EFFECT OF BALANCED INHIBITION OF DNA AND PROTEIN SYNTHESIS ON PROLIFERATIVE ACTIVITY OF LYMPHOCYTES IN VITRO AND IN VIVO

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Lymphocytes isolated from the peripheral blood of rabbits were irradiated in vitro in doses of 200, 400, and 1000 R and cultured with phytohemagglutinin (PHA) at 37°C for 48 h. In separate experiments 60 min before and 30 min after irradiation the cells were treated with cycloheximide, an inhibitor of protein synthesis, but this had no significant effect on survival of the irradiated cells. The ability of lymphocytes from the popliteal lymph nodes of the irradiated mice to proliferate after injection of PHA into the footpad of one hind limb also was investigated. Injection of cycloheximide or puromycin into the footpad of one hind limb immediately after irradiation of the animals increased the proliferative activity of lymphocytes from the lymph nodes of that limb by 50-100% compared with the contralateral limb. Cytosine arabinoside, an inhibitor of DNA synthesis was ineffective under these conditions.

KEY WORDS: blast transformation of lymphocytes; inhibitors of DNA and protein synthesis.

It was shown previously that balanced inhibition of DNA and protein synthesis in bacteria increases their ability to survive by comparison with cells in which only DNA synthesis or only protein synthesis is inhibited [3, 4]. After x-ray irradiation it is mainly DNA synthesis in the cells that is inhibited, and protein synthesis remains unchanged for a long time [6]. It can tentatively be suggested that disturbance of the balance of macromolecular synthesis contributes to increased cell death. By changing the balance between DNA and protein synthesis, it has been found possible to control the radiosensitivity of cells [5].

The object of this investigation was to study the effect of balanced inhibition of protein synthesis on restoration of the proliferative activity of mammalian lymphocytes after irradiation in vitro and in vivo.

EXPERIMENTAL METHOD

Lymphocytes were isolated from the peripheral blood of chinchilla rabbits in a one-step Ficoll-Urografin density gradient (density 1.078) by Boyum's method [7]. Cells ($1 \cdot 10^6$) were irradiated in doses of 200, 400, and 1000 R and then cultured with 16 μg phytohemagglutinin (PHA) for 48 h at 37°C in Eagle's medium with glutamine and antibiotics. In separate experiments, 60 min before and 30 min after irradiation of the cells cycloheximide, an inhibitor of protein synthesis, was added to the cultures. The intensity of DNA and protein synthesis was determined after addition of [^{3}H]thymidine and [^{14}C]glycine to each sample, 4-16 h before the end of culture, each in a dose of 1 mCi. The samples were washed on filters with TCA [1, 8] and radioactivity counted in a scintillation spectrophotometer (Intertechnique, France). The index of balance between DNA and protein synthesis was expressed as the ratio between the velocities of these syntheses. This ratio in control (intact) cells was taken to be 1. To determine the ability of the lymphocytes to take part in the blast transformation reactions under the influence of PHA in vivo an experimental model was developed, using regional lymph nodes from (CBA $\times$ C57BL)F_1 mice. PHA, in a dose of 100 μg in 0.1 ml, was injected into the footpad of one hind limb of the animals, and 0.1 ml medium No. 199 was injected into the opposite footpad as the control. The mice were killed 48 h later, the popliteal lymph nodes were removed separately, cell suspensions were prepared from them, and each sample was treated for 3 h with 1 mCi of [^{3}H]thymidine. After incubation at 37°C the contents were precipitated on filters and washed with 5% TCA. The intensity of the reaction was expressed as a coefficient of stimulation, determined as the ratio between the number of counts per minute of the experimental lymph node to the quantity of label taken up by cells of the control lymph node. To de-

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TABLE 1. Effect of Balanced Inhibition of DNA and Protein Synthesis on Ability of Rabbit Lymphocytes, Stimulated by PHA in vitro, to Survive

Dose of irradiation, R	Dose of cycloheximide, μg	Level of synthesis rel. to control, %		Index of balance	Ability of lymphocytes to survive after addition of cycloheximide	
		DNA	protein		60 min before irradiation	30 min after irradiation
0	0	100	100	1	41,9±4,0	43,3±3,7
200	0	61,99	100	0,62	33,1±2,2	33,4±6,0
0	0,45	100	62	1,61	39,0±5,4	39,0±5,4
200	0,45	61,99	62	1	34,1±4,3	33,6±3,3
400	0	45,3	100	0,45	24,3±1,0	30,0±3,0
0	0,90	100	45	2,22	39,0±3,2	39,0±3,2
400	0,90	45,3	45	1	33,2±6,3	25,2±4,1
1000	0	29,3	100	0,29	28,5±2,5	22,5±1,2
0	1,1	100	29	3,45	38,0±4,5	38,0±4,5
1000	1,1	29,3	29	1	23,6±4,1	17,7±2,5

