Abstract

Two novel B cell populations were characterized in peripheral blood of patients with common variable immunodeficiency and healthy controls were observed using flow cytometry in the study supported by the grant IGA MZ ČR NKT11414-3. These B cell populations were defined as $CD19^{+}CD27^{-}CD21^{+}CD38^{low}CD24^{+}IgM^{+}$ FO I and $CD19^{+}CD27^{-}CD21^{+}CD38^{low}CD24^{++}IgM^{++}$ cells.

Since none of found populations has ever been described, the aim of this thesis was to characterize these populations with focus on analysis of variable regions of the heavy chains of immunoglobulins and genes coding proteins participating in the process of $V_HD_HJ_H$ formation (Rag 1, Rag 2, and TdT) produced by cells of these populations.

Flow cytometry, single cell sorting, single-cell RT-PCR, IgVH, Rag 1, Rag 2, and TdT specific PCR amplification and cycle sequencing were employed to perform the molecular analysis in individual B lymphocytes. Both populations in two patients with common variable immunodeficiency, two healthy controls, and in two patients with autoimmune diseases – rheumatoid arthritis and systemic lupus erythematosus (as the disease control) – were examined. Finally, the statistical analysis was used to evaluate the differences in expression of variable regions of the heavy chains of immunoglobulins and in Rag1 and 2, and TdT.

Unusual and heavily biased usage of V_H, D_H, and J_H, genes having zero or extremely low mutational frequencies was identified in all analysed B cells. In addition, in FO I and FO II B cells of patient with RA a predominance of VH1-69 gene potentially coding autoantibodies was found. Interestingly, production of IgG isotype and TdT was detected suggesting active ongoing process of $V_HD_HJ_H$ rearrangement.

Since the populations did not express surface marker CD27 they were originally considered as naive B cells. In this regard, absence of mutations, longer CDR3 regions and significant presence of non-productive $V_HD_HJ_H$ rearrangements (namely in controls) suggests rather for naive nature of these cells that could. Another possibility is that this population could represent transient antigen unexperienced B cell stages and/or B cells being precursors of B cells of marginal zone. In the case of patient with rheumatoid arthritis, FO I and FO II B cells might be a population subjected to negative selection escaping this control checkpoint and being a founder of autoreactive B cells.

In addition, our findings may support the hypothesis that observed B cell populations may play a certain role in the regulation of homeostasis in immune system in healthy people, which can be impaired in patients with immunodeficiency (depressed immune system) and autoimmune disease (hyperactivated immnune system).

These findings seem to be very interesting and further research should be carried out to describe these populations more precisely which could contribute to better understanding of their role in physiological protective immune functions and their contribution to pathological conditions.

Key words:

B lymphocytes, FO I B cells, FO II B cells, rheumatoid arthritis, systemic lupus erythematosus, common variable immunodeficiency, CD antigens, flow cytometry, single-cell RT-PCR, VDJ rearrangement, TdT, Rag 1, Rag 2