THE ALLOGENEIC EFFECT IN INBRED MICE

I. Experimental Conditions for the Enhancement of Hapten-Specific Secondary Antibody Responses by the Graft-*Versus*-Host Reaction*

By DAVID P. OSBORNE, Jr.‡ and DAVID H. KATZ

(From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115)

(Received for publication 15 May 1972)

In recent studies (1–3) it has been demonstrated that the passive transfer of immunocompetent allogeneic lymphoid cells to guinea pigs previously primed with 2,4-dinitrophenyl-ovalbumin (DNP-OVA)[1] results in the increased synthesis of anti-DNP and anti-OVA antibodies in the absence of further antigenic challenge, and markedly enhances anti-DNP responses to an appropriately timed challenge with DNP coupled to an unrelated carrier, bovine gamma globulin (BGG). This phenomenon has been termed the "allogeneic effect" and has several salient features. First, the phenomenon reflects and requires the development of a transient graft-*versus*-host (GVH) reaction in the lymphoid organs of the primed host (1). The existence of a concomitant host rejection reaction is not only not required, but appears to play little if any role (3). Secondly, the lymphoid cells of the host must be primed before the administration of allogeneic cells. Numerous attempts to demonstrate an enhanced primary response by the prior administration of allogeneic cells have failed, and, indeed, the primary response is usually suppressed under these circumstances (1–3a). Finally, the allogeneic effect has been shown to replace the requirement for carrier-specific helper T cells in the development of hapten-specific anamnestic antibody responses manifested by the primed lymphoid cells of the host (2).

The studies presented in this and the accompanying paper (4) were undertaken in inbred mice to elucidate further the phenomenon of the allogeneic effect, in that, as a model, the mouse provides greater flexibility in manipulating cell populations and histocompatibility differences. In the experiments described here, we show that: (*a*) the general features of the allogeneic effect

* This investigation was supported by Grant AI-10630 from the National Institutes of Health, U.S. Public Health Service.

‡ Supported by Surgical Training Grant GM-2019 from the National Institutes of Health, U.S. Public Health Service.

[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; CAF_1, (BALB/c × A/J)F_1 hybrid mice; D-GL, copolymer of D-glutamic acid and D-lysine; DNP, 2,4-dinitrophenyl; GVH, graft-*versus*-host; KLH, keyhole limpet hemocyanin; OVA, ovalbumin; TAA, taka-amylase A.

in mice parallel, in general, those demonstrated in the guinea pig and are critically related to factors such as time interval between cell transfer and challenge, dose of allogeneic cells employed and route of administration, and magnitude of histocompatibility differences between donor and host; (*b*) the allogeneic effect in mice, as in guinea pigs (1–3), effectively replaces the requirement for carrier-specific helper T cells; and primed B lymphocytes which are not integral participants in the active GVH reaction, but rather are bystanders to such a reaction, do not appear to develop enhanced antibody production as a result of the allogeneic effect.

Materials and Methods

Proteins and Chemical Reagents.—Hen ovalbumin (OVA) 5× recrystallized and bovine gamma globulin (BGG) were obtained from Pentex Biochemical, Kankakee, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Biomarine Supply Co., Venice, Calif. Taka-amylase A (TAA), a purified and recrystallized amylase from the bacterium *Aspergillus oryzae*, was kindly provided by Dr. T. Hamaoka in our laboratory. The copolymer of D-glutamic acid and D-lysine (D-GL) was obtained from Pilot Chemicals, Inc., Watertown, Mass. The isomer had an average molecular weight of 115,000 and a ratio of G:L of 60:40. All other chemical reagents used were, in general, identical with those described in previous related studies (5).

Hapten-Carrier Conjugates.—The following 2,4-dinitrophenyl (DNP) conjugates were prepared as previously described (5, 6): DNP_9-KLH, DNP_{32}-BGG, DNP_8-OVA, and DNP_9-TAA. The preparation of DNP_{37}-D-GL has been described in detail elsewhere (2). Subscripts refer to the average number of moles of DNP per mole of carrier.

Immunizations and Cell Transfers.—Mice of the inbred lines BALB/c, A/J, and (BALB/c × A/J)F_1 hybrids (CAF_1) were obtained from Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were also obtained from West Seneca Laboratories, Buffalo, N.Y. All mice were used at 8–12 wk of age. The general scheme of the basic experimental protocol is outlined diagrammatically in Fig. 1. In general, primary immunization with hapten-carrier conjugates was carried out with 100 μg of aqueous DNP-KLH plus 10^8 *Bordetella pertussis* organisms (Eli Lilly & Co., Indianapolis, Ind.) intraperitoneally. 2 wk later, DNP-KLH-primed mice were injected intraperitoneally or intravenously with varying numbers of allogeneic or semiallogeneic splenic lymphoid cells. Such transferred cells were obtained from single cell suspensions, prepared and washed in minimal essential medium (Eagle), from spleens of nonimmune donor mice. At varying intervals after cell transfer, recipients and control mice which had not received cell transfers were secondarily challenged with a DNP-carrier conjugate administered in aqueous form intraperitoneally. All animals were bled just before the challenge (day 0) and 7 (day 7) days later, and anti-DNP antibody determinations were performed as described below.

Measurement of Anti-DNP Antibodies.—Serum anti-DNP antibody levels were determined by a modified Farr technique (7, 8) using DNP-^{3}H-ε-amino-*N*-caproic acid (5). Using standard curves constructed for individual mouse strains in a manner identical to that described previously for inbred guinea pigs (5), percentage of binding was converted into amount of anti-DNP antibody in micrograms per milliliter of serum.

