THE ALLOGENEIC EFFECT IN INBRED MICE

IV. REGULATORY INFLUENCES OF GRAFT-VS.-HOST REACTIONS ON HOST T LYMPHOCYTE FUNCTIONS*

BY DAVID P. OSBORNE, JR.‡ AND DAVID H. KATZ

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

(Received for publication 6 June 1973)

Since the description of the in vivo allogeneic effect in inbred guinea pigs (1–3) and more recent extension of the system to inbred mice (4–7), considerable attention has been focused on this phenomenon as a model of the regulatory mechanisms of activated T lymphocytes on immune responses in vivo (for reviews, see references 8 and 9). The allogeneic effect, as first described, demonstrated that the transfer of allogeneic immunocompetent lymphoid cells to animals previously primed with a hapten-carrier conjugate, such as 2,4-dinitrophenyl (DNP)-keyhole limpet hemocyanin (KLH), markedly enhanced the antihapten antibody response to an appropriately timed secondary challenge with that hapten coupled to an unrelated carrier. This phenomenon has been shown to require the initiation of an active graft-vs.-host (GVH) reaction in the lymphoid tissues of the primed host (1) and to reflect the direct interaction of donor T lymphocytes recognizing foreign histocompatibility determinants on primed host B lymphocytes, irrespective of the presence of host T cells (5). There appeared to be an absolute requirement that the host hapten-specific lymphocytes be primed before establishing the GVH reaction, and attempts to demonstrate an enhanced primary antibody response to a wide variety of immunogens were unsuccessful (1–4). More recently, in our own and other laboratories (10–12) the capacity of the allogeneic effect to markedly enhance primary antibody responses, usually of the IgG class, to a very restricted group of functionally thymus-independent antigens has been clearly demonstrated.

In the present studies, we have examined the possible regulatory effects of interactions of allogeneic donor T cells with T lymphocytes of the host. Of the various specific functions performed by mature T lymphocytes, we have assessed the capacity of carrier-primed T cells to exert a specific helper function with hapten-primed B lymphocytes in producing an antihapten antibody

*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.
1Abbreviations used in this paper: BGG, bovine gamma globulin; CFA, complete Freund's adjuvant; GVH, graft-vs.-host; KLH, keyhole limpet hemocyanin.
response. By employing a variety of carrier molecules and priming regimens, we demonstrate that the transfer of allogeneic lymphoid cells during the course of priming of helper T lymphocytes can significantly increase the functional capacity of such T cells when assayed in a cooperative cell transfer system with DNP-specific B lymphocytes. This enhancement of T cell activity is dependent upon the number of allogeneic cells transferred and the time-course of the resultant GVH reaction. Indeed, conditions of too great or prolonged allogeneic stimulation result in suppression rather than enhancement of helper cell function. Moreover, this phenomenon reflects the interaction of allogeneic T lymphocytes with the host T lymphocytes during the phase of carrier priming, and can be manifested on either primed or virgin T cell populations.

**Materials and Methods**

**Proteins and Hapten-Protein Conjugates.**—Bovine gamma globulin (BGG) was obtained from Pentex Biochemicals, Kankakee, Ill. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Bio-Marine Supply Co., Venice, Calif. The following 2,4-dinitrophenyl (DNP) conjugates were prepared as previously described (13, 14): DNP<sub>14</sub>-KLH (the subscript refers to the average number of moles of DNP per 10,000 mol wt units of KLH) and DNP<sub>32</sub>-BGG (subscript refers to the average number of moles of DNP per mole of BGG).

**Animals, Immunizations, and Cell Transfers.**—Mice of the inbred lines A/J and (BALB/c × A/J)F<sub>1</sub> hybrids (CAF<sub>1</sub>) were obtained from Jackson Laboratory, Bar Harbor, Maine. Inbred A/St mice were purchased from West Seneca Laboratories, Buffalo, N. Y. All mice were immunized or used as recipients between 8 and 12 wk of age.

DNP-primed spleen cells were prepared by immunizing CAF<sub>1</sub> mice intraperitoneally (i.p.) with 100 µg of either DNP-KLH or DNP-BGG emulsified in complete Freund's adjuvant ([CFA] Difco Laboratories, Detroit, Mich.) at least 1 mo before cell transfer. Carrier-primed spleen cells were prepared by immunizing CAF<sub>1</sub> mice with KLH or BGG either with or without the administration of parental A/J allogeneic spleen cells, as outlined in the body of this paper. Both DNP-primed and carrier-primed spleen cells were prepared as single-cell suspensions, washed in minimal essential medium (Eagle's), mixed to a final concentration of 25 × 10<sup>6</sup> hapten-primed cells and 10 × 10<sup>6</sup> carrier-primed cells, and incubated with an appropriate concentration of stimulating antigen for 15 min at 4°C. This mixture was then injected intravenously into 550 R irradiated syngeneic CAF<sub>1</sub> mice. All experimental mice were bled 7 days after cell transfer and anti-DNP antibody determinations were performed as described below.

