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Identification of CLL stereotyped BCR in splenic B cell subsets using Next Generation Sequencing analysis

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Chronic Lymphocytic Leukemia (CLL) is characterized by the accumulation in the blood, bone marrow and lymphoid tissues of monoclonal B lymphocytes with a distinctive phenotype characterized by the expression of CD5 and CD23 antigens and a low expression of surface Immunoglobulins (sIG). The course of the disease is heterogeneous with some patients showing an aggressive form and a second group with a more stable disease; these groups are well defined by the absence or presence of somatic mutation on the IGHV gene, respectively.

In CLL clones the rearrangements of the B-cell Receptor (BCR) IGV regions exhibit distinctive features. In spite of the enormous diversity that can be created in the IGHV region up to 30% of CLL clones exhibit very similar (stereotyped) BCR which have been categorized in Subsets. Of these, 19 are most represented therefore are defined as "major Subsets". The identification of stereotyped receptors is codified on the basis of HCDR3 features in the context of the use of certain IGHV genes.

The above observations sustain the notion of an antigen drive role in CLL ontogeny. In addition, stimulation via BCR appears to be critical for leukemic cell survival/proliferation as suggested by the possibility to inhibit CLL growth by modern inhibitors of BCR-associated kinases. Furthermore, has been reported existence of a BCR homotypic interaction between BCR epitopes leading to receptor de-clustering and allowing the activation of autonomous signaling. Thus, this homotypic BCR–BCR interaction provides a further mechanism of CLL cell stimulation.

Whether CLL-like stereotyped BCR are present in the B-cell repertoire of healthy subjects remains largely unexplored. We addressed this issue in splenic B lymphocyte subpopulations and used a high throughput sequencing technique to collect IGV gene rearranged sequences. In particular, we separated and analyzed follicular mantle B cells (sIgDbright IgM+ CD38-CD27-), germinal center (GC) B cells (CD38+, sIgD-, CD24-), marginal zone B cells (sIgDlow, sIgM+, CD38-) and switched memory B cells (sIgD-sIgM-CD38-). In addition, we focus on IGV rearrangements using IGHV1 family genes because these are the most represented among the major subsets identified in CLL. Our study shows that CLL stereotyped receptors can be traced in a sizeable proportion of most of splenic B-cell subpopulations indicating that these receptors are part of the normal B-cell repertoire.