C2. **Evaluation of the B-cell compartment in human peripheral blood as a tool for the diagnosis of primary immunodeficiencies**

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Important technological advances have been achieved in the last decades in flow cytometry instrumentation and reagents, which have increased both the multiparameter capabilities of flow cytometry immunophenotyping and the potential number of cells being analysed per sample. Together, such advances have facilitated the identification of low represented cell populations and their detailed immunophenotypic characterization.

Among other cells, characterization of bone marrow, peripheral blood and tonsil/lymph node B-lymphocytes has specifically benefited from these technological advances. Therefore, at present, multicolor combinations can be built which allow for very detailed characterization of the B-cell populations present in the above types of samples in normal vs. disease (e.g. primary immunodeficiency) states.

For many years it is well-established that B-cell maturation from early CD34+ hematopoietic precursors to mature immunocompetent B-lymphocytes in human occurs in the bone marrow. As an end result, both immature/transitional and naïve B-lymphocytes are released to the peripheral blood; these cells will circulate in peripheral blood until they will enter the different lymphoid tissues (e.g. lymph nodes). At these lymphoid tissues, usually in association with T-cell help, naïve B-lymphocytes further undergo proliferation, hypersomatic mutation and potentially also immunoglobulin class switch together with maturation to memory B-lymphocytes and/or antibody-producing plasma cells, in case they would encounter their specific antigen. The newly-generated memory B-cells and plasma cells recirculate through peripheral blood to the lymph nodes, spleen, mucosa-associated lymphoid tissues (MALT) and the bone marrow, where they will potentially develop further effector functions. Therefore, during B-cell differentiation, peripheral blood acts as a crossroad where recently produced naïve, as well as memory B-lymphocytes and plasma cells arrive. Therefore, multiple different compartments of B-lineage cells can be identified in such samples, including steady state peripheral blood.

Each B-cell population typically displays unique immunophenotypic features that allow clear cut discrimination among distinct maturation stages. As an example, B-cell precursors frequently express CD10 and CD38 in association with variable amounts of CD20. Immature/transitional B-cells strongly express CD20 and IgM, they are clearly positive for CD38 and CD10 but at lower levels, and they display variable levels of CD5. Naïve B-cells typically lack CD10 and CD38, as well as CD27 and they coexpress SmIgM and SmIgD. Most memory B-cells in normal individuals show positivity for CD27 in association with expression of a single immunoglobulin heavy chain isotype: either IgM or IgD, or IgA, IgG or IgE. In contrast, after the germinal center reaction, plasma cells increase the expression of CD38, they progressively decrease SmIg expression, and acquire plasma cell-associated markers like CD138. Thereby, construction of antibody panels based on the above markers allows for detailed dissection of the different B-cell compartments in peripheral blood. Additional markers are typically required for the dissection of early B-cell (e.g. NuTdT, CD19, CD22, CyCD79a and CD34) and late plasma cell (e.g. CD19 and CD56) maturation in the bone marrow, and to evaluate the germinal center B-cell reaction (e.g. CD44 in addition to CD10 and CD38).

In recent years, the EuroFlow Consortium has initiated a new Working Group aiming at the development of new tools for the assessment of lymphoid maturation and cell compartments, through the characterization of patients with immunological disorders such as primary immunodeficiencies, based on previously developed EuroFlow tools and concepts.

In this presentation, we will review the immuno-phenotypic features of the distinct subsets of B-cells and plasma cells present in bone marrow, peripheral blood and tonsils, as a reference for the detection of altered maturation patterns and immunophenotypic profiles in primary immunodeficiency patients.

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