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Molecular analysis of B-cell dysfunction in patients with chronic lymphocytic leukemia (CLL)

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western hemisphere. Infectious complications are a major cause of death in CLL patients due to an immunodeficiency. The underlying mechanisms of the immunodeficiency in CLL patients are only partially understood, especially the role of the remaining non-malignant B-cells in the impaired humoral immune response is still unclear. With this project we aim to elucidate the impact of the normal residual B-cells (NRB) in CLL patients on the humoral immune defect of CLL patients.

First, we performed a detailed analysis of B-cell subsets in 45 CLL patients and 12 age-matched healthy controls by flow cytometry. After isolation of peripheral blood mononuclear cells (PBMCs) by Ficoll density centrifugation, we performed a negative selection by immunomagnetic depletion of ROR1+ CLL cells in order to enrich the small subset of NRBs. Besides a general reduction of non-tumor-B-cells compared to healthy age-matched controls, we found a significant reduction of naïve B-cells among the NRBs of CLL patients while class-switched CD27+ memory B-cells were overrepresented in CLL patients.

Next, we aimed to find out if the remaining class-switched memory B-cells were still functional. In assays addressing the proliferation capacity (eFluor staining) and the ability for IgG secretion (IgG EliSpot), the NRB cells were able to proliferate and secrete IgG, but needed a stronger stimulation compared to B-cells from healthy donors. In order to identify the involved pathways, we sorted naïve B-cells as well as IgM+ and IgG+ memory B-cells of CLL patients and healthy donors by flow cytometry and performed RNA sequencing before and after 24 h of strong stimulation (CpG, CD40L-HA, anti-HA, anti-Ig). Before stimulation all subsets of B-cells from CLL patients showed a differential gene expression compared to B-cells from healthy donors, however after 24 h of maximum stimulation the gene expression profiles were very similar in both groups. Pathway analysis revealed that the NRB cells of CLL patients were in a stage of inactivity or dormancy.

In summary, NRBs in CLL patients are not only quantitatively reduced, they also show a skewed subset distribution towards class-switched memory B-cells. The remaining NRB cells in CLL patients are still able to react, but require a stronger stimulation to overcome dormancy. The reasons for this inactivity are subject to our current investigations.