A unique immune signature in patients with active rheumatoid arthritis but normal C-reactive protein levels suggests an altered pathogenic mechanism

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Background: An atypical subgroup of patients with seropositive rheumatoid arthritis (RA) with active disease, but normal levels of acute phase protein C-reactive protein (CRP) was identified using ultrasound (US) to assess joint erosions and disease activity (significant Power Doppler) . We questioned whether these patients had delayed diagnosis or were undertreated. Understanding the underlying immune pathology in this subgroup could aid therapeutic targeting in patients whose needs are currently unmet.

Methods: 44 RA patients with active synovitis (≥1 joint with PD) were recruited, 29 nCRP (≤5mg/L) and 15 hCRP (>5mg/L). Peripheral blood mononuclear cells (PBMCs), serum and clinical data were collected. Blood was also collected from 18 age and sex matched healthy controls (HC). PBMC immunophenotyping was performed using flow cytometry. Serum cytokines were assessed using Cytometric Bead Array. Plasma was subjected to SOMAscan™ Proteomic Assay. Serum amyloid A (SAA) and serum IL-6/IL-6Rα complex were measured using ELISA.

Results: Patients with nCRP had an increased erosion accrual rate compared to hCRP patients (p=0.022) reflecting more disease-associated joint damage; other clinical and laboratory parameters were not significantly different including expression of acute phase reactant serum amyloid A which was increased in both patient groups compared to HCs (p=<0.05). Serum cytokines IL-6, IL-1β, IL-10, IL-12/IL-23, IL-17A, IL-17F and IL-21 and serum soluble IL-6/IL-6Rα complex were elevated in both patient groups compared to HC (p=<0.001). Furthermore, significant positive correlations existed between serum cytokine levels in the hCRP patients, but these were lost in nCRP patients. In particular, the positive correlation between IL-6 and IL-1β in hCRP patients (p=<0.001, r=0.5047) was absent in nCRP patients. Since IL-6 and IL-1β trigger CRP production the results suggested a defect in cytokine (potentially IL-6) signaling or an IL-6-independent disease mechanism.

nCRP patients had an anti-inflammatory immune cell phenotype with significantly increased $Foxp3^{+}CD161^{+}$Tregs (p=0.014) which have been negatively correlated with CRP levels and increased Treg-suppressive capacity. Alternatively, hCRP patients had an activated T-cell phenotype including increased central memory T-cells (p=0.024). However, no significant differences in *ex vivo* CD4+ T cell cytokine production were identified between the two patient groups matching the serum cytokine profiles. Strikingly proteomic analysis identified significant increases in complement components (C3b and C5) and SAP in hCRP compared to nCRP patients indicating decreased complement activation in the nCRP patients.

Conclusion: This study stratifies distinct patient subgroups using detailed immunophenotyping and proteomic signatures which could translate to improved patient-specific therapies.

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