PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 470–474 (1974)

Carrier-Determined Tolerance in Various Strains of Mice: The Role of Isogenic IgG in the Induction of Hapten Specific Tolerance[1] (37833)

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(Introduced by R. S. Schwartz)

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Adult animals treated with dinitrophenyl (DNP) attached to a nonimmunogenic carrier no longer respond to the same antigenic determinant attached to an immunogenic carrier protein (1). Among the various carrier proteins studied, isogeneic IgG has the greatest efficiency in the induction of tolerance of DNP (2). Therefore, this form of hapten specific tolerance is carrier determined. DNP isogeneic IgG, by itself, even in complete Freund's adjuvant, is a poor immunogen in isogeneic mice, yet it is a powerful tolerogen following immunization with dinitrophenyl keyhole limpet hemocyanin (DNR-KLH) (2). In view of this observation, it is likely that carrier-determined tolerance can be induced in any individual regardless of its capacity to respond to the antigen DNP-KLH. The results of the present experiments support this assumption and confirm the role of DNP isogeneic IgG in the induction tolerance of DNP.

Materials and Methods. Animals. Six-to-seven-week-old mice belonging to 11 different strains of mice (A/HeJ, A/Jax, C57BI/6, C57BI/10, BALB/c, DBA, NZB, CBA, C3H, AKR, SJL) were used. The mice were obtained from Jackson Laboratories, Bar Harbor, ME. A total of over 500 animals were studied.

Tolerogen.[2] 2,4-Dinitrobenzene sulfonic acid sodium salt (Eastman Kodak, twice recrystallized) was bound to isogeneic IgG which was separated from the serum (purchased from Jackson Laboratory, Bar Harbor, ME) of each strain of mice by block electrophoresis as previously described (2).

To test the influence of the dose of the tolerogen, the same preparation at a lower dose (50 μg) than the one usually given (200 μg $DNP_{9.6}$-SJL IgG) was given to two different strains of mice, AKR and SJL.

Hemolytic plaque assay. Anti-DNP plaque-forming cells (anti-DNP-PFC) were assayed by detecting their cross-reaction with trinitrophenol-coated (TNP) sheep red cells as previously described (2). TNP-coated sheep red cells were used because until recently methods using DNP-coated sheep red cells had been unreliable. Indirect plaque-forming cells were revealed using an anti-mouse gamma globulin serum (shown to react with γ_1, γ_2, and γ_{2b} mouse γ-globulin on immunoelectrophoresis) after treatment with specific anti-μ serum (gift of Dr. Robert McIntyre) to absorb the direct PFC, as

[1] These studies were supported by U. S. Public Health Service Grant No. AI 09823 from the Immunology Branch, National Institute of Allergy and Infectious Disease, and, in part, by Grant No. 7-32 from the Massachusetts Chapter of the Arthritis Foundation.

[2] The various preparations of tolerogen were: DNP_{11}, A/HeJ IgG; DNP_{12}, A/Jax IgG; DNP_9, C57B1/6 IgG; DNP_9, C57B1/10 IgG; $DNP_{11.6}$, BALB/c IgG; $DNP_{12.9}$, DBA IgG; DNP_{10}, NZB IgG; DNP_9, CBA IgG; DNP_{11}, C_3H IgG; DNP_7, AKR IgG; DNP_{14} SJL IgG.

The following antigenic preparations were used: DNP_{85}-KLH in all strains of mice for the direct anti-DNP-PFC assay. For the indirect anti-DNP-PFC, three different preparations were used: DNP_{50}-KLH for SJL, CBA, C57B1/10, and AKR mice; DNP_{72}-KLH for A/HeJ, C_3H, and NZB mice; and DNP_{86}-KLH for BALB/c, A/Jax, DBA, and C57B1/6 mice.

described by Pierce (3).

Statistical analysis. The geometric means were taken for each group of PFC, and the data were analyzed according to the Student's *t* test.

Results. To induce tolerance of DNP, a single iv injection of 0.2 mg of DNP isogeneic IgG was given in 11 different strains of mice. Immediately thereafter, these mice together with untreated control animals were immunized with 0.2 mg of DNP-KLH given in complete Freund's adjuvant intraperitoneally. Five days later, the anti DNP-PFC were assayed in their spleen. This experiment was repeated at least twice with five animals in each group for all strains of mice (in strains of AKR, CBA, SJL, the experiments were repeated 3–5 times). A summary of the results is given in Figs. 1 and 2.

