The estimation of B7 family molecule expression on dendritic cells generated from CLL patients

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Abstract

T cell and B cell activation is dependent upon signals delivered through the antigen-specific T or B cell receptors. The ultimate immune response is determined by costimulatory signals which are supplied through molecules that belong to B7 family. The molecules B7.1 (CD80) and B7.2 (CD86) and interactions with their receptors CD28 and CTLA-4 constitute costimulatory and coinhibitory system which regulate immune response. This signals promote initial lymphocytes activation and regulate self-tolerance. The B7 ligands, B7-H1, B7-DC, B7-H3 and B7-H4 are expressed on profesional antigen presenting cells. This molecules are expressed both in lymphoid and non-lymphoid tissues. B7-H4 is recently identified molecule from B7 family. It appears that B7-H4 protein is expressed in many cancers such as breast, ovarian and lung cancer and it may influence tumor-specific T-cell responses in cancer patients. B7 family members may play an important role in the development of autoimmune and immunodeficiency diseases. Understanding of pathways, mechanisms of action and functions of these ligands may contribute to the development of novel strategies for the treatment of immune-mediated diseases.

Dendritic cells (DCs) are the most effective antigen presenting cells (APC) and play a crucial role in the initiation and regulation of immune responses against a variety of antigens, including tumor-specific antigens.

Key words: *B7 family molecule, dendritic cells, CLL.*

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Introduction

In patients with chronic lymphocytic leukemia (CLL), both malignant B cells as well as normal T cells exhibit functional dysfunctions. CLL is a malignancy characterized by accumulation of clonal CD5+ lymphocytes that are inefficient in antigen presentation. Also T-cell compartment demonstrate defects, which increase the risk of infections, hinder immune responses and elimination of leukemic cells. T cells are activated by antigen presenting cells (APCs) with the involvement of two signaling ways. First signal is induced by T-cell receptor (TCR) which recognizes MHC peptide complexes. Second signal derives from costimulatory molecules present on the surface of APCs which influence with the ligands on the T-cell surface. The costimulatory receptors are the members of B7 family molecules. A major function for the costimulatory molecules: CD80 and CD86 and their receptors: CD28 and cytotoxic T lymphocyte antigen (CTLA-4), is to provide positive or negative cosignaling at the initiation of T-cell response [1]. The discovery and characterization of new molecules belonging to this immunoglobulin superfamily that regulates T cell activities is very intensely investigated area of immunology [2]. More recently identified B7 homologue molecules: B7-H1, B7-H2, B7-H3 and B7-H4 represent a class of molecules with much more diverse functions in the adaptive immune system [3-5]. These molecules are broadly expressed in many tissues and cells. B7-H1 molecule participate in the inhibition of TCR-mediated proliferation and cytokine production. The

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B7-H1 pathway is involved in the negative regulation of some immune responses and may play an important role in the regulation of peripheral tolerance. B7-H2 molecule activation appears to play roles in T cell dependent B cell activation and T helper (Th) cell differentiation. B7-H4 mRNA could be detected broadly in normal human tissues, however immunohistochemical analysis does not reveal positive staining for B7-H4 protein in any tissues and organs from healthy individuals. In contrast, cell surface expression of B7-H4 is found in a variety of human cancers including 31% of lung cancer and 85% of ovarian cancer.

In this study we estimated the expression of CD80, CD86, B7-H1, B7-H2 and B7-H4 molecules on dendritic cells generated from patients with CLL. Dendritic cells (DCs) are the most effective antigen presenting cells (APC) and play a crucial role in the initiation and regulation of immune responses against a variety of antigens, including tumor-specific antigens.

Material and methods

Peripheral blood was obtained from 30 untreated patients with CLL (0-1 Rai stadium) and 10 healthy donors.

Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation in density gradient (Gradisol L, Aqua Medica, Lodz). Vitality of PBMCs were estimated by trypan blue solution (Trypan Blue Solution 4%). Only samples which the percentage of viable cells over 95% were used to further experiments. Monocytes were isolated from PBMCs by magnetic separation (MACS, Miltenyi Biotec, Germany) using anti-CD14 MicroBeads. CD14+ cells were used to generate dendritic cells. Monocytes were cultured in RPMI 1640 (PAA, Austria) supplemented with 2% human serum albumin (Baxter, USA) and antibiotics: penicillin, streptomycin and neomycin (Sigma, Germany) at 37°C, 5% CO_2 in culture dishes (BD Falcon, USA). 1000 U/ml of GM-CSF (Leukine, Berlex USA) and 500 U/ml of IL-4 (Miltenyi Biotec, Germany) were added to the culture medium on day 1,3 and 5. The maturation of DCs was induced by addition of TNF-α (50 ng/ml) (Miltenyi Biotec, Germany) and autologous tumor cells lysates on day 6. On the 7th day of the culture of mature dendritic cells were obtained. We evaluated the percentage of cells with an expression and mean fluorescent intensity (MFI) of surface markers, such as: CD1a, CD14, CD45, CD80, CD83, CD86, B7-H1, B7-H2, B7-H4.

Tumor cells lysates were obtained by magnetic separation of DC19 positive cells using anty-CD19 MicroBeads (MACS, Miltenyi Biotec, Germany). CD19+ tumor cells were exposed to 5 cycles of freezing (–80°C) and thawing. Homogenate obtained in this way was centrifuged and filtered. 100 µl of protein was added to the 1 ml of cell culture. We used spectrophotometer (Beckman, USA) to the estimation of protein level.

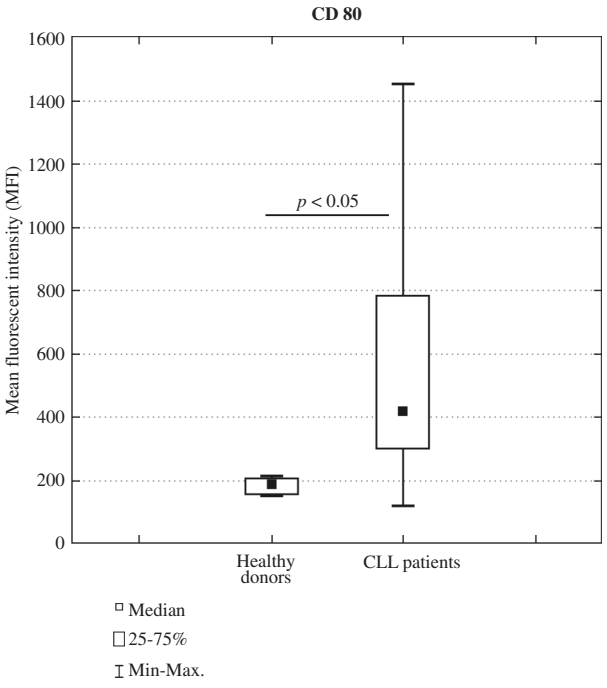

Fig. 1. The estimation of CD 80 mean fluorescent intensity (MFI) on DCs generated from CLL patients and healthy donors

Results and discussion

The immune homeostasis is a dynamic process that is regulated by the balance between activation and inhibition of various signals [6, 7].

CD80 and **CD86** molecules present on APCs (DCs) bind to the CD28 on T cell surface and transmit second signal necessary to the activation of lymphocytes [1]. This mechanism results in secretion of IL-2 and proliferation of T lymphocytes. Moreover, linking of CD80 with CD28 molecule mainly influence development of T helper type 1 immune response. Whereas CD86 molecule plays predominantly role in the induction of T helper type 2 immune response. We noticed that expression of CD80 molecule was significantly higher on DCs generated from CLL patients comparing to the healthy donors. Increase of CD80 expression on DCs could contribute to strong promotion of cell response and to increase of anti-tumor immunity.

