Tissue inflammation signatures point towards resolution in adhesive capsulitis

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SIR, adhesive capsulitis (frozen shoulder) is a remarkable example of a severe, yet self-limiting, inflammatory and fibrotic condition affecting the shoulder joint capsule. Patients experience pain and restricted shoulder motion for up to 3 years, severely limiting activities and disrupting quality of life [1]. The disease mechanisms are poorly understood and there are no truly effective therapies for symptomatic patients. The pathological features of adhesive capsulitis are reported to include leukocyte and myeloid infiltration, fibroblast accumulation and increased vascularity [2]. However, the distinct inflammatory pathways and the phenotypes of tissue resident stromal cells active in disease remain to be identified, and may inform why the condition ultimately spontaneously resolves. In this case study, we use contrasting manifestations of established shoulder disease in similarly aged patients to advance understanding of why inflammation is frequently self-limiting in adhesive capsulitis but persists in shoulder rotator cuff tendon tears. We therefore investigated tissue inflammation signatures using previously validated markers [3, 4] to identify the phenotypes of macrophages and fibroblasts in samples from patients with adhesive capsulitis, comparing them with tissues from patients with shoulder rotator cuff tendon tears and with normal rotator cuff tendons. We also investigated if adhesive capsular tissues expressed proresolving receptors mediating resolution of inflammation.

The adhesive capsulitis cohort consisted of 12 female and 4 male patients aged between 43-72 undergoing arthroscopic capsular release surgery as part of the NIHR-HTA programme funded UK FROST study [5]. Adhesive capsulitis patient tissues were compared with those from similarly aged patients with torn supraspinatus tendons undergoing surgical debridement and repair (n=11). Healthy supraspinatus tendons were collected from patients undergoing shoulder stabilisation surgery (n=3). Tissues were collected under research ethics from the Oxford Musculoskeletal Biobank (09/H0606/11) and NRES Committee, Newcastle and North Tyneside (14/NE/1176). Full informed consent according to the Declaration of Helsinki was obtained from all patients. Collected tissues were processed for RNA isolation and histology. RT-qPCR and immunohistochemistry were performed using previously published protocols [3] to identify activation markers for macrophages and fibroblasts and proresolving receptors in collected tissues.

Inflammation signatures differed between tissues collected from adhesive capsulitis compared to tendon tear patients. Adhesive capsulitis tissues showed reduced expression of NFκB response genes including *TNF-alpha, IL6* and *IL8* compared to tissues from tendon tear patients (Figure 1A-C, p=0.001, 0.05 and 0.004 respectively). Adhesive capsulitis tissues showed increased *IL10, CD14*, *CD163,* and *C1QA* mRNA expression compared to torn tendons (Figure 1D-G, p=0.001, 0.005, 0.002, and 0.002 respectively). Fibroblast activation markers Podoplanin (*PDPN*), *CD106* (VCAM-1), CD248 and FAP were highly expressed in adhesive capsulitis and torn tendons compared to healthy tendons (Figure 1 H-K). However, the fibroblast activation marker *CD90* was significantly reduced in adhesive capsulitis compared to healthy and diseased tendon tissues (Figure 1L p=0.01 and p<0.0001 respectively). Immunostaining supported increased CD163, PDPN, CD106, FAP and reduced CD90 in tissue sections from adhesive capsulitis patients (Figure 1M). Proresolving receptors mediating resolution of inflammation including ALX/FPR2, CMKLR1 and GPR32 were highly expressed in adhesive capsulitis tissues (Figure 1N).

Investigating common shoulder diseases in similarly aged patients presents a unique opportunity to understand why inflammation ultimately resolves in adhesive capsulitis but persists in tendon tears. We identify tissues from patients with adhesive capsulitis differentially express markers of macrophage and fibroblast activation compared to those from patients with shoulder rotator cuff tendon tears. Adhesive capsular tissues showed reduced NFκB response genes and increased *IL10* compared to tendon tears, suggestive of a resolving inflammatory milieu. In support of this, increased CD163 suggests macrophages in adhesive capsulitis exhibit a glucocorticoid receptor activation signature, associated with dampening inflammation and tissue repair [6]. Fibroblast activation markers PDPN, CD106 and FAP were highly expressed in both conditions, however CD90 was significantly reduced in adhesive capsulitis compared to tendon tears. CD90 (Thy1) is expressed by pathogenic synovial fibroblasts from Rheumatoid Arthritis patients with a pro-inflammatory and invasive phenotype [7, 8]. The current study suggests the phenotypes of fibroblast subsets populating diseased shoulder tissues differ between self-limiting and persistent inflammation. CD90 therefore represents an important pathogenic marker and possible molecular checkpoint regulating persistent stromal mediated inflammation in common soft tissue disease of the joint. The identification of proresolving receptors ALX/FPR2, CMKLR1 and GPR32 suggests proresolving pathways mediating resolution of inflammation are active in adhesive capsulitis. These proresolving proteins were highly expressed in adhesive capsulitis compared to our previous study on patients with established shoulder tendon tears [3]. Collectively, these findings provide novel insight into the disease mechanisms underpinning self-limiting inflammation in adhesive capsulitis, identifying proresolving receptors, macrophage and fibroblast activation signatures that point towards a resolving inflammatory milieu. Improved understanding

of the biological mechanisms governing successful resolution of inflammation will inform the development of new therapeutic strategies targeting stromal mediated inflammation. These therapies are required to accelerate disease resolution in symptomatic adhesive capsulitis patients and in other common soft tissue diseases of the joint.