Statistical Analysis.—Serum antibody levels were logarithmically transformed and means and standard errors calculated. Group comparisons were made employing Student's *t* test. In those mice in which no specific antigen binding could be detected in the serum, a value of 0.10 μg/ml was arbitrarily assigned to allow logarithmic transformation of the data.

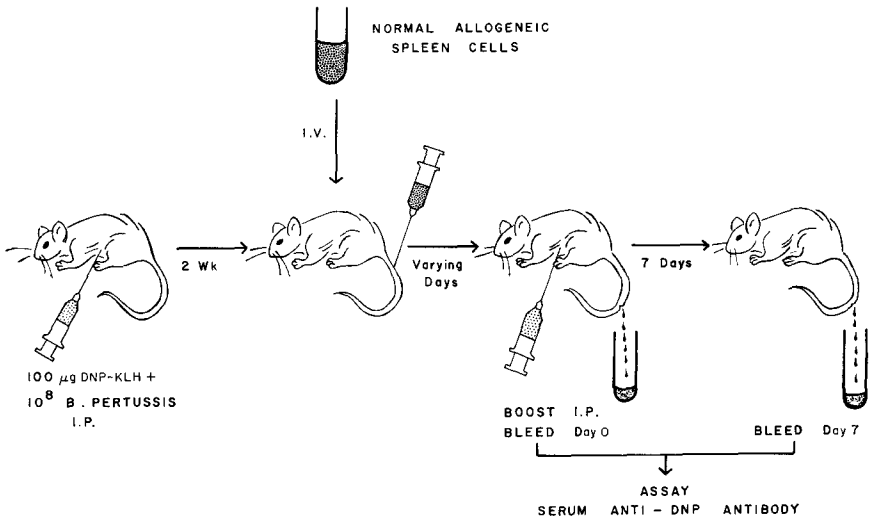

FIG. 1. Model I: transfer of allogeneic lymphoid cells into primed host. Experimental protocol for the elicitation of the allogeneic effect in inbred mice. See Materials and Methods for explanation.

RESULTS

Elicitation of the Allogeneic Effect in Inbred Mice.—Groups of CAF_1 mice, primed 2 wk previously with DNP-KLH, were injected intraperitoneally with 25 or 50 $\times$ 10^6 spleen cells from nonimmunized parental A strain donors. 6 days later, these recipient mice and groups of similarly primed control mice which had received no allogeneic cell transfer were subjected to secondary challenge intraperitoneally with either 100 μg of DNP-KLH or varying doses (10, 50, or 100 μg) of the heterologous conjugate, DNP-BGG.

As illustrated in Fig. 2 and Table I, all mice challenged with the homologous conjugate, DNP-KLH, displayed comparable secondary anti-DNP antibody responses irrespective of whether or not they had received parental donor cells. This observation, which was consistent in many such experiments, probably reflects the essentially maximal stimulation induced by the homologous DNP carrier. On the other hand, a striking contrast is evident among the groups of mice subjected to secondary challenge with DNP-BGG. Hence, whereas comparatively little or no secondary anti-DNP response to DNP-BGG was obtained in control animals, a very clear allogeneic effect reflected by, in some instances, markedly enhanced antibody responses was observed in recipients of parental spleen cells. Elicitation of the effect, and its magnitude, was related to the dose of DNP-BGG administered as well as the number of cells transferred. Thus, the most striking enhancement occurred in the recipients of 50 $\times$ 10^6 cells that were challenged with 50 μg of DNP-BGG, conditions which resulted in a secondary anti-DNP response of even greater magnitude than that elicited with DNP-KLH. A significant allogeneic effect,

though lesser in magnitude, was also obtained in recipients of 25 × 10^6 cells challenged with 50 μg of DNP-BGG and in recipients of both cell doses challenged with 100 μg of DNP-BGG. Animals challenged with 10 μg of DNP-BGG, on the other hand, failed to display significantly enhanced secondary

