Dexamethasone Effects on Activation and Proliferation of Immune Memory T Cells

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Dose-dependent effects of dexamethasone on activation and proliferation of donor immune memory T cells ($CD45RO^+$) were studied. Activation of memory T cells associated with IL-2 production and membrane expression of CD25 molecule was resistant to dexamethasone. Proliferative activity of memory T cells associated with membrane expression of CD71 molecule was highly sensitive to dexamethasone. Hence, glucocorticoid hormones can maintain the clonal balance in the lymphoid tissue without preventing realization of the immune memory mechanism.

Key Words: *dexamethasone; memory T-cells; cell activation; interleukin-2*

According to modern concepts, the efficiency of immune response to antigens of different nature (bacterial, viral, tumor, *etc.*) is determined by generation of antigen-specific immune memory cells [11]. $CD4^+$ and $CD8^+$ memory T cells are present in the organism without the antigen and their counts are replenished by homeostatic proliferation [9].

Failure of the mechanisms responsible for the formation of immunological memory underlies the development of some diseases [9]. This necessitates the search for effective methods for its regulation aimed in some cases at stimulation and in others at inhibition of immune processes. It is known that glucocorticoids are capable of modulating the immune system cells [8,10]. Dexamethasone (Dex), a synthetic glucocorticoid, is widely used in clinical practice for the treatment of immunopathological processes [7,10].

We studied the dose-dependent effects of Dex on activation and proliferation of immune memory T cells *in vitro*.

MATERIALS AND METHODS

The study was carried out on cells from 18 donors, 6 women and 12 men aged 20-39 years. Mononuclear cells (MNC) were isolated from heparin-treated venous blood by centrifugation in ficoll-urografin density gradient (ρ=1.077 g/cm^3). The $CD45RO^+$ T cells were isolated from MNC by immunomagnetic separation (MACS MultiStand; MidiMACS Separator, LS Columns, Miltenyl Biotec) using magnetic particles charged with antibodies to CD45RO molecule (MicroBeads, Miltenyl Biotec). The level of the target cell fraction was at least 95%. Isolated lymphocytes (10^6/ml) were then cultured in Iskov medium (Sigma) containing 0.5% human serum albumin (Microgen), 5×10^{-5} M mercaptoethanol (Acros Organics), and 30 μg/ml gentamicin with different concentrations of Dex (KRKA) or without it (control) for 48 h at 37°C in a humid atmosphere with 5% CO_2. Antibiotin particles with biotinilated antibodies to CD2, CD3, and CD28 molecules served as T cell activators (T Cell Activation/Expansion Kit human, Multenyl Biotec).

Culturing variants were as follows: intact sample (control), sample with T cell activator, samples with T-cell activator and Dex in concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M.

The content of IL-2 in culture supernatants was measured by enzyme immunoassay using test systems according to the instruction (Vector-Best).

The content of T cells expressing CD25 and CD71 molecules was measured by flow cytofluorometry on a GuavaEasyCyto™Plus (Millipore) with antibodies labeled with fluorescent dyes (Sorbent, e-Bioscience). Viability of the studied cultures was

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evaluated using GuavaViaCount reagents and software (Millipore).

The data were statistically processed by Statistica 7.0 software. The means (M) and standard deviations (s) were calculated. The significance of differences was evaluated by the Mann–Whitney U test. The differences were considered significant at $p<0.05$.

RESULTS

By the end of incubation (48 h), the level of viable CD45RO$^+$ cells in control samples was 69.72±11.28%. Mitogen significantly reduced (by 1.4 times) the levels of viable lymphocytes. Dexamethasone in a high concentration (10^{-5} M) reduced the negative effect of stimulation to the values similar to those in intact samples. In lower concentrations (10^{-6} and 10^{-7}), Dex virtually did not change the viability of activated T cells.

Activation of T cells is inseparable from their production of IL-2 and expression of CD25 molecule (IL-2 receptor a-chain) on their surface [12]. Interleukin-2 is one of the key cytokines triggering the proliferation of T cells [13]. Activation of memory T cells led to increase of their production of IL-2 (7.7 times; Fig. 1). Dexamethasone in a concentration of 10^{-5} M reduced significantly the production of IL-2. Lower concentrations of the hormone were inessential for the studied parameter. Activation of memory T cells led to an increase in the levels of CD25$^+$ cells (4.9 times) in this population (Fig. 2). Addition of Dex in a concentration of 10^{-5} M to stimulated lymphocytes led to a decrease of CD25$^+$ cell content by 36%. Lower concentrations of the hormone caused no effect of this kind.

CD71 molecule is a transferrin receptor. As a rule, this molecule is expressed on proliferating cells [4]. Stimulation led to an increase (1.6 times) in the counts of CD71$^+$ cells (Fig. 3). Addition of Dex in all the studied concentrations to the culture reduced the level of CD71$^+$ cells by 30%.

According to our data, stimulation of memory T-cells, induced by particles simulating the activity of antigen-presenting cells, reduced the percentage of viable lymphocytes in the culture. This reduction could result from activation apoptosis, developing along with the unfolding proliferative reaction [1]. On the other hand, the increase of cell viability in stimulated cultures, caused by addition of 10^{-5} Dex, could be explained by glucocorticoid inhibition of stimulation-induced apoptosis of T cells via suppression of fasL expression on membranes [7].

