Aberrations inFunctional Activity and Immunophenotype of Antigen-Presenting Cells in B – Cell Chronic Lymphocytic Leukemia

Tsertsvadze Tamar

Nina Kulikova, Nino Porakishvili

E-mail :tamar.tsertsvadze@tsu.ge

Department of Biology, Iv. Javakhishvili Tbilisi State University, University street.13, 0143, Tbilisi, Georgia

Chronic Lymphocytic Leukemia (CLL) presents with clonal expansion and accumulation of CD5+CD19+CD23+ cells in peripheral lymphoid organs and tissues and in bone marrow.There is increasing evidence that CLL cellsreceive microenvironmental signals which support their growth, survival and expansion *in vivo*. Since CLL has been suggested to beantigen/ antigens driven (although currently unknown) elucidation of both – the antigen itself and this antigen-binding receptor/receptors is a question of great importance, that will help to reveal the mechanisms of CLL immunopathogenesis, which, subsequently, will lead to the identification ofoptimal therapeutic or even disease preventive approaches for this currently incurable disease.

The recognition of this hypothetical CLL-inductive antigen should be performed by different populations of antigen-presenting cells, while the most probable receptors for its recognition are highly conserved members of Toll-like receptors (TLRs) family, that are expressed on the surface of all types of antigen-presenting cells. Therefore, studying the mechanisms of CLL immunopathology, with an especial attention should be studied not only the percentage of antigen-presenting cells, but also their immunophenotyped and functional characteristics. Two main types of peripheral blood antigen-presenting cells are both derived from monocytes: macrophages and monocyte-derived dendritic cells.Based on abovementioned, first of all we have studied immunophenotyped and functional characteristics of this particular cell type. At the first stage of our research we have revealed a phagocytic function deficiency in monocytes from CLL patients. Simultaneously we have been studying monocytes' phenotype by evaluation the surface expression of Fcγ-receptors: FcγRI (CD64) and FcγRII (CD32). A decrease inFcγRI (CD64) expression level, compared to control group of age-matched healthy individuals, has been detected. While the level of FcγRII (CD32) appeared to be similar in patients- and healthy individuals groups.

At the following step of our research, we have decided to study the phenotype profile of B cell, taking into account, that B cells population is an another population of antigen-presenting cells, the most numerous one in case of CLL, as a clonal expansion of this particular population takes place during the course of the disease. We have looked at CD32 surface expression in B cells and also, in parallel, considering, that CD32 is a negative regulator of BCR signaling pathway, we also studied the surface expression of another negative regulator of BCR-signalling pathway: CD5 receptor. CD32 expression in B cells appeared increase in patients and healthy individuals (Donors: 20,74±4,658 **%**, B-CLL Cells: 59,47±12,279**%**; MFI : Donors: 111,1 ±11, B-CLL Cells: 249,1 ± 89,79). The same time a positive correlation between CD32 and CD5 surface expression has been revealed (corr.coef.=0,97). Abovementioned reflects overexpression of BCR negative regulators in CLL cells. Based on our previous results, showing a decreased surface expression of Toll-like receptors family member – CD180 in B lymphocytes from CLL patients [Porakishvili et al., 2005], we presumed reasonable to evaluate CD180 expression level in another population of antigen-presenting cells: monocytes. Like the situation seen in B cells from CLL patients, in monocytes similarly the expression level of CD180 appeared to be decreased (% in control group: 80.72 ± 2.85, **%**, B- CLL patients: 38.86 ± 4.559 **%**, P< 0.0001; MFI: in control group: 83.94 ± 4.710, B- B- CLL patients:: 34.34 ± 3.867,P< 0.01).

It should be mentioned, that in peripheral blood of CLL patients neither B lymphocytes, nor monocytes are proliferating. Meanwhile, as it has been already mentioned above, proliferation plays an important role in the pathogenesis of CLL, but it is restricted to so called “proliferative centers”, which are situated in lymph nodes or bone marrow. Also another important restriction, affecting the research, is the fact, that CLL cells *in vitro* apoptose intensively and because of this it appeared impossible to evaluate CD32 and CD180 expression on longer time-points.

Based on abovementioned, we decided to continue our research on a CLL model system: MEC1 cell line, which was established from EBV-seropositive CLL patient”s lymphocytes and is widely used as a model for CLL pathogenesis studies. Therefore, at the last stage of our research, we have studied CD32 and CD180 expression level at different time-points in case of actively cycling MEC1 cells . According the obtained data, CD32 expression level appeared to be increased in time: being 11±3.8% at 24h after re-seeding and reaching 36±1.3% at 96h. Our results have revealed, that CD180 expression on MEC1 cells is not stable: fluctuations are seen at different time-points. At this moment we don’t have any explanation of the detected phenomenon. At the further stages of our research we plan to investigate the effect of CD180 signalling on pro-survival and proliferative mechanisms, as it has been demonstrated , that BCR ligation causes the abrogation of CD180-ligation induced signal transduction pathway, which gives us a background to hypothesize, that CD180 and BCR are using similar signalling pathway. To check this hypothesis we assume to evaluate CD180 and/or IgM ligation effect on proliferation and apoptosis levels. This will enable a better understanding of the microenvironmental stimulithat lead to the expansion of the leukaemic cells and progression of the disease, in order to develop newtherapies for this currently incurable disease.