Brief Definitive Report

THE IMMUNE RESPONSE OF ALLOPHENIC MICE TO 2,4-DINITROPHENYL (DNP)-BOVINE GAMMA GLOBULIN

I. Allotype Analysis of Anti-DNP Antibody*

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The question of whether or not lymphoid cells can cooperate across a histocompatibility difference barrier has been studied in several laboratories. Using an adoptive transfer system, Katz et al. (1) first showed that T cells from (low responder × high responder) F_1 mice, primed to the terpolymer L-glutamic acid, L-lysine, L-tyrosine (GLT), could collaborate with 2,4-dinitrophenyl (DNP)-primed B cells from a high responder, but not a low responder strain, in response to DNP-GLT. The response to GLT is under *H-2*-linked Ir gene control. In contrast, studies with mouse bone marrow chimeras have shown that T cells can interact with *H-2*-histoincompatible B cells in response to antigens not under Ir gene control (2–4). Another type of chimera, the allophenic mouse, has been used to study possible histoincompatible cell interactions to a number of antigens, including DNP-L-glutamic acid, L-lysine, L-alanine; L-glutamic acid, L-alanine, L-tyrosine; L-glutamic acid, L-lysine, L-phenylalanine; and poly-L (Tyr, Glu)-poly D,L-Ala-poly-L-Lys[T,G)-A–L] (5–9). The response to each of these antigens is under *H-2*-linked Ir gene control.

It was initially reported (8, 9) that in allophenic mice containing both high and low responder cells, the antibody to (T,G)-A–L was of both the high and low responder allotype. This was interpreted to mean that high responder T cells had cooperated with low responder B cells across a histocompatibility difference barrier in the environment of the allophenic mice. However, Press and McDevitt (10) have recently reported that additional and more accurate analyses of these allophenic mouse sera failed to detect any anti-(T,G)-A–L antibody of the low responder allotype. Moreover, in an experiment using bone marrow chimeras, there was no low responder allotype antibody produced in response to (T,G)-A–L(10).

The present study was undertaken to test the immune response of allophenic mice to an antigen, DNP-bovine gamma globulin ($DNP_{56}BGG$), known to be controlled by genes both inside and outside the *H-2* complex (11, 12).[1] When high and low responder cells to $DNP_{56}BGG$ are present in allophenic mice, only antibody of the high responder allotype is produced. The results suggest that

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cell cooperation in allophenic mice cannot occur across a histocompatibility difference barrier in response to an antigen whose genetic control is at least partially within the *H-2* complex.

Materials and Methods

Mice. The inbred strains A and C57BL/6 were purchased from The Jackson Laboratory, Bar Harbor, Maine. The F_1 hybrids (A × C57BL/6) were bred in our laboratory. The allophenic mice were produced as previously described (5).

Immunization. The immunization schedule was according to Newton and Warner (11). Mice received 50 μg of $DNP_{56}BGG$ in 1 mg $Al(OH)_3$ (dry weight) administered intraperitoneally on days 1, 29, and 57. The animals were bled, sacrificed, and their spleens were excised on day 64.

Spleen Cell Composition of Allophenic Mice. After spleen excision, a single cell suspension was prepared, the lymphocytes were isolated on a Ficoll-Hypaque density gradient, and the proportion of C57BL/6 parental cells was determined by a trypan blue dye exclusion microcytotoxicity test (6). The anti-C57BL/6 serum was produced by the injection of C57BL/6 spleens into A mice to eliminate any possible cross-reaction with A parental type spleen cells.

Assays for DNP Antibody. Two different radioimmunoassay procedures were used to measure serum antibody levels. The first was a modification of the procedure of Green et al. (13). In this procedure, [^{3}H]DNP-lysine synthesized in our laboratory by the method of Stupp et al. (14) was used as the antigen, and $(NH_4)_2SO_4$ was used to precipitate the antibody-hapten complex (11). The second procedure was according to Freed et al. (12). In this procedure ^{125}I-DNP-bovine serum albumin (BSA), synthesized in our laboratory by the method of Greenwood and Hunter (15), was used as the antigen, and rabbit anti-mouse immunoglobulin (Miles Laboratories Inc., Elkhart, Ind.) was used to precipitate the antibody-antigen complex. Values <5% binding in this assay are insignificant.

Allotype Analysis of DNP Antibody. Antibody to the b allotype (C57BL/6) was prepared according to Herzenberg and Herzenberg (16). Analysis using normal sera and myeloma proteins in double gel diffusion showed that the antiserum was monospecific for the b allotype.

The immunoglobulin fraction was isolated from the anti-allotype serum by ammonium sulfate salt fractionation, and coupled to cyanogen bromide-activated Sepharose 4B by the method of Cuatrecasas (17). A 1-ml column of immunoadsorbent was poured and equilibrated with 0.10 M sodium phosphate buffer, pH 7.0, containing 1.0 mg/ml BSA as a carrier. The sera were assayed for anti-DNP antibody before application to the column. 10 μl of immune sera were applied, and the column was incubated at room temperature for 1 h to ensure complete binding. Then, 5 ml of buffer was passed through the column and collected. This was called the "passed fraction". Elution of the antibody bound to the column was accomplished using 5 ml of 0.10 M acetic acid, pH 3.1, containing 1.0 mg/ml BSA. This fraction, called the "eluted fraction", was neutralized with NaOH to pH 7.0–7.2. Both fractions were then concentrated using a Minicon B-15 (Amicon Corp., Lexington, Mass.) to a final volume of 0.5 ml (a 1:50 dilution of the original serum sample). The passed and eluted fractions were then assayed for anti-DNP antibody activity, as described above.

Results

Table I summarizes data collected in our laboratory which shows that A mice are high responders and C57BL/6 mice are low responders to $DNP_{56}BGG$. Data is also shown for (A × C57BL/6)F_1 hybrid mice.

