DRUG-INDUCED IMMUNOLOGICAL TOLERANCE TO SHEEP'S RED CELLS IN MICE OF DIFFERENT STRAINS

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Immunological tolerance to sheep's red cells was induced in mice of various strains (CBA, C57BL/6, CC57BR, C3H, DBA/2) by combined injection of a large dose of antigen and cyclophosphamide into the animals. After a test injection of erythrocytes the number of 19S-antibody-forming cells was determined in the spleens of the mice by the local hemolysis in gel method. The degree of tolerance developing was shown to depend on the animals' genotype, and mice of strain DBA/2 were found to be the most sensitive to its induction. No correlation was found between the level of immunological reactivity to sheep's red cells in intact mice of different strains and the degree of its inhibition as a result of the induction of tolerance.

KEY WORDS: immunological tolerance; cyclophosphamide; genotype.

Interest in the study of the genetic control of immune reactions included among the manifestations of cellular and humoral immunity has risen steadily in recent years [4]. However, the role of genetic factors in the induction of immunological tolerance has still received little study. The available data are concerned chiefly with tolerance to serum proteins in adult mice; such investigations have shown that the observable interlinear differences can be connected with inherited variations of the blood complement level [6, 7] and activity of macrophages [8, 10].

The object of this investigation was to determine whether differences exist between different strains of mice in the degree of drug-induced immunological tolerance to sheep's red cells.

EXPERIMENTAL METHOD

Experiments were carried out on adult male CBA, C57BL/6, CC57BR, C3H, DBA/2, and (CBA × C57BL/6)F_1 mice weighing 20-26 g. Tolerance was induced by combined injection of a large dose of sheep's red cells (RBC) and cyclophosphamide (CP) in accordance with the scheme drawn up previously [2]: intraperitoneal injection of $6.2 \cdot 10^9$ RBC followed, 42-46 h later, by intraperitoneal injection of CP. Two doses of CP were used: 50 and 100 mg/kg. The test injection of RBC ($5 \cdot 10^8$ RBC intravenously) was given after 10 days, and the number of 19S-antibody-forming cells (AFC) in the spleens of the animals was determined 4 days later. Intact mice and mice receiving CP only in the corresponding dose without any accompanying injection of RBC served as the control. The results were subjected to statistical analysis with the aid of various criteria [1, 5].

EXPERIMENTAL RESULTS

The results are given in Tables 1 and 2.

As Tables 1 and 2 show, injection of CP alone caused no significant change in the immunological reactivity of the animals, and their immune response differed only a little from that of the intact mice. A significant decrease in the response was observed only in CC57BR mice (Table 1; $P = 0.034$). After combined treatment with RBC and CP the immune response of the animals of all strains was sharply reduced compared with the

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TABLE 1. Tolerance to RBC in Mice of Different Strains Using CP in a Dose of 100 mg/kg

Strain of mice	Number of 19S-AFC in spleens after test injection of antigen		
	tolerant mice	mice receiving CP	intact mice
CBA	2312; 1,72% (1416–3776) n=15	134 300 (104 700–172 100) n=13	167 500 (135 500–207 000) n=15
CC57BR	5957; 3,97% (2392–15 000) n=10	150 000 (104 100–216 000) n=10	227 500 (190 100–272 300) n=9
DBA/2	1175; 1,22% (726–1902) n=10	96 160 (80 540–114 800) n=14	122 500 (93 540–160 300) n=15
C57BL/6	6266; 4,93% (1583–24 800) n=6	127 100 (91 520–176 400) n=6	89 540 (48 310–166 000) n=5
(CBA×C57BL/6)F_1	1758; 0,91% (948–3258) n=8	192 800 (162 600–228 600) n=9	269 200 (244 900–295 800) n=10

Legend. Here and in Table 2: 1) geometric mean values with confidence limits for $P < 0.05$ shown in parentheses. 2) Level of immune response of tolerant mice given as percentage of its level in mice receiving CP only.

TABLE 2. Tolerance to RBC in Mice of Different Strains Using CP in a Dose of 50 mg/kg

Strain of mice	Number of 19S-AFC in spleens after test injection of antigen		
	tolerant mice	mice receiving CP	intact mice
CBA	1096; 0,95% (650–1849) n=11	114 800 (101 600–129 700) n=12	107 600 (88 720–130 600) n=11
DBA/2	546; 1,36% (391–762) n=23	40 180 (28 320–56 890) n=26	32 580 (21 180–50 120) n=24
C3H	2275; 1,3% (1007–5140) n=12	121 900 (95 280–156 000) n=18	106 200 (76 380–147 600) n=16
C57BL/6	1274; 7,13% (605–2683) n=13	17 860 (10 000–31 920) n=18	27 860 (19 630–39 540) n=17

control. Meanwhile, in absolute terms the number of AFC in the spleens of the tolerant animals of the various strains differed. Dispersion analysis showed that the strains of the tolerant animals significantly ($P < 0.01$) affected the levels of their immune responses (the result was exactly the same when the "specific" immunoreactivity of the animals was calculated, i.e., the number of AFC per 10^6 nucleated spleen cells; and for that reason these results are not shown in Tables 1 and 2).

