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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 Feburary 2000, at the Ludwig Boltzmann Institute for Cytokine Research (Head: Prof. J. Schwarzmeier) in Vienna, Austria.

Report:

During the period from the begining 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" Insitute 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 normaly present on B lymphocytes, monocytes/machrophages, 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 manifestation 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 presented normaly 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 determinate 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 immidiately 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 considerd statistically relevant.

Results and discussion

Experimental data show significant reduction of CD3 positive cells in B-CLL patients, indipendently 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

0/1, 2 and 3/4. 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 changes 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.

Cumulative results, in more details, are displayed in the Table 1. and 2.

Conclusion

In the B-CLL patients we found higher proportion of CD85 positive T lymphocytes which accompanied progression of disease as showed by comparison with a group of healthy laboratory staff.

From_the results obtained we can not state if the higher level of CD85 expression on T lymphocytes is of benefit or is a harmful event in immune response and regulation of tumor progression. The most likely explanation for the

Table1. 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 ly**	**CD8+CD85+ on T ly**
HEALTHY DONORS (n=7)	65,24	18,89	60,45	31,26	8,24	6,66
B-CLL patients (n=54)	8,84 *** [1]	34,32 *** [1]	43,71 * [1]	44,90 ns [1]	8,66 ns [1]	18,73 * [1]
B-CLL patients stage RAI 0-1 (n=7)	11,38 *** [1]	31,88 * [1]	72,11 ns [1]	23,47 ns [1]	11,95 ns [1]	7,61 ns [1]
B-CLL patients stage RAI 2 (n=17)	12,22 *** [1] ns [2]	36,64 ** [1] ns [2]	54,13 ** [1] ns [2]	42,12 ns [1] ns [2]	10,94 ns [1] ns [2]	13,95 * [1] ns [2]
B-CLL patients stage RAI 3-4(n=30)	6,29 *** [1] ns [2] ns [3]	34,22 *** [1] ns [2] ns [3]	37,38 *** [1] ** [2] *** [3]	52,13 ** [1] ns [2] ** [3]	8,29 ns [1] * [2] * [3]	20,83 ** [1] ns [2] ns [3]

*** 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 treshold 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 immuneregulatory 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

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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

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

ns **not significant**