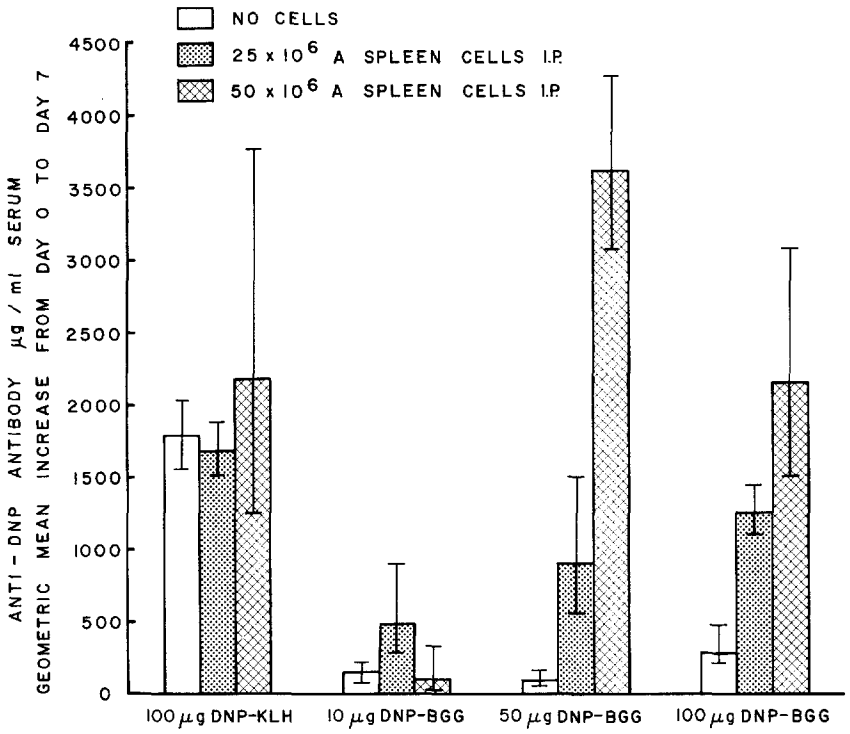

FIG. 2. Elicitation of the allogeneic effect in DNP-KLH-primed CAF_1 recipients of parental A strain lymphoid cells. CAF_1 mice, primed 2 wk previously with 100 μg DNP-KLH plus 10^8 *B. pertussis*, were injected intraperitoneally with 25 or 50 × 10^6 spleen cells from nonimmunized A strain donor mice. Control DNP-KLH-primed CAF_1 mice (open bars) did not receive allogeneic cells. 6 days after cell transfer groups of five mice received secondary challenge intraperitoneally with either 100 μg of DNP-KLH or varying doses (10, 50, or 100 μg) of DNP-BGG. The increase in geometric mean serum anti-DNP antibody levels from just before secondary challenge to 7 days later is illustrated for the respective groups.

anti-DNP antibody responses regardless of the dose of parental cells injected. Comparable results were obtained in studies not shown in which parental BALB/c cells were transferred to primed CAF_1 mice.

Effect of Varying the Route of Administration of Allogeneic Lymphoid Cells.—2 wk after primary immunization with DNP-KLH, groups of CAF_1 and BALB/c mice were injected either intravenously or intraperitoneally with varying numbers of spleen cells from nonimmunized A donors. 6 days later, recipients of allogeneic cells and similarly primed control mice of each

strain which had received no cell transfer were challenged with 100 μg of DNP-BGG intraperitoneally.

The results are summarized in Table II. Both recipient strains displayed enhanced secondary anti-DNP antibody responses as a result of allogeneic

TABLE I

Elicitation of the Allogeneic Effect in DNP-KLH-Primed CAF_1 Recipients of Parental A Strain Lymphoid Cells

Protocol*		Secondary challenge	Anti-DNP antibody (μg/ml)‡		
Group	No. of allogeneic cells transferred		Day 0	Day 7	Boost
A	None	100 μg	59.7	1858.0	1798.3
B	25×10^6	DNP-KLH	58.0	1747.0	1689.0
C	50×10^6		64.0	2448.0	2384.0
D	None	10 μg	110.5	279.3	168.8
E	25×10^6	DNP-BGG	25.0	532.0	507.0
F	50×10^6		46.9	403.6	356.7
G	None	50 μg	23.8	117.4	93.6
H	25×10^6	DNP-BGG	53.8	942.0	888.2
I	50×10^6		84.0	3725.0	3641.0
J	None	100 μg	56.6	363.9	307.3
K	25×10^6	DNP-BGG	21.0	1287.0	1266.0
L	50×10^6		45.6	1982.4	1936.8

* CAF_1 mice, primed 2 wk earlier with 100 μg DNP-KLH plus 10^8 *B. pertussis*, were injected intraperitoneally with 25 or 50×10^6 spleen cells from normal A strain donor mice. Control DNP-KLH-primed CAF_1 mice received no allogeneic cell transfer. 6 days after cell transfer, groups of mice received secondary challenge with a DNP-carrier conjugate as indicated above.

‡ The data are expressed as geometric means of groups of five mice. Boost represents the increase in mean antibody levels from the day of secondary challenge (day 0) to 7 days later (day 7). A comparison of the geometric mean boost values gave the following results: comparison of group A with groups B and C yielded a P value of $0.80 > P > 0.70$. Comparison of group D with groups E and F yielded P values of $0.10 > P > 0.05$ and $0.20 > P > 0.10$, respectively. Comparison of group G with groups H and I yielded P values of $0.02 > P > 0.01$ and $0.001 > P$, respectively. Comparison of group J with groups K and L yielded P values of $0.025 > P > 0.02$ and $0.02 > P > 0.01$, respectively.

cell transfer, although the optimal conditions for obtaining the effect clearly differed between them. Thus, CAF_1 mice manifested enhanced anti-DNP antibody responses after transfer of allogeneic cells either intraperitoneally or intravenously, the latter route being most effective. BALB/c recipients, on the other hand, displayed enhanced secondary responses only when allogeneic

cells were injected intravenously; cells administered intraperitoneally were ineffective at the cell doses employed.

The magnitude of the allogeneic effect was also related to the numbers of

TABLE II

The Effect of Varying the Route of Administration of Allogeneic Lymphoid Cells on the Elicitation of the Allogeneic Effect in DNP-KLH-Primed CAF_1 and BALB/c Mice

Protocol*				Anti-DNP Antibody (μg/ml)‡		
Recipient strain	Group	No. of allogeneic A/J cells transferred and route	Secondary challenge	Day 0	Day 7	Boost
DNP-KLH-primed CAF_1	A	None		12.2	97.0	84.8
	B	5×10^6 i.p.	100 μg	13.4	144.9	131.5
	C	25×10^6 i.p.	DNP-BGG	13.4	224.0	210.6
	D	5×10^6 i.v.		12.1	150.6	138.5
	E	25×10^6 i.v.		42.2	362.0	319.8
DNP-KLH-primed BALB/c	F	None		3.1	10.8	7.7
	G	5×10^6 i.p.	100 μg	3.4	3.2	−0.2
	H	25×10^6 i.p.	DNP-BGG	5.4	3.7	−1.7
	I	5×10^6 i.v.		4.1	16.3	12.2
	J	25×10^6 i.v.		5.3	33.9	28.6

* CAF_1 and BALB/c mice, primed 2 wk earlier with DNP-KLH, were injected intraperitoneally or intravenously with 5 or 25×10^6 spleen cells from normal A strain donor mice. Control DNP-KLH-primed CAF_1 and BALB/c mice received no allogeneic cell transfer. 6 days after cell transfer all mice were boosted with 100 μg of DNP-BGG.

