Ultraviolet Photomodification of the Functional State of Leukocytes Under Conditions of Experimental Endotoxemia

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Ultraviolet irradiation of peripheral blood in therapeutic doses corrects the immunosuppressive effect of staphylococcal toxin. The main immunoregulatory effect of ultraviolet irradiation is associated with the production of endogenous immune transmitters by blood cells such as interleukin-1 and tumor necrosis factor. Ultraviolet irradiation increases the content of $CD3^+$ and $CD4^+$ lymphocytes and restores the natural killer activity. The last parameter is proposed as a convenient criterion of the efficiency of quantum hemotherapy.

Key Words: *blood ultraviolet irradiation; immunocompetent cells; immunoregulation; interleukin-1; endotoxemia*

Ultraviolet irradiation of the blood (UVIB) and its components occupies an important place in quantum therapy widely used in the clinics. The necessity of immunocorrection is the most usual indication for UVIB [7,8]. It has been recently proposed that clinical effect of ultraviolet (UV) irradiation is based on its immunoregulatory action. Despite a great body of clinical data, the immediate effect of UV on immunocompetent cells in patients with endotoxemia (pyoseptic diseases, mechanical and thermal injuries) remains unclear.

The aim of the present study was to examine the effect of UVIB on immunocompetent cells of peripheral blood in endotoxemia.

MATERIALS AND METHODS

The following model was used to estimate the immunomodulatory effect of UVIB in endotoxemia. The upper layer of erythrocyte mass (about 50 ml), which is removed upon conservation of donor erythrocytes, was divided into three portions: portion 1 was intact control; staphylococcal toxin (N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, series 28-1) was added to portions 2 and 3 in a standard dose of 50 μl/ml [1] and incubated for 1 h at 37°C. After incubation, portion 3 was exposed to UV irradiation in a special apparatus for UVIB in a dose equal to therapeutic dose. Thus, functional state of immunocompetent cells was assessed in the following three samples: control, staphylococcal toxin, staphylococcal toxin+ UVIB.

Functional activity of phagocytes was assessed in the nitro blue tetrazolium test (Sigma). The basal and zymosan-stimulated neutrophil activity indices were determined [5]. Lymphocytes were isolated on a Ficoll-Verografin gradient. Expression of surface cell antigens CD3 (T lymphocytes) CD4 (T helpers), CD8 (T suppressors), CD22 (B lymphocytes), CD25 (interleukin-2 receptor), and DR (activation marker) were evaluated in the lymphocytotoxic test using monoclonal antibodies (IKO series, MBS, Moscow) [9].

Natural killer activity was assessed using K562 targets [2]. Production of tumor necrosis factor (TNF) and interleukin-1 by peripheral blood mononuclears was measured after a 24-h incubation in RPMI-1640

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TABLE 1. Effect of UV Irradiation of the Blood on Functional Parameters of Leukocytes under Conditions of Experimental Endotoxemia

Parameter	Initial value	Toxin	UVIB
$CD3^+$ cells, %	61.7±3.2	72.5±5.4	71.2±4.0*
$CD4^+$ cells, %	32.2±2.3	32.8±3.8	44.9±4.8*,**
$CD8^+$ cells, %	20.4±2.3	20.9±3.4	21.2±2.4
$CD22^+$ cells, %	20.6±3.9	16.9±3.6	20.7±2.9
DR^+ cells, %	28.7±4.0	26.2±3.6	28.8±1.6
$CD25^+$ cells, %	27.1±2.2	28.1±1.8	28.2±1.7
Natural killer activity, %	64.1±2.5	45.6±3.5*	67.2±2.2**
NDP, arb. units	39.7±3.4	65.2±6.3*	51.2±8.2
Neutrophil activity index, arb. units:			
basal	0.11±0.02	0.09±0.01	0.07±0.02
stimulated	0.45±0.03*	0.35±0.05*	0.35±0.06*
TNF, ng/ml:			
basal	0.92±0.23	0.80±0.29	1.05±0.25
stimulated	4.09±0.82*	1.32±0.70*	2.81±0.94
Interleukin-1, pg/ml:			
basal	120±69	88±60	95±65
stimulated	1745±688*	354±133**	2450±714***

Note: *Denotes considerable differences between the basal and stimulated activity. $p<0.05$: *compared with the initial value, **compared with the toxin-treated portion.

medium under basal conditions and after pyrogenal stimulation (5 μg/ml). The content of cytokines was measured by enzyme-enhanced immunoassay (Protein Contour, St. Petersburg). The content of nucleoprotein degradation products (NDP) in lymphocytes was measured as described previously [3].

RESULTS

No considerable changes in phagocytic activity and expression of the differentiation receptors in lymphocytes were found in the portion treated with the toxin and exposed to UVIB.

The immunodepressive effect of staphylococcal toxin manifests itself as suppression of the production of endogenous immunomodulators such as TNF and especially interleukin-1 and as inhibition of natural killer activity. This is accompanied by accumulation of NDP in peripheral blood mononuclears (Table 1).

Irradiation of toxin-treated leukocyte suspension stimulated expression of CD3 (T lymphocyte) and CD4 (T helpers) receptors and restored TNF and interleukin-1 production by peripheral blood mononuclears and slightly decreased the content of NDP (Table 1).

Enhanced expression of T-cell receptors probably results from either a direct effect of UVIB on lymphocyte membrane or modified production of immunomodulators. The last assumption is supported by the fact that the observed phenotypical changes in lymphocytes are similar to those induced *in vitro* by the hydra peptide morphogen, an acute phase mediator secreted in mammalian by hypothalamic neurons and exhibiting anabolic and immunostimulating effects [6]. The slight reduction in the content of NDP probably results from a mitogenic (direct or indirect) action and/or possible repair effect of UVIB.

Thus, our experiments showed that the main immunoregulatory effect of UVIB is associated with the production of endogenous immunomediators, in particular, interleukin-1 and TNF by blood cells. The enhanced secretion of lympho- and monokines by a small population of UV-irradiated circulating mononuclears initiates systemic reactions leading to a considerable clinical effect [4]. It seems interesting to apply the proposed experimental model for evaluation of the production of other cytokines and for choosing the mode of UVIB in a certain pathology. Of special interest is the possibility of using NDP as a signal parameter. The fact that UVIB normalizes the reduced natural killer activity in experimental endotoxemia allows the use of this parameter for determining indications and diagnostic monitoring of the efficiency of UV irradiation of the blood.

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