IFCC Professional Scientific Exchange Programme Expression of CD85, a killer-cell inhibitory receptor (KIR) molecule on T cells in B-chronic lymphocytic leukemia (B-CLL)

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Zoran Siftar, Clinical Chemist, stayed as a short term scholar, December 1999 till February 2000, at the Ludwig Boltzmann Institute for Cytokine Research (Head: Prof. J. Schwarzmeier) in Vienna, Austria.

Report:
During the period from the beginning of December 1999 till the end of February this year, I had opportunity to work on the project “Expression of CD85, a killer-cell inhibitory receptor (KIR) molecule on T cells in B-Chronic lymphocytic leukemia (B-CLL)” in collaboration with Prof. Schwarzmeier’s team from the “Ludwig Boltzmann” Institute for Cytokine Research,AKH, in Vienna. Working together on project at host laboratory we produced interesting results listed below in the text.

Introduction
In the recent past years, the receptors on the cells of human immune system which recognized MHC class I molecules on target cells and upon ligation deliver the inhibitory signals into the cells, thus maintaining negative regulation of humoral and cell’s response to virus-infected cells or eventually present tumor cells, have been discovered. So far, at least ten receptors have been found clustered in several related families encoded by genes at chromosome 19q13.4. Among them, CD85 molecule, recently recognized as ILT2 inhibitory receptor, belongs to Immunoglobulin-like transcript (ILT) family. It is normally present on B lymphocytes, monocytes/macrophages, dendritic cells, most of NK cells, and subsets of T lymphocytes. Upon ligation to a MHC class I molecule, CD85 inhibits NK and T-cell mediated cytotoxicity and cytokine production1.

In our study we evaluated the expression of CD85 molecule on T lymphocytes and their subsets in B-chronic lymphocytic leukemia (B-CLL). There are indications that deregulated functions of T cells in this disorder may contribute to the neoplastic proliferation of B cells.

Materials and methods
Expression of CD85 molecule on T lymphocytes in B-CLL was evaluated by flowcytometric method. For this purpose B-CLL patients with different clinical manifestations staged according to RAI from zero (0) to four (4), were chosen. Experiment was done either on the fresh blood lymphocytes or on the frozen peripheral blood mononuclear cells (PBMCs) taken from seven (7) healthy laboratory donors and nineteen (19) B-CLL patients. In some cases, patients were presented by two or more samples collected in different periods of disease giving the final number of 54 B-CLL samples. Because the CD85 is present normally on almost all mononuclear cells, the specific immune CD3-gating was performed for measuring the expression of this molecule on T cells more precisely. Multicolor method used in the survey allowing determination of CD85 bearing T cells and subsets, simultaneously. To enumerate T lymphocytes and subsets monoclonal antibodies CD3-Cyp5, CD4-PE, CD8-PE were used, with anti CD85-FITC for enumeration the cells carrying CD85 molecule, all from DAKO.

To determine the background staining IGG1-FITC/IGG1-PE/CD3-Percp combination of antibodies was included, together with CD4-FITC/CD8-PE/CD3-PerCp for enumeration of double positive (CD4+CD8+) and double negative (CD4-CD8-) T lymphocytes, all from B.Dickinson. Lyse/no wash procedure for sample preparation was done thus minimizing the loss of cell of interest and keeping them close to physiological conditions as much as possible. Acquisition was performed immediately after preparation finished on FACScan flowcytometer, B.Dickinson. Statistical analysis was done using Origin statistic’s package for PC, version 4.0, and P<0.05 was considered statistically relevant.

Results and discussion
Experimental data show significant reduction of CD3 positive cells in B-CLL patients, independently of the stage of disease as compared to the group of laboratory staff (p<0.001). The expression of CD85 on T lymphocytes has significantly higher levels in a group of B-CLL patients (p<0.001), but there is no difference between patients stratified according to RAI stage of disease into group of
Elevation of CD85 expression on T lymphocytes found in B-CLL patients is related to elevation of CD8+CD85+ population of T cells (0.01<p<0.05). This is true for the group of more advanced forms of disease; RAI 2 and RAI 3/4, but not for RAI stage 0/1. Comparative analyses showed no significant difference between each stage-separated group. CD4+ population of T lymphocytes was reduced in our group of B-CLL patients and this change is accompanied by progression of the disease. In RAI stage 3/4 the level of CD4+ population differs from other tested group at the significance level of p<0.001 and 0.01<p<0.001, respectively. Additionally, we looked at the influence of chemotherapy on the expression of CD85 molecule on T lymphocytes in patients staged as RAI 2 and RAI 3/4, but no difference was seen.

