antigenic kinship with the B subunit of colerogen. When prospects for the use of the B subunit for construction of a vaccine preparation are discussed, it must be noted that difficulties connected with the obtaining of these subunits from the whole toxic protein can be resolved only by the use of a strain that produces only the B subunit. This approach not only will facilitate the process of obtaining the subunit, but will also significantly increase its yield in the form of the purified product, by the use of affinity chromatography. Under these circumstances the purified product will be completely identical in molecular weight and immunochemical properties with LTB obtained by the traditional method of purification. The use of the B subunit as a component of a vaccine against diarrheal diseases will be the subject of our forthcoming research.

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EFFECT OF REGULATORY CELLS INDUCED BY INFLUENZA VIRUS DURING ADOPTIVE TRANSFER

A. P. Volgarev, L. P. Maiorova, and A. A. Smorodintsev

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The differential role of various factors of immunity in protection of the organism has been demonstrated on a model of influenza infection in mice, protected by blood sera for cells of the lymphoid-macrophagal system, obtained from immunized syngeneic mice [4, 5].

The model of adoptive transfer is widely used in immunology to assess the protective role of different cells of the lymphoid-macrophagal series. By means of this method the antiviral protective effect of cytotoxic T lymphocytes has been confirmed [9] and the role of macrophages in modification of the virus antigen in the inductive phase of the immune response in virus infections has been established [2]. However, during induction of the immune response to a test antigen complex mechanisms of intracellular relationships have been found in recipients [10]. Under these circumstances, the resultant effect of cellular cooperation is not always equal in value. Not only the absolute numbers of interacting cells, but also the ratio between them are decisive factors [8]. Recording of the immune response in the recipient also depends on the time of observation [14].

Department of Virology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.

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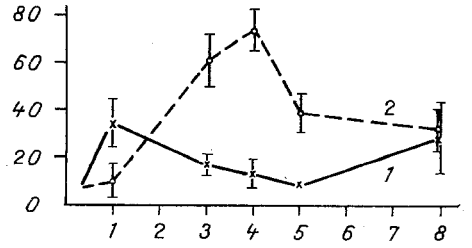

Fig. 1. Protective effect of cellular and humoral factors of immunity. Abscissa, Time after immunization of donor mice (in days); ordinate, percentage protection of recipient mice. 1) Serum; 2) splenocytes.

The aim of this investigation was to study the protective effect of humoral and cellular factors of immunity in experimental influenza infection in mice, with analysis of the functional activity of the regulatory subpopulations of lymphocytes, especially in the early stages of observation.

EXPERIMENTAL METHOD

Influenza A/PR/8/34 (HON1) virus was used. Experiments were carried out on male C56BL/6 mice aged 6-8 weeks, obtained from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR. Donor mice were immunized by a single intraperitoneal injection of virus in a dose of 10^6 EID_{50}/0.2 ml, and they were killed under ether anesthesia 1, 3, 4, 5, and 8 days after immunization by total exsanguination through the subclavian artery. Preparation of the cellular and humoral factors of protection and the method of their use were described in detail previously [6]. The number of spleen cells injected was 10^7.

Experimental influenza infection was used in recipient mice 24 h before the main experiment by intranasal infection with the virus in a dose of $10^{4.5}$ EID_{50}/0.2 ml. The parameters of protection were calculated by estimating the intensity of involvement of lung tissue 7 days after infection.

The concentration of anti-influenzal antibodies in the blood sera was determined by the hemagglutination inhibition test, in a micromodification, and by the neutralization test on chick embryos. Serum interferon was assayed by titration with a coefficient of 2 and by culture *in vitro* for 24 h at 36°C in an atmosphere of air with 5% CO_2, on the basis of inhibition of the cyotoxic action of vesicular stomatitis virus (100 CPD_{50}), cultured on L-929 mouse fibroblasts. Serum from mice immunized with Newcastle disease virus, with an activity of 1280 units/ml was used as the reference preparation. The test was carried out in micropanels from the Moscow experimental factory.

Regulatory activity of the splenic lymphocytes was studied by two methods.

1. Adoptive intraperitoneal transfer of 10^7 spleen cells from donor mice in 1 ml of medium 199 into intact recipient mice. The recipients were immunized intraperitoneally at the same time with homologous native virus, in a dose of 512 HU/0.2 ml in a volume of 1 ml. The recipient mice were exsanguinated 14 days after transfer of the cells and the level of serum antibody accumulation was determined. Experiments with intraperitoneal immunization of the recipients and simultaneous intraperitoneal application of spleen cells of intact mice and intraperitoneal injection of virus alone served as the control.