‡ The data are expressed as geometric means of groups of four mice. Boost represents the increase in mean antibody levels from the day of secondary challenge (day 0) to 7 days later (day 7). A comparison of geometric mean antibody levels on day 7 of group A with its respective experimental groups yielded P values of:

(*a*) group A with group B, $0.80 > P > 0.70$,
(*b*) group A with group C, $0.20 > P > 0.10$,
(*c*) group A with group D, $0.30 > P > 0.20$,
(*d*) group A with group E, $0.01 > P > 0.005$.

A comparison of geometric mean antibody levels on day 7 of group F with its respective experimental groups yielded P values of:

(*a*) group F with group G, $0.40 > P > 0.30$,
(*b*) group F with group H, $0.05 > P > 0.025$,
(*c*) group F with group I, $0.20 > P > 0.10$,
(*d*) group F with group J, $0.10 > P > 0.05$.

donor cells transferred. In the present experiment the dose of 25×10^6 allogeneic cells was considerably more effective than 5×10^6 cells in both CAF_1 and BALB/c recipients. On the other hand, doses higher than 25×10^6 cells, particularly when the cells are administered intravenously, usually result in suppression of antibody production (unpublished observations). Based on these observations, subsequent experiments were performed utilizing the 25×10^6 cell dose and the intravenous route of administration.

Effect of Varying the Time Interval Between Allogeneic Cell Transfer and Secondary Challenge.—In the preceding experiments, an interval of 6 days between allogeneic cell transfer and secondary challenge was employed since this had been found to be the optimal time interval in the guinea pig system. The present experiment was carried out to elucidate the optimal time interval in the mouse system.

DNP-KLH-primed CAF_1 mice were injected intravenously with 25×10^6 parental A strain lymphoid cells. At varying intervals after cell transfer, recipient mice and primed control animals which had received no allogeneic cells were challenged with either 10 μg of DNP-KLH or 50 μg of DNP-BGG intraperitoneally.

The results are summarized in Table III. When secondary challenge was made with the homologous antigen, DNP-KLH, control mice as well as allo-

TABLE III

Effect of Varying the Time Interval Between Transfer of Allogeneic Lymphoid Cells and Secondary Challenge on the Magnitude of the Allogeneic Effect in CAF_1 Mice

Protocol*				Anti-DNP antibody (μg/ml)‡		
Group	No. of allogeneic A/J cells transferred	Time interval	Secondary challenge	Day 0	Day 7	Boost
A	None	1 day	50 μg DNP-BGG	139.0	208.0	69.0
B	25×10^6			46.5	1049.0	1002.5
C	None	2 days		30.1	161.0	130.9
D	25×10^6			46.5	3210.0	3163.5
E	None	3 days		13.0	212.0	199.0
F	25×10^6			25.0	818.0	793.0
G	None	1 day	10 μg DNP-KLH	40.8	2242.0	2201.2
H	25×10^6			55.0	2415.0	2360.0
I	None	2 days		24.8	1366.0	1341.2
J	25×10^6			39.0	5230.0	5191.0
K	None	3 days		62.0	4029.0	3967.0
L	25×10^6			108.0	3500.0	3392.0

* CAF_1 mice, primed 2 wk earlier with DNP-KLH, were injected intravenously with 25×10^6 spleen cells from normal A strain donor mice. Control DNP-KLH-primed CAF_1 mice received no allogeneic cell transfer. At varying time intervals after cell transfer, groups of control and experimental mice received secondary challenge with either DNP-BGG or DNP-KLH.

‡ The data are expressed as geometric means of groups of four mice. Boost represents the increase in mean antibody level from the day of secondary challenge (day 0) to 7 days later (day 7). A comparison of geometric mean boost values gave the following results:

(*a*) comparison of group A with group B yielded $0.20 > P > 0.10$,
(*b*) comparison of group C with group D yielded $0.001 > P$,
(*c*) comparison of group E with group F yielded $0.10 > P > 0.05$,
(*d*) comparison of group G with group H yielded $0.90 > P > 0.80$,
(*e*) comparison of group I with group J yielded $0.20 > P > 0.10$,
(*f*) comparison of group K with group L yielded $0.60 > P > 0.50$.

geneic cell recipients displayed, as expected, very good secondary anti-DNP antibody responses. The magnitudes of such secondary responses were comparable between the control and experimental groups at the 1- and 3-day intervals; however, when DNP-KLH was administered 2 days after cell transfer, recipients of allogeneic cells displayed considerably higher secondary responses than corresponding controls. The response to the heterologous antigen, DNP-BGG, offers a striking contrast in that allogeneic cell recipients manifested markedly enhanced anti-DNP antibody production, as compared with controls, at all time intervals employed. The magnitude of the allogeneic effect is, indeed, related to the time interval between transfer and secondary challenge as evidenced by the considerably higher response observed in mice challenged 2 days after transfer. It is noteworthy that additional studies indicate that at intervals of 6 days or longer the effect of allogeneic cell transfer tends to diminish rather than enhance anti-DNP antibody production.

Enhancement of Secondary Responses to DNP Conjugates of Various Heterologous Carrier Molecules.—The capacity of a transient GVH reaction to augment secondary anti-DNP antibody responses to a heterologous carrier-DNP conjugate indicates that the allogeneic effect replaces the requirement for carrier-specific helper cells to participate in such reactions (1–3). Indeed, this has been amply demonstrated in previous studies in inbred guinea pigs (2). The present experiment was designed to confirm this critical point in mice utilizing several different carrier molecules known to vary considerably in immunogenic strength. Thus, BGG and OVA were employed as carriers of moderate to high immunogenicity while TAA was employed as a carrier of relatively low immunogenicity in these mice. The copolymer of D-GL was employed as a nonimmunogenic carrier molecule based on previous observations with this substance in guinea pigs (2) and mice (unpublished observations). Indeed, under ordinary circumstances of administration to either nonimmune or DNP-primed guinea pigs (2) or mice (unpublished observations), the DNP conjugate of D-GL induces a profound state of DNP-specific B cell tolerance.

