This work was supported by Czech Ministry of Health - NR/9082-4 and Pfizer EU ARTICULUM Fellowship.

AB03

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

T. Dallosa,b,c, M. Krivošíkovab,c, M. Chorząź-Massalska, E. Warnawin,b, E. Zająć, W. Rudnicka, A. Radzikowskaa, W. Maśliński

a 2nd Department of Paediatrics, Comenius University, Bratislava, Slovak Republic, b Institute of Immunology, Medical Faculty, Comenius University, Bratislava, Slovak Republic, c Department of Pathophysiology and Immunology, Institute of Rheumatology, Warsaw, Poland, d National Institute of Rheumatic Diseases, Piesiány, Slovak Republic

Background: Mesenchymal stem 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 following 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 (70.11±7.28% and 54.65±11.83% viable cells, respectively) as compared to controls (35.13±13.83%, p < 0.001, Kruskal-Wallis ANOVA), the effect being more prominent in RA-MSCs (p < 0.05, Tukey-Kramer test). Both RA-MSCs and OA-MSCs displayed expression of BAFF mRNA and protein. We did not observe a convincing enhancement of BAFF mRNA expression by TNF-α. Blocking BAFF signalling by specific BAFF and BAFF-R antibodies, reduced the survival of B-cells by 20%, but did not abrogate the positive effect of MSCs on B-cell survival.

Conclusions: MSC interaction with B-cells may provide additional stimuli for lymphocyte survival via an as yet unidentified factor and therefore contribute to the pathogenesis of RA. BAFF, though produced by MSCs, is of minor importance in this setting. Further studies to identify the molecular basis of our observation are warranted.

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

* This work was supported by grant MRTN-CT-2004-005693 while Tomáš Dallos, Monika Krivošíková and Elizabeth Záňová were Marie-Curie fellows at the Department of Pathophysiology and Immunology of the Institute of Rheumatology, Warsaw, Poland.

AB04

Synovial glycosidases in joint diseases


a Department of Genetics, Cell and Immunobiology, Semmelweis University, Medical School, Budapest, Hungary, b Inflammation Biology and Immunogenomics Research Group, Hungarian Academy of Sciences-Semmelweis University, c Department of Rheumatology, Semmelweis University, Medical School, Budapest, Hungary, d Department of Orthopedic Surgery, Szeged, Hungary

We have shown earlier that certain synovial fluid exoglycosidases are predictors of rheumatoid arthritis and are capable of depleting the articular cartilage in glycosaminoglycans.

In the current study we investigated the expression of several glycosidases and glycosidase-like molecules including hexosaminidase (Hex), glucuronidase (Gus), hyaluronidase (Hyal), klotho and the chitinase-like human cartilage glycoprotein 39 (Hc-gp39) in synovial fluid and membrane samples as well as synovial fibroblast strains of patients with osteoarthritis (OA) and rheumatoid arthritis (RA).

The gene expression of the chitinase like Hc-gp 39 was by far the highest among the tested genes both in synovial fibroblasts and synovial membrane samples. HexA gene was characterized by the second strongest gene expression, followed by the expression of HexB, GusB, Hyal1 and KLOTHO in a decreasing sequence of order. The only significant difference was found in the gene expression of Hyal1 of the RA and OA 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 fibroblast 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, TNF-α, IL-6 and IL-1β) in rheumatoid arthritis bone marrow preferentially promote activation of T-cells*

E. Warnawin, A. Radzikowska, T. Burakowski, W. Maśliński

Institute of Rheumatology, Warsaw, Poland

* This work was supported by grant MRTN-CT-2004-005693 while Tomáš Dallos, Monika Krivošíková and Elizabeth Záňová were Marie-Curie fellows at the Department of Pathophysiology and Immunology of the Institute of Rheumatology, Warsaw, Poland.