The system of IL-2 with receptor plays the key role in stimulation and triggering of the T cell proliferative response [14]. The expression of CD25 molecule (IL-2 receptor a-chain) on lymphocytes is

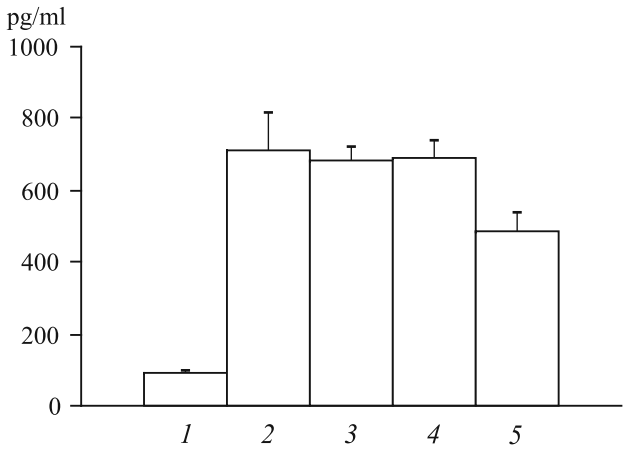

Fig. 1. Production of IL-2 in CD45RO$^+$ lymphocyte cultures. Here and in Figs. 2, 3: 1) control; 2) stimulation; 3) 10^{-7} Dex; 4) 10^{-6} M Dex; 5) 10^{-5} M Dex.

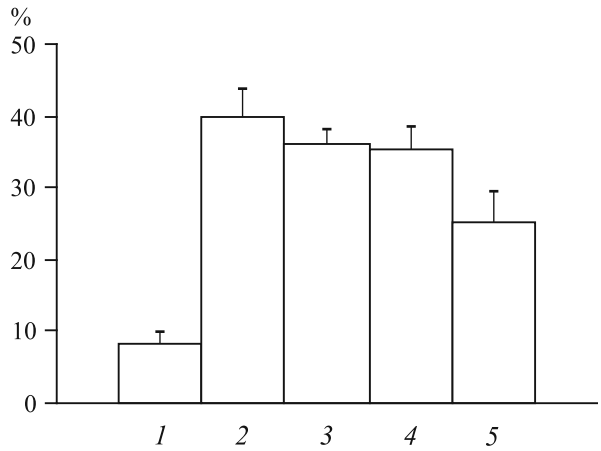

Fig. 2. Content of CD25$^+$ lymphocytes in T cell culture.

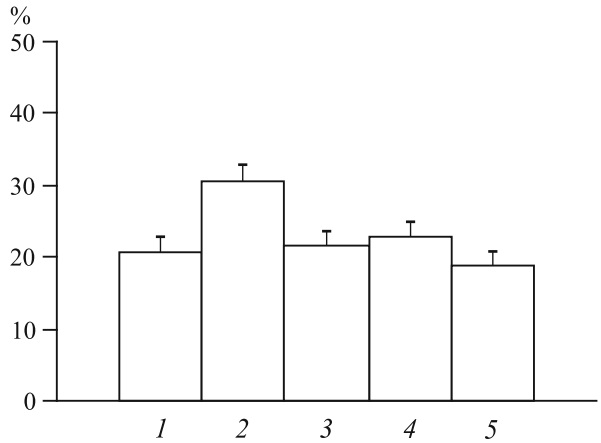

Fig. 3. Content of CD71$^+$ lymphocytes in T cell culture.

associated with IL-2-dependent stage of immune response [5,13]. The immune memory cells are in the G_1 phase, this promoting their more rapid start of the IL-2-dependent phase of immune response [6]. As expected, the increase in the level of CD25$^+$ cells in

cultures of mitogen-stimulated T cells was associated with their more intense production of IL-2. According to our data, reduction of IL-2-dependent response to activation developed only after addition of Dex in a rather high concentration (10^{-5} M) into the culture. Interestingly that stimulated naive $CD45RO^+$ T-cells in parallel experiments demonstrated sensitivity to lower concentrations of the hormone as well (the data not presented). Hence, the results were in line with a previous hypothesis on the glucocorticoid resistance of functional activation of memory T-cells [2,3].

Transferrin receptor CD71 provides the entry of iron ions essential for mitotic processes into active cell and is detected on the majority of proliferating cells [15]. Addition of Dex in concentrations of 10^{-7}-10^{-5} M led to a reduction of the number of cells expressing CD71 marker. These data suggest that the antiproliferative effect of glucocorticoid hormones may be (at least partially) due to not their suppressive effect on the functional activation of memory T-cells associated with their production of IL-2. Presumably, the role of glucocorticoid hormones in immunogenesis is primarily aimed at suppression of excessive growth of memory T-cells and at maintenance of the clonal cell balance in the lymphoid tissue. On the other hand, Dex resistance of memory T-cells activation may create prerequisites for rapid realization of their functional potential during the development of secondary immune response.

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