CAF_1 mice, primed 2 wk previously with DNP-KLH, were injected intravenously with 25×10^6 lymphoid cells from nonimmune A strain donors. 2 days after cell transfer, groups of recipient mice and similarly primed control animals which received no allogeneic cells were challenged with either the homologous conjugate, DNP-KLH (10 μg), or with one of the heterologous carrier conjugates intraperitoneally.

The results are summarized in Table IV and depicted graphically in Fig. 3. Secondary challenge with DNP-KLH elicited, as expected, very good anti-DNP antibody responses in control mice. As in the preceding experiment, the secondary responses to DNP-KLH of allogeneic cell recipients were enhanced roughly fourfold over controls. However, even more striking effects were obtained in allogeneic cell recipients challenged with heterologous carrier conjugates. Thus, recipients of allogeneic cells manifested markedly enhanced anti-DNP antibody responses, as compared with controls, to 50 μg doses of

TABLE IV

Enhancement of Secondary Responses of DNP-KLH-Primed CAF_1 Mice to DNP Conjugates of Various Heterologous Carrier Molecules after Transfer of Parental A Strain Lymphoid Cells

Group	Protocol*		Anti-DNP antibody (μg/ml)‡		
	No. of allogeneic cells transfered	Secondary challenge	Day 0	Day 7	Boost
A	None	10 μg	11.9	559.3	547.4
B	25×10^6	DNP-KLH	24.6	2211.5	2186.9
C	None	50 μg	24.2	40.7	16.5
D	25×10^6	DNP-BGG	17.3	106.4	89.1
E	None	50 μg	14.0	14.8	0.8
F	25×10^6	DNP-OVA	24.8	124.8	100.0
G	None	50 μg	8.2	8.0	−0.2
H	25×10^6	DNP-TAA	6.0	100.9	94.9
I	None	10 μg	12.9	0.2	−12.7
J	25×10^6	DNP-D-GL	12.1	158.9	146.8
K	None	1.0 μg	20.2	12.4	−7.8
L	25×10^6	DNP-D-GL	14.4	93.5	79.1

* CAF_1 mice, primed 2 wk earlier with DNP-KLH, were injected intravenously with 25×10^6 spleen cells from normal A strain donor mice. Control DNP-KLH-primed CAF_1 mice received no allogeneic cell transfer. 2 days after cell transfer, groups of control and experimental mice received secondary challenge with a DNP-carrier conjugate as indicated above.

‡ The data are expressed as geometric means of groups of four mice. Boost represents the increase in mean antibody levels from the day of secondary challenge (day 0) to 7 days later (day 7). A comparison of geometric mean boost values of the respective groups with their controls yielded P values of:

(*a*) group A with group B, $0.40 > P > 0.30$,
(*b*) group C with group D, $0.40 > P > 0.30$,
(*c*) group E with group F, $0.025 > P > 0.02$,
(*d*) group G with group H, $0.10 > P > 0.05$,
(*e*) group I with group J, $0.001 > P$,
(*f*) group K with group L, $0.005 > P > 0.001$.

either DNP-BGG, DNP-OVA, or DNP-TAA. Most impressive and significant were the responses of allogeneic cell recipients to both 10- and 1-μg doses of the conjugate, DNP-D-GL. Hence, the capacity to elicit enhanced secondary anti-DNP responses with heterologous carrier conjugates of varying degrees of immunogenicity, and particularly with a normally tolerogenic substance, as a result of the allogeneic effect provides compelling evidence that this phenomenon replaces the function of carrier-specific helper T cells.

Failure to Observe the Effect When Primed B cells are Bystanders to the Allo-

geneic Cell Interaction.—In the initial studies of the allogeneic effect in guinea pigs it was clearly demonstrated that mediation of the phenomenon required the existence of an active GVH reaction (1). Existence of a host rejection response alone was not sufficient, irrespective of its magnitude (1). Moreover, a host rejection response is not even required as evidenced by the capacity to

