IMMUNODEPRESSIVE ACTION OF CYCLOPHOSPHAMIDE ON DIFFERENT STRAINS OF MICE

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The immunodepressive effect of cyclophosphamide (CP) was studied on mice of three strains (BALB/c, CBA, DBA/2) immunized with sheep's red cells (RBC). When the optimal immunizing dose of antigen was used (5×10^8 RBC) the strongest immunodepression was observed in the DBA/2 mice, but when a large dose of RBC was used (6.2×10^9) the strongest effect was observed in DBA/2 and CBA mice. The action of CP was shown to depend on the dose of antigen injected: In BALB/c mice the decrease in the number of antibody-forming cells was the same with both doses of RBC, in the DBA/2 mice an increase in the dose of antigen led to weakening of immunodepression, but in CBA mice immunodepression was intensified (provided that sufficiently large doses of CP were used). Determination of the rate of oxidative hydroxylation of CP by the mouse liver microsomes showed it to be comparatively low in DBA/2 and CBA mice and much higher in BALB/c mice. It is suggested that the differences in the immunodepressive action of CP thus revealed could be due to differences in the sensitivity of the target cells and (or) differences in its metabolism in mice of different strains.

KEY WORDS: *cyclophosphamide*; *immunodepression*; *genotype*.

The investigation of the genetic control of the immune response is one of the most rapidly developing and promising lines of research in modern immunology and immunogenetics. Evidence is now available that genetic factors play an important role in reactions of cellular and humoral immunity [3, 11, 15, 17]. However, some immunological phenomena have been inadequately studied from this point of view, notably drug-induced immunological tolerance. Only a few investigations have been undertaken and these have demonstrated the existence of interlinear differences in the degree of tolerance induced in adult mice with the aid of immunodepressants [13]. The mechanisms of these differences are still largely unexplained, but it is evident that they can be determined not only by differences in the response of mice of different lines to the antigen, but also by differences in the effectiveness of the immunodepressant used for mice of the same strain.

The sensitivity of mice of different strains to the immunodepressive action of cyclophosphamide (CP), one of the most effective immunodepressive and cotolerogenic agents, was studied in the investigation described below [4, 16, 18].

EXPERIMENTAL METHOD

Inbred male CBA, DBA/2, and BALB/c mice (obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR), weighing initially 18-25 g, were used. Experiments to study microsomal oxidation of CP were carried out on mice deprived of food for 24 h before the experiment began [8]. Sheep's red cells (RBC) were used as the antigen and in all the experiments the Soviet preparation of CP, cyclophosphan, was used.

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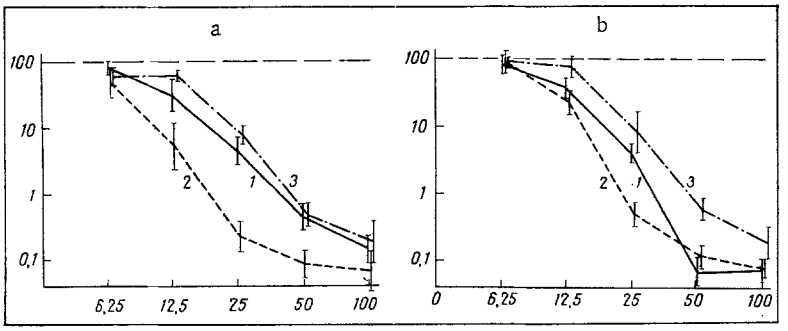

Fig. 1. Immunodepressive activity of CP after immunization of mice of different strains with RBC in doses of 5×10^8 (a) and 6.2×10^9 (b). Here and in Fig. 2: abscissa, dose of CP (in mg/kg); ordinate, number of 19S-AFC (in % of control). 1) CBA, 2) DBA/2, 3) BALB/c mice.

The immune response in mice was assessed from the number of 19S-antibody-forming cells (AFC) in the spleen, estimated by the local hemolysis in gel method [14]. The rate of oxidative hydroxylation of CP by the mouse liver microsomes was determined by the method described in [5]. The increase in the rate of oxygen consumption after the addition of CP was expressed as a percentage of the level of microsomal respiration after the addition of NADPH and was taken as the rate of oxidative hydroxylation of CP.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of CP on the immune response was studied in mice receiving the optimal immunizing dose of antigen (5×10^8 RBC, intravenously); CP was injected intraperitoneally in a dose of 6.25-100 mg/kg, 24 h after immunization. As was shown previously [1], an interval of 24 h between injections of the antigen and CP results in the maximal immunodepressive effect when the number of 19S-AFC in the spleen is counted 4 days after immunization. The results of these experiments are summarized in Fig. 1a.

As Fig. 1a shows, DBA/2 mice were the most sensitive to the immunodepressive action of CP; differences from mice of the other strains were revealed with all all doses of CP used except 6.25 mg/kg (a dose giving very weak immunodepression). The sensitivity of the mice of the other two strains was about equal, although significant differences were found for them with individual doses of CP (Fig. 1a).

According to data in the literature, the immunodepressive effect of some preparations depends on the dose of the antigen used for immunization. In particular, it has been shown [10] that CP depresses the immune response more strongly when large doses of antigen are used. To induce tolerance with CP, it is essential that a large dose of RBC be given [2, 6, 9]. In this connection it was interesting to discover if there are interlinear differences in the action of CP when a larger dose of RPC is used.

