INHIBITION OF DNA SYNTHESIS IN LYMPHOCYTES BY UV-IRRADIATED PLASMA

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KEY WORDS: UV irradiation; mitogen induced proliferation of human lymphocytes; plasma

The mechanisms of the photoimmunologic reactions responsible for the therapeutic effect of a new method of treatment, i.e., autotransfusion of UV-irradiated blood, are currently being intensively studied. Analysis of data in the literature suggests that the final, resultant effect of photohemotherapy is due to structural and functional changes in the cells and plasma components, induced by UV light in whole blood [7]. It has been shown, for instance, that UV radiation *in vitro* causes certain photoconversions of the components of blood serum [1, 5, 6], and modifies the functions of platelets [12] and cells of the lymphoid-macrophagal series [9, 10, 13]. UV irradiation affects all immunocompetent cells, namely macrophages and T- and B-lymphocytes. During irradiation of whole blood in the "Izol'da" apparatus, the authors demonstrated by the blast transformation of lymphocytes test (BTLT) that the cells of a concrete donor exhibit individual sensitivity to the action of UV radiation, and that B-lymphocytes are more resistant to UV rays than T-cells [2]. This last conclusion is in full agreement with views expressed by other workers [11].

To continue previous studies of photo-induced changes found in whole blood, it was decided to investigate the effect of UV-irradiated plasma blast transformation on intact human peripheral blood lymphocytes.

EXPERIMENTAL METHOD

The procedure of irradiation of heparinized blood from healthy donors was described by the authors previously [2]. The blast transformation reaction was set up on lymphocytes, isolated in a Ficoll-Verografin density gradient, by a radiometric micromodification of the method [3], using immunologic test plates from "Medpolimer" (Leningrad). The medium for cell culture included 15% autologous plasma, in two versions: platelet-enriched (PEP) and platelet-free plasma. Platelets were removed by centrifugation at 400g for 20 min. The final concentration of the mitogens was 5 μg for concanavalin A (con A, from Sigma, USA), 5 μg for pokeweed mitogen (PWM, from "Sigma"), and phytohemagglutinin (PHA-P) (from "Difco," USA) was added in a dilution of 1:64. The DNA-synthetic activity of the lymphocytes was determined by measuring the uptake of ^{3}H-thymidine. Interferon activity in the plasma was determined on a transplantable culture of human Hep-2 cells against vesicular stomatitis virus and expressed in international units (IU), allowing for the degree of sensitivity of the test system used to the USSR national reference preparation of human leukocytic interferon P_9 (N. F. Gamaleya

TABLE 1. Effect of Plasma from UV-Irradiated Blood in Intensity of BTLT (M ± m)

Blast transformation of lymphocytes index, cpm	Test plasma (n = 13)		P
	unirradiated	irradiated	
Spontaneous DNA synthesis	209±21	154±16	>0,05
In presence of:			
PHA	15 158±1 650	8638±1075	<0,01
con A	4 599±866	2414±236	<0,05
PWM	6 004±426	5343±429	>0,05

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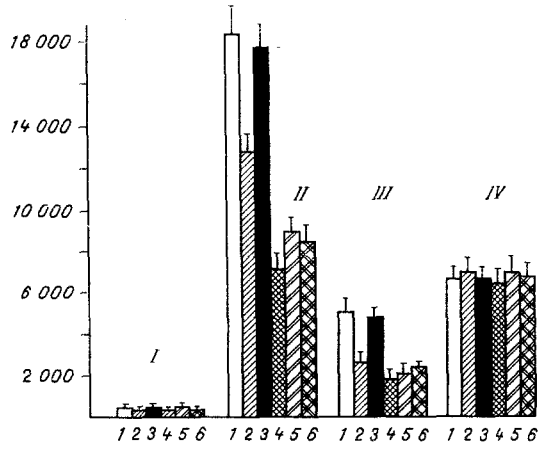

Fig. 1. Antiproliferative effect of plasma irradiated after sedimentation of blood cells. Ordinate, level of ^{3}H-thymidine incorporation into lymphocytes (in cpm). I) Spontaneous DNA synthesis in lymphocytes; II) response to PHA; III) response to con A; IV) response to PWM. 1) Control (autologous plasma from unirradiated blood); 2) irradiated PEP; 3) unirradiated plasma without platelets; 4) plasma irradiated without platelets; 5) Hanks' solution without irradiation of cells; 6) the same, after irradiation of cells.

Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow). The significance of the results was determined by Student's t test.

