AB06
Mesenchymal stem cells from bone marrow of rheumatoid arthritis patients provide survival signals to T- and B-cells in vitro

Tomasz Dallos 1,2, Monika Krivosikova 1, Lukasz Luszczynska 1, Magdalena Chorazy-Massalska 1, Ewa Warnawin 1, Weronika Rudnicka 1, Wlodzimierz Maslinski 1
1 Department of Pathophysiology and Immunology, Institute of Rheumatology, Spartanska 1, 02-637 Warsaw, Poland, 2 University Children’s Hospital, Limbova 1, Bratislava, Slovak Republic

Introduction: Although the role of bone marrow as a primary lymphoid organ has been appreciated for long time, recent data indicate that site exerts important properties of the secondary lymphoid organ as well. Indeed, it has been shown that efficiency of antigen presentation by dendritic cells in bone marrow exceeds several fold that of lymph nodes. Based on our recent results showing that both T- as well as B- cells compartments in bone marrow of rheumatoid arthritis (RA) patients exerts significant differences in comparison to osteoarthritis (OA), we hypothesized that RA- bone marrow cells contribute to the pathogenesis of RA. Bone marrow derived mesenchymal stem cells (MSC), unique cell type that can differentiate into several cell types, including osteoclasts, chondrocytes, muscle cells and adipocytes. In addition, MSC isolated from healthy donors exert strong anti-proliferative effects on in vitro co-cultured T-cells. In the present preliminary study we compared the effects of RA fibroblast-like synoviocytes (RA-FLS) and RA bone marrow derived mesenchymal cells (RA-MSC) on survival of healthy donor peripheral blood mononuclear cells (PBMC), and purified subpopulations of T- and B-cells in vitro.

Materials and methods: Bone marrow RA-MSC and RA-FLS were isolated from RA patients undergoing hip replacement surgery, and cultured in vitro for 2-5 passages. Washed cell were co-cultured with healthy donor PBMC or purified (using magnetic beads) CD3+ T-cells or CD20+ B-cells for 48-72 hours. Cell survival was analysed using 7-amino-actinomycin D (7ADD) labeling and flow cytometric analysis. Expression of analysed genes (T-cell growth factor IL-15 and B-cell activating factor, BAFF) in RA-FLS and RA-MSC were done using RT-PCR.

Results: It has been observed that co-culture of RA-FLS as well as RA-MSC enhances PBMC, and T- and B-cell survival. Based on our previous results with RA-FLS, we suggested that RA-MSC provide T-cells survival factors to T-cells via IL-15. Preliminary results indicate that RA-MSC exert expression of mRNA encoding IL-15. Experiments evaluating complexity of bone marrow OPG-RANKL system in biological assays are underway.

Conclusions: Our results show expression of RANKL and OPN on different populations of bone marrow mononuclear cells and PBMCs isolated from patients with RA and OA.

AB07
Soluble and surface expression of RANKL and osteoprotegerin in bone marrow from rheumatoid arthritis patients

Anna Radzikowska, Tomasz Burakowski, Wlodzimierz Maslinski
Department of Pathophysiology and Immunology, Institute of Rheumatology, 02-637 Warsaw, Poland

Background: Rheumatoid arthritis is a chronic inflammatory polyarthritis characterized by persistent inflammation of the synovium and destruction of the joint. The juxtaarticular erosion develops at the interface between articular cartilage and bone. Osteoclasts are the main cell type responsible for focal bone loss in RA. The activation and differentiation of osteoclasts requires the presence of RANKL that signals via receptor RANK. RANKL activity is blocked by OPG that binds to RANKL, preventing it from binding to RANK. RANKL exists as membrane-bound and soluble isoforms.

Objectives: to compare levels of cell-surface expressed RANKL and OPG on different populations of bone marrow mononuclear cells and PBMCs isolated from patients with RA and OA.

Methods: Bone marrow samples were obtained during routine total hip replacement surgery. PBMCs were isolated from peripheral blood of patients with RA and OA. Sodium citrate was used as an anticoagulant. To assess the real surface expression of those molecules (not bound to OPG, RANKL or RANK) cells were pre-incubated in low pH glycine buffer followed by washing in PBS. The surface expression of RANKL and OPG was determined by flow cytometry using specific antibodies.

Results: The freshly isolated CD3+ and CD33+ cells from peripheral blood expressed higher levels of RANKL than cells isolated from bone marrow. Expression of OPG was more frequent on CD3+ and CD33+ T-cells from peripheral blood than from bone marrow from RA patients. Both ligands were expressed mainly on CD4+ T cells, with only minor expression on CD8+ cells. There were no differences between surface expressed OPG or RANKL between RA and OA patients either in bone marrow or peripheral blood. Interestingly, our previous studies indicated higher levels of soluble RANKL in bone marrow from RA than from OA. The opposite, i.e. higher level of OPG was observed in bone marrow of patients with OA. Further experiments evaluating complexity of bone marrow OPG-RANKL-RANK systems in biological assays are underway.

Conclusions: Our results showed expression of RANKL and OPG on the surface of monocytes/macrophages and CD4+ T from peripheral blood and bone marrow. However, ratio of soluble OPG/RANKL in bone marrow better reflects situation in vivo, where microenvironment favors bone degradation in RA patients.

AB08
The levels of BAFF in serum, bone marrow and synovial fluid from patients with rheumatoid arthritis

E. Zanova 1,2, U. Musalowicz, W. Rudnicka 1, E. Kontny 1, A. Radzikowska 1, J. Rovensky 2, W. Maslinski 1
1 Department of Pathophysiology and Immunology, Institute of Rheumatology, Warsaw, Poland, 2 National Institute of Rheumatic Disease, Piestany, Slovakia

The B cell-activation factor (BAFF), a member of the tumor necrosis family, is expressed by several types of cells including monocytes/macrophages, dendritic cells and T lymphocytes. BAFF regulates B cell homeostasis and immunoglobulin production through its receptors BCMA, TACI and BAFF-R. BAFF-triggered signaling might