Investigation of Cell Surface Chemokine Receptors CCR1 and CCR2 in Peripheral Blood Mononuclear Cells of Patients with Rheumatoid Arthritis, Osteoarthritis and Chronic Lymphocytic Leukemia

Irina Spaka [1], *Artjoms Spaks* [1], *Anda Kadisa* [1, 2, 4], *Peteris Studers* [3], *Marina Soloveichika* [4], *Alla Rivkina* [1, 2, 4], *Sandra Lejniece* [2, 4], *Modra Murovska* [1], *Irina Holodnuka* [1]

[1] *Rīga Stradiņš University, A. Kirchenstein Institute of Microbiology and Virology, Latvia*
[2] *Rīga Stradiņš University, Department of Internal Diseases, Latvia*
[3] *Rīga Stradiņš University, Research Laboratory of Traumatology and Orthopedics, Latvia*
[4] *Rīga Eastern Clinic University Hospital, Latvia*

Introduction. Chemokines and their receptors regulate the proper function of the immune system. Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder. Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder (LPD) characterized by the accumulation of monoclonal B cells in the blood, bone marrow, and secondary lymphoid tissues. The composition of the peripheral blood (PB) cell populations and their activation state reflect the immune status of an individual and are characteristic for autoimmune and lymphoproliferative diseases. CCR1 and CCR2 are well studied in T-cell and macrophage-mediated immune-responses; however, the role of these CCRs in B cells, B cell lymphoproliferative and autoimmune diseases (as RA) is largely unknown.

Aim. The aim of this study is to analyze cell surface expression of CCR1 and CCR2 on PB mononuclear cells (B-, T-, monocytes, and granulocytes) of healthy individuals and patients with RA, osteoarthritis (OA), and CLL and to correlate their expression with the activation marker CD38.

Material and methods. The phenotype analysis of PB mononuclear cells (PBMC) was performed by polychromatic flow cytometry (pFC), based on FSC/SSC scatter and expression pattern of CD19, CD10, CD38 (an marker of cell activation), CD27, CD5, CD191 (CCR1) and CD192 (CCR2) markers. Cells were scored using a FACSAriaII analyzer equipped with 3 lasers, and the data were processed using Diva6.2 software (BD, USA). PB samples were collected from 10 healthy individuals (HI), 10 patients diagnosed with RA, 9 OA patients, and 16 patients diagnosed with CLL (10 of the Rai clinical stages I/II and 6 of the stage III).

Results. Four PBMC populations were analyzed in PB samples: monocytes, granulocytes, T-lymphocytes ($CD19^-$), and B-lymphocytes. Three major subpopulations of B cells were detected: $CD19^+CD10^-CD27^-CD38^{+/-}CD5^{+/-}$ naïve B cells – 29.3% (RA), 45.7% (OA), 56.8% (HI); $CD19^+CD10^-CD27^{++}CD38^{++}CD5^-$ memory B cells – 54.8 (RA), 23.5% (OA), 28.4% (HI); $CD19^+CD10^-CD27^+CD38^{+/-}CD5^-$ plasmatic B cells – 5.4% (RA), 9.4% (OA), 3.1% (HI). Compared to the healthy individuals, marked increase numbers were found: of plasmatic B cells in OA patients (3 times) and memory B cells in RA patients (2 times).

Of note is that the number of the CCR1 and CCR2-positive B cells was higher 1.5–2.0 times in PB of OA patients, and 4–5 times in RA patients, compared to the HI.

CLL cells ($CD19^+CD5^+CD10^-$) of patients with the Rai stages I/II were $CD38^-$ and did not express CCR1 and CCR2 either. In contrast, in 3 Rai stage III CLL patients, the 43%, 74%, and 87% of leukemic cells expressed CD38 as well as CCR1 and CCR2 (25–50% of the cells).

In PB of healthy individuals and patients with RA, OA, and CLL, the cell surface expression of CCR1 and CCR2 was exclusively detected on activated CD38+ cells.

Conclusion. The frequency of activated B cells has been found to be increased in RA and CLL III/IV Rai stage. CCR1 and CCR2 may have diagnostic (in RA versus OA) and prognostic (in CLL) relevance.