EXPERIMENTAL RESULTS

Addition of autologous PEP, obtained after irradiation of whole blood, to the culture medium was followed by a change in the parameters of the lymphocyte blast transformation reaction: significant inhibition of DNA-synthesizing activity of the unirradiated lymphocytes occurred in the presence of PHA and of con A. With respect to spontaneous DNA synthesis a similar tendency was observed, but the response of the lymphocytes to PWM did not differ from the control values (Table 1).

Considering the antiproliferative action of interferon, it was necessary to determine its concentration in the test samples of plasma obtained after irradiation of whole blood. No interferon activity could be detected in any of the samples studied (19 IU/ml).

In connection with the recording of the above changes it was necessary to determine the components of the blood (cell or serum) whose photoconversions were responsible for the observed phenomenon. To rule out the influence of cellular factors the following procedure was adopted: the plasma was replaced by Hanks' solution and the blood cells were irradiated under similar conditions. Addition of the supernatant in this case did not inhibit blast transformation of the lymphocytes (Fig. 1). On the addition of PEP, irradiated after sedimentation of the cells, inhibition of BTLT in the presence of PHA and con A was still present. The results indicate that inhibition of mitogen-induced DNA synthesis in the lymphocytes was caused by photoconversions of plasma components.

During UV-irradiation of blood plasma photoperoxidation of its lipids is known to occur [4]. Accumulation of products of free-radical oxygenation of lipids is accompanied by inhibition of blast transformation of lymphocytes [8], and under these circumstances the platelets destroy these products [1]. It can thus be postulated that the addition of plasma, irradiated after removal of the platelets, ought to be accompanied by enhancement of the phenomenon observed. In fact, on the addition of such plasma, inhibition of DNA synthesis in the presence of PHA and con A increased. The response of the lymphocytes to PWM in this case also was not inhibited, which confirms the view that B-lymphocytes have higher UV-resistance. This suggests that T- and B-lymphocytes may differ in the activity of their antioxidant systems.

The results are thus evidence that UV irradiation of whole blood modifies the functions of immunocompetent cells not only as a result of the direct action on them, but also due to photoconversions of serum components. The possibility cannot be ruled out that the therapeutic effect of autotransfusion of irradiated blood is the integral result of interaction between cellular and serum factors.

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EFFECT OF INTENSIVE PHYSICAL EXERCISE ON MACROPHAGAL FUNCTIONS

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The functional state of phagocytic cells of the reticuloendothelial system (RES) and, in particular, its chief compartment — the mononuclear phagocytic system (MPS), essentially determines resitance to trauma, blood loss, burn toxemia, various forms of circulatory shock, and so on [4]. After treatment of animals with various substances stimulating RES function (microaggregated human albumin, glyceryl trioleate, quinones, a combination of estrogens and glucocorticoids), in many cases tolerance to stress increased, whereas after blockade or depression of the phagocytic activity of the resident macrophages (Mph) mortality increased [10]. It was shown previously that after sudden cooling (to −7°C) [9], acute physical exercise [1], and administration of hydrocortisone in a dose of 125 mg/kg [5, 6], the clearing function of the RES was abruptly depressed, with a corresponding lowering of resistance to stress; the ingestive function of the RES was depressed after acute stress, moreover, because of depression of the phagocytic function of the Lupffer cells (KC) of the liver, whereas clearance of the blood of pulmonary interstitial Mph showed a compensatory increase. Meanwhile, the functional activity of Mph from different compartments of the MPS during stress has received little study.

TABLE 1. Number of Monocytes in Blood, PMph, AMph, and Sinusoidal Cells of the Liver in Control and after IPE (M ± m)

Experimental conditions	PE				BAW		
	total number of leukocytes	absolute number of monocytes	total number of cells	PMph	total number of cells	AMph	number of sinusoidal liver cells per 1000 hepatocytes
	·10⁹/liter		·10⁶				
Control	6,8±0,8 (6)	0,7±0,09 (6)	14,5±1,1 (15)	14,2±1,0 (15)	9,2±0,8 (5)	8,2±0,4 (5)	335,2±8,2 (7)
IPE	4,8±0,6 (6)	0,4±0,05* (6)	12,0±1,8 (6)	11,8±1,0* (6)	12,1±0,9* (5)	11,0±1,0* (5)	326,9±5,2 (7)

Legend. *p < 0.05 compared with control. Here and in Tables 2 and 3, number of animals given in parentheses.

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