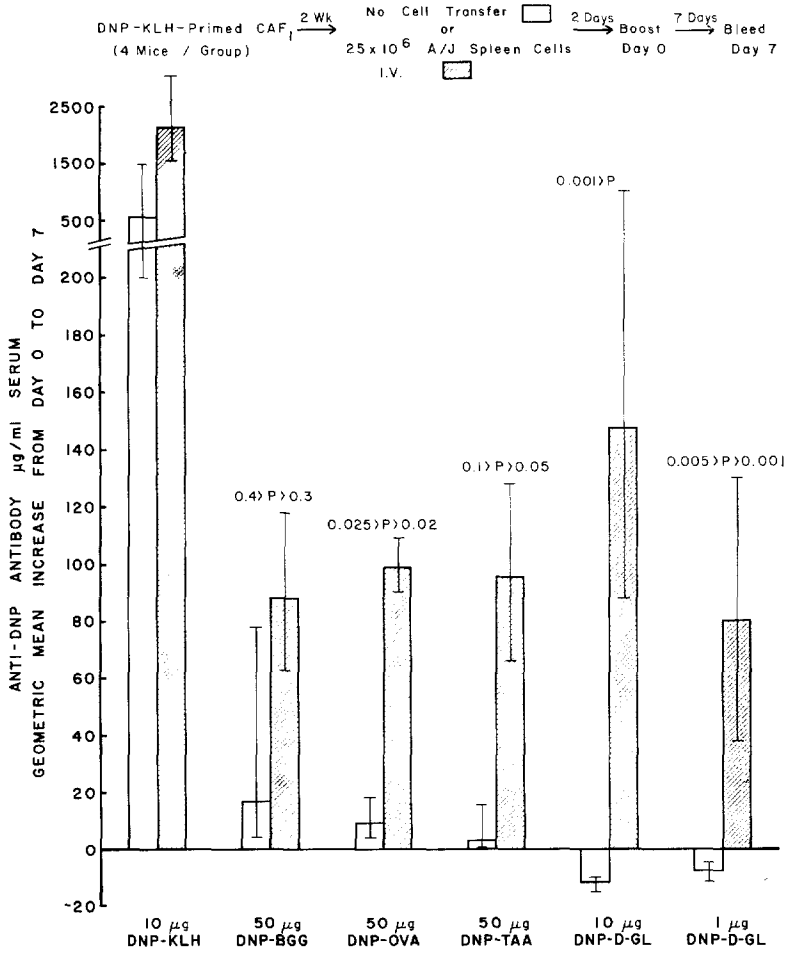

FIG. 3. Enhancement of secondary responses of DNP-KLH-primed CAF_1 mice to DNP conjugates of various heterologous carrier molecules after transfer of parental A strain lymphoid cells. CAF_1 mice, primed 2 wk previously with 100 µg of DNP-KLH plus 10^8 *B. pertussis*, were injected intravenously with 25×10^6 spleen cells from normal A strain donor mice (shaded bars). Control DNP-KLH-primed CAF_1 mice (open bars) did not receive allogeneic cells. 2 days after cell transfer, groups of control and experimental mice received secondary challenge with a DNP-carrier conjugate as indicated above. The increase in geometric mean serum anti-DNP antibody levels from just before secondary challenge to 7 days later is illustrated for the respective groups.

elicit the phenomenon in primed F_1 hybrid recipients of parental donor cells, as shown previously in guinea pigs (3) and in the present studies in mice. This point suggests that more than antigenic stimulation, per se, by histocompatibility antigens must take place for the allogeneic effect to be expressed.

TABLE V

Failure to Observe the Allogeneic Effect When DNP-KLH-Primed BALB/c B Lymphocytes Are Not an Integral Participant in the Allogeneic Cell Interaction

Protocol*			Anti-DNP antibody (μg/ml)‡
Group	Secondary challenge	Irradiated recipient of DNP-KLH-primed BALB/c cells	Day 8 after transfer
A	None		6.8
B	10 μg DNP-KLH	BALB/c	410.6
C	100 μg DNP-KLH		508.5
D	100 μg DNP-BGG		5.6
E	None		2.4
F	10 μg DNP-KLH	A/J	301.7
G	100 μg DNP-KLH		358.4
H	100 μg DNP-BGG		0.2

* 25×10^6 BALB/c spleen cells, primed 2 wk earlier with 100 μg DNP-KLH in complete Freund's adjuvant, were injected intraperitoneally into irradiated syngeneic BALB/c and allogeneic A/J recipients. The same day groups of mice either received no secondary challenge or were injected intraperitoneally with a DNP-carrier conjugate as indicated above.

‡ The data are expressed as geometric means of serum anti-DNP antibody levels 8 days after secondary challenge of groups of six mice. A comparison of geometric mean antibody levels gave the following results:

(*a*) comparison of group A with group D yields $0.80 > P > 0.70$,
(*b*) comparison of group C with group D yields $0.001 > P$,
(*c*) comparison of group E with group H yields $0.001 > P$,
(*d*) comparison of group G with group H yields $0.001 > P$,
(*e*) comparison of group B with group F yields $0.40 > P > 0.30$,
(*f*) comparison of group C with group G yields $0.30 > P > 0.20$.

The present study was performed to determine whether or not the same circumstances condition the phenomenon in mice.

DNP-primed spleen cells from BALB/c donor mice, which had been primed intraperitoneally 2 wk earlier with 100 μg of DNP-KLH in complete Freund's adjuvant, were injected intraperitoneally into groups of irradiated syngeneic or allogeneic (A strain) recipients. Individual mice received 25×10^6 primed spleen cells from a common single cell suspension pool. Immediately after cell transfer, groups of recipients were challenged with either the homologous conjugate, DNP-KLH, or 50 μg of DNP-BGG. Control mice received no secondary challenge.

The results, summarized in Table V, illustrate two important points: first, no allogeneic effect was obtained under these circumstances as evidenced by the

failure to induce antibody production with DNP-BGG in the irradiated allogeneic recipients of primed cells. As expected, no secondary response to DNP-BGG was obtained in the syngeneic combination in contrast to the very brisk anamnestic response elicited by DNP-KLH. The second point of note was the slightly depressed antibody responses to DNP-KLH observed in the allogeneic combination. We do not place any importance on this, however, since the differences were not statistically significant.

It would appear from this study, in conjunction with the other evidence cited above, that the primed B cell must be more intimately involved in the allogeneic cell interaction in order for a demonstrable stimulatory effect on antibody production to occur. Clearly, a mere bystander status to the active cell interaction is not sufficient.[2]

DISCUSSION

Since the breakthrough observations of Claman et al. (9) which demonstrated the requirement for cooperative interaction between T and B lymphocytes in development of humoral immune responses, delineation of the mechanism of this interaction has been the subject of intense investigation (for review, see reference 10). The demonstration that induction of a transient graft-*versus*-host reaction can, under appropriate circumstances, markedly enhance specific antibody production in a previously primed animal (1) has provided a useful model with which to approach this problem. Thus, the allogeneic effect, whose properties are discussed briefly in the introduction, and studies of other investigators using somewhat different systems (11, 12), have provided strong evidence for a hypothesis which considers T cell regulation of B cell function to be mediated through release of a soluble nonspecific factor(s) from appropriately activated T lymphocytes (10).