2. The study of changes in the level of mitogen-induced proliferation of test cultures of lymphocytes from intact animals in the presence of lymphocytes obtained from infected mice at different times after infection [7]. In this case concanavalin A (from Sigma, USA) in a final concentration of 20 µg/0.1 ml per sample, mitomycin C (from Serva, West Germany) in a dose of 40 µg/ml, and ^{3}H-thymidine (specific activity 1 TBq/mmole, 0.074 mBq/0.1 ml per sample, from Izotop, USSR) were used.

The results of the study of proliferative activity of the test cultures, when cocultured with lymphocytes of infected animals, were expressed as percentages.

The numerical results were subjected to statistical analysis by Student's t test [3].

EXPERIMENTAL RESULTS

Maximal protective action of the blood sera was observed 1 and 8 days after immunization of the donor mice (Fig. 1). Spleen cells, removed on the 3rd-4th day after immuniza-

TABLE 1. Characteristics of Blood Sera of Donor Mice

Time after immunization, days	Sera contained	
	interferon, units/0.1 ml	antiboides (geometric mean titers, reciprocals)
1	64	<4
4	12	35
8	<4	320

TABLE 2. Modulating Effect of Donor Splenocytes (M±m)

Time after infection of donors, days	Effect of transferred splenocytes	
	geometric mean titers of serum antibodies (reciprocals) in recipients	changes in proliferation of lymphocyte test cultures, %
2	299±63	—
3	275±57	+137,9±11,2
5	71±34	—54,7±5,1
7	—	—62,5±7,9
14	23±17	—81,3±8,4
Control	110±28	—50,1±4,7

tion of the donor mice, gave significantly greater protection to the recipient mice than cell suspensions obtained at later times. It was found that the protective effect of the cells exceeded that of the blood sera.

Characterization of the humoral and cellular factors of protection included determination of the titer of antiviral antibodies, the interferon concentration, and regulatory activity of the spleen cells of the donor mice. On the 8th day after immunization a significant increase in antibody formation was observed compared with the first day (Table 1). Meanwhile the interferon concentration in the animals of this group showed a negative trend. The results of the study of regulatory activity of the splenic lymphocytes of the donor mice are shown in Table 2. The level of antibody formation in the recipients, receiving spleen cells taken from the donor mice on the 2nd and 3rd days after their immunization, was significantly higher ($P < 0.01$) than the control and, conversely, after injection of spleen cells taken on the 14th day, it was significantly lower ($P < 0.01$). Similar data on the modulating effect of lymphocytes from the donor mice were obtained *in vitro*. The proliferative activity of the test cultures was increased if the cocultured cells were obtained on the 3rd day after immunization, and it was inhibited if the donors' cells were obtained on the 5th and 7th days. Consequently, spleen cells taken from donor mice on the 2nd and 3rd days possessed helper activity, whereas cells taken on the 5th, 7th, and 14th days possessed suppressor activity.

In the present investigation the study of the protective effect of humoral and cellular factors of immunity in mice with experimental influenza infection was combined with characterization of the donor's blood sera and spleen cells. The protective effect of the cells followed a definite time course and was stronger than the protective action of the sera. The latter were characterized by a high interferon concentration on the first days, but by an increase in the titer of anti-influenzal antibodies in the later stages. Assessment of the activity of the regulatory subpopulations of lymphocytes in these investigations was phenomenologic in character. However, the results are in full agreement with those obtained by other workers [12, 13]. Times of involvement of regulatory subpopulations of lymphocytes established *in vitro* were completely confirmed by the results of experiments *in vivo* in an adoptive transfer system, i.e., induction of the regulatory subpopulations of lymphocytes established *in vitro* was valid also for the system *in vivo*. Consequently, our data showed that the effectiveness of protection of the recipients or, in other words, the intensity of the immune response in the recipient, depends on the concrete functional state of the transferred donors' cells. It must be noted in this connection that the trend of the immunization process is characterized by a cyclic pattern of regulatory activity of the lymphocytes. This cyclic pattern of helper activity of the T cells was demonstrated in experiments on mice immunized with sheep's red blood cells [11]. It was shown that the concrete times of activation of the helper T cells depend on the immunizing dose of antigen. This fact deserves great attention, for it opens up definite prospects for concrete and pathogenetically based pharmacological intervention [1] and the plotting of a scientifically-based graph of prevention vaccinations.

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