

# ABSTRACT

The immune system will maintain the integrity of the organism from harmful non-malicious recognizes and protects the body against exo- and endogenous toxic substances and together with the nervous and endocrine system are among regulatory systems of the organism.

Recently the evidence has supported the emerging concept of different B cell subpopulations to play a direct or indirect role in a pathogenesis of spectrum of disorders. However, so far the knowledge has been limited mainly in the way of how the specific differentiation stages of B lymphocytes are involved in pathogenesis of diseases and how course of disease, stage, and eventually different treatment can affect B cell homeostasis.

This work is focused on the study of peripheral CD27<sup>+</sup> B lymphocytes (one of the least explored human B lymphocytes) in healthy controls and patients with various immunopathologies, in this case we present representative results for patients with inflammatory bowel disease. Using polychromatic flow cytometry we examined 31 of peripheral blood samples, including 14 controls, 7 patients with Crohn's disease (CD) and 5 with ulcerative colitis (UC). In 6 patients with CD, we were able to perform immunophenotyping also 2 hours after intravenous administration of infliximab, and in one patient 14 days after drug administration.

Memory B lymphocytes characterized as CD19<sup>+</sup>CD27<sup>+</sup>, CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup> and CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>IgM<sup>+</sup> were in patients with CD present always in increased frequencies as compared to controls ( $20.6 \pm 13.58$ ,  $17.61 \pm 13.48$ ;  $88.60 \pm 20.56$  vs.  $11.75 \pm 26.47$   $11.25 \pm 26.50$ ,  $66.82 \pm 22.60$ , respectively), which well corresponds to the active untreated stadium of the disease.

However, other analyzed CD27<sup>+</sup> B cell subpopulations, CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>CD38<sup>+</sup>, CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>CD24<sup>-</sup>CD21<sup>+</sup> and CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup> were in peripheral blood of patients with CD deprived as compared with controls. Interestingly, in the case of CD19<sup>+</sup>CD20<sup>+</sup>CD27<sup>+</sup>IgM<sup>-</sup> B lymphocytes this reduction in comparison to controls was even statistically significant ( $11.10 \pm 20.62$  vs. CD  $39.40 \pm 25.05$  KO,  $p = 0.0234$ ). Patients with UC displayed different immunophenotypes in comparison to CD, which might support

generally assumed consensus that ulcerative colitis based on clinical features represents independent and distinct clinical unit.

Current work was drafted as a pilot study, which should indicate trends and possible future direction of research. Understanding the different aspects of the behavior of the B lymphocyte subpopulations in the future may provide useful markers allowing to evaluate the course and prognosis of the disease, or serve as a useful diagnostic marker for certain diseases.

**Keywords:** Immune system, B lymphocytes, antibodies , CD molecules , immunopathology , flow cytometry.