The present studies were undertaken to establish the optimal conditions for demonstrating the allogeneic effect in inbred mice. Although such details have been previously determined in inbred guinea pigs (1, 3), it seemed possible, if not likely, that certain critical factors might differ considerably from species to species. Moreover, establishment of this phenomenon in mice offers the great advantage of pursuing the model in the species in which the most is known about identifying and separating distinct T and B cell populations. We have shown that the transfer of immunocompetent allogeneic lymphoid cells into recipient mice that were previously primed with DNP-KLH permits the development of enhanced secondary anti-DNP antibody responses to a

[2] It should be pointed out that the variation in magnitude of anti-DNP responses that is evident in the data tables herein reflect the varying extent to which mice were initially primed from one experiment to the next, i.e. in experiments where mice were better primed (as evidenced by higher day 0 antibody levels), the magnitude of the secondary responses was greatest. However, since each experiment was internally controlled, the validity of interpretation is not affected by these differences.

DNP-conjugate of an unrelated carrier molecule. The capacity to elicit this effect is determined by several critical experimental factors.

One such factor appears to be the route of administration of allogeneic cells. Thus, DNP-KLH-primed CAF_1 recipients of parental A strain lymphoid cells manifested better enhanced secondary anti-DNP responses to the optimal dose of DNP-BGG when 25×10^6 allogeneic cells were injected intravenously than when the same number of allogeneic cells were administered intraperitoneally 6 days before secondary challenge. In another experiment presented herein, a very striking allogeneic effect on the secondary response to 50 μg of DNP-BGG was obtained in such mice after intraperitoneal transfer of 25 or 50×10^6 allogeneic cells. Nonetheless, additional studies have generally confirmed the greater efficacy of the intravenous route when CAF_1 mice are used as recipients of parental donor cells. In the case of DNP-KLH-primed BALB/c recipients of allogeneic lymphoid cells, the effect on the response to DNP-BGG challenge 6 days later was obtained only when the intravenous route was employed; transfer of cells intraperitoneally, in the doses used, failed to enhance anti-DNP antibody production.

The magnitude of the allogeneic effect was also related to the numbers of donor cells transferred. In DNP-KLH-primed CAF_1 recipients of intraperitoneally administered A strain donor cells, secondary anti-DNP responses were considerably greater when 50×10^6 cells were give than when 25×10^6 cells were transferred 6 days before challenge with 50 μg of DNP-BGG. In the case of intravenously transferred allogeneic cells, 25×10^6 cells were considerably more effective than 5×10^6 cells in both CAF_1 and BALB/c recipients. It appears, moreover, that when the intravenous route is used, more careful attention to cell dose is required. Thus, doses of allogeneic cells above 25×10^6 per recipient, when injected intravenously, have generally resulted in suppression of anti-DNP antibody production (unpublished observations). Although the precise explanation for this latter point has not yet been defined, two possibilities appear most likely: (*a*) the GVH reaction, occurring in the appropriate strength in lymphoid organs of the host, diminishes the number of DNP-primed precursor B cells available to respond to secondary antigenic stimulation. This may result either because such B cells have been among the targets of specific action by T lymphocytes of the donor population, or because they fall victim to nonspecific cytotoxic factors produced during such reactions. The fact that large numbers of cells ($100–200 \times 10^6$) administered intravenously across a strong *H-2* barrier ($H\text{-}2^b \rightarrow H\text{-}2^d$) results in a marked depression in the secondary anti-DNP response to the original priming DNP conjugate (unpublished observations) may support this notion. (*b*) A vigorous GVH reaction may result in the release of factors in quantitatively sufficient amounts to suppress nonspecifically certain parameters of B cell function. In earlier experiments in guinea pigs, it was found that at certain times during

the course of the GVH reaction, one parameter of B cell function (i.e. antibody production) was suppressed while induction of DNP-specific memory, reflected in the response to an antigenic challenge made at a later time, was significantly increased (3). These two possibilities are not mutually exclusive and, indeed, may both operate depending on the existing circumstances (3a).

Another relevant factor in the elicitation of the allogeneic effect in mice, shown by these experiments, is the time interval employed between cell transfer and secondary challenge. When the allogeneic cells are transferred intravenously, the magnitude of enhancement of anti-DNP responses was optimal when secondary antigen challenge was administered 2 days after cell transfer. In the case of secondary responses to DNP-BGG, enhancement was also observed at intervals of 1, 3, and 6 days but at a level of three- to fourfold less than that obtained at 2 days. Moreover, the secondary responses to the homologous antigen, DNP-KLH, which were generally not affected when intervals of 1, 3, or 6 days were employed, were considerably increased when challenge was made 2 days after cell transfer. These observations differ from those made in guinea pigs where the 6 day interval was clearly optimal (1). It is quite possible, however, that the optimal time interval in mice may differ according to the strength of the histocompatibility differences between donor and host strains being studied.

