MECHANISM OF THE PROTECTIVE ACTION OF ANTITOXIN IN CELL CULTURES IN VITRO

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Treatment of a culture of guinea pig peritoneal macrophages with diphtheria antiserum and subsequent removal of the serum by washing lead to the appearance of a culture resistant to diphtheria toxin. A factor capable of transmitting toxin resistance to a fresh culture was discovered in the culture fluid.

Interest of research workers in the role of reticulo-endothelial cells in the immunological response has recently increased [3]. However, a no less important problem, namely the role of these cells in passive immunity, still remains unsolved. If foreign antigens are injected into an animal, they may be ingested by phagocytes and undergo certain changes. Data in the literature indicating the formation of Fab-fragments in the body and the participation of cells of the reticulo-endothelial system in this process [2] nevertheless do not answer the fundamental problems, such as: what is the fate of the "ingested" antibodies; can they or the products of their catabolism participate in the defense of the organism; what is the role of phagocytes in this process? It is evident that these problems can be solved only at the cellular level.

The objective of the present investigation was to study ingestion of antitoxin by peritoneal macrophages in vitro and its subsequent antibody role.

EXPERIMENTAL METHOD

Experiments were carried out on a 24-h culture of guinea pig peritoneal macrophages. The macrophages were obtained by the usual method. Medium No. 199 with the addition of 20% bovine serum and antibiotics (penicillin and streptomycin, 100 units/ml of each) was used for cultivation.

Diphtheria antitoxic serum of brand Diaferm-3 (DAT) and diphtheria toxin (1 MLD = 0.0036 ml) were used in the experiments. Both components were diluted with medium No. 199 immediately before addition to the cell culture in a volume of 1.5 ml. Toxin was used in the experiment in a dose causing death of 60-70% of cells in 24 h. The serum was added in a dose of 36 or 72 a.u. to the flask containing the cell culture.

DAT was added to a 24-h culture of macrophages and the culture was then incubated at 37°C for 7 h. At various times after the beginning of incubation with DAT the culture was washed to remove serum (frequently rinsed with buffer solution) and the toxin was added. In some experiments the cell culture was in contact with DAT for 5, 30 or 60 min, after which the serum was washed off, fresh medium No. 199 was added, and cultivation allowed to continue. At various time intervals (from 5 min to 7 h) thereafter the culture fluid was poured off and toxin added. The decanted culture fluid in some experiments was transferred to flasks with a fresh 24-h culture of macrophages, and this was then tested for its resistance to toxin. The results were assessed by counting the number of viable cells after contact for 24 h with the toxin. The control for determination of the percentage of surviving cells consisted of intact cultures of macrophages. The index of resistance was then determined as the ratio between the percentage of dying cells in the culture treated with toxin only (taken as 1) to the percentage of dying cells in the culture treated with DAT and toxin.

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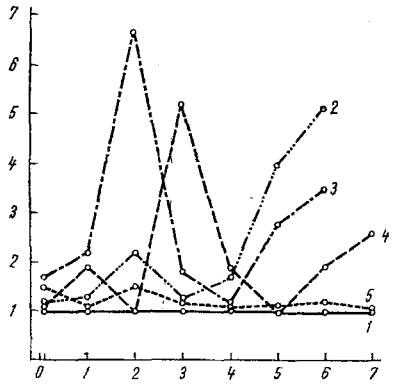

Fig. 1

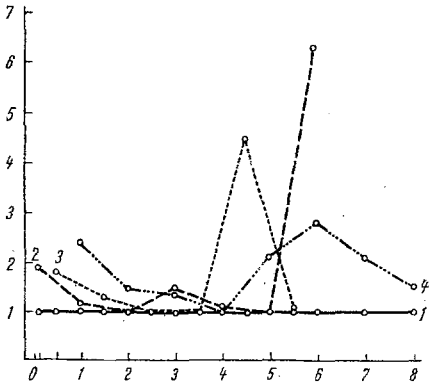

Fig. 2

Fig. 1. Resistance of culture of macrophages to diphtheria toxin during constant contact with DAT: 1) culture of macrophages not treated with DAT; 2, 3, 4) cultures treated with DAT, incubated at 37°C; 5) culture treated with DAT for 5 min at 37°C and then kept at 4°C until the end of the experiment. Abscissa, time of incubation with DAT (in h); here and in Figs. 2 and 3, index of resistance.

Fig. 2. Resistance of culture of macrophages to diphtheria toxin during incubation of cells with DAT for 5 (2), 30 (3) and 60 min (4). 1) Culture not treated with DAT. Abscissa, here and in Fig. 3, duration of cultivation of macrophages (in h).

