EFFECT OF STEM CELL PROLIFERATION INHIBITING FACTOR ON DEVELOPMENT OF THE IMMUNE RESPONSE TO VIRAL ANTIGEN

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The solution to the problem of regulation of the humoral immune response involves a study of the mechanisms of proliferation and differentiation of the polypotent hematopoietic stem cell (PHSC), which is the common precursor for erythrocytes, leukocytes, and lymphocytes. An increase in the number of splenic colony-forming units (CFUs) in the bone marrow [8] and in their proliferative activity [13] has been demonstrated in the early stages after immunization of mice by various antigens. In this connection the study of the immunomodulating effect of factors modifying the proliferative activity of PHSC is an urgent task, for these cells may also possess immunomodulating properties. This suggestion was confirmed by the writers previously: the stimulating effect of a proliferation inhibiting factor (PIF), inhibiting proliferative activity of PHSC, on the formation of the immune response to sheep's red blood cells (SRBC) in an adaptive transfer system, was demonstrated [10]. It was also found that transfer of bone marrow cells (BMC) from mice with high proliferative activity of their CFUs (after injection of testosterone propionate), after incubation of the cells with PIF, leads to increased formation of antibody-forming cells (AFC) in the recipients' spleens compared with the results of transfer of BMC incubated without the factor.

The aim of this investigation was to study the effect of PIF on the course of experimental viral infection and on development of the immune response to viral antigens.

EXPERIMENTAL METHOD

Noninbred male and female mice weighing 15-20 g BALB/c mice obtained from the animal house of the Vektor Research and Production Combine (Kol'tsovo, Novosibirsk), and (CBA × C57BL/6)F_1 hybrids obtained from the "Stolbovaya" nursery, were used. PIF was obtained from hog rib bone marrow extract, for it has been shown to be species-nonspecific [11]. After centrifugation the supernatant was fractionated by successive passage through membranes (Amicon, Diaflo) with diminishing pore diameter, the fraction with mol. wt. of 50-100 kilodaltons being isolated. In a dose of 1 mg per mouse (calculated as dry residue) the factor was injected intraperitoneally [12] on the day of the first immunization and again on the 3rd and 6th days after infection with virus. Coxsackie A13 virus (strain Flores) was chosen for immunization, and cultured in L-41 cells; the titer of the virus was 6.0 TCD_{50}/0.1 ml. The virus was injected intraperitoneally in a volume of 1.0 ml. Immunization was carried out in accordance with the program developed previously and the virus was injected 3 times at intervals of 2 days [3]. The presence of virus-neutralizing antibodies in the serum was determined on the 9th day [2]. To produce experimental influenza, influenza A/Aichi (H3N2) virus, obtained from the D. I. Ivanovskii Institute of Virology, was used; the titer of the virus was 4.0 log ID_{50}/0.05 ml. The virus was introduced intranasally into the mice under superficial anesthesia, in a dose of 10 ID_{50}/0.05 ml. PIF was injected in a dose of 1.0 mg per mouse 1 h before and 24 and 48 h after infection. The efficacy of treatment was judged from the difference between mortality levels in the experimental and control groups, and also the mean length of survival of the animals in the two groups [4]. The immune

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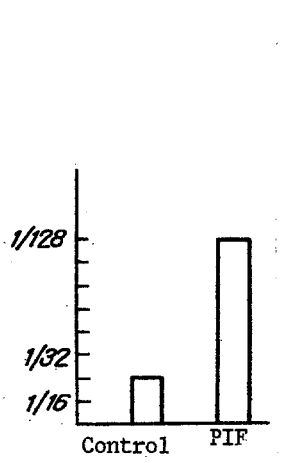

Fig. 1

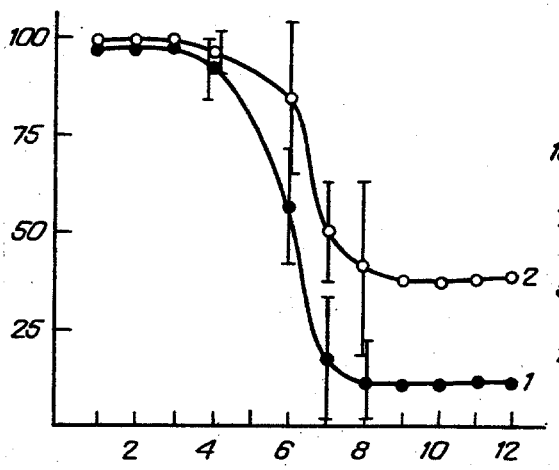

Fig. 2

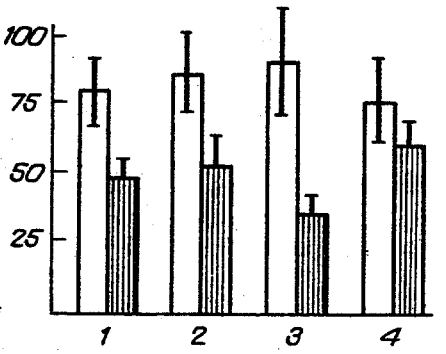

Fig. 3

Fig. 1. Effect of PIF on production of virus-neutralizing antibodies to Coxsackie A13 virus (Flores strain).

