at sites of inflammation, particularly at sites of joint destruction, might be implicated in the process of aggressive fibroblast behavior contributing to the pathogenesis of rheumatoid arthritis.

Keywords: S100A4; rheumatoid arthritis; apoptosis; matrix degrading enzymes; synovial fibroblasts

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AB03

Bone marrow derived mesenchymal stromal cells provide survival signals to B-cells in vitro — no major role for BAFF

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Background: Mesenchymal stromal cells (MSCs) are a unique cell type that has strong anti-proliferative effects on co-cultured activated T and B-cells in vitro. Based on our observation of significant differences between rheumatoid arthritis (RA) and osteoarthritis (OA) bone marrow B-cell compartments, we hypothesized that RA bone marrow MSCs may contribute to the pathogenesis of RA by enhancing B-cell survival.

Objectives: To compare the effect of RA and OA bone marrow derived MSCs (RA-MSCs, OA-MSCs) on the survival of healthy donor purified B-cells.

Methods: RA-MSCs (n=7) and OA-MSCs (n=5) were isolated from patients undergoing hip replacement surgery, and cultured in vitro for 2-5 passages. Washed cells were co-cultured with CD20+ B-cells for 60 hours in 17 different co-culture experiments. Cell survival was analyzed using 7-amino-actinomycin D (7AAD) labelling and flow-cytometric analysis and compared to the survival of B-cells cultured without MSCs (n=8). Expression of B-cell activating factor (BAFF) mRNA and protein was determined by RT-PCR and flow-cytometry after labelling with BAFF-specific antibodies.

Results: We observed that the presence of both RA-MSCs and OA-MSCs in the cultures significantly enhanced B-cell survival. MSCs without MSCs (n=7) and OA-MSCs (n=5) were isolated from patients undergoing hip replacement surgery, and cultured in vitro for 2-5 passages. Washed cells were co-cultured with CD20+ B-cells for 60 hours in 17 different co-culture experiments. Cell survival was analyzed using 7-amino-actinomycin D (7AAD) labelling and flow-cytometric analysis and compared to the survival of B-cells cultured without MSCs (n=8). Expression of B-cell activating factor (BAFF) mRNA and protein was determined by RT-PCR and flow-cytometry after labelling with BAFF-specific antibodies. We found that while synovial fibroblasts appeared the primary source of the β-D-N-acetyl-glucosaminidase and β-D-galactosaminidase enzymes, they produced relatively low amounts of β-D-glucuronidase. There was no significant difference in the activities associated with the synovial membrane and synovial fibroblast of OA and RA patients.

Synovial membrane homogenates were characterized by high β-D-N-acetyl-glucosaminidase, β-D-N-acetyl-galactosaminidase and β-D-glucuronidase expression as compared to the synovial fluid samples. We found that while synovial fibroblasts appeared the primary sources of the β-D-N-acetyl-glucosaminidase and β-D-N-acetyl-galactosaminidase enzymes, they produced relatively low amounts of β-D-glucuronidase. There was no significant difference in the activities associated with the synovial membrane and synovial fibroblast of OA and RA patients.

Using fluorescent substrates of β-D-glucuronidase we found stronger enzyme activities in OA fibroblasts as compared to those isolated from patients with RA. Furthermore, we found that β-D-glucuronidase activity was associated with microparticles found in the supernatants of synovial fibroblasts of both RA and OA patients. We also tested if cytokines, implicated in the pathomechanism of RA, regulated the expression of the above enzymes. While IL-17 had no effect, TNF-alpha markedly upregulated the expression of the KLOTHO gene.

AB05

Proinflammatory cytokines (IL-15, TNFα, IL-6 and IL-1β) in rheumatoid arthritis bone marrow preferentially promote activation of T-cells

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