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PHOTOMODIFICATION OF HUMAN IMMUNOCOMPETENT BLOOD CELLS

V. A. Krylenkov, R. P. Ogurtsov,
M. A. Osmanov, and V. E. Kholmogorov

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Research into the mechanisms of action of optic radiation (OR) on blood has recently assumed great practical importance [1, 8]. The study of photomodification of immunocompetent cells (lymphocytes) is particularly promising: the basic function of immunity, namely ensuring the stability of the antigenic structure of the organism, is realized through them. The immune system, as we know, is a component of the adaptation-protection mechanisms which maintain homeostasis of the human and animal body. There is information in the literature to show that UV radiation, as the biologically most active part of radiation in the optic range, induces modification of certain functions of lymphoid cells [7, 10, 12].

In the investigation described below, processes of photomodification of lymphoid cells in human blood, developing immediately after exposure to OR and also in the late stages after irradiation, were investigated by methods of spontaneous and immune rosette formation and the blast transformation test, combined with treatment with the antooxidant α-tocopherol (TP) (the results of the investigation were recorded 90 min and 3 days after irradiation).

EXPERIMENTAL METHOD

Human blood (from men aged 25-40 years) was used. Lymphocytes were isolated from stabilized blood (TsOLIPK-7B stabilizer, final concentration 1%) by centrifugation in a Ficoll-Verografin density gradient [13]. The cell suspension, in a concentration of $2 \cdot 10^6$ cells/ml, was irradiated with OR (80% of the radiant energy lay within the 280-365 nm waveband) in buffered physiological saline (0.15 M NaCl, pH 7.4), in a volume of 2 ml with continuous mixing [3]. The lethal effect was estimated in accordance with the fraction of cells stained with trypan blue (0.2% solution of the dye, from Merck, West Germany). T lymphocytes were counted as the number of cells forming "active" E rosettes (E-FRC) [15]. B-lymphocytes form identifiable EAC-rosettes (EAC-FRC) [9]. The formation of secondary lipid peroxidation (LPO) Products (malonic dialdehyde) was estimated by their reaction with 2-thiobarbituric acid (TBA-active products) [6]. To assess the functional state of the lymphocytes (ability to proliferate, blast-transformation) in the late stages after irradiation spontaneous and stimulated DNA synthesis was determined (the cells were labeled with ^{3}H-thymidine, 23-26 Ci/mmole, from Izotop, USSR) [5]. Phytohemagglutinin (PHA) was used as the mitogen (FGA-P, 8.5 μg/ml, from Difco, USA). The antioxidant TP (M. V. Lomonosov Institute of Fine Chemical Technology, Moscow), dissolved in ethanol, was added to the cell suspension or to the blood before irradiation or immediately thereafter, in a final concentration of 10^{-7} M. The ethanol concentration in the cell suspension or in the blood did not exceed 1%.

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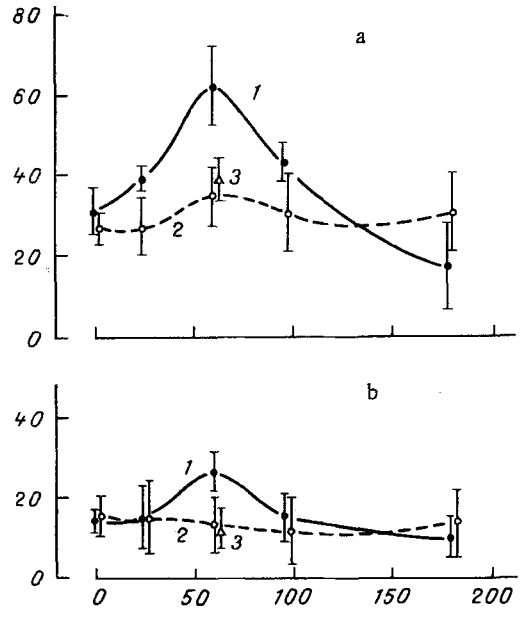

