THU0055

AN ANATOMICALLY DISTINCT PATHOGENIC FIBROBLAST SUBSET DRIVES INFLAMMATION IN ARTHRITIS


Background: Fibroblasts are key effector cells in the persistence of synovial inflammation and joint damage. It is not yet known whether specific subsets of synovial fibroblasts exist, and if so, whether they are responsible for the distinct fibroblast-mediated features observed in inflammatory arthritis, such as invasion of cartilage, bone damage, and persistence of inflammation.

Objectives: Here we identify and describe the biology of a functionally distinct pathogenic fibroblast cell type marked by the co-expression of Thy1.2 and Podoplanin (Pdpn), that is responsible for persistence of synovial inflammation.

Methods: We used the serum transfer arthritis (STA) model to induce joint inflammation. To identify putative subsets of fibroblasts we used flow cytometry using established markers of stromal cells, on enzymatically digested synovial tissue. These cells were localised in tissue sections using immunohistochemistry or immunofluorescence staining. Proliferation of subsets in vivo during the time course of STA was determined by BrdU incorporation studies. Transcriptomic analysis was performed using ultra-low input RNA sequencing on flow sorted populations of cells and single cell analysis performed on CD45 negative gated cells.

Results: Using FAP as a biomarker of activated synovial fibroblasts we were able to identify and define distinct subsets of synovial fibroblasts based on their co-expression of Thy1.2 and Pdpn. These subsets reside in distinct compartments of the synovial microanatomy including the lining layer (LL), sub-lining layer (SL) and a subset of pericytes. We found that FAP+ cells within the SL are highly proliferative and their expansion in cell number positively correlates with inflammation. Global deletion of FAP+ mesenchymal cells in the synovium using a FAP-DTR mouse attenuated synovial inflammation; protected against erosive bone damage and lead to reduced leukocyte accumulation as a result of reduced chemokine and cytokine production by synovial fibroblasts within the membrane. Collectively these data suggest a pathogenic pro-inflammatory role for these cells.

THU0056

14–3–3 IS A MOLECULAR SWITCH REGULATING MACROPHAGE POLARISATION IN INFLAMMATORY ARTHRITIS

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Background: Functional heterogeneity is a hallmark of macrophages, which can be classified into 2 major phenotypes with opposite role in inflammation termed M1 (classically activated) and M2 (alternatively activated) macrophages. Progranulin (PGRN), a multiple functional growth factor, binds to TNF receptor 2 (TNFR2) and activates the protective and anti-inflammatory pathway in inflammatory arthritis. In addition, 14–3–3ε is a critical downstream mediator of PGRN regulation of macrophage polarisation.

Objectives: In this study, we examined whether 14–3–3ε regulated macrophage polarisation and if so, whether this was important for PGRN’s anti-inflammatory action in inflammatory arthritis.

Methods: LysMCre and 14–3–3ε/F−/F+ mice were obtained from Jackson Laboratory. We found that 14–3–3ε deficiency enhanced M1 while inhibited M2 polarisation (figure 1a, b). Interestingly, PGRN showed reverse effects on macrophage polarisation. In addition, PGRN’s effects were largely lost in 14–3–3ε deficient BMDMs (figure 1a, b). Together, these data indicate that 14–3–3ε is a critical downstream mediator of PGRN regulation of macrophage polarisation.

Macrophage-specific 14–3–3ε contributes to control of inflammatory arthritis and is critical for PGRN’s anti-inflammatory action. We then explored the role of macrophage-specific 14–3–3ε in inflammatory arthritis and whether PGRN’s anti-inflammatory activity depended on 14–3–3ε in vivo. We established CIA in 14–3–3εF/F (serve as WT) and 14–3–3ε−/− mice, followed by i.p. injection of recombinant PGRN. The clinical arthritis score demonstrated that 14–3–3ε−/− mice displayed increased severity of CIA compared with WT CIA. In addition, PGRN’s protective effects against inflammatory arthritis was compromised in 14–3–3ε−/− mice (figure 1c), suggesting that 14–3–3ε is critical downstream mediator of PGRN’s anti-inflammatory effects. In addition, FACS analysis showed that total numbers of F4/80 cells were not different in all WT and knockout CIA mice. However, analysis of CD45+CD11b+ cell population in spleen demonstrated a significant increase in mean fluorescence intensity (MFI) of iNOS and a significant decrease of CD206+ cells in PBS treated 14–3–3ε−/− CIA mice as compared with PBS treated WT CIA mice; moreover, there was a significant decrease of iNOS MFI while a dramatic increase of CD206+ cells in PGRN-treated WT mice compared to that in PBS-treated mice. Further PGRN-mediated effects on macrophage polarisation was lost in 14–3–3ε−/− CIA mice (figure 1d, e). Collectively, these results indicate that PGRN skews macrophage towards M2 polarisation to resolve inflammation and this effect depends on 14–3–3ε.