RESTORATION OF THE IMMUNE RESPONSE BY MACROPHAGES IN MICE AFTER ADMINISTRATION OF IMMUNODEPRESSANTS

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The possibility of restoring the primary immune response in mice immunized with bacteriophage T2 after administration of various immunodepressants (actinomycin D, olivomycin, rifampicin, chloramphenicol, chloroquine, histone F2a, and bacterial lipopolysaccharide) was studied. For this purpose peritoneal macrophages from intact syngeneic donors were transferred simultaneously with injection of the antigen into the recipients following administration of the immunodepressants. The immunodepressive effects of most of the substances studied could be completely compensated in this way. Macrophages can thus be regarded as one of the sites of action of the immunodepressants studied. The results also indicate a role of the macrophages in the induction of the primary immune response to bacteriophage T2 (synthesis of phage-neutralizing antibodies).

Key words: primary immune response; immunodepressants; macrophages.

The action of immunodepressants on the macrophagal stage of immune responses has so far received little study. In their analysis of the mechanism of the immunodepressive activity of some chemical substances, Fontalin et al. [1] directed attention to macrophages as a possible "critical point" selectively attacked by different immunodepressants. Other workers [5, 7, 8] succeeded in restoring the lost ability to synthesize antibacterial antibodies in sublethally irradiated mice by injecting them with syngeneic peritoneal macrophages incubated with shigellas. Frisch and Wilson [6], in experiments with the crossed syngeneic cell transfer, found selective sensitivity of macrophages and spleen cells to various immunodepressants. Londner et al. [9] compensated the immunodepressive effect of an RNA preparation in both the primary and the secondary immune response by the transfer of syngeneic macrophages.

In the investigation described below the mechanism of the immunodepressive action of certain substances inhibiting the primary immune response was studied in mice immunized with bacteriophage T2 [2-4].

EXPERIMENTAL METHOD

The following substances were used as immunodepressants: transcription inhibitors (actinomycin D 250 μg/kg, olivomycin 1 mg/kg, rifampicin 200 mg/kg), a translation inhibitor (chloramphenicol 100 mg/kg), and also substances with a more complex mechanism of action (chloroquine, 50 mg/kg, the F2a histone fraction 50 mg/kg, and lipopolysaccharide 10 mg/kg).

Mice of inbred lines C3H and CBA were used in the experiments with transfer of macrophages. Peritoneal macrophages were obtained by flushing out the peritoneal cavity of donor mice with Hanks's solution containing 1% calf serum and heparin 4 days after the animals received an injection of 10% proteose-peptone. The resulting cell suspension, containing 90% of macrophages, was sedimented by centrifuging in the cold (10 min, 1000 rpm) and resuspended in the same volume of medium to give a cell concentration of 2×10^7 /ml. The recipient mice (experimental group) received an intraperitoneal injection of 0.5 ml of this suspension together with 0.5 ml of antigen (bacteriophage T2 in a concentration of 2×10^{11} particles/ml) 24 h

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after they received the corresponding immunodepressants. The control animals received the same immunodepressants, but instead of macrophages they were injected with Hanks's solution containing the antigen. In a parallel series a group of animals was immunized with the same antigen but without receiving the immunodepressants (this group is subsequently called the "immune" group). The intensity of the primary immune response was studied 15 days later by the bacteriophage neutralization test with individual mouse sera. The phage-neutralizing activity of the sera was estimated with the aid of the index

$$\lg \frac{P_0 - P}{P},$$

where P_0 is the number of sterile plaques in the subculture from the control sample; P the number of sterile plaques in the subculture from the sample treated with the immune serum. The phage-neutralizing activity of the sera from the animals of the control and experimental groups was compared with the level of antibody formation in the animals of the "immune" groups. For this purpose the differences between the corresponding logarithms of the ratios were calculated:

$$\left[\lg \frac{P_0 - P}{P}\right]_c - \left[\lg \frac{P_0 - P}{P}\right]_i \text{ and } \left[\lg \frac{P_0 - P}{P}\right]_e - \left[\lg \frac{P_0 - P}{P}\right]_i.$$

The statistical treatment of the experimental data was carried out by the method of dispersion analysis.

EXPERIMENTAL RESULTS

The results of the main experiments are illustrated in Fig. 1, in which the above-mentioned differences between the logarithms of the ratios are shown graphically. All the biologically active substances tested had a marked immunodepressive action on the primary response. Chloroquine was the weakest of the immunodepressants tested.