For the experiments described in this paper, 13 A ↔ C57BL/6 allophenic mice were immunized with $DNP_{56}BGG$. Table II shows that the population of mice included a range of mixtures of parental spleen cell types. The allophenic mice studied were not assessed for total serum allotype, but rather for spleen cell composition to determine their degree of chimerism. However, another study on C57BL/6 ↔ (CBA × CBA/H-T6) allophenic mice (18) has shown that the percentage of each parental cell type is the same when assayed either by spleen cell composition or by total serum allotype composition. The response to

TABLE I

Response of Inbred Strains of Mice to Immunization with $DNP_{56}BGG$

Mouse strain	No. of mice tested	ABC_{33} ± SEM*	Classification
A	26	80 ± 5	High responder
C57BL/6	31	10 ± 2	Low responder
(A × C57BL/6)F_1	10	97 ± 15	High responder

* Data are the reciprocal of the serum dilution for 33% binding of 10^{-8} M [^{3}H]DNP-lysine. ABC_{33}, antigen binding capacity at 33% antigen bound.

TABLE II

Response of A ↔ C57BL/6 Allophenic Mice to Immunization with $DNP_{56}BGG$

Mouse	% C57BL/6 SWBC*	ABC_{33}‡
170	1	32
171	1	88
159	11	128
191	12	100
172	52	100
173	59	65
162	63	43
160	72	26
187	84	0
168	95	33
157	95	3
158	97	9
167	106	47

* Data are the percentage of C57BL/6 spleen white blood cells (SWBC) analyzed by cytotoxicity testing as described in the text. All assays were done in duplicate

‡ Data are the reciprocal of the serum dilution for 33% binding of 10^{-8} M [^{3}H]DNP-lysine. All assays were done in duplicate. ABC_{33}, antigen binding capacity at 33% antigen bound

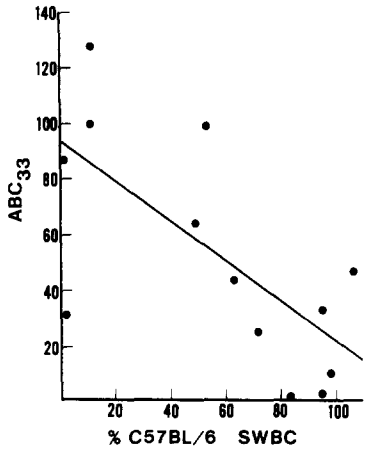

FIG. 1. Correlation of the antigen binding capacity at 33% antigen bound (ABC_{33}) to the percentage of C57BL/6 SWBC. The line is the least squares linear regression through all the data points. Number of mice = 13; $r = -0.68$; $P < 0.01$.

immunization was variable. In general, those mice with a higher proportion of low responder (C57BL/6) spleen cells produced less anti-DNP antibody in response to immunization with $DNP_{56}BGG$. This point is illustrated in Fig. 1.

Five of the mice listed in Table II were chosen for further experimentation. These mice were assayed for the presence of the low responder b (C57BL/6) allotype in the total anti-DNP antibody population, as is shown in Table III. Despite the fact that a significant proportion of C57BL/6 cells were present in

TABLE III

*Allotype Analysis of the Anti-DNP Antibody Produced by Inbred and Allophenic Mice in Response to Immunization with $DNP_{56}BGG$**

Mouse	% Antigen bound before anti-C57BL/6 column	% Antigen bound by passed fraction	% Antigen bound by eluted fraction
A (pooled)	27 ± 4	24 ± 6	2 ± 0
C57BL/6 (pooled)	20 ± 3	6 ± 2	16 ± 6
170	32 ± 3	29 ± 5	4 ± 3
171	37 ± 1	38 ± 1	5 ± 3
191	40 ± 3	32 ± 8	3 ± 2
172	38 ± 5	37 ± 10	2 ± 2
162	33 ± 0	28 ± 1	4 ± 1

* All data are the percent of antigen (^{125}I-DNP-BSA) bound by a 1:50 serum dilution. Data are the average of duplicate determinations ± SD.

some of the mice, none of them produced any significant antibody of the low responder (b) allotype.

Discussion

Allophenic mice provide an environment in which histoincompatible cells can coexist in a single animal. Since the cells coexist from a very early stage of development, allophenic mice are potentially chimeric in all their tissues and organs. They are therefore termed primary chimeras and are different from secondary chimeras, such as bone marrow chimeras, which are chimeric in only their lymphomyeloid system (19). The mechanisms for the establishment and maintenance of tolerance in allophenic mice or in bone marrow chimeric mice are unknown. However, it is clear that functional tolerance of the histoincompatible cells does exist in both types of mice.

In the present study, we have shown that the presence of two histoincompatible cell types in allophenic mice, one from a high responder strain, and one from a low responder strain to $DNP_{56}BGG$, is not sufficient to allow the low responder cells to produce anti-DNP antibody. It is not known if the genes controlling the immune response to $DNP_{56}BGG$ are expressed at the level of the T cell, the B cell, the macrophage, or all three. Low responder (C57BL/6) B cells can make anti-DNP antibody when the DNP group is on a carrier other than BGG (20). Thus, the inability to respond is probably at the T-cell or T cell-B cell interaction level. However, as is shown in the present study, in allophenic mice containing high responder T cells capable of recognizing the carrier and low responder B cells capable of recognizing the hapten, antibody is not produced by the low responder B cells. A likely explanation is that the high responder T cells and low responder B cells cannot cooperate across a histocompatibility difference barrier in response to an antigen with genetic control residing in immune response gene(s) in the *H-2* complex. This interpretation would be in full agreement with the recently reported data on the (T,G)-A – L system (10).

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