**Measurement of Anti-DNP Antibody.**—Serum anti-DNP antibody levels were determined by a modified Farr technique (15, 16) using [³H]-DNP-ε-amino-N-caproic acid (13). Using standard curves constructed for individual mouse strains in a manner identical with that described previously for inbred guinea pigs (13), 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 geometric means and standard errors calculated. Group comparisons were made employing Student's t test.

**RESULTS**

The Enhancing Effect of Allogeneic Cell Transfer on Preparation of BGG Helper Cells.—In this initial experiment helper cells were prepared in CAF<sub>1</sub> mice, as outlined in Fig. 1, by an initial immunization with BGG in CFA
DAVID P. OSBORNE, JR. AND DAVID H. KATZ

Fig. 1. The enhancing effect of allogeneic cell transfer on the priming of BGG-specific helper cells. CAF1 mice were primed with BGG according to the various regimens listed above. 10 × 10⁶ spleen cells from these mice were transferred intravenously together with 25 × 10⁶ DNP-KLH-primed CAF1 spleen cells and 50 µg of DNP-BGG into 550 R irradiated CAF1 mice. 7 days later the recipient mice were bled and their sera assayed for anti-DNP antibody. The data are expressed as geometric means of groups of five mice. A comparison of various groups yielded the following P values: (a) group I with group II, P = 0.0112; (b) group I with group III, P = 0.00003; (c) group I with group IV, P = 0.0012; (d) group II with group III, P = 0.0030; (e) group II with group IV, P = 0.2136.

followed 7 days later by a boosting immunization of soluble BGG either with or without a simultaneous intravenous injection of 25 × 10⁶ parental A/J spleen cells. Thus, carrier-primed donor mice in group II were immunized with 100 µg of BGG in CFA and boosted 7 days later with 50 µg of soluble BGG. In group III, the latter immunization was accompanied by the intravenous transfer of 25 × 10⁶ parental spleen cells from unimmunized A/J donors. Group IV cell donors received no initial injection of BGG in CFA, but were primed with 50 µg of soluble BGG i.p. and simultaneously given 25 × 10⁶ normal A/J spleen cells. Spleen cells from normal CAF1 donor mice substituted for BGG-primed helper cells in group I. 4 days after immunization with soluble BGG and allogeneic cell transfer, spleen cells from these groups of mice were transferred together with DNP-KLH-primed spleen cells plus antigen into irradiated recipients. 7 days later these mice were bled and levels of serum anti-DNP antibody were measured.

The results are illustrated in Fig. 1. Control mice that received only 25 × 10⁶ DNP-KLH-primed spleen cells and were challenged with 20 µg of DNP-KLH
developed a marked anamnestic response, producing a mean of 9,692 μg/ml of anti-DNP antibody. Mice in group I, which received normal rather than BGG-primed spleen cells, made a negligible response to 50 μg of DNP-BGG. Mice in group II, which received BGG-primed helper cells in addition to the DNP-KLH-primed spleen cells, made significantly higher levels of anti-DNP antibody in response to challenge with DNP-BGG, clearly demonstrating the “helper effect” of specifically primed T lymphocytes (8). However, when an allogeneic cell transfer was added to the priming regimen of helper cells at the time of boosting with soluble BGG (group III) there was a marked increase in the helper function of these spleen cells as reflected by the threefold higher magnitude of response to DNP-BGG in recipients of such cells. This difference is highly significant and clearly demonstrates that allogeneic spleen cells can have an enhancing effect on the priming of carrier-specific helper T cells. Furthermore, as shown in group IV, a single exposure to 50 μg of soluble BGG together with 25 × 10^6 allogeneic spleen cells 4 days before harvest of donor spleen cells resulted in the production of an appreciable number of helper cells capable of cooperating with DNP-primed cells. This finding could reflect one of two possibilities: either (a) the presence of an expanded population of BGG-specific helper cells, or (b) the carry-over to the irradiated CAF1 recipients of significant numbers of allogeneic A/J spleen cells that are then capable of abrogating the requirement for specific helper T cells in the response of DNP-KLH-primed spleen cells to challenge with DNP-BGG, i.e., the allogeneic effect on primed B cells (1-4). The following experiment was designed to answer these questions.