The phage-neutralizing activity of the sera from the recipients of the macrophages exceeded by a statistically significant degree the level of the antibodies in the animals of the corresponding control groups receiving the inhibitors only. The primary response in animals of the experimental groups receiving macrophages immediately after the inhibitors was close to the level of the primary response in the intact mice immunized with phage. Estimation of the significance of the differences in antibody formation in the animals of these two groups showed that the immunodepressive effects of most of the preparations studied were completely compensated as a result of the transfer of syngeneic intact macrophages. The exception was the immunodepressive effect of rifampicin, which was reduced by half but not completely abolished after the transfer of the macrophages (Fig. 1).

A special series of experiments showed that the injection of syngeneic macrophages in a dose of 1×10^7 cells into intact mice simultaneously with antigen was not itself reflected at all in the intensity of the primary immune response. Consequently, in the present experiments the macrophages did not intensify the primary response but simply restored the ability of the animal to respond in the usual way to injection of the antigen.

In the experiments with transfer of purified macrophages the technique of separating cells adherent and nonadherent to glass, widely used to separate macrophages from lymphocytes [10], was employed. The modification of the method used to prepare the macrophages for transfer was that the cells were washed off with medium No. 199 containing 10% calf serum and heparin. The resulting cell suspension was transferred in volumes of 5 ml to flat-walled flasks and incubated for 2 h at 37°C. During this time the macrophages adhered to the surface of the glass, after which the lymphocytes could be removed together with the medium. The monolayer of macrophages was carefully removed from the surface of the glass by means of a glass spatula with a rubber tip and resuspended in one-tenth of the original volume of medium (0.5 ml), so that the cell concentration was increased to 2×10^7 cells/ml. The viability of the macrophages (as shown by the trypan blue test) was not thereby reduced.

A series of control spot tests yielded results similar to those described above. After the transfer of the purified macrophages the primary immune response was also fully restored after its inhibition by the F2a histone fraction, lipopolysaccharide, chloramphenicol, and actinomycin D.

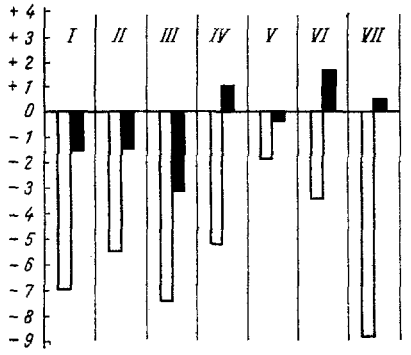

Fig. 1. Restoration by syngeneic macrophages of the primary immune response in mice after administration of immunodepressants: I) actinomycin D; II) olivomycin; III) rifampicin; IV) chloramphenicol; V) chloroquine; VI) F2a histone fraction; VII) lipopolysaccharide. Unshaded columns show immune response after administration of immunodepressants; shaded columns show immune response after administration of immunodepressants and macrophages. Ordinate, difference between logarithms of ratios (see Experimental Method); zero line represents immune response of control immunized mice.

It was deemed necessary to check the possibility of restoring the primary immune response by syngeneic macrophages in the case of immunodepression induced by the antimetabolite 6-azauridine. The immunodepressive effects of 6-azauridine cannot be mediated through macrophages, for it is effective only if administered 24 h after the antigen, when the basic functions of the macrophages in the uptake and processing of the antigens have already been completed. Transfer of the macrophages 24 h after injection of 6-azauridine had no effect whatsoever on its immunodepressive action.

The series of control experiments increased the certainty that the restoration of the primary immune response, when inhibited by the substances tested, could in fact be attributed to the replacement transfer of syngeneic macrophages. The effectiveness of the transfer of macrophages purified by culture in vitro rules out in this case any role of lymphocytes in the restoration of antibody formation. The experiments with 6-azauridine showed that the transfer of macrophages cannot abolish immunodepression unless it is connected with some damage to the macrophagal stage.

The connection established between the immunodepressive effects of the test agents and the disturbance of macrophagal functions induced by them agrees closely with the ability of the same agents, demonstrated by the writers previously, to inhibit the functional activity of macrophages in tissue culture [4].

Since the immune response can be restored by transfer of syngeneic macrophages there are grounds for regarding the macrophages as one point of action of the immunodepressants tested. This does not rule out the possibility of other points of action, which could be discovered by administering them in different doses and at different times relative to the antigen. The results may also serve as proof of the role of the macrophages in the induction of the primary immune response to bacteriophage T2.

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