Figure 1

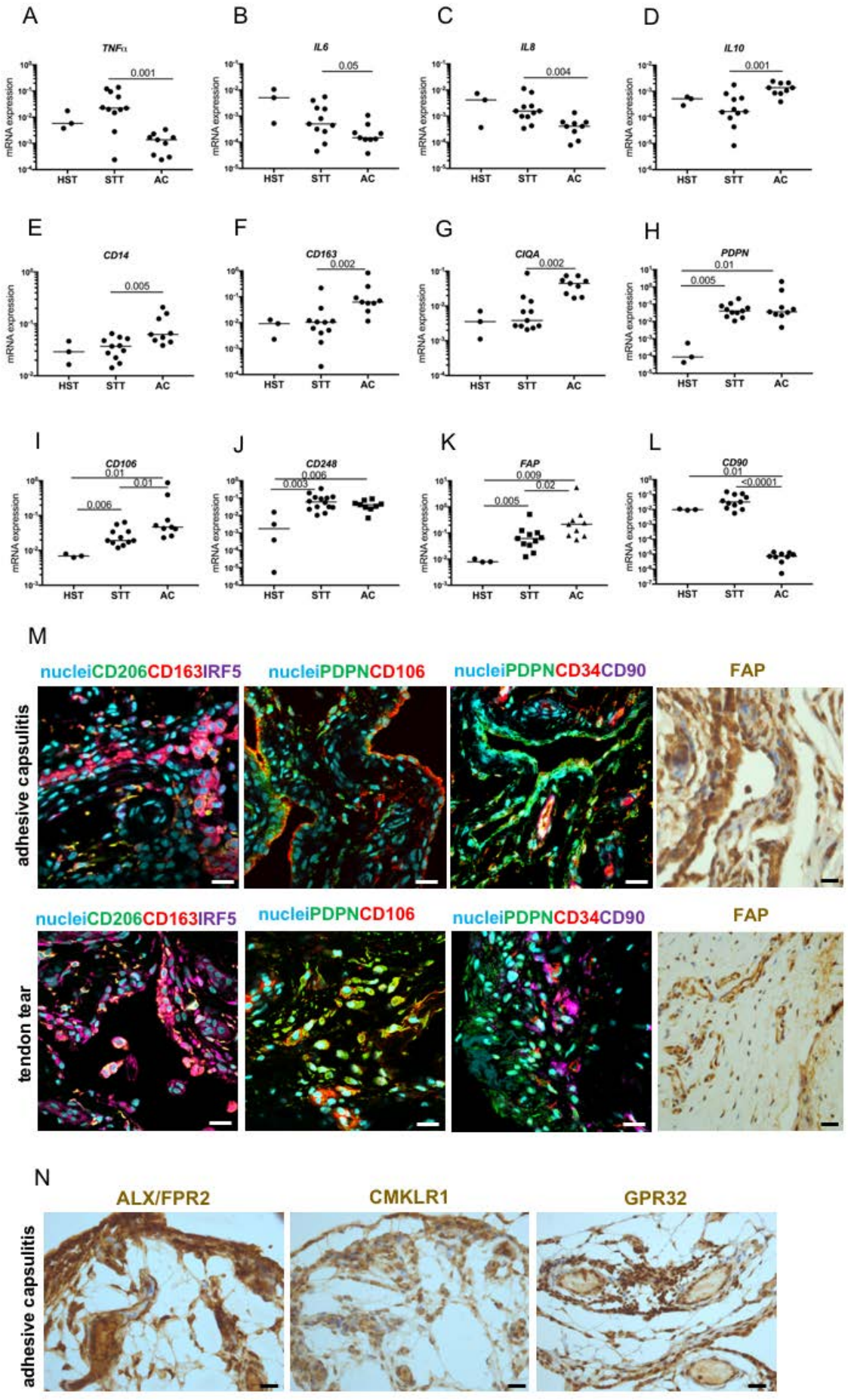

Figure 1. Activation of macrophages and fibroblasts and the presence of proresolving receptors point towards a resolving inflammatory milieu in tissues from patients with adhesive capsulitis. Healthy shoulder tendons (HST) were collected from patients undergoing shoulder stabilisation surgery (n=3),

diseased shoulder tendons were collected from patients undergoing surgery to repair a supraspinatus tendon tear (STT) (n=11). Tendon tissues were compared with capsular tissues collected from patients undergoing arthroscopic capsular release surgery for adhesive capsulitis (AC, n=9). Log transformed mRNA expression was determined for NFκB response genes (A-C), anti-inflammatory cytokine *IL10* (D), myeloid activation (E-F), complement activation (G) and fibroblast activation markers (H-L). Statistically significant differences were calculated using pairwise Mann-Whitney U tests. Gene expression is normalized to β-actin; bars represent median values. (M) Representative images of sections of adhesive capsulitis tissues stained for markers of macrophage (CD206, CD163, IRF5) and fibroblast activation (PDPN, CD106, CD90, FAP). Cyan (POPO-1) and haematoxylin represent nuclear counterstain. Scale bar, 20μm. (N) Representative images of immunostaining (brown) for proresolving receptors in sections of adhesive capsulitis tissues. Proresolving receptors ALX/FPR2, CMKLR1 and GPR32 are highly expressed in adhesive capsular tissues. Nuclear counterstain is haematoxylin. Scale bar, 20μm.

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