RATE OF DEVELOPMENT OF IMMUNOLOGICAL MEMORY AND CHARACTERISTICS OF THE SECONDARY IMMUNE RESPONSE IN MICE OF STRAINS WITH HIGH AND LOW REACTIVITY

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Immunological memory for sheep's red blood cells develops in mice of strains CBA and DBA/2 and (CBA × C57BL/6)F_1 hybrids 24 h after injection of a small dose of the antigen, but 48 h after injection in C57BL/6 mice. The level of the secondary immune response in CBA, C57BL/6 and F_1 hybrids is significantly higher than in DBA/2 mice. Maximal production of antibody-forming cells in the spleen of the CBA mice is observed after two injections of small doses of the antigen. By contrast to this, to obtain a marked immune response in the case of adoptive transfer of spleen cells of C57BL/6 mice a second injection of a large dose of antigen is required.

KEY WORDS: *immunological memory; secondary immune response; genotype.*

There is much evidence in the literature to show that animals of different inbred lines differ in their ability to respond to the same antigen. In particular, mice of different strains have been shown to differ in the level of their primary immune response during immunization by sheep's red blood cells (SRBC); mice of strain CBA belong to the "strongly" and mice of strains C57Bl/6 and DBA/2 to the "weakly" reactive types [2].

The object of this investigation was to study the pattern of formation and realization of immunological memory in mice of the above strains.

EXPERIMENTAL METHOD

Adult male mice of strains CBA, C57BL/6, and DBA/2, and also (CBA × C57BL/6)F_1 hybrid mice, weighing 20-25 g, were used. To study the rate of development of ability to give a secondary immune response and its intensity, SRBC were injected intravenously in a dose of 10^6 cells twice with different intervals between injections. Four days after the second injection of antigen the number of 19S-antibody-forming cells (AFC) in the spleen of the mice was determined by the local hemolysis in agar method [4].

To study the secondary immune response in adoptive transfer, 50×10^6 spleen cells of mice receiving 10^6 or 10^8 SRBC were transplanted into syngeneic recipients, irradiated or treated with cyclophosphamide, together with the antigen, as described in the previous paper [1].

The results were subjected to statistical analysis by Student's t test (with calculation of the geometric mean and confidence intervals at $P < 0.05$).

EXPERIMENTAL RESULTS

Data on the development of immunological memory are given in Table 1.

As Table 1 shows, mice of strain CBA were able to give a marked secondary response to repeated injection of the antigen 24 h after sensitization by a small dose of red cells. The number of AFC in their spleen was significantly greater than after a single injection of the total dose of SRBC ($2 \cdot 10^6$). In the C57BL/6 mice, with an interval of 24 h between injections, the immune response was practically the same as after a single injection of the total dose,

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TABLE 1. Rate of Formation of Immunological Memory in Mice of Different Strains

Interval between injections of SRBC, days	Number of AFC in spleen of mice of strains			
	CBA	C57BL/6	(CBA×C57BL/6) F_1	DBA/2
1	52 600 (38 990—70 960) $n=20$	1 914 (927—3 954) $n=20$	88 720 (62 810—125 300) $n=6$	2 624 (1 143—6 026) $n=16$
2	59 840 (41 780—85 700) $n=21$	40 180 (31 190—51 760) $n=27$	158 100 (106 400—235 000) $n=5$	12 450 (5 794—26 730) $n=19$
3	99 770 (39 170—152 400) $n=10$	102 600 (79 620—132 100) $n=8$	234 400 (134 600—408 300) $n=4$	18 320 (11 910—28 180) $n=6$
Control 1	8 128 (3 917—16 870) $n=17$	464 (154—1 403) $n=15$	—	245 (127—473) $n=12$
Control 2	4 285 (2 489—7 379) $n=23$	3 639 (2 046—6 471) $n=21$	27 610 (18 710— 40 740) $n=5$	298 (173—512) $n=19$

Legend. Control 1) Single injection of total dose ($2 \cdot 10^6$ SRBC) 4 days before determination of number of AFC; control 2) 6 days before determination. Here and in Tables 2 and 3, n = number of animals.

TABLE 2. Dependence of Number of AFC in Spleen of Mice on Dose of First Injection of Antigen and Interval Between Injections

Dose of SRBC at immunization		Strain	Interval between injections of SRBC, days	
primary	secondary		7	21
10^6	10^6	CBA	61 940 (45 920—83 560) $n=16$	110 900 (77 800—158 100) $n=10$
		C57BL/6	42 360 (24 770—72 440) $n=18$	30 830 (9 162—103 800) $n=11$
10^8	10^6	CBA	2 023 (1 225—3 342) $n=16$	5 395 (2 673—10 890) $n=9$
		C57BL/6	4 977 (2 698—9 183) $n=14$	15 920 (10 990—23 070) $n=16$
—	10^6	CBA	4 436 (2 259—8 511) $n=20$	
		C57BL/6	$\leqslant$51 (35—74) $n=24$	

and not until 48 h after the first injection of SRBC was a large number of AFC, comparable with that found in the CBA mice, formed in response to the second injection. (CBA × C57BL/6)F_1 hybrids had the same ability as the CBA mice for rapid development of immunological memory. In DBA/2 mice, two injections of SRBC at an interval of 1 day led to accumulation of AFC in a number one order of magnitude greater than in the control. Meanwhile, the absolute level of the secondary immune response in the mice of this strain was considerably lower than in animals of the other strains, even if the interval between injections was increased to 2 or 3 days.

