IMMUNOCORRECTIVE ACTION OF VITAMIN A IN STRESS

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There is much clinical and experimental evidence to show that exposure to severe and prolonged stress may induce a whole range of immunologic disturbances: lymphocytopenia, hypoplasia of the thymus, depression of the function of T-lymphocytes, macrophages, natural killer cells, and so on [1-3]. Resistance to tumors [9, 19] and resistance to viral and bacterial infections and parasitic diseases [12, 17] also are depressed under these circumstances. In recent years research has been published in which attempts have been made to use certain substances to prevent stress-induced disturbances of activity of the immune system [9, 10], but this problem is still very far from solution. In this communication we give data on the possibility of using vitamin A as an effective immunicorrective agent in stress. The basis for these investigations was the results of the writers' previous investigations into the immunostimulating properties of vitamin A [6-8] and its ability to restore the immune status in patients with chronic pneumonia and lung cancer — diseases accompanied by marked disturbances of reactivity. Besides, evidence of the antistressor properties of vitamin A obtained on various pathophysiological, but not immunologic models, described in the literature [13, 16], also was taken into account.

EXPERIMENTAL METHOD

Experiments were carried out on 160 male CBA mice weighing 16-18 g. The experiments were repeated twice. Stress was induced in the animals by immobilizing them in the supine position, with all four limbs fixed for 6 h. Data in the literature and the results of our previous experiments indicate that during the first 3-5 days of such immobilization, marked immunologic disturbances develop. For the 2 days before induction of stress and on the day of stress, the mice received by mouth an oily solution of vitamin A (retinol acetate) in a daily dose of 3000 I.U. Mice of the control groups received the oily solvent of vitamin A. The animals were either immunized with sheep's red blood cells (SRBC) 24 h after stress for future determination of antibody-forming cell (AFC) production by Jerne's method, or they were killed for the other investigations. Splenocytes from the mice were used for the blast-transformation test with mitogens, and also for investigations to study natural killer cell activity. To study the formation of blast cells 10^6 splenocytes were cultured for 3 days in medium RPMI-1640 (Flow Laboratories, USA) with the addition of antibiotic, 10% embryonic calf serum (Flow) and mitogen — either phytohemagglutinin (PHA) or concanavalin A (con A). The mitogens were obtained from Serva (West Germany) and were used in a dose of 20 µg to 10^6 cells. ^{3}H-thymidine was added to the samples in a dose of 1 µCi 6 h before the end of culture, after which specific activity was counted on an Intertechnique counter (France). When natural killer cell activity was determined, cells of murine T-cell lymphoma IAC-1, maintained by serial passages in vitro, were used as the target cells. RPMI-1640 culture medium (Flow), containing 10% embryonic calf serum (Flow), 2 mM glutamine, 1 mM HEPES buffer, and monomycin, was used. To label the target cells, 100 µCi of ^{51}Cr (Amersham International, England) was added to 1 ml of culture medium containing $4 \cdot 10^6$-$5 \cdot 10^6$ cells. The cells were incubated for 1 h at 37°C, washed three times with medium, and the concentration adjusted to $2 \cdot 10^5$ cells/ml. In wells in round-bottom plates $2 \cdot 10^4$ target cells in 100 µl of medium were added to 100 µl of effectors. All experiments were carried out with three different ratios of effector to target — 100:1, 50:1, and 25:1. The mixture of effector cells and target cells was incubated for 4 h at 37°C in an atmosphere with 5% CO_2. The plates were then centrifuged at 100g for 5 min, and a sample of

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TABLE 1. Effect of Stress and Vitamin A on Some Immunologic Parameters in Mice (M ± m)

Parameter	Experimental conditions		
	stress	stress + vitamin A	control
Number of leukocytes in 1 mm^3 peripheral blood	4456±456*	6935±390**	6645±840
Lymphocytes, %	35,5±6,0*	54,0±4,2**	56,0±4,2
Weight of thymus, mg	30,5±4,0*	50,1±4,8**	65,0±5,5
Weight of spleen, mg	661,5±4,0*	79,9±3,8	85,2±7,3
Phagocytosis by macrophages:			
phagocytic number	26,3±1,3*	38,0±2,6**	44,4±1,3
phagocytic index	2,9±0,06*	3,2±0,2	3,7±0,1

Legend. *) Statistically significant differences between control group and "stress" group; **) between "stress" group and "vitamin A + stress" group.

