Molecular Mechanisms of the Effect of Interleukin-2 on Apoptosis of Blood Lymphocytes

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The effects of recombinant IL-2 on apoptosis of lymphocytes of healthy donors were studied in *in vitro* experiments. It was shown that the inductive and inhibitory effects of IL-2 on apoptotic process depend on the dose of the cytokine and cell microenvironmental conditions. Culturing of lymphocytes with recombinant IL-2 increases the percent of cells with reduced transmembrane potential, reduces the content of intracellular proteins Bcl-2, Bcl-X_L и Bax, and increases the level of Bad. The proapoptotic effect of this cytokine is realized with participation of nuclear transcription factor NF-κB and transcription factor P53.

Key Words: *apoptosis; interleukin-2, proteins of the Bcl-2 family; nuclear transcription factor NF-κB; P53*

The involvement of many types of cells, subcellular elements, and organ system predetermines the formation of complex mechanisms of regulation of immune reactivity at both the local and organism levels. The central role in this process is played by the cytokine network [3], a system of cell transmitters and their receptors, including IFN, CSF, transforming growth factors, chemokines, and IL.

IL-2 is a potent activator of proliferation and differentiation of immunocompetent cells; it participates in the maturation of their precursors from stem cells and in the regulation of apoptosis, *i.e.* programmed cell death [7]. However, the modulating effect of this transmitter on the apoptotic program has a dual nature [10]. To explain the choice between pro- or antiapoptotic effect of IL-2, a feed-back theory of the regulation of T cell apoptosis was proposed, according to which this cytokine increases the sensitivity of T cells to apoptosis [1]. Further fate of the cells depends on the level of antigenic and co-stimulating influences. In the absence of these influences, production of IL-2 and its cell receptor is suppressed and the cell undergoes apoptosis due to deficit of the cytokine. This mechanism limits the immune response after death of the pathogen. Apoptosis of T cells can be reduced or abolished by cytokines, whose receptors have common γ-chain (IL-2, IL-4, IL-7, IL-15, and IL-21) [2]. Excessive antigenic stimulation leads to antigen-induced apoptosis of T cells. It is caused by antigen binding with the receptor, is mediated by the influence of Fas-ligand and TNF-α on activated T cell, and is blocked by c-FLIP, whose expression is suppressed by IL-2 [14].

Despite the great number of experimental and clinical studies in this field, the molecular mechanisms regulating apoptosis via IL-2-trigered transduction are poorly studied. For instance, the dependence of apoptosis on the dose of the cytokine, stage of cell differentiation, and microenvironment conditions remains unclear.

Here we studied molecular mechanisms of the effect of IL-2 on apoptosis of blood lymphocytes.

MATERIALS AND METHODS

For detection of the dose-dependent effect of IL-2 on apoptosis, lymphocyte from 20 healthy donors (11

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men and 9 women aged 18-40 years) isolated on Ficoll-Paque density gradient (ρ=1.077 g/cm^3; Pharmacia) were cultured in incomplete nutrient medium and in a medium containing recombinant human IL-2 in a concentration range of 0.015-1.000 ng/ml for 18 h at 37°C and 5% CO_2. For evaluation of the antiapoptotic effect of IL-2, blood lymphocytes were incubated in complete nutrient medium in the presence of apoptosis inductor dexamethasone (10^{-4} mol/ml, KRKA) and recombinant IL-2 (0.025-0.050 ng/ml) for 18 h at 37°C and 5% CO_2.

Apoptosis was evaluated by flow cytofluorometry [12]. After culturing, the cells were resuspended in an annexin buffer containing annexin V labeled with FITC and propidium iodide and incubated for 15 min in darkness at room temperature. Cell suspension samples were analyzed on an Epics XL laser flow cytometer (Beckman Coulter).