Formation of the immunological memory is evidently complete 2-3 days after sensitization. This was shown by the fact that lengthening the interval to 1 or 3 weeks caused no increase in the level of the secondary immune response (Table 2).

It will be clear from Table 2 that when comparatively large doses of SRBC (10^8) were used for primary immunization, the secondary immune response tested after 7 days was much weaker in the mice of both strains studied, in agreement with results described previously [1]. In mice of strain C57BL/6 these differences disappeared as the interval between injections of SRBC was lengthened to 3 weeks.

TABLE 3. Secondary Immune Response of Mice of Strains with Low and High Reactivity Under Conditions of Adoptive Transfer of Spleen Cells

Dose of SRBC at immunization		Strain	
primary	secondary	CBA	C57BL/6
10^8	10^6	11 610 (5 681—23 010) $n=16$	7 621 (2 301—25 230) $n=9$
10^8	10^8	56 750 (35 240—91 410) $n=14$	106 200 (84 920—133 700) $n=10$
10^6	10^6	111 400 (87 900—141 300) $n=16$	8 810 (4 797—16 220) $n=11$
10^6	10^8	93 970 (77 620—113 800) $n=10$	108 100 (64 710—180 700) $n=10$
—	10^6	4 093 (1 774—9 441) $n=20$	21 (8—51) $n=5$
—	10^8	30 620 (20 320—46 130) $n=22$	8 356 (4 550—15 350) $n=14$

Legend. Interval between primary and secondary immunization 7 days. When increased to 3 weeks the results were the same and they are not therefore shown in this table.

In the second part of the investigation the character of the secondary immune response in CBA and C57BL/6 mice was studied during adoptive transfer of their spleen cells 1 and 3 weeks after primary immunization. The results of these experiments are summarized in Table 3.

They show that the height of the secondary immune response in the CBA mice depends more on the size of the first dose of antigen. The maximal effect was obtained after sensitization of the spleen cells of the donor mice with 10^6 SRBC, regardless of the dose used for the secondary stimulus. A different pattern was observed in the C57BL/6 mice. The main factor in them, determining the height of the secondary immune response, was the size of the dose of SRBC injected together with the transplanted cells: maximal accumulation of AFC took place when 10^8 SRBC were used as the secondary stimulus, regardless of the sensitizing dose of antigen.

These results clearly differed not only from those obtained with CBA mice in adoptive transfer, but also from the experiments in which the secondary immune response was studied in situ (Table 2). Under these conditions, in the C57BL/6 mice, just as in the CBA mice, two injections of small doses of the antigen gave a fully developed immune response, which was stronger than after the 10^8-10^6 SRBC scheme with an interval of 7 days.

The following conclusion can be drawn from these results. According to data in the literature [2], CBA mice have a higher level of primary immune response to SRBC than C57BL/6 and DBA/2 mice. This is revealed after a single immunization of the animals with small (10^6 or $2 \cdot 10^6$ SRBC) and large (10^8 with adoptive transfer) doses of the antigen (see Tables 1-3).

The study of the rate of development of immunological memory and the intensity of the secondary immune response showed that CBA mice have high reactivity with respect to both indices, whereas in the animals of the other two strains there is evidently a "defect" in relation to one of these indices. In C57BL/6 mice development of ability to give a secondary immune response is delayed by 24 h compared with animals of the other strains, but later they respond by intensive AFC formation to the second injection of antigen just like CBA mice. Immunological memory is formed in DBA/2 mice 24 h after sensitization (just as in CBA mice), but the level of their secondary immune response is significantly lower than in CBA and C57BL/6 mice (Table 1).

The experiments with adoptive transfer of the secondary immune response showed that in CBA mice a characteristic feature is complete agreement between the response of the spleen cells in situ and in vivo in the recipients. In both cases maximal AFC production is achieved following two injections of small doses of SRBC. Spleen cells of C57BL/6 mice in adoptive transfer give a marked immune response only after a second injection of a large dose of the antigen, by contrast with what is observed in situ. The impression is obtained that certain extrasplenic "potentiating" factors participate in the realization of the secondary immune response of C57BL/6 mice in situ, but these are lost during transplantation

of spleen cells into the recipient. Under these circumstances stronger antigenic stimulation is required to induce a high secondary immune response of the transplanted cells. The possibility likewise cannot be ruled out that the distinctive nature of the secondary immune response of the C57BL/6 mice is due to differences in the migration properties of their immunocompetent cells, a view supported by the results obtained on investigation of their primary immune response [3].

The results described in this paper thus show that whether mice belong to strains characterized by low or high initial reactivity to an antigen is reflected in the pattern of formation and realization of their immunological memory. The differences between strains revealed by these experiments are evidently determined not only by the state of the population of immunocompetent cells of the reacting organ (the spleen), but also by other factors, whose regulatory effect is manifested only in the intact organism.

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