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Tissue inflammation signatures point towards resolution in frozen shoulder

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Key message: Proresolving receptors, macrophage and fibroblast activation point towards a resolving inflammatory milieu in frozen shoulder

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SIR, 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 joint 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 frozen shoulder 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 frozen shoulder but persists in shoulder rotator cuff tendon tears. We therefore investigated inflammation signatures, characterising the phenotypes of macrophages and fibroblasts in tissue samples from patients with frozen shoulder, comparing them with tissues from patients with shoulder rotator cuff tendon tears and with normal rotator cuff tendons. We also investigated if frozen shoulder tissues expressed proresolving receptors mediating resolution of inflammation.

The frozen shoulder 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 [3]. Frozen shoulder 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 [4] to identify activation markers for macrophages and fibroblasts and proresolving receptors in collected tissues.

Inflammation signatures differed between tissues collected from frozen shoulder compared to tendon tear patients. Frozen shoulder 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). Frozen shoulder tissues showed increased *CD14*, *CD163, IL10* and *C1QA* mRNA expression compared to torn tendons (Figure 1D-G, p=0.005, 0.002, 0.001 and 0.002 respectively). Fibroblast activation markers Podoplanin (*PDPN*) and *CD106* (VCAM-1) were highly expressed in frozen shoulder and torn tendons compared to healthy tendons (Figure 1 H-I). However, the fibroblast activation marker *CD90* was significantly reduced in frozen shoulder compared to healthy and diseased tendon tissues (Figure 1J p=0.01 and p<0.0001 respectively). Immunostaining supported increased CD163, PDPN and CD106 and reduced CD90 expression in tissue sections from frozen shoulder patients (Figure 1K). Proresolving receptors mediating resolution of inflammation including ALX, CMKLR and GPR32 were highly expressed in frozen shoulder tissues (Figure 1L).

Investigating common shoulder diseases in similarly aged patients presents a unique opportunity to understand why inflammation ultimately resolves in frozen shoulder but persists in tendon tears. We identify tissues from patients with frozen shoulder differentially express markers of macrophage and fibroblast activation compared to those from patients with shoulder rotator cuff tendon tears. Frozen shoulder 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 frozen shoulder tissues exhibit a glucocorticoid receptor activation signature, associated with dampening inflammation and tissue repair [5]. Fibroblast activation markers PDPN and CD106 were highly expressed in both conditions, however *CD90* was significantly reduced in frozen shoulder compared to tendon tears. CD90 (Thy1) is expressed by pathogenic synovial fibroblasts from Rheumatoid Arthritis patients with a pro-inflammatory and invasive phenotype [6, 7]. 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, CMKLR and GPR32 suggests proresolving pathways mediating resolution of inflammation are active in frozen shoulder. These proresolving proteins were highly expressed in frozen shoulder compared to our previous study on patients with established shoulder tendon tears [4]. Collectively, these findings provide novel insight into the disease mechanisms underpinning self-limiting inflammation in frozen shoulder, 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 frozen shoulder patients and in other common soft tissue diseases of the joint.