Table 1. Comparison of the surface markers expression on T lymphocytes and subsets in the samples from healthy donors and B-CLL patients staged according to RAI classification

| MEDIAN of surface markers expression in percents (%) | CD3 | CD85 on T lymphocytes | CD4 on T lymphocytes | CD8 on T lymphocytes | CD4+CD85+ on T lymphocytes |
|--------------------------------------------------|-----|-----------------------|----------------------|----------------------|---------------------------|
| HEALTHY DONORS (n=7)                              | 65.24 | 18.89 | 60.45 | 31.26 | 8.24 | 6.66 |
| B-CLL patients (n=54)                             | 8.84 *** | 34.32 *** | 43.71 * | 44.90 ns | 8.66 ns | 18.73 * |
| B-CLL patients stage RAI 0-1 (n=7)                | 11.38 *** | 31.88 * | 72.11 ns | 23.47 ns | 11.95 ns | 7.61 ns |
| B-CLL patients stage RAI 2 (n=17)                 | 12.22 *** | 36.64 ** | 54.13 ** | 42.12 ns | 10.94 ns | 13.95 * |
| ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 |
| B-CLL patients stage RAI 3-4 (n=30)               | 6.29 *** | 34.22 *** | 37.38 *** | 52.13 ** | 8.29 ns | 20.83 ** |
| ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 |

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| ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 | ns ^2 |

* 0.01<p<0.05; ** 0.001<p<0.01; *** p<0.001; ns not significant; compared towards: ^1 healthy donors; patients in ^2 stage RAI 0-1; ^3 stage RAI 2.
CD85 presentation on T lymphocytes after activation is that the CD85 molecule establishes a threshold for T lymphocyte activation, including both major subsets, CD4+ and CD8+ T cells. Probably, in the disease as B-CLL, it is not the major event responsible for ineffective immunoregulatory function of T cells.

However, it contributes downmodulating ongoing low avidity T-cells -malignant B-lymphocytes interactions which may be represented by manifold and/or of many interaction lines. Thus, additional investigations are needed to fully evaluate the role of CD85 on T cells in B-chronic lymphocytic leukemia.

References

Table 2. Comparison of surface markers expression on T lymphocytes and subsets in the samples from B-CLL patients in RAI 2 and RAI 3-4 determined before and after therapy administration

|                  | CD3 | CD85 on T lymphocytes | CD4 on T lymphocytes | CD8 on T lymphocytes | CD4+CD85+ on T lymphocytes | CD8+CD85+ on T lymphocytes |
|------------------|-----|-----------------------|----------------------|----------------------|-----------------------------|----------------------------|
| stage RAI 2      |     |                       |                      |                      |                             |                            |
| before therapy   | 9,30| 23,50                 | 49,64                | 41,20                | 7,38                        | 10,86                      |
| (n=9)            |     |                       |                      |                      |                             |                            |
| stage RAI 2      |     |                       |                      |                      |                             |                            |
| after therapy    | 17,38| 41,73                 | 58,56                | 43,09                | 16,17                       | 19,27                      |
| (n=8)            |     |                       |                      |                      |                             |                            |
| stage RAI 3-4    |     |                       |                      |                      |                             |                            |
| before therapy   | 3,50| 33,65                 | 38,34                | 46,22                | 8,39                        | 17,24                      |
| (n=9)            |     |                       |                      |                      |                             |                            |
| stage RAI 3-4    |     |                       |                      |                      |                             |                            |
| after therapy    | 8,04| 34,78                 | 36,67                | 54,19                | 8,19                        | 21,81                      |
| (n=21)           |     |                       |                      |                      |                             |                            |

ns not significant

Acknowledgements

I thank my colleagues from "Ludwig Boltzmann" Institute for Cytokine Research for technical assistance and Prof J. Schwarzmeier for helpful discussion. The study was supported by a grant from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

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