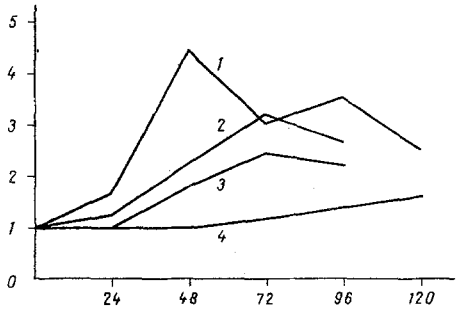

Fig. 1

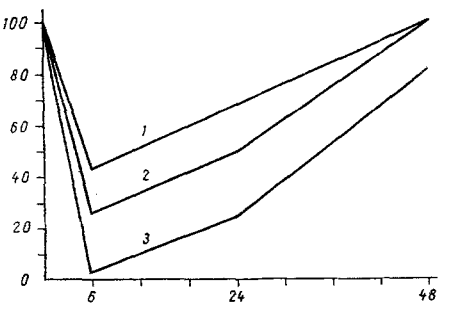

Fig. 2

Fig. 1. Effect of whole-body irradiation on ability of mouse lymphocytes to take part in blast transformation reactions under the influence of PHA. 1) Control; 2) irradiation 100 R, 3) 200 R, 4) 400 R. Abscissa, time (in h); ordinate, coefficient of stimulation.

Fig. 2. Inhibition of protein synthesis in popliteal lymph node cells with time depending on dose of inhibitors. 1) Cycloheximide 50 μg and puromycin 25 μg; 2) cycloheximide 100 μg and puromycin 55 μg; 3) cycloheximide 200 μg. Abscissa, time (in h); ordinate, percentage of synthesis relative to control.

termine protein synthesis, different concentrations of cycloheximide and puromycin were injected into the footpad of one hind limb of mice. At various time intervals the popliteal lymph nodes were removed separately, [^{14}C]glycine was added for 4 h at 37°C, and the contents were precipitated with 5% TCA on filters. The possibility of restoring the proliferative activity of the lymphocytes was tested after irradiation of the mice in doses of 100 and 200 R. Various doses of inhibitors of protein synthesis were injected in 0.1 ml into the footpad of one hind limb 15-20 min after irradiation, and 0.1 ml of medium No. 199 was injected into the opposite footpad as the control. PHA, in a dose of 100 μg, was then injected 20-30 min later into both footpads. The animals were killed after 48 h, the popliteal lymph nodes were removed separately, and incorporation of [^{3}H]thymidine into the cells was investigated. The animals and cells were irradiated in all experiments on the ÉGO-2 apparatus (dose rate 110.53 R/min). The significant differences between the results were assessed by Student's t-test.

EXPERIMENTAL RESULTS

In the experiments of series I an attempt was made to balance protein synthesis relative to DNA synthesis, which was inhibited by irradiation, by means of a selective inhibitor, cycloheximide, and to determine the ability of the cells under these conditions to undergo transformation by PHA. The experimental results are given in Table 1. They show that inhibition of protein synthesis down to the level of DNA synthesis in the cells (index of balance 1) had no effect on the ability of the cells to survive after culture. The uptake of [^{3}H]-thymidine also was virtually the same in control and experimental samples. Protein synthesis, incidentally, was inhibited in the lymphocytes throughout the period of culture (48 h). In the experiments of series II the

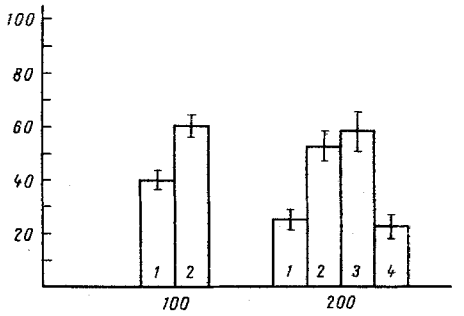

Fig. 3. Restoration of proliferative activity of lymphocytes from popliteal lymph nodes of irradiated animals by inhibitors of protein synthesis. 1) Irradiated animals; 2) irradiated and treated with cycloheximide; 3) irradiated and treated with puromycin; 4) irradiated and treated with cytosine arabinoside. Irradiation with 100 R corresponded to a dose of cycloheximide of 50 μg; irradiation with 200 R corresponded to 100 μg cycloheximide, 55 μg puromycin, and 2.5 mg cytosine arabinoside. Abscissa, doses of irradiation (in R); ordinate, percentage stimulation relative to unirradiated control.