Figure 1

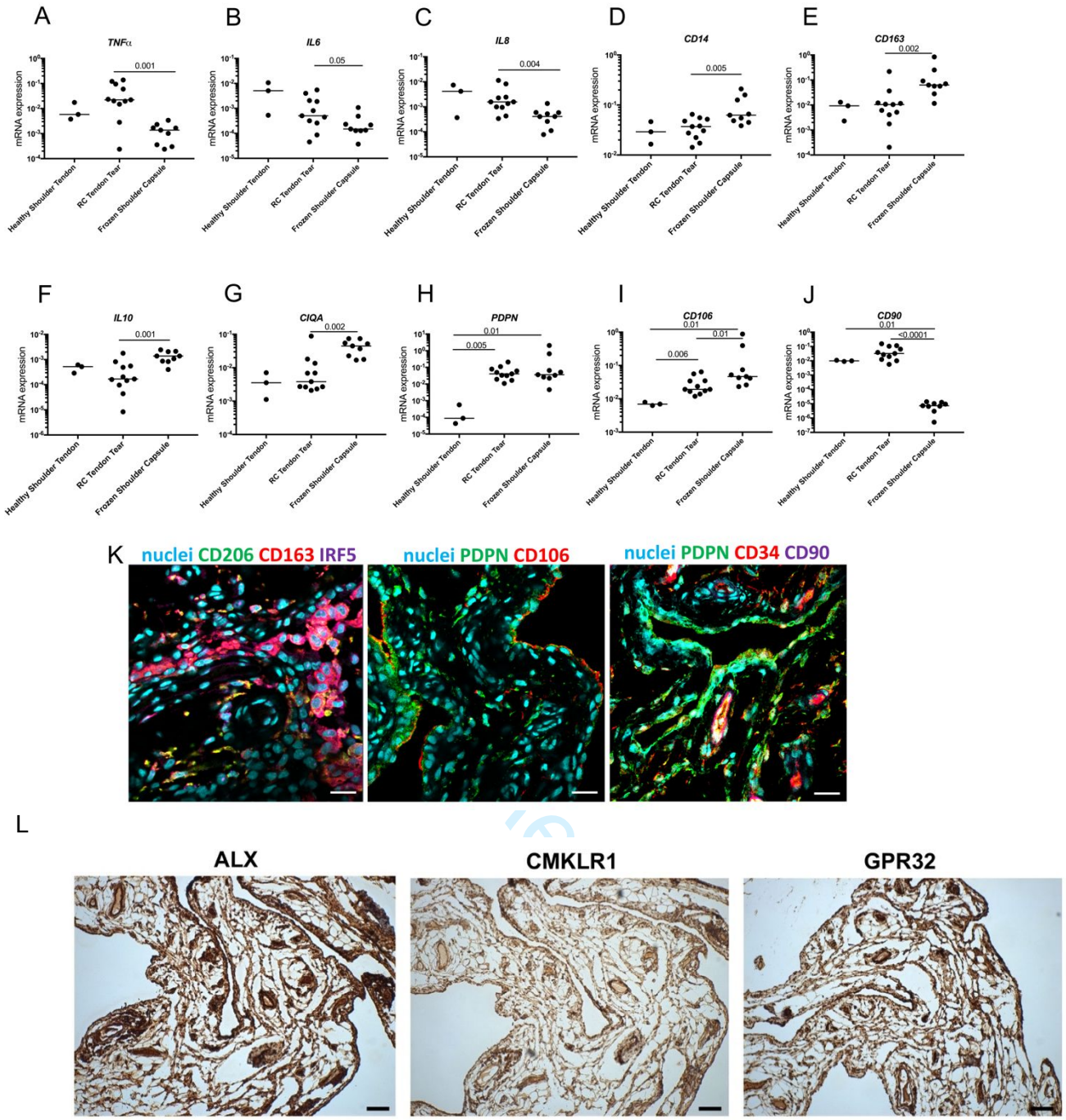

Figure 1. Activation of macrophages and fibroblasts and the presence of proresolving receptors point towards a resolving inflammatory milieu in tissues from patients with frozen shoulder. Tissues were collected from healthy patients undergoing shoulder stabilisation surgery (n=3, healthy supraspinatus tendon), patients undergoing surgery to repair a supraspinatus rotator cuff (RC) tendon tear (n=11) or arthroscopic capsular release surgery for Frozen Shoulder (n=9). mRNA expression was determined for NFκB response genes **(A-C)**, myeloid activation **(D-E)**, anti-inflammatory cytokine **(F)**, complement activation **(G)** and fibroblast activation markers **(H-J)**. Statistically significant differences were calculated using pairwise Mann-Whitney U tests. Gene expression is normalized to β-actin; bars represent median values. **(K)** Representative immunofluorescence images of sections of Frozen Shoulder tissues stained for markers of macrophage (CD206, CD163, IRF5) and fibroblast activation (PDPN, CD106, CD90). Cyan represents POPO-1 nuclear counterstain. Scale bar, 20μm. **(L)** Representative images of immunostaining (brown) for proresolving receptors in sections of frozen shoulder tissues. Proresolving receptors ALX, CMKLR1 and GPR32 are highly expressed in frozen shoulder. Nuclear counterstain is haematoxylin. Scale bar, 100μm.

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