10 µl of supernatant was withdrawn from each well. The samples were counted on a RackGamma-II gamma-counter (LKB, Sweden). When spontaneous release of ^{51}Cr was estimated, instead of effectors, 100 µl of medium was added to the targets, when the maximal yield of ^{51}Cr was determined, the cells were destroyed by the addition of 100 µl of a 4% solution of the detergent Triton X-100. The percentage of lysis (the cytotoxicity index — CI) was determined by the equation

$$CI = \frac{a-c}{b-c} \cdot 100,$$

where a denotes the experimental yield of ^{51}Cr, b the maximal yield of ^{51}Cr, and c the spontaneous release of ^{51}Cr. As a rule, the spontaneous release of ^{51}Cr did not exceed 10-15%. When the phagocytic activity of the macrophages was studied, cells were flushed out of the peritoneal cavity of the mice with medium 199, the cell mixture was resuspended, after which, under sterile conditions, the suspension of macrophages was poured in volumes of 2 ml into test tubes in which coverslips were placed, and the tubes and their contents were incubated for 2 h at 37°C. After adhesion of the cells to the surface of the coverslip the culture medium was removed and replaced by fresh, to which $5 \cdot 10^6$ microbial cells of a 24-h culture of *Staphylococcus aureus* (strain Zhaev) were added. After incubation for 1 h the coverslips were removed and washed with physiological saline; the adherent cells were fixed with methyl alcohol and stained with azure-eosin by Romanovsky's method. To calculate phagocytic activity the percentage of cells taking part in phagocytosis (the phagocytic number) and the number of bacteria ingested by one phagocyte (the phagocytic index) were determined. The effect of stress and vitamin A on generation of antigen-specific suppressors was estimated by the method in [5]: mice of the control group and animals receiving vitamin A were immunized 24 h after exposure to stress with SRBC, and 2 weeks later, $5 \cdot 10^7$ splenocytes from each mouse were injected intravenously into intact mice, which were simultaneously immunized with SRBC. Control animals received splenocytes from unimmunized mice. Jerne's test was then carried out; the level of antigenically specific suppression was expressed as a percentage of the control.

EXPERIMENTAL RESULTS

Information on the effect of stress on some immunologic parameters is given in Table 1. It will be clear from Table 1 that exposure to stress for 6 h caused atrophy of the thymus, leuko- and lymphocytopenia, and suppression of phagocytic activity (a decrease in the values of both phagocytic number and phagocytic index). The severity of the disturbances which developed will be noted. For instance, the weight of the thymus, the number of lymphocytes in the peripheral blood, and the phagocytic activity of the peritoneal macrophages were all depressed by 33-50% under the influences of stress. Administration of vitamin A under these circumstances almost completely abolished the immunologic disturbances induced by stress, and restored these parameters to virtually their original values.