Fig. 1

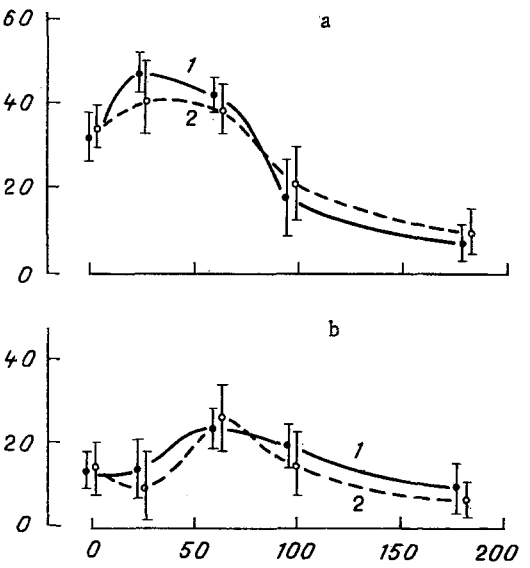

Fig. 2

Fig. 1. Effect of OR (1) and TP (10^{-7} M) combined with OR (2) on ability of human peripheral blood lymphoid cells to form "active" E-rosettes (a) and EAC-rosettes (b). Abscissa, dose of OR (in J/m^2); ordinate, number of RFC (in % of number of cells examined); 3) TP was added to the cell suspension immediately after irradiation. RFC were determined immediately after irradiation.

Fig. 2. Effect of OR (1) and TP (10^{-7} M), added 90 min after treatment with OR (2) on ability of human peripheral blood lymphoid cells to form "active" E-rosettes (a) and EAC-rosettes (b). RFC counted 90 min after irradiation. Legend as to Fig. 1.

A special series of experiments showed that the presence of the antioxidant in an unirradiated suspension of lymphoid cells or in blood did not modify the ability of the cells to form rosettes with sheep's red blood cells (SRBC) or their reaction to mitogens. These observations confirm results obtained by the writers previously [10]: antioxidants do not modify the cytotoxic action of monospecific antisera against unirradiated lymphoid cells.

The numerical results were subjected to statistical analysis by the Student–Fisher test, with a level of significance of $p = 0.05$. Values of the arithmetic means and confidence intervals obtained by analysis of the results of 7-12 series of experiments are given in Figs. 1 and 2 and in Table 1.

EXPERIMENTAL RESULTS

Exposure of the lymphocytes to OR in doses of LD_0-LD_4 (20-60 J/m^2) increased the ability of these cells to form "active" E-rosettes (by 8-31%, by 1.3-2 times) and EAC-rosettes (by 10-11%, by 1.6-1.7 times; Fig. 1) with SRBC. Under these circumstances the content of TBA-active products in the medium surrounding the irradiated cells was doubled (compared with the initial level in the unirradiated control). Increased ability of lymphocytes, irradiated in nonlethal doses, to form E- and EAC-rosettes was still preserved 90 min after their irradiation (Fig. 2); in this case, moreover, the maximum of E-rosette formation was shifted toward the smaller dose (20 J/m^2).

A further increase in the dose of OR (up to 180 J/m^2, LD_{20}) led to a fall in the ability of the irradiated lymphocytes to form E- and EAC-rosettes to the level of the unirradiated control, when the rosettes were formed either immediately after irradiation or after incubation of the cells for 90 min in darkness. Under these circumstances, no further increase in the concentration of TBA-active products could be observed in the intercellular medium after irradiation.

TP, added to the cell suspension before or immediately after exposure to OR, prevented the increase in concentrations of E- and EAC-RFC in the irradiated lymphoid cell suspension

TABLE 1. Level of DNA Synthesis in Human Peripheral Blood Lymphocytes after Irradiation of these Cells with OR in Vitro and in the Composition of the Blood in Experiments in Vivo (M ± m)

Type of procedure	Dose, J/m²	Incorporation of ^{3}H-thymidine, cpm	
		without mitogen	stimulation by PHA
Irradiation of lymphocytes in vitro	Unirradiated cells	4669±1927	17 462±9277
	20	4247±1847 (4703)*	4 470±1905 (17 055)*
	40	3361±1475 (4651)**	1 669±550 (17 813)**
	60	763±441	533±207
Irradiation of blood	Unirradiated cells	5997±2271	25 736±9092
	80	6289±2540 (4001)*	16 850±4969 (24 891)*
	160	5642±2088 (3687)**	13 635±5336 (23 950)**
	320	3485±433 (3120)*	5 120±3938 (19 712)*

Legend. *) Lymphocytes or blood were irradiated in the presence of TP; **) TP was added to the cells or blood immediately after irradiation. Values in parentheses are levels of DNA synthesis in lymphocytes irradiated and incubated with the antioxidant, and obtained on the basis of two series of experiments.