The results of our study showed that B7-H4 molecule was significantly higher expressed on DCs generated from CLL patients in comparison to the healthy donors.

mRNA encoding B7-H4 is widely distributed in human peripheral tissues. The expression of B7-H4 cell surface protein is generally absent in most normal human somatic tissues, apart from epithelial cells, kidney, lung and pancreas. It was shown that expression of this molecule is higher in many human cancers. High levels of B7-H4 were found in ovarian carcinoma cells [8, 9]. Kryczek *et al.*

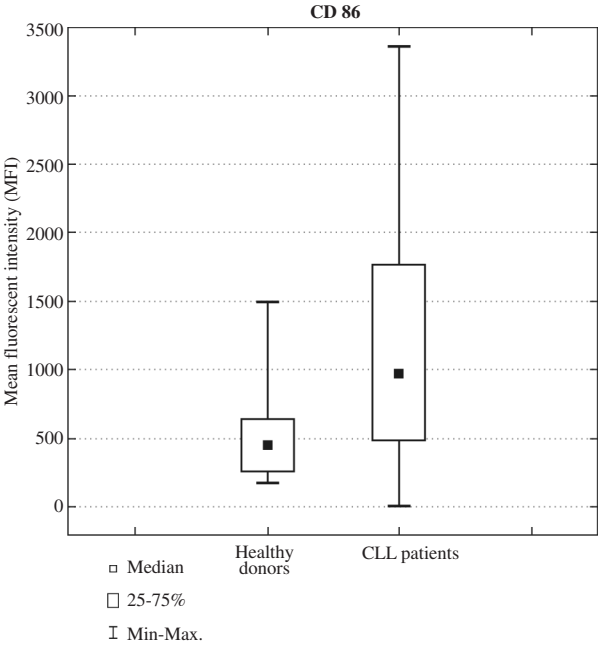

Fig. 2. The estimation of CD 86 mean fluorescent intensity (MFI) on DCs generated from CLL patients and healthy donors

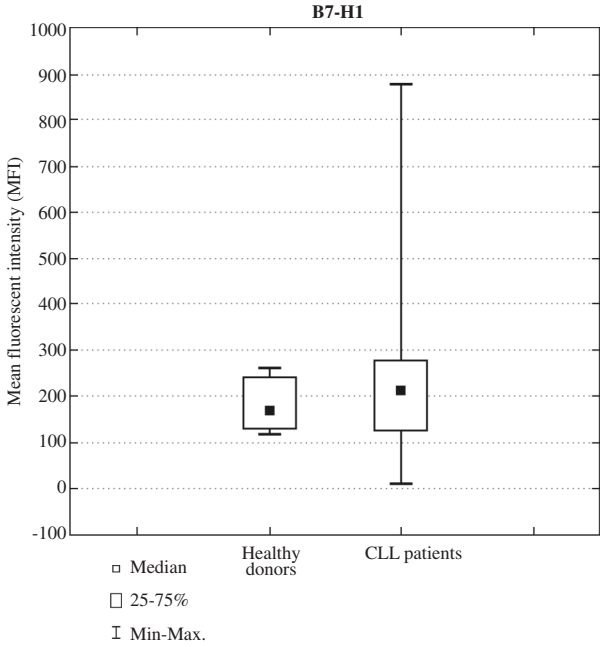

Fig. 3. The estimation of B7-H1 mean fluorescent intensity (MFI) on DCs generated from CLL patients and healthy donors