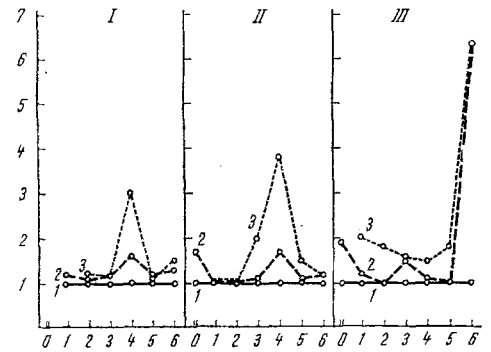

Fig. 3. Dynamics of appearance of the "protective factor" in culture fluid: 1) resistance of culture not treated with DAT; 2) resistance of culture treated with DAT for 60 min (I) or 5 min (II and III); 3) resistance of culture of macrophages treated with culture fluid obtained from previous culture (curve 2).

EXPERIMENTAL RESULTS

Previous investigations showed that treatment of a transplantable culture of human amniotic cells (line A_1) with diphtheria antiserum caused a substantial increase in the resistance of the cells to the cytotoxic action of diphtheria toxin. It was found that the quantity of serum adsorbed by the cells bore no relationship to the resistance of these cells to the toxin. It was naturally interesting to study these relationships in a culture of cells specialized for phagocytosis, pinocytosis, and active intracellular digestion. The experiments showed that treatment of macrophages with DAT increased the resistance of the cells to toxin (Fig. 1, 2, 3). The dynamics of the protective effect was cyclic in character, with alternation of rises and falls of resistance. Although this cyclic tendency in the manifestation of the protective effect continued (Fig. 1), the character of the cycles was not the same in frequently repeated experiments (only some of them are illustrated). This applies both to the level of acquired resistance and to the time of its appearance. This fact evidently can be attributed to individual fluctuations in the functional activity of the macrophages obtained from different guinea pigs.

In the experiments of series II the cells were in contact with DAT for only 5, 30, or 60 min (Fig. 2). During the first few hours the resistance of the cells fell to the level of resistance of the untreated culture (Fig. 2). However, after incubation for 4 h the resistance rose again to reach a much higher level than initially. If the results of experiments in which the cell culture was constantly in contact with DAT are summarized (Fig. 1), two maxima of the resistance of the macrophage culture can be seen. The first maximum occurred 2-3 h after the beginning of incubation with the serum, the second after 5-6 h. In the case of temporary contact with DAT (Fig. 2), only one maximum can be seen, namely the "second" maximum

of resistance at the same time after the beginning of incubation (5-6 h). It can evidently be postulated that adsorption of DAT on the cells similar to that demonstrated on a transplantable culture of amniotic cells plays an important role in the formation of the "first" peak. However, this process does not take place passively but evidently requires the active participation of macrophages. The reasons are as follows. 1) During incubation of the culture in a refrigerator (4°C) no maxima of resistance occurred (Fig. 1, 5). At reduced temperatures the adsorption of protein on cell membranes is known to take place unchanged [5], but at the same time the intracellular digestion of protein is substantially inhibited [4, 6]. 2) Immediately after the rise of resistance there was a sharp fall, in some cases to the level of resistance of the cell culture not treated with serum. Where the index of resistance was 2.5 or more, the difference from the control was statistically significant. This fall in resistance is difficult to explain, especially if it is remembered that an excess of DAT was constantly present in the culture fluid.

The "second" maximum of resistance is undoubtedly associated with the active functioning of the macrophages and is perhaps due to destruction of the adsorbed DAT. Evidence of the necessity for active participation of macrophages in this process is also given by the experiment in which, after contact for 5 min with DAT at 37°, the culture was placed in a refrigerator (Fig. 1, 5). It is interesting to note that the height of the maximum of resistance was practically the same with constant contact as with temporary contact with DAT (Figs. 1 and 2). Hence, if destruction of DAT took place, about 4 h was required for the process. It was after incubation for this period that the "second" maximum of resistance appeared. It could be suggested that some sort of factor (perhaps a fragment of antibody) is liberated into the culture fluid, where it may neutralize the cytopathogenic action of the diphtheria toxin. Accordingly experiments were carried out which showed that in the period of recovery of resistance of the cell culture (previously treated for 5 min with DAT), i.e., after incubation for 4-5 h, a factor appeared in the culture fluid which, if added to a fresh culture, communicated a high degree of resistance to it (Fig. 3). Under these conditions the protective effect in the absolute majority of cases was higher than the level of resistance of the culture from which the culture fluid had been taken. However, this factor disappeared from the culture fluid within 1 h (Fig. 3, I and II). This factor communicated maximal resistance to the cells after contact for 5 min. Prolongation of this contact to 1 h or more led to a decrease in resistance down to the level of resistance of the untreated culture. The reason for the rapid disappearance of the factor has not yet been explained. It can only be postulated that the factor either was very rapidly adsorbed by the cells or that it was inactivated. Neutralization of the toxin by the culture fluid is ruled out because in every case the culture fluid was decanted before addition of the toxin. It must be emphasized that, by contrast with DAT, this factor exerted its protective action very rapidly and, probably, completely because no subsequent maxima of resistance such as were found in the case of treatment with DAT occurred.

The results thus demonstrate the active role of macrophages in the mechanism of the protective action of DAT. It is expressed not only as ingestion of the serum, but also as its modification to a fragment capable of communicating high and specific resistance to untreated cells.

LITERATURE CITED

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