As expected, the normal immune response varies greatly among the various strains of mice. The highest responders were the A/Jax (500 ± 51 PFC/10^6) and AKR mice (333 ± 48 PFC/10^6); the lowest responders were the SJL (73 ± PFC/10^6) and C57B1/10 mice (78 ± 10 PFC/10^6). Between these two extremes, there were also other differences which were statistically significant. For example, DBA mice were higher responders than SJL or C57B1/10 yet lower responders than the A/Jax.

Tolerance to DNP was induced in all

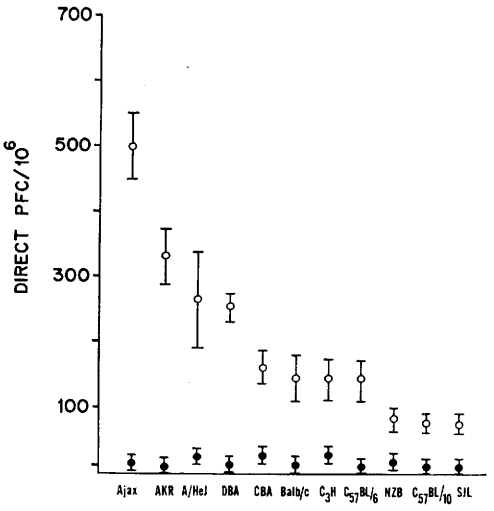

FIG. 1. Carrier-determined tolerance to DNP in terms of the direct PFC/10^6 spleen cells. ⊼ Immunity (±SE); ⊼ tolerance (±SE). Each point, both for tolerance and immunity, represents the geometric mean of a group of 10 animals. The order of the strain of mice was chosen arbitrarily according to the magnitude of the immune response. Thus, the highest to the lowest strain of mice are presented in decreasing order.

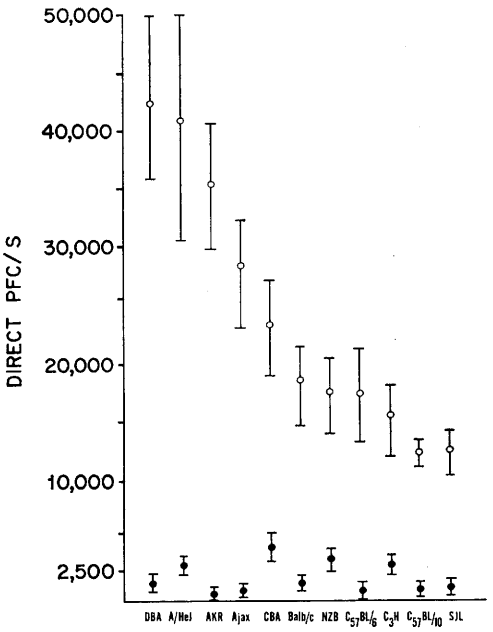

FIG. 2. Carrier-determined tolerance to DNP in terms of the direct PFC/spleen. ⊼ Immunity (±SE); ⊼ tolerance (±SE). The order of the strain of mice was chosen arbitrarily according to the magnitude of the immune response from the highest to the lowest responder mice. Note that the order of the strain is not the same as the order of the strain presented in Fig. 1, where the immune response was measured in PFC/10^6 spleen cells. The reason is twofold: (1) the spleen weight varies from mouse strain to mouse strain, and (2) the nonspecific weight increases as a result of the injection of the antigen in complete Freund's adjuvant which also varies from strain to strain. For example, the A/Jax, the first and the highest responders as measured in PFC/10^6 spleen cells, are the fourth when measured in direct PFC/spleen. The reason is that nonimmunized A/Jax mice have a very low spleen weight (41 ± 4 g) as compared to unimmunized DBA mice (71 ± 7 g). In immunized A/Jax, the spleen weight remains low (42 ± 4 g); in contrast, it increased in immunized DBA (123 ± 6 g). Each point, both for tolerance and immunity, represents a group of 10 animals.