Perhaps the most intriguing aspect of the allogeneic effect is the apparent obviation of the requirement for participation of carrier-specific helper T cells in the development of DNP-specific secondary responses (1–3). This has been confirmed in the present studies in mice in which a secondary anti-DNP response was obtained in animals undergoing a GVH reaction after challenge with DNP-D-GL. This particular DNP conjugate is not only nonimmunogenic but also induces a profound state of DNP-specific tolerance when administered under normal conditions to guinea pigs (2) or mice (unpublished observations). It is presumed, and some evidence exists,[3] that functional T cells with specificity for D-GL do not exist in these animals. The capacity to elicit an anti-DNP antibody response with DNP-D-GL in the presence of a GVH reaction provides, therefore, compelling evidence that, indeed, the allogeneic effect circumvents the required participation of carrier-specific helper cells in the anti-hapten antibody response. As suggested earlier (2), it is not unlikely that this stimulation of antibody-forming cell precursors by a normally nonimmunogenic substance during the allogeneic effect may well have significant pathogenetic implications. It is conceivable, for example, that potent nonspecific stimuli of the immune system analogous to the GVH reaction, such as might occur during certain infectious processes, may stimulate the development of autoantibodies to host antigens which may be tolerated

[3] Katz, D. H., W. E. Paul, and B. Benacerraf. 1972. Carrier function in anti-hapten antibody responses. VI. Establishment of experimental conditions for either inhibitory or enhancing influences of carrier-specific cells on antibody production. Manuscript submitted.

as a result of unresponsiveness in the helper cell population. Under the influence of such potent stimuli, direct activation of precursors of antibody-forming cells by host antigens may occur.

Finally, the demonstration that no enhancement of the secondary anti-DNP response occurred after passive transfer of DNP-KLH-primed BALB/c spleen cells into irradiated allogeneic recipients bears directly on questions concerning the nature of the cellular interactions involved in this phenomenon. Thus, although a presumably strong one-way allogeneic cell interaction (i.e. the reaction of donor T lymphocytes against histocompatibility antigens of the host, but not vice versa) existed, the response of primed B lymphocytes, in this case members of the donor cell population, was not appreciably affected. Taken in context with similar observations reported previously in guinea pigs (1, 3), this finding suggests that the primed B cell must be an integral participant in the GVH reaction and not a mere bystander to the allogeneic cell interactions. Indeed, in the accompanying paper (4) we provide proof of this point by demonstrating the elicitation of the allogeneic effect on primed B cells from which isologous T cells have been depleted.

SUMMARY

The administration of allogeneic lymphoid cells to 2,4-dinitrophenyl keyhole limpet hemocyanin (DNP-KLH)-primed mice prepares such recipients for markedly enhanced secondary anti-DNP antibody responses to a DNP conjugate of a heterologous carrier. This allogeneic effect phenomenon, reflecting the development of a graft-*versus*-host (GVH) reaction, was first described in guinea pigs and has been extended in the present studies to inbred mice. The expression of the allogeneic effect in mice is dependent upon critical factors such as the number and route of administration of allogeneic cells, the time interval between cell transfer and secondary challenge, and the strength of histocompatibility differences between the donor and the host. The transient GVH reaction established by the transfer of allogeneic cells obviates the requirement for carrier-specific helper T cells in secondary anti-DNP responses, as evidenced by the ability to elicit such responses with DNP-D-GL, a substance which presumably does not stimulate effective T cell helper function. These studies also demonstrate that primed B cells which are not an integral part of the active GVH reaction fail to produce enhanced levels of antibody.

The authors are deeply indebted to Professor Baruj Benacerraf for his advice and encouragement during the course of these studies. We thank Mr. Herbert Levine for expert technical assistance and Miss Karen Ellis for her excellent secretarial assistance in the preparation of the manuscript.

BIBLIOGRAPHY

1. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1971. Carrier function in anti-hapten antibody responses. III. Stimulation of antibody synthesis and facilitation of hapten-specific secondary antibody responses by graft-*versus*-host reactions. *J. Exp. Med.* **133**:169.

2. Katz, D. H., J. M. Davie, W. E. Paul, and B. Benacerraf. 1971. Carrier function in anti-hapten antibody responses. IV. Experimental conditions for the induction of hapten-specific tolerance or for the stimulation of anti-hapten anamnestic responses by "nonimmunogenic" hapten-polypeptide conjugates. *J. Exp. Med.* **134:**201.
3. Katz, D. H., W. E. Paul, and B. Benacerraf. 1971. Carrier function in anti-hapten antibody responses. V. Analysis of cellular events in the enhancement of antibody responses by the "allogeneic effect" in DNP-OVA-primed guinea pigs challenged with a heterologous DNP-conjugate. *J. Immunol.* **107:**1319.
3a. Katz, D. H. 1972. The allogeneic effect on immune responses: Model for regulatory influences of T lymphocytes on the immune system. *Transplant. Rev.* In press.
4. Katz, D. H., and D. P. Osborne, Jr. 1972. The allogeneic effect in inbred mice. II. Establishment of the cellular interactions required for enhancement of antibody production by the graft-*versus*-host reaction. *J. Exp. Med.* **136:**455.
5. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J. Exp. Med.* **132:**261.
6. Benacerraf, B., and B. B. Levine. 1962. Immunological specificity of delayed and immediate hypersensitivity reactions. *J. Exp. Med.* **115:**1023.
7. Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. *J. Infect. Dis.* **103:**329.
8. Green, I., B. Benacerraf, and S. H. Stone. 1969. The effect of the amount of mycobacterial adjuvants on the immune response of strain 2, strain 13, and Hartley strain guinea pigs to DNP-PLL and DNP-GL. *J. Immunol.* **103:**403.
9. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. Exp. Biol. Med.* **122:**1167.
10. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T lymphocytes on B cell responses to antigen. *Adv. Immunol.* **15:**1.
11. Sulica, A., R. Tarrab, and M. Sela. 1971. Stimulation *in vitro* with protein carrier of antibodies against a hapten. *Science* (*Wash. D.C.*) **171:**1165.
12. Hartmann, K.-U. 1970. Induction of a hemolysin response in vitro. Interaction of cells of bone marrow origin and thymic origin. *J. Exp. Med.* **132:**1267.