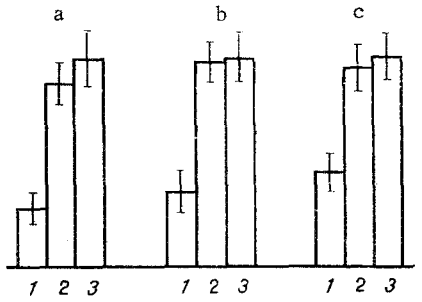

Fig. 1

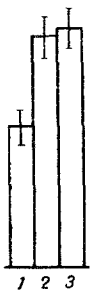

Fig. 2

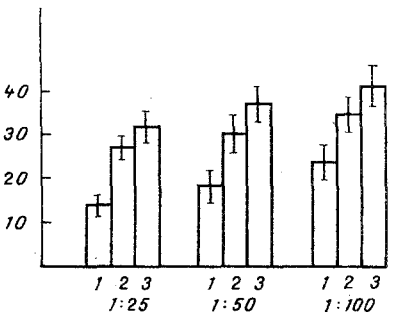

Fig. 3

Fig. 1. Effect of immobilization stress and vitamin A on production of antibody-forming cells of SRBC (a) and on blast-transformation of lymphocytes during their stimulation by PHA (b) and con A (c). Here and in Fig. 3: 1) stress; 2) stress + vitamin A; 3) control. Here and in Fig. 2, data given as percentages of control, taken as 100%.

Fig. 2. Effect of stress and vitamin A on generation of antigenically specific suppressor cells. 1-2) Number of AFC during immunization with SRBC, with addition of suppressor cells taken from animals exposed to stress, exposed to stress and receiving vitamin A, and receiving the oily solvent of vitamin A.

Fig. 3. Effect of stress and vitamin A on natural killer cell activity. Ordinate, CI (%). 1:25, 1:50, and 1:100 — ratios of target to effector cells.

Data showing the effect of stress and vitamin A on AFC production in response to SRBC and on the mitogenic response of the lymphocytes when stimulated by PHA and con A are given in Fig. 1. Exposure to the stressors as a rule sharply depressed (by 50-67%) the humoral and cellular immune response, whereas administration of vitamin A restored immunoreactivity virtually completely. To explain the possible mechanism of depression of ARC production during stress and the normalizing action of vitamin A, experiments were carried out to study the activity of antigenically specific suppressors under the influence of the above factors. The results of these observations are given in Fig. 2, from which is follows that stress caused intensification of generation of antigenically specific suppressors, whereas vitamin A had an inhibitory action on this process.

In the experiments to study natural killer cell activity, unlike in the other series of experiments vitamin A was given in accordance with two schedules: in a daily dose not only of 3000 I.U., but also of 300 I.U., on account of the results of previous investigations, which showed that a dose of 300 I.U. normally causes marked stimulation of natural killer cell activity, whereas the 10-times-larger dose has no effect [7]. It was also observed in the present investigation that administration of vitamin A in a daily dose of 3000 I.U. had no antistressor action, whereas a dose of 300 I.U. was highly effective. As Fig. 3 shows, with all three ratios of effector to target used, vitamin A significantly weakened stress-induced disturbances of natural killer cell activity.

These investigations thus showed that vitamin A is an effective immunocorrector in stress. The mechanisms of this effect have not been fully explained, but a number of soundly based suggestions can now be made. We know that during stress production of interleukin-2, one of the key immunoregulatory factors, is inhibited [9]. Meanwhile under normal conditions vitamin A stimulates interleukin-2 production [14]. Data showing that vitamin A stimulates proliferation of lymphoid cells are in full agreement with these facts [11, 15]. Vitamin A probably competes with the action of stressors at the stage of synthesis and (or) secretion of interleukin-2, with the result that immunocompetence is preserved. Vitamin A under normal conditions, as we showed previously [8], inhibits generation of antigenetically specific suppressor cells. During exposure to stressors activating this cell subpopulation, because of the opposite direction of its action, vitamin A limits the intensity of suppressor cell activation. The immunocorrective action of vitamin A against activated macrophages can also be explained at the interleukin-2 level, but another possibility may be mentioned: phagocytosis is an energy-dependent process, and vitamin A stimulates reactions of glycolysis [4], high activity of which is essential for adequate phagocytic activity of the macrophages.

The results of these investigations point directly to the desirability of using the marked immunocorrective effects of vitamin A in the course of investigations on human subjects, who, by virtue of their activity, are exposed to various important stressors.

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