TABLE I.[a]

Mouse strain	"Tolerant" PFC		"Background" PFC	
	PFC/10^6 spleen cells (± SE)	PFC/spleen (± SE)	PFC/10^6 spleen cells (± SE)	PFC/spleen (± SE)
A/HeJ	22.5 (4.2)	2805 (599)	5.9 (0.7)	693 (95)
A/Jax	11.3 (2.3)	682 (150)	6.2 (0.7)	317 (53)
C57B1/6	3.6 (0.5)	471 (66)	2.1 (0.1)	152 (15)
C57B1/10	3.5 (0.9)	535 (146)	1.1 (0.4)	112 (57)
BALB/c	8.8 (2.8)	1292 (412)	6.8 (2.2)	788 (111)
DBA	8.5 (2.0)	1187 (315)	7.0 (0.6)	680 (98)
NZB	16.2 (3.0)	3485 (638)	44 (14)	5849 (1728)
CBA	23 (4.3)	4353 (912)	5.5 (1.5)	510 (126)
C_3H	23.8 (4.3)	2847 (555)	14.4 (2.6)	2064 (504)
AKR	3.3 (0.8)	395 (96)	4.5 (1.6)	355 (145)
SJL	3.4 (0.5)	588 (111)	3.1 (0.4)	503 (113)

[a] The results represent the geometric mean of 10 tolerant animals and 5 normal nonimmunized control mice for the "background" PFC. Note that the tolerant PFC for the NZB mice were below the background PFC though it was not statistically significant.

strains of mice to a high degree. Table I shows that tolerant mice produced direct PFC in numbers approximately equal to the background PFC. In only two strains (A/HeJ and CBA) the number of PFC was statistically significantly higher than the background. It is also apparent from Figs. 1 and 2 that there is no correlation between the degree of suppression and the magnitude of the immune responses.

Since tolerance was easily induced by 200 μg of DNP isogeneic IgG, a lower dose (50 μg) was also tried. Two strains of mice were chosen, AKR and SJL, because they differed widely in their direct PFC immune response (Fig. 1). Table II shows that tolerance was induced at both doses of tolerogen, though suppression of the immune PFC was slightly better with the higher dose. Although the results are still qualitative and further analysis is required to obtain quantitative results, the data suggest that this slight dosage effect was not related to the magnitude of the normal immune response of the two strains used.

The same tolerogen preparations at the same dose (0.2 mg) used to suppress the direct anti-DNP PFC were also used to induce tolerance in terms of the indirect anti-DNP PFC. As before, the experimental animals, together with untreated control animals, were immunized with 0.2 mg of DNP-KLH given intraperitoneally in complete Freund's adjuvant. The indirect PFC was done on Day 6 since preliminary experiments in several strains of mice have shown that this day was the peak of the indirect anti-DNP-PFC response. The results are summarized in Fig. 3.

Profound suppression occurred in all strains. In 6 of the 11 strains, no indirect plaques were found, and in the remaining five strains the number of indirect PFC was very low.

Although the immune response in terms of the indirect anti-DNP-PFC was somewhat lower and more uniform in the various strains of mice than the direct anti-DNP-PFC, there was also no correlation between the ability to be rendered tolerant of DNP and the magnitude of the immune response.

Discussion. The main finding is that tolerance of the haptenic determinant DNP can be induced in all strains of mice regardless of the capacity of these animals to form an immune response to the same hapten. This was the case both for the direct and the indirect PFC (Figs. 1–3). It should be stressed that the manner by which tolerance is induced in this system is unique because (a) the hapten (DNP) is, by itself, neither tolerogenic nor antigenic (2), and (b) the molecule to which the hapten is bound (IgG) is a native host immunoglobulin of which the host is naturally tolerant. Consequently, this experimental model is closely

TABLE II. Influence of Dose of Tolerogen.[a]

Mice No.	Tolerogen	Direct PFC/10^6 spleen cells (± SE)	Direct PFC/spleen (± SE)
AKR			
10	None	333 (48)	35046 (5704)
6	200 μg DNP_7, AKR IgG	4.3 (1.6)	610 (210)
5	50 μg DNP_9, SJL IgG	16 (2.5)	1483 (101)
SJL			
10	None	73 (11)	12518 (2248)
8	200 μg DNP_{14}, SJL IgG	3.4 (0.5)	588 (111)
5	50 μg DNP_9, SJL IgG	4.5 (2.2)	2084 (953)

All mice were given the indicated dose of tolerogen prior to the immunization with 0.2 mg of DNP-KLH in CFA. The variation of PFC/10^6 spleen cells as compared to PFC/spleen is due to a variation of spleen weight not only from strain to strain but also from one experiment to another.

related to the study of natural tolerance of "self" antigen. We call this phenomenon carrier-determined tolerance, since tolerance of the hapten is dependent on the carrier to which the hapten is covalently bound (2). Others have shown that partial or complete hapten specific tolerance can be induced *in vivo* (4–7).

