

ABSTRACT

B-lymphocytes are lymphoid cells, which are a part of the adaptive/innate immune system and generate antibodies. Recently, many studies have supported hypothesis that different rather minor B-lymphocyte subpopulations may play a direct and indirect role in immunopathogenesis in human pathologies such as Crohn's disease (CD).

The aim of current study was therefore to investigate distribution of frequencies of B lymphocyte subpopulations (from transient to mature effector B cell stages) in peripheral blood of healthy subjects (CO), patients with Crohn's disease (CD) and ulcerative colitis (UC).

Thus, using 11-colour flow cytometry we have analysed 30 blood samples of individuals, including 14 healthy controls, 11 patients with Crohn's disease and 5 with UC. In 6 patients with CD we have had an opportunity to analyze blood samples collected 2 hours after an administration of anti-TNF therapy.

Higher frequencies of memory B-lymphocytes ($CD19^+CD27^+$, $CD19^+CD20^+CD27^+$ and $CD19^+CD20^+CD27^+IgM^+$) were found in patients with CD as compared to COs. ($20.06\pm13.58\%$; $17.61\pm13.48\%$; $88.60\pm20.56\%$ vs. $11.75\pm26.47\%$; $11.25\pm26.50\%$; and $66.82\pm22.60\%$), in case of $CD19^+CD20^-CD27^-IgM^+$ B-lymphocytes the difference was statistically significant ($57.15\pm17.21\%$ in CD vs. $19.59\pm31.79\%$ in CO; $p=0.0341$), which is in accordance with active stage of the disease.

On the other hand, frequencies of naive B-lymphocytes ($CD19^+CD20^-CD27^-IgM^-$ a $CD19^+CD20^+CD27^-IgD^-IgM^-$) were found to be significantly reduced in CD patients compared with COs ($57.15\pm 17.21\%$ for CD vs. $83.56\pm26.66\%$ for CO; $p=0.0373$; a $2.73\pm 13.41\%$ pro CD vs. $7.82\pm24.98\%$ pro CO; $p=0.0531$).

Patients with UC in all cases displayed different immunophenotype resembling rather COs suggesting that UC represents distinct disease unit.

In summary, monitoring of B-lymphocyte subpopulations can be useful in the future as an indicator of the activity of autoimmune diseases such as Crohn's disease and of response to biological anti-TNF therapy. In addition, our results suggest that it will be possible to present the immunophenotype data of individual patients as simple graphical schema easily

readable for clinicians and to create “heat” maps to group patients with similar clinical features.

Keywords: immunophenotype, polychromatic flow cytometry, B lymphocyte, B cell subpopulations, Crohn’s disease, anti-TNF therapy