(Fig. 1). If, however, TP was added to the lymphocyte suspension 90 min after they were irradiated, its presence had virtually no effect on the character of dose-dependence of E- and EAC-rosette formation, studied at the same stage of postradiation incubation of the cells (Fig. 2).

Photomodification of lymphocytes, which are the principal immunocompetent cells in the delayed-type hypersensitivity reaction [4], can characterize the response of these cells to a mitogen (the blast-transformation reaction). It follows from Table 1 that the spontaneous level of DNA synthesis, characterizing the viability of lymphocytes obtained in vitre, fell significantly after exposure to OR in the maximal dose only (60 J/m²), whereas the ability of the irradiated cells to undergo PHA-activation of DNA synthesis fell significantly even after the minimal dose (20 J/m²). A similar decline in the response to the mitogen while the viability of the cells was preserved was observed also in the case of irradiation of whole human blood. Under these circumstances the decline of the response to PHA by lymphocytes irradiated in vitro and in the composition of the blood (in vivo) was prevented by TP, added to the cells both before and immediately after irradiation (Table 1).

Exposure to OR in nonlethal doses thus leads to an increase in the number of E- and EAC-RFC, to decline of the response of the hymphocytes to PHA, and to an increase in the content of TBA-active products in the irradiated population of human blood lymphocytes. The antioxidant TP prevents changes in expression of the membrane markers E- and EAC-RFC and the decline in the response to PHA in human blood lymphocytes, when added both before and immediately after irradiation of these cells in vitro or in blood. Doses of OR causing an equally effective depression of the response of the blood lymphocytes to PHA after irradiation of these cells in vitro were 8-16 times less than doses of OR used to irradiate whole blood.

Inhibition of the dark stage of the process of intravital photomodification of immunocompetent blood cells by the antioxidant points to an essential contribution of LPO to this phenomenon. The fact that the enhanced expression of membrane markers in the irradiated lymphocytes after incubation in darkness for 90 min could not be reduced by the antioxidant suggests that the process of self-oxidation which develops in the dark period [15] gave rise to irreversible changes in the receptor structure of the lymphocytes. It is from this point of view that the publication [11], which gives data showing that the character of death of cells irradiated with lethal doses of OR is the same as the character of their response to mitogens, must be considered. In the present case the effect of OR in nonlethal doses was investigated, and this suggests only the reversible modification of specific receptors for PHA, the existence of which has been stated by Ling [2].

Meanwhile the possibility cannot be ruled out that the decline of the response to the mitogen in irradiated hymphocytes is due to the activity of suppressor cells from the lymphocyte population irradiated in vitro or in the composition of the blood. The definite similarity in the character of the response to the mitogen in lymphocytes irradiated in vitro and in the composition of photomodified blood, must also be emphasized, for it suggests that the intervital response of cells to the mitogen is relatively independent of photometabolites in irradiated blood plasma.

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SENSITIZATION TO INFLUENZA A_2 VIRUS IN COMBINED EXPERIMENTAL INFECTION

S. Gönczi

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Influenzal infection can significantly aggrevate bronchial asthma or provoke its onset. Vaccination against influenza can also exacerbate asthma. After viral infection, resistance to secondary Gram-negative infection is reduced. Experimental data on the mutual effect of these two infections are contradictory [1, 3, 5, 7, 9-12].

The aim of this investigation was to develop a model of combined immunization to influenza virus and *Haemophilus influenzae* (HI), for the latter is very often found in healthy people. The effect of infection due to HI on the degree of sensitization to influenza virus was studied.

EXPERIMENTAL METHOD

Noninbred guinea pigs weighing 250-300 g were used. In the experiments of series I a method of viral vaccination of the guinea pigs was used and the doses of viral antigens not inducing a reaction in intact animals (n = 38) were determined in various tests. Strain A_2 Victoria 35/72 H_3N_2 was used as the influenza vaccine. After preliminary propagation on chick embryos the strain of influenza virus was adapted (N. A. Andreeva) to guinea pigs by intranasal infection. The virus was reisolated from nasal washings of the animals on the 4th-5th day after infection. The animals were immunized with influenza vaccine once, intra-

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