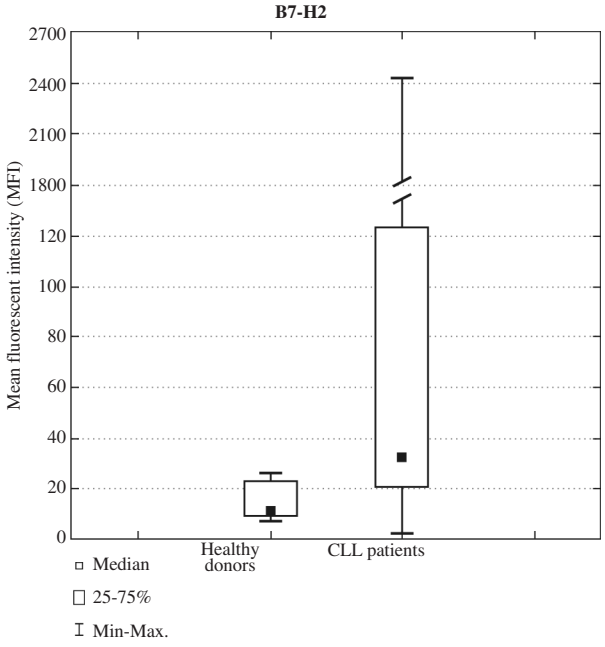

Fig. 4. The estimation of B7-H2 mean fluorescent intensity (MFI) on DCs generated from CLL patients and healthy donors

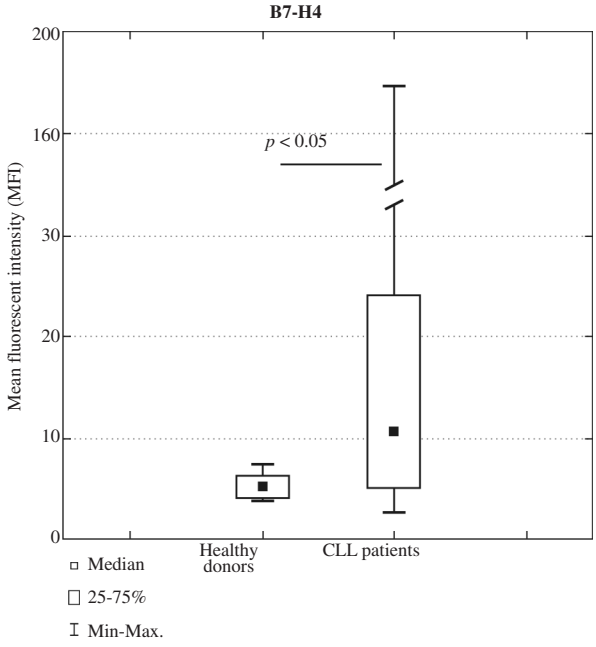

Fig. 5. The estimation of B7-H4 mean fluorescent intensity (MFI) on DCs generated from CLL patients and healthy donors

showed that ovarian tumors express intracellular B7-H4. Moreover B7-H4 is present on the surface of ovarian tumor associated macrophages and may contribute to tumor progression and it may inhibits TAA-specific immunity. In the tumor microenvironment high concentrations of IL-6 and Il-10 were found which strongly stimulate macrophage B7-H4 expression. It seems that tumor-conditioned macrophage impair TAA-specific T cell immunity through

B7-H4 signaling. However, the receptor for B7-H4 has not been discovered yet. Initially B- and T-lymphocyte attenuator (BTLA) was suggested to be a receptor for B7-H4 [10]. BTLA is structurally and functionally similar with the other two T cell inhibitory receptors, cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1). Although other studies do not confirm the observations [11, 12]. IL-6 and IL-10 can stimulate monocytes, macrophages, and myeloid DCs to express B7-H4. High level of this molecule could be downregulated by granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 [13-15]. In our study we demonstrate that monocyte-derived dendritic cells (MDDC) grown in the presence of GM-CSF and IL-4 also have high level of B7-H4 molecule. It may be one of the aspect which is responsible for defects in initiating of immune response in CLL patients. These data showed that *in vivo* blocade of endogenous B7-H4 by specific mAb promotes T-cell response. This indicates an inhibitory role for B7-H4 [16]. Overexpression of B7-H4 at both the mRNA and protein level was also found in ductal and lobular adenocarcinoma of breast cancers [17-19]. B7-H4 is also expressed on renal cell carcinoma [20] and lung cancer.

Further study are needed to identify the inhibitory receptor for B7-H4. Increased expression of B7-H4 protein observed in a many pathogenic conditions, like: cancer and inflammation may be an opportunity to design of new immunotherapeutic ways.

Acknowledgments

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