ability of the lymph nodes of the mice to proliferate under the influence of PHA in vivo and the effect of whole-body irradiation on this ability were studied. As Fig. 1 shows, irradiation not only reduced the coefficient of stimulation, but also delayed the beginning of the reaction. Irradiation of the animals in a dose of 400 R virtually completely abolished the stimulation effect for 2, 3, and 4 days. Not until 120 h after injection of PHA was some increase in the uptake of [^{3}H]thymidine observed in the experimental lymph nodes compared with the controls. The results of experiments to study inhibition of protein synthesis in the popliteal lymph node cells of the mice by the selective agents cycloheximide and puromycin are shown in Fig. 2. Clearly the maximal effect of the inhibitors was reached 6 h after the injection, and this was followed by gradual recovery of protein synthesis in the cells. Investigation of the effect of transient inhibition of protein synthesis in the lymphocytes of the popliteal lymph nodes of the irradiated animals on their transformation by PHA revealed more intensive (by 50-100%) proliferative activity of these cells than of the cells of the contralateral limb into which medium No. 199 had been injected as the control. The results of these experiments are illustrated in Fig. 3. The inhibitor of DNA synthesis was ineffective under these conditions.

The results thus indicate that a short period of inhibition of protein synthesis in irradiated cells increases their resistance to irradiation. Inhibition of protein synthesis in rabbit lymphocytes in culture for 48 h in vitro, on the other hand, was not followed by improvement in the survival of the cells or by an increase in their proliferative activity. Prolonged inhibition of protein synthesis evidently prevents the normal course of repair processes. The results of investigation of the blast transformation reaction of mouse lymphocytes in vivo are in agreement with those obtained in cultures of bacterial cells [5] and in suspensions of rat thymocytes [2], reflecting the need for reversible inhibition of protein synthesis in irradiated cells to ensure their better survival and to stimulate the course of repair processes. The future discovery of the optimal duration of inhibition of protein synthesis can make an important contribution to our understanding of the role of interconnection between macromolecular DNA and protein synthesis in increasing the radioresistance of cells.

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CHANGES IN DEPENDENCE OF LYMPHOCYTE REACTIVITY TO PHYTOMITOGENS ON ENDOGENOUS HORMONAL FACTORS IN EXPERIMENTAL BACTERIAL PROSTATITIS

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Dependence of lymphocyte blast transformation reactions (BTR) on phytohemagglutinin P, concanavalin A, and the mitogen pokeweed on the plasma 11-hydroxycorticosteroid (11-HCS) and testosterone (T) levels and the urinary excretion of 17-ketosteroids was studied in 11 dogs with experimental urogenic (urethrogenic) prostatitis produced by means of a pathogenic staphylococcus isolated from a patient with chronic prostatitis. Before the experiment, multiple correlation was found between hormonal factors and BTR indices, but this was upset 1 month after infection of the animals and restored 2 months after infection. Before the experiment the association was expressed mainly by direct correlation with 11-HCS, but 2 months after the experiment, by negative correlation with T. The homeostatic character of the hormonal-lymphoid dependence relative to T is suggested.

KEY WORDS: reactivity of lymphocytes; endogenous hormonal factors; experimental prostatitis.

When androgenic saturation of the body is reduced, direct correlation is found between the indices of the lymphocyte blast transformation reaction (BTR) to phytohemagglutinin P (PHA) and testosterone (T) excretion in patients with chronic prostatitis and sterility [4]. On the other hand, castration of healthy animals can increase the immune response as a result of an increase in the number of T lymphocytes [5]. Exogenous T in vitro depresses the reactivity of normal lymphocytes to PHA [1, 9, 11]. Conflicting results have been obtained in the study of correlation between the circadian rhythm of the cortisol level and BTR to PHA in the healthy organism [8, 10].

The object of the present investigation was to study the dependence of lymphocyte reactivity on hormonal factors by the use of a model of an isolated bacterial affection of an androgen-dependent organ – the prostate. Considering that the body is a multiple-factor self-regulating system in which the resultant effect on the lymphoid system is exerted by a combination of hormonal factors, dependence of the BTR indices to PHA, to concanavalin A (con A), and to pokeweek mitogen (PWM) on the plasma 11-hydroxycorticosterone (11-HCS) and T levels and of the urinary excretion of 17-ketosteroids (17-CS), reflecting the activity of T metabolism, was estimated.

EXPERIMENTAL METHOD

Experiments were carried out on 11 young sexually mature male dogs in which an isolated urogenic (urethrogenic) bacterial prostatitis was produced by the methods described previously [2], by infection with

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