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Differential activation of complement in rheumatoid arthritis

Background An atypical subgroup of patients with seropositive rheumatoid arthritis (RA) has been identified with active disease but normal levels of the acute phase protein C-reactive protein (CRP), considered an accurate marker of disease activity. Previously we identified that patients with normal CRP (nCRP) during flares of RA had an altered immunological profile, had diagnostic delays and seemed to respond less well to conventional treatment compared to RA patients who mounted an appropriately high CRP (hCRP) response. Activation of the complement pathway has been associated with disease activity in RA patients and it is suggested both the presence of autoantibodies and activation of complement are required during the inflammatory processes that lead to joint damage. CRP plays a crucial role in the regulation of complement activation via both the alternative and classical pathways, either through interaction with C4 binding protein or degrading C3 fragment-C3b to C3i via factor H. However, it is not known whether these pathways are altered in RA patients with nCRP compared with hCRP during active flares.

Objectives To investigate how altered CRP response may differentially regulate C3 cleavage in RA patients with nCRP compared to hCRP levels during flares of RA.

Methods 24 RA patients with active synovitis were recruited, defined by ≥ 1 joint with Power Doppler detected by US, 15 had normal (n)CRP (≤ 5 mg/L) and 9 had high (h)CRP (> 5 mg/L) levels. Serum and detailed clinical data were collected. 18 age and sex matched healthy donors (HCs) were also analysed. Serum was subjected to SOMAScan Proteomic Assay. Complement components were analysed by Western blot following 1:400 serum dilution and assessed for C3/C3a and albumin expression. Densitometric analysis was applied to the Western blots and the C3a values were normalised against albumin, resultant values were expressed as fold change from HC. Results were correlated with clinical and disease features using linear regression curves in Prism.

Results Proteomics identified differential expression of complement components in serum from hCRP compared to nCRP patients; specifically a significant upregulation of alternative complement pathway factors (eg Factors I, H and B) was seen in hCRP patients and a downregulation of kallistatin, an inhibitor of the classical pathway in nCRP patients. Average C3 cleavage product was 4.8 (0.18–14.4) for hCRP and 3.05 (0.28–6.6) for nCRP, both significantly higher compared to HCs ($p < 0.01$ HC vs nCRP, $p < 0.05$ HC vs hCRP). The levels of C3 cleavage were then correlated against ESR, CRP and anti- Anti-cyclic citrullinated peptide (CCP) levels in both sets of patients. In hCRP patients, strong correlations ($R^2 > 0.5$) were observed for C3 vs ESR ($p < 0.05$), C3 vs CRP ($p < 0.03$) and ESR vs CRP ($p < 0.0003$) but no correlation was found between C3 and levels of anti-CCP antibodies ($R^2 = 0.208$, $p = 0.255$). In contrast nCRP patients demonstrated a strong correlation between C3 levels vs anti-CCP antibodies ($R^2 = 0.53$, $p < 0.05$) whilst no correlation was seen with CRP or ESR levels ($R^2 = 0.0004$, $p = 0.236$).