Fig. 2. Effect of PIF on course of experimental influenza in BALB/c mice. Abscissa, time of experiment (in days); ordinate, survival rate of animals (in %): 1) control, 2) PIF.

Fig. 3. Effect of PIF on AFC production in spleens of mice immunized with SRBC: 1) control, 2) PIF on 4th day; 3) control, 4) PIF on 5th day. Unshaded columns – 5% SRBC, shaded columns – 50% SRBC.

response to SRBC (in a concentration of 5% and 50%) was estimated from the number of AFC in the spleen on the 4th and 5th days after immunization [9]. PIF (0.1 g/mouse of the chromatographically purified preparation) was injected 3 times: 1 day before SRBC, on the day of immunization, and 1 day thereafter. The molecular weight of the purified preparation was 17 kilodaltons.

EXPERIMENTAL RESULTS

The experiments showed that PIF stimulated production of neutralizing antibodies to Coxsachie A13 virus. The antibody titer in the blood serum of mice receiving PIF together with immunization was 1:128 compared with 1:32 in the control group. Thus a fourfold increase in titer of virus-neutralizing antibodies was observed under the influence of PIF (Fig. 1).

In the model of experimental influenza, death of the animals in the control group was observed starting from the 4th day after infection (Fig. 2), and reached its peak by the 7th day of the experiment, when 16.7 ± 6.8% of the animals remained alive, and the percentage of surviving mice remained at this level until the end of the period of observation. In the group of mice receiving PIF, starting from the 6th day after infection, mortality of the mice decreased compared with the control group. On the 7th day of the experiment (the time of maximal mortality of the mice) the difference between the survival rates of the mice in the experimental and control groups became statistically significant. At the late stages of infection (8-14 days) this difference became a little less marked, but it still continued to be present until the end of the experiment. Thus PIF, injected into mice therapeutically and prophylactically, prevented death of animals infected with influenza virus. The treatment given lengthened the mean survival time of the mice compared with the control group (9.5 and 7.4 days respectively).

An immunostimulating effect of PIF also was found when sheep's red blood cells (SRBC) were used as the antigen. Figure 3 shows that PIF had no effect when 5% SRBC were used. No stimulating effect of PIF likewise was observed on the 4th day after injection of the larger dose of antigen (50% SRBC). However, there was a sharp increase in the number of AFC in response to injection of 50% SRBC on the 5th day after immunization. An increase in the dose of antigen

evidently led to enhanced proliferation of PHSC, making it more sensitive to the inhibitory action of PIF. The delayed effect of PIF observed may also have been due to a change in parameters of differentiation and migration of PHSC, which are indissolubly connected with proliferation.

The negative correlation between proliferative activity of PHSC and parameters of the immune response was observed previously: an increase in proliferative activity of PHSC against the background of inhibition of antibody formation and blast transformation in response to T- and B-cell mitogens on injection of infectious antigens of *Bordetella pertussis* [1], and in mixed infection with mycoplasmas and viruses [6]. Our earlier results indicate strong correlation between the immunodepressive effect of procedures such as hypoxia and injection of testosterone propionate and phenylhydrazine, and their stimulating action on proliferative activity of PHSC [5, 7]. On the basis of data showing shortening of the duration of the cell cycle in response to stimulation of CFUs proliferation [14], it has been suggested that when the temporal parameters of the cell cycle are shortened, differentiation of CFUs takes place predominantly in the erythroid direction, at the expense of cells of other branches of hematopoiesis, including lymphoid cells, thereby leading to depressed activity of the immune system.

It can be tentatively suggested that injection of PIF against the background of development of an immune response to viral and nonviral antigens depresses the proliferative activity of PHSC, and stimulates differentiation toward myelopoiesis. This change in the direction of differentiation of precursors of hematopoiesis is reflected in potentiation of the immune properties of the host. It may thus be expected that the magnitude of the immune response is corrected by agencies aimed at modifying the proliferative powers of the hematopoietic stem cell.

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