FGFR1 inhibitor, (250nM). Medium conditioned by re-cut and loaded cartilage contained bFGF (as assessed by ELISA) and activated ERK in isolated monolayer chondrocytes. This activation was inhibited in the presence of neutralising antibodies to bFGF. bFGF mediates a rapid graded ERK activation in articular cartilage over a wide range of loads. This may be relevant to cartilage function during normal or excessive weight bearing. Increased matrix turnover, which is observed in osteoarthritic cartilage, may be in part mediated by bFGF.

M126

Expression and function of Monocyte Chemoattractant Protein-3 (MCP-3) in Scleroderma

Voon H. Ong, Lowri A. Evans, Vineeth Rajkumar, Xu Shiwen, David J. Abraham, Carol M. Black, Christopher P. Denton

Centre for Rheumatology, Royal Free Hospital, London NW3 2QG, UK

We have previously shown that monocyte chemoattractant protein-3 (MCP-3), a CC chemokine, is overexpressed in the type 1 light skin mouse model (Tsk1) of scleroderma (SSc). The present study extends this by examining expression and function of MCP-3 in human SSc. Immunohistochemical studies were performed on skin biopsies from patients with early stage (n=14) and established diffuse cutaneous SSc (dcSSc; n=8), limited cutaneous SSc (lcSSc; n=6) and healthy controls (n=11). All SSc patients demonstrated an increased expression of MCP-3, while all healthy controls demonstrated no staining. The remaining had Sc170 or U1RNP specific antibodies in equal proportions. In the dcSSc subset, 52% of the patients carried anti-Sc170, 9% had anti-RNA polymerase I or III reactivity. There was significantly greater MCP-3 expression in skin biopsies from patients with early dcSSc compared to established dcSSc or lcSSc, with dermal and vascular localization observed in 10 (70%) early stage patients and 2 (25%) patients with established dcSSc. This staining was absent from healthy control skin. Mononuclear inflammatory infiltration, associated with MCP-3 expression, was identified in 14 (64%) dcSSc samples. Additional specific immunostaining for MCP-3 was also observed in the lower epidermal layer of controls and SSc biopsies. To investigate whether this chemokine might be profibrotic as well as promoting leucocyte trafficking, MCP-3 induced activation of collagen gene expression was examined in a transient transfection assay using human proα2(1)collagen promoter-reporter constructs in dermal fibroblasts. There was promotion (90%basal expression±SEM) of collagen reporter gene expression (152±14%, p<0.05 Student’s unpaired t-test) with a threshold MCP-3 concentration of 200ng/ml. This effect was partially blocked by a pan-specific anti-TGFβ antibody (mean±SEM, % change 31.0±6.7%, p<0.05, Student’s unpaired t-test). Our results confirm overexpression of MCP-3 in early stage SSc and suggest a potential for activating extracellular matrix gene expression via induction of TGFβ, in addition to its recognised effects on leucocyte trafficking.

M127

Relative importance of different human aPL derived heavy and light chains in the binding of aPL to cardiolipin

Ian P.Giles MRCP1,2; Joanna Haley BSc1,2; Sylvia Nagl PhD3; David S.Latchman DSc, FRCPath1; Pojen P Chen PhD4; Reginald U. Chukwuocha PhD1; David A.Isenberg MD, FRCP1 and Anisur Rahman PhD, MRCP1,2

1 Centre for Rheumatology, Department of Medicine, University College London, UK
2 Medical Molecular Biology Unit, Institute of Child Health, University College, London, UK.
3 BBSRC Centre for Structural Biology, Department of Biochemistry and Molecular Biology, University College London, UK.
4 Department of Medicine, Division of Rheumatology, University of California, Los Angeles, USA

Introduction Previous studies have suggested the importance of somatic mutations and arginine, asparagine and lysine residues in the complementarity determining regions (CDR’s) of antiphospholipid antibodies (aPL) implicated in the pathogenesis of the antiphospholipid antibody syndrome. The relative contributions of the heavy and light chains of aPL in binding to cardiolipin (CL) were assessed by pairing the heavy and light chains of two IgG, β2GPI dependent aPL (IS4 and CL24) with different partner chains from other IgG, β2GPI independent aPL (UK4) and anti-DNA antibodies (B3 & 33H11). Subsequently chimeric light chains combining CDR’s derived from different antibodies were tested in the same way.

Methods Heavy (VH) and light (VL) chain variable sequences from these antibodies were cloned into expression vectors containing appropriate γ, λ or κ constant region cDNA. This allowed transient expression of whole IgG molecules in COS-7 cells. These were harvested and tested for the ability to bind CL and DNA by ELISA. Results Whole IgG was produced from 19 heavy/light chain combinations. IS4 Vh was dominant in conferring the ability to bind CL. The identity of the VH region paired with IS4 VH was important in determining the strength of binding to CL. IS4 Vh contains multiple arginine residues in CDR3, which may have accumulated due to antigen driven selection. It is likely that these arginine residues may interact with CL. Tests on chimeric light chains confirmed that arginine in B3 VL, CDR1 are also likely to enhance binding to CL, whereas an arginine in UK4 VL CDR3 blocks binding. Computer models of the heavy/light chain combinations supported these hypotheses

Conclusion Whole IgG molecules capable of binding CL were produced by in-vitro expression in COS-7 cells. Arginine residues play important roles in binding to CL and double stranded DNA. However, different patterns of mutation to arginine are associated with binding to each of these antigens and the positions occupied by the arginine residues are critical.