In the experiments of series II, RBC were injected intraperitoneally into mice in a dose of 6.2×10^9 and CP was injected intraperitoneally 42-46 h later, i.e., the scheme used was that which, according to previous findings [2], is optimal for inducing tolerance. The number of 19S-AFC in the spleen was determined on the fifth day after injection of RBC. The results are given in Fig. 1b.

It will be clear from Fig. 1b that the relations between mice of the highly sensitive DBA/2 strain and the BALB/c mice still continued (the degree of relative immunodepression in the DBA/2 mice was higher in this case also). However, CBA mice under these conditions were more sensitive than BALB/c, and after injection of large doses of CP the depression of the immune response was compensurate with that found in mice of the highly sensitive DBA/2 strain.

Comparison of the response of the mice of CP after immunization with different doses of RBC (Fig. 2) showed that the character of immunodepression differed in mice of different strains. In BALB/c mice (Fig. 2a) the level of depression of the immune response was the same regardless of the dose of antigen; this was found with all doses of CP sufficient to produce marked immunodepression. In DBA/2 mice (Fig. 2b) the degree of depression of AFC formation was higher when a smaller dose of antigen was used (12.5 and 25 mg/kg). CBA mice

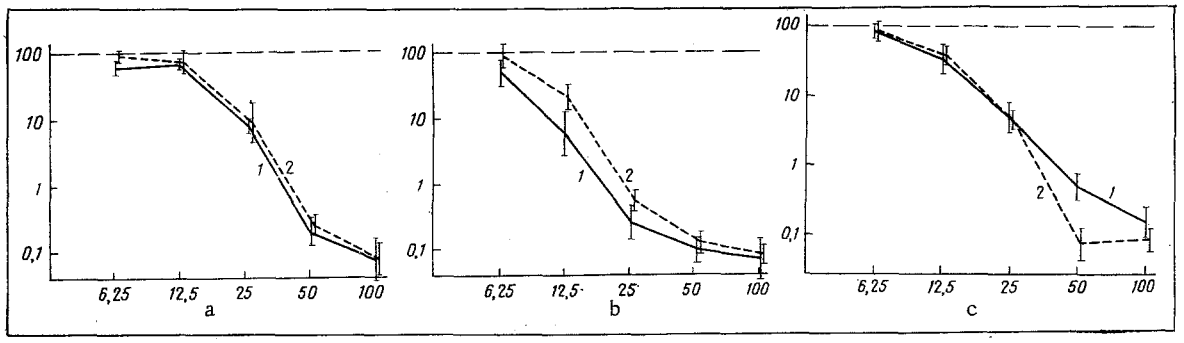

Fig. 2. Immunodepressive action of CP as a function of dose of RBC in mice of different strains: a) BALB/c, b) DBA/2, c) CBA mice; 1) 5×10^8 RBC, 2) 6.2×10^9 RBC.

(Fig. 2c) were characterized by a different type of relationship: no significant difference in depression of the immune response when small doses of CP were used, but more marked depression in the case of a large dose of antigen provided that the dose of CP injected was large enough (50 and 100 mg/kg).

In the final series (III) the starting point was the evidence in the literature [7, 12] of activation of CP by membrane NADPH-dependent enzyme systems of the endoplasmic reticulum of the liver. It was important to discover the rate of activation of CP in mice of different strains. The results showed that the rate of oxidative hydrolylation of CP in DBA/2 and CBA mice is low and virtually identical (10 ± 1.2 and 11 ± 0.74, respectively), whereas the rate of CP metabolism in the BALB/c mice was relatively high (38 ± 3.3).

The main results of those experiments was that interlinear differences were found in the immunodepressive activity of CP in mice. These differences were manifested in two ways: If a fixed does of antigen was given sensitivity to the action of CP was found to differ, and if the dose of antigen was increased, sensitivity could either be reduced or increased, or it could be unchanged depending on the genotype of the animals. Results obtained in other works [10], indicating only that the degree of the immunodepressive action of CP depends on the dose of antigen, can therefore be regarded as simply a special case and not as a general rule.

In the discussion of the possible causes of the differences thus revealed in the immunodepressive activity of CP for mice of different strains it must be made clear that they were not connected with the level of the primary immune response to RBC; the number of AFC in the spleen on the fourth day after injection of 5×10^8 RBC into intact animals was virtually the same in the mice of all three strains: CBA 83,560 (range from 51,760 to 134,900), DBA/2 82,410 (55,980 to 121,300), BALB/c 72,280 (59,570 to 87,770).

Negative correlation was found when the degree of immunodepression was compared with the intensity of CP activation in the liver of mice of the different strains. For instance, BALB/c mice, characterized by a high degree of CP activation, were less sensitive to the immonodepressive action of CP than mice of the other strains. This difference was particularly marked when BALB/c and DBA/2 mice were compared (Fig. 1). This result is to some extent paradoxical, for we know [7, 12, 19] that it is not CP as such which possesses cytotoxic activity, but the products of its metabolic conversion. Possibly CP is activated in DBA/2 (and also CBA) mice not only in the liver, but in other organs also. The possibility likewise cannot be ruled out that immunocompetent target cells differ in their sensitivity to the action of active metabolites of CP in mice of different strains.

The explanation of the mechanisms of interlinear differences in the action of CP evidently necessitates further research and, in particular, the study of the activity of this immunodepressant at the cellular level and a detailed analysis of the processes of CP activation in mice of different genotypes.

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