The percent of lymphocytes with reduced mitochondrial transmembrane potential was evaluated using MitoScreen reagents (BD Pharmigen); the key reagent of this kit, JC-1, in live cells exists in the form of monomers and aggregates (green and red fluorescence, respectively). In case of impairment of mitochondrial membrane integrity, JC-1 cannot form aggregates and fluoresces green (which corresponded to FL-1 channel of the flow cytofluorometer).

The contents of Bcl-2 family proteins, nuclear transcription factor κB (NF-κB), and transcription factor P53 were assayed by Western blotting. To this end, lysing duffer (Helicon), 0.1% bromphenol blue (Helicon), 15% glycerol (Helicon), 0.02% β-mercaptoethanol (Helicon), and a mixture of proteinase inhibitors (Sigma Aldrich) were added to isolated lymphocytes. The proteins were separated by molecular weights by electrophoresis (10 V/cm) in 5% and 10% SDS-PAAG. Molecular weight markers 14.3-220.0 kDa (Fermentas) were used. The proteins were then transferred onto nitrocellulose membrane (Bio-Rad) and successively incubated with TTBS (0.05% Tween-20 in PBS) with 5% defatted dry milk, with primary antibodies to Bcl-2, Bcl-X_L, Bax, Bad, NF-κB, and P53 (Sigma Aldrich). Then, peroxidase-labeled secondary antibodies (Biosource) and a substrate for horseradish peroxidase on the basis of tetramethylbenzidine were added. The content of the test protein was evaluated by the ratio of the corresponding signal to the signal of glyceraldehyde-3-phosphate dehydrogenase, G3PDH (Chemicon).

Normal distribution of the quantitative data was verified using Kolmogorov–Smirnov test. The median (Me) and the upper and lower quartiles (Q_1-Q_3) were calculated for each sample. The significance of differences between independent groups was evaluated using nonparametric Kruskal–Wallis test. Mann–Whitney test was used for paired comparisons of the results in the studied groups.

RESULTS

The problem of dose-dependent effect of cytokines on programmed cell death is now actively discussed [1]. To confirm the hypothesis on the dose-dependent effects of cell transmitters on the realization of lymphocyte apoptosis we used different concentrations of recombinant IL-2 (from 0.015 to 1.000 ng/ml). The results of *in vitro* study showed that recombinant IL-2 exhibits its proapoptotic effect on lymphocytes in a concentration of 0.100 ng/ml incubation medium. Further increase in IL-2 dose led to an increase in the number of phosphatidylserine-expressing lymphocytes (Fig. 1).

The inhibitory effect of IL-2 on the tanatogenic program was evaluated on the model of apoptosis induced by glucocorticoid (dexamethasone). Addition of dexamethasone to the incubation medium in a concentration of 10^{-4} M increased the number of annexin-positive lymphocytes by 5 times. Simultaneous addition of dexamethasone and recombinant IL-2 in a dose of 0.025 ng/ml 2-fold suppressed the process of programmed cell death compared to the corresponding parameter in glucocorticoid-containing medium (Fig. 2). Increasing the concentration of recombinant IL-2 in the incubation medium to 0.100 ng/ml resulted in further decrease in the number of apoptotic lymphocytes. Thus, we observed a dual effect of IL-2 on the realization of programmed cell death depending on the dose of the cytokine and microenvironmental conditions.

A possible mechanism underlying the blockade of glucocorticoid-induced apoptosis by recombinant IL-2