The results in Tables 1 and 2 show that intralinear differences in the level of the immune response occurred also in the intact animals, in agreement with observations of other workers [4], and also in mice receiving CP alone. It could accordingly be postulated that differences in the level of the immune response of the tolerant mice of different strains were due to genetically determined differences of immunoreactivity under normal conditions. However, calculation of correlation between the height of the immune response of the tolerant and intact animals of different strains showed that its level was not significant ($P > 0.05$ by the use of Spearman's rank correlation coefficient [1]). This is also clear from a comparison of the results of the experiments on mice of different strains. For instance, the number of AFC in the spleen of intact DBA/2 and C57BL/6 mice or of mice of the same strains receiving CP did not differ significantly, whereas in tolerant mice of those strains the difference was significant ($P = 0.014$; Table 1). Conversely, the immune response of control DBA/2 mice was significantly lower than that of (CBA × C57BL/6)F_1 hybrids ($P < 0.001$ for both intact mice and mice receiving CP), whereas the differences in the tolerant animals were not statistically significant. Similar comparisons can also be made among the data in Table 2.

When these results are assessed the fact will be noted that tolerance arising in mice following administration of CP in a dose of 50 mg/kg was stronger than when a dose of 100 mg/kg was given. This somewhat paradoxical finding can be explained in several ways. First, seasonal fluctuations in general immunoreactivity of the animals used may play a role (investigations with a dose of 100 mg/kg of CP were carried out in the fall, those with a dose of 50 mg/kg at the end of winter or beginning of spring). As Tables 1 and 2 show, the number of AFC in the control animals varied considerably even in the same strain (especially in C57BL/6 and DBA/2 mice), so that the larger number of AFC in the control could correspond to a higher level of the immune response in the tolerant animals.

Another possibility connected with differences in the mechanisms of induction and maintenance of tolerance created with the aid of CP likewise cannot be ruled out. It was shown previously [3] that, under certain conditions, antibodies with blocking properties appear in the blood streams of tolerant animals and that the presence of these antibodies is one of the factors which determines the lowered immunoreactivity in drug-induced tolerance. In experiments which form part of a special investigation the writers showed that CP, in a dose of 50 mg/kg, inhibits the production of 19S-AFC just as effectively in the mice of certain strains in response to injection of a large dose of RBC as CP in a dose of 100 mg/kg. At the same time, it can be postulated that the formation of blocking antibodies, evidently of a different class than the 19S antibodies [3], is depressed by a lesser degree when the dose of the immunodepressant is reduced, and that this is reflected in a lowered response of the mice to the test injection of antigen.

Despite these variations in the immune response of the tolerant mice receiving CP in different doses, an attempt can be made to distinguish "alternative" strains. To judge from the absolute number of AFC in the spleen, DBA/2 mice are more "sensitive" to the induction of tolerance: Their response was significantly lower than the immune response of tolerant mice of other strains (except the F_1 hybrids). It is difficult to determine the most "resistant" strain by this method of assessment, and only after analysis of the relative level of AFC in the tolerant mice compared with the control (expressed as a percentage in Tables 1 and 2) can it be deduced that strain C57BL/6 is "alternative" in relation to strain DBA/2. However, this is a problem for further investigation; the experimental results so far obtained enable it to be concluded with confidence only that tolerant mice of strain DBA/2 occupy a special position, characterized by the minimal number of AFC in the spleen.

The results given in this paper are thus evidence of interlinear differences between mice as regards tolerance to RBC induced with the aid of CP. These differences, under the experimental conditions used, are not so marked as, for example, in the case of tolerance induced in mice by deaggregated proteins [8, 9, 11]. It is possible that, by varying the conditions of induction of tolerance (dose of antigen, dose of immunodepressant, interval between their injection), a greater difference could be obtained in the degree of tolerance arising in mice of different genotypes.

Analysis of causes of the differing sensitivity of mice of the strains used to the induction of tolerance is a difficult matter, bearing in mind the fact that tolerance in each particular case arises as the result of the combined action of two factors: antigens and immunodepressants. It is evident that one way in which to investigate this problem further would be by studying the role of each of these factors. Preliminary experiments have shown that mice of different strains differ in their sensitivity to the immunodepressive action of CP, and this could be the factor which determines differences in the degree of tolerance produced in them.

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