T cells from SSc patients had already significant higher levels of IL-17 as compared to healthy donors (2182±722.2 vs 1053±263.6 pg/ml, mean±SEM, $p<0.05$). Furthermore, in co-culture system of CD4 T cells with monocytes or myeloid dendritic cells, CXCL4 treatment induced IL-17 production upon triggering by superantigen ($p<0.05$). Moreover, when monocyte-derived dendritic cells were differentiated in the presence of CXCL4, they orchestrated significantly increased levels of IL-17, IFN-γ, and proliferation by CD4 T cells (all $p<0.05$).
Conclusions: Altogether, we demonstrate that CXCL4 boosts pro-inflammatory cytokine production especially IL-17 by human CD4 T cells, either by acting directly or indirectly via antigen presenting cells. This indicates that targeting CXCL4 may potentially alleviate immune responses in Th17-mediated rheumatic diseases such as systemic sclerosis.
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AB0166 INTERLEUKIN-6-POLARISED MACROPHAGES PROMOTE DERMAL MYOFIBROBLAST DIFFERENTIATION

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Background: Macrophages and fibroblasts are key effector cell types present in scleroderma tissue[1]. While the effect of scleroderma fibroblast conditioned medium on macrophages has been previously studied[2], less is known about the effect of scleroderma macrophages on fibroblasts. Interleukin-6 (IL-6) is an important mediator of fibrosis and is overexpressed in scleroderma sera and cells such as skin fibroblasts, monocytes and endothelial cells[3]. Given the increase in IL-6 levels in scleroderma and presence of the IL-6 receptor on the cell surface of macrophages, we are now investigating the phenotype of macrophages exposed to IL-6. Here we present our work into the paracrine function of IL-6-treated macrophages in stimulating fibroblasts using a media transfer approach.