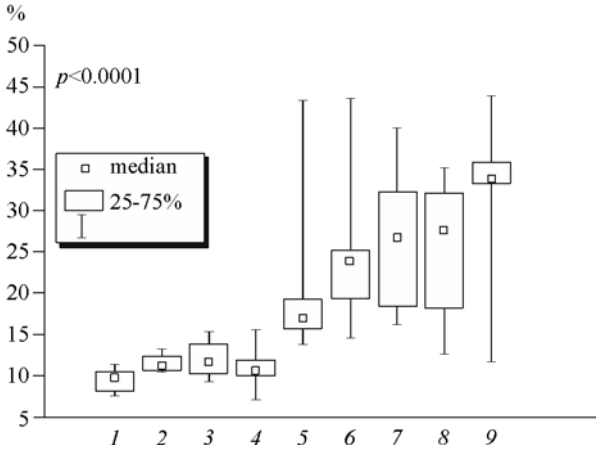

Fig. 1. Number of apoptotically changed lymphocytes after culturing in a medium containing different concentrations of recombinant IL-2. *1*) intact lymphocyte culture. Lymphocyte cultures incubated with recombinant IL-2 in different concentrations: *2*) 0.015 ng/ml, *3*) 0.025 ng/ml, *4*) 0.050 ng/ml, *5*) 0.100 ng/ml, *6*) 0.200 ng/ml, *7*) 0.350 ng/ml, *8*) 0.500 ng/ml, *9*) 1.000 ng/ml. Here and on Fig. 2: *p* is the significance of differences between the groups (Kruskal–Wallis test).

is its capacity to initiate the proliferative cascade by JAK/STAT, PI3K, or MAPK signal pathways converging on the regulation of the expression of bcl-2 gene; up-regulation of bcl-2 gene expression results in cell survival and proliferation [6]. This assumption is confirmed by a well-known capacity of glucocorticoids to inhibit the synthesis of antiapoptotic [2] and stimulate the synthesis of proapoptotic Bcl-2 family proteins [6,15], which leads to activation of the apoptotic program in the cell [11]. Moreover, we previously showed that binding of STAT5 and AP 1 proteins activated by IL-2 to glucocorticoid receptor prevents the formation of complexes of these receptors with cofactors CBP/p300 or SRC-1a, which blocks the transduction of the proapoptotic signal [3]

Mitochondrial factors (cytochrome C, AIF, calcium ions, Apaf-1, *etc.*) also can mediate the effect of cytokines on programmed cell death [8]. Using fluorescent indicator of mitochondrial membrane integrity JC-1 we detected a significant increase in the relative content of lymphocytes with reduced mitochondrial transmembrane potential during their incubation with recombinant human IL-2 to 7.61% (6.73-9.29%) compared to 3.70% (3.61-3.74%) in intact culture ($p<0.05$). This fact probably attests to the involvement of mitochondria into realization of apoptosis mediated by this cytokine. At the same time, this parameter during culturing in the medium containing recombinant IL-2 and dexamethasone was significantly lower than in the cytokine-containing medium without dexamethasone (7.61% (6.73-9.29%) and 2.69% (2.35-3.63%), respectively; $p<0,05$).

The release of apoptosis regulators from intermembrane space of mitochondria into the cytosol is regulated by Bcl-2 family proteins; the balance between these proteins determines the choice between life and death for the cell [6]. We measured the content of Bcl-2 family proteins in lymphocytes during exposure to recombinant IL-2 and revealed decreased

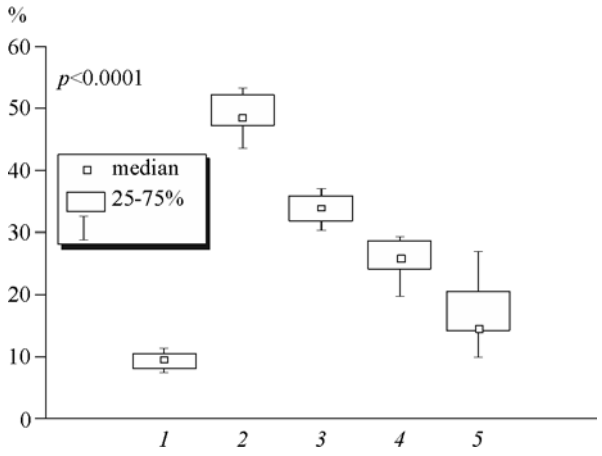

Fig. 2. Number of apoptotically changed lymphocytes after culturing in a medium containing dexamethasone and different concentrations of recombinant IL-2. *1*) intact lymphocyte culture; 2) lymphocytes cultured in a medium with dexamethasone. Lymphocyte cultures incubated with dexamethasone and recombinant IL-2 in different concentrations: *3*) 0.025 ng/ml; *4*) 0.050 ng/ml; *5*) 0.100 ng/ml.

levels of antiapoptotic proteins Bcl-2 and Bcl-X_L and proapoptotic protein Bax and increased content of proapoptotic protein Bad (Table 1).