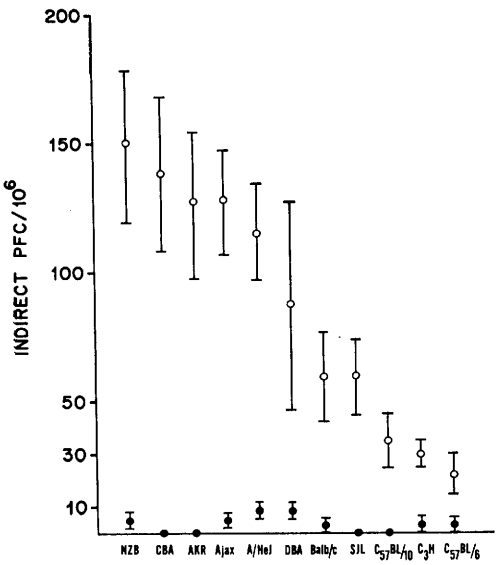

FIG. 3. Carrier-determined tolerance to DNP in terms of the indirect PFC/10^6 spleen cells. ⊺ Immunity (±SE); ⊺ tolerance (±SE). Each point, both for tolerance and immunity, represents the geometric mean of a group of 10 animals. The order of the strain was chosen according to the magnitude of the immune response. Note that the order is different than the one chosen for the direct PFC/10^6 spleen cells.

The ease with which tolerance was induced by this system in all strains of mice contrasts with the reports of others using heterologous protein tolerogens (8). For example, Golub and Weigle found that while trace amounts of nonaggregated HGG were sufficient to render C57B1/6 mice tolerant, BALB/c mice did not become unresponsive to doses as high as 10 mg (9). They found that this apparent difference between strains was due to the presence of immunogenic aggregates in the tolerogenic preparation. When the tolerogen was free of these aggregates, both strains were rendered tolerant at the same dose of tolerogen. As these workers rightly pointed out, the two strains differed not in their ability to become tolerant but differed in their ability to handle the immunogenic contaminant.

A priori, it might be expected that there would be either a direct or inverse relationship between the magnitude of the immune response of a particular mouse strain and the ease of tolerance induction in that strain. No such relationship can be shown (Figs. 1–3) under the present experimental conditions (constant doses of tolerogen and antigen and constant time of assay in all strains of mice). Whether a variation in the amount of tolerogen used would result in a difference in the degree of unresponsiveness in various strains of mice is unknown. However, in two different strains of mice (AKR and SJL) which vary widely in their immune response (one was high-AKR, the other low-SJL), a

fourfold reduction in the dose of tolerogen resulted only in a slight dosage effect of the degree of unresponsiveness, which, again, was unrelated to the magnitude of the immune response in these two strains of mice.

It is worth noting that unresponsiveness to DNP was also induced in NZB mice. In this strain, conflicting results have been reported as far as the induction of tolerance to protein antigens is concerned. Some found the NZB resistant to the induction of tolerance to unaggregated bovine or human gamma globulin (10), while others found that NZB mice can be rendered tolerant to unaggregated gamma globulin (11). Our results support the latter.

This uniformity of the susceptibility of 11 strains of mice to the induction of tolerance might be explained as follows: Recent data by others suggest that the number of antigen-binding cells in both high- and low-responding strains are the same prior to immunization (12, 13). The tolerogen will render this population of cells tolerant regardless of the ability of the antigen to stimulate this population of antigen-binding cells.

Finally, the finding that the magnitude of the immune response, which is known to be on a genetic basis, is not related to the susceptibility to tolerance raises the question whether tolerance also has a genetic basis.

Summary. Eleven strains of mice were injected intravenously with a single dose (0.2 mg) of DNP isogeneic IgG. Immediately thereafter, they were challenged intraperitoneally with the same dose of 0.2 mg DNP-KLH in complete Freund's adjuvant. The immune response of DNP was determined by the direct and indirect anti-DNP-PFC. Tolerance of DNP was induced in all strains of mice including the NZB, both in terms of direct and indirect PFC. No correlation was found between tolerance and immunity of a single antigenic determinant suggesting that these two immune phenomena might behave independently of each other.

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Received July 12, 1973. P.S.E.B.M., 1974, Vol. 145.