During stress exposure, Bcl-2 and Bcl-X_L are located in mitochondrial membranes and close the channels, thus preventing the release of proapoptotic factors from the intermembrane space [8]. A possible mechanism of this process is heterodimerization of Bcl-2 with proapoptotic protein Bax, which leads to blockade of its channel-forming capacity [1].

The regulation of Bcl-2 family protein activity is mediated by transcription factors NF-κB and P53 modulating the expression of the corresponding genes at the transcription levels [1,6]. We found that culturing of lymphocytes with recombinant IL-2 in the proapoptotic dose 0.1 ng/ml was accompanied by a significant increase in NF-κB content (Table 1). Similar inductive effect of IL-2 was previously demonstrated

TABLE 1. Content of Bcl-2 Proteins and Transcription Factors NF-κB and P53 in Blood Lymphocytes after Incubation with Recombinant IL-2 in a Dose of 0.1 ng/ml

Intracellular proteins, arb. units	Intact lymphocyte culture	Lymphocytes incubated with 0.1 ng/ml recombinant IL-2
Bcl-2	1.26 (0.98-1.45)	0.77 (0.76-0.92)*
Bcl-X_L	1.90 (1.59-1.98)	1.47 (1.29-1.58)*
Bax	0.97 (0.87-1.04)	0.45 (0.42-0.59)*
Bad	0.57 (0.51-0.59)	1.02 (1.01-1.24)*
NF-κB	0.48 (0.36-0.52)	0.92 (0.78-1.09)*
P53	1.48 (1.42-1.65)	0.91 (0.80-1.26)*

Note. The data are presented as medians and upper and lower quartiles. *$p<0.05$ compared to the same parameter in intact lymphocyte culture.

on three cell systems, including Jurkat cells, murine B lymphocytic BA/F3 cells, and primary human resting T cells [7]. Most authors consider this transcription factor as an intracellular second messenger mediating transduction of the proliferative signal of IL-2 from the plasma membrane to cell nucleus [11,15]. However, some experiments demonstrated the possibility of NF-κB-mediated activation of P53 leading to transcription of proapoptotic proteins [9]. It is evident that the decrease in the level of non-phosphorylated P53 in lymphocytes cultured in the presence of recombinant IL-2 (Table 1) can attest to intensification of the formation of its active phosphorylated form. This process can be mediated by activation of MAP kinase JNK and NF-κB protein involved in phosphorylation of N-terminal domain of P53 [7].

At the same time, both these transcription factors require P300 co-activator for proper interaction with the corresponding promoter sequences and the amount of P300 in the nucleus is limited. Therefore, competitive interactions between P53 and NF-κB for binding with this protein for induction of pro- or antiapoptotic signal cannot be excluded [5]. However, the participation of P300 kinase in signal transduction from IL-2 was demonstrated [10], which can indirectly attest to the involvement of P53 and NF-κB into the realization of apoptosis mediated by this cytokine.

Thus, our experiments showed that the modulating effect of recombinant IL-2 on the apoptotic program is determined by the dose of the cytokine and culturing conditions. The molecular mechanisms underlying the proapoptotic effect of IL-2 on blood lymphocytes are mediated by activation of transcription factors P53 and NF-κB and shifts in the balance between the anti- and proapoptotic Bcl-2 family proteins towards the latter. Further study of the mechanisms of realization of apoptosis upon exposure to IL-2 can help to develop a molecular technology of selective regulation of programmed cell death of immunocompetent cells applicable for pathogenetically substantiated correction of diseases associated with disturbances in transduction of stimuli initiating or inhibiting apoptotic cell death.

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