The Enhancing Effect of Allogeneic Cell Transfer on the Preparation of KLH Helper Cells.—In this experiment, helper cells were primed with KLH precipitated with aluminum potassium sulfate (alum) rather than BGG emulsified in CFA, to insure that the phenomenon described was not limited to the BGG helper system and that it was not significantly influenced by the presence of CFA. As outlined in Fig. 2, five different immunization schemes were employed to prepare KLH-primed helper cells (groups II–VI), which were tested in a double adoptive transfer system with spleen cells from DNP-BGG-primed donor mice. Thus, 10 × 10^6 KLH-primed helper cells, or normal spleen cells in the case of group I, 25 × 10^6 DNP-BGG-primed hapten-specific spleen cells, plus 100 μg of DNP-KLH were transferred intravenously into individual 550 R irradiated syngeneic CAF1 mice. On day 7 serum anti-DNP antibody levels were determined. The results are shown in Fig. 2.

Control mice that received 25 × 10^6 DNP-BGG-primed spleen cells and

---

3 The conversion values from percent binding to micrograms per milliliter are relative rather than absolute in the case of the homologous boost controls in these studies, since wide discrepancies in average antibody affinity from those of experimental groups are not accounted for by this assay.
Fig. 2. The enhancing effect of allogeneic cell transfer on the priming of KLH-specific helper cells. CA1F mice were primed with KLH according to the above regimens. 10 X 10^6 spleen cells from these mice were transferred intravenously together with 25 X 10^6 DNP-BGG-primed CA1F spleen cells and 100 μg of DNP-KLH into 550 R irradiated CA1F mice. 7 days later the recipient mice were bled and their sera assayed for anti-DNP antibody. The data are expressed as geometric means of groups of five mice. A comparison of various groups yielded the following P values: (a) group I with group II, P = 0.0006; (b) group I with group III, P = 0.0004; (c) group I with group IV, P = 0.0001; (d) group I with group V, P = 0.0525; (e) group I with group VI, P = 0.0007; (f) group II with group III, P = 0.0144; (g) group II with group IV, P = 0.2523; (h) group II with group V, P = 0.0778; (i) group II with group VI, P = 0.4203.

10 X 10^6 normal spleen cells produced a mean of 4,229 μg/ml of anti-DNP antibody after challenge with the homologous conjugate DNP-BGG, whereas, recipients of the same cell mixture (group I) made no response to the heterologous conjugate, DNP-KLH. Spleen cells from CA1F mice immunized with 100 μg of KLH (alum) i.p. and boosted 1 wk later with 50 μg of soluble KLH (group II) provided significant helper activity when mixed with DNP-BGG-primed CA1F spleen cells and challenged with DNP-KLH. The addition of allogeneic spleen cells to the priming regimen at the time of boost with KLH resulted in a striking fourfold increase in helper activity after challenge with DNP-KLH (group III). This again demonstrates the capacity of allogeneic cells to enhance the specific priming of helper T cells. The possibility that this...
enhancement is due, in any part, to carry-over of allogeneic cells to irradiated recipients is ruled out by the results shown for group V, in which CAF1 mice received only \(25 \times 10^6\) parental A/J spleen cells and at no time were exposed to KLH. The level of anti-DNP antibody response to DNP-KLH when spleen cells from these mice were mixed with DNP-BGG-primed spleen cells was not significantly different from the low response noted when normal CAF1 spleen cells were employed in place of specific KLH-primed helpers (group I). Spleen cells harvested from mice only 4 days after i.p. immunization with 50 \(\mu\)g of soluble KLH, a highly immunogenic molecule, conferred significant helper activity (group VI); indeed they were as active as helper cells from primed and boosted mice (group II). Finally, mice receiving allogeneic cells and soluble KLH 4 days before harvest of helper cells (group IV) also provided a significant level of helper activity, but this was not appreciably greater than that of cells from similarly primed donor mice that did not receive allogeneic cells (group VI).

Preparation of BGG Helper Cells with a 4 Day Priming Regimen.—One surprising observation in both of the preceding experiments was that a 4 day exposure to antigen, with or without allogeneic cells, could result in significant levels of helper activity (Fig. 1, group IV; Fig. 2, groups IV and VI). The following experiment, illustrated in Fig. 3, was designed to investigate this effect more thoroughly utilizing varying conditions of BGG priming.

As illustrated in Fig. 3, mice primed with BGG (CFA) and boosted 7 days later with soluble BGG (group II) produced helper cells with high levels of specific functional helper activity. Donor mice that received only allogeneic cells (group IV) or only soluble BGG (group V) yielded spleen cells with only meager helper activity. However, when allogeneic cells and soluble BGG were given together (group III), spleen cells harvested 4 days later manifested a fourfold higher degree of helper activity. In contrast to the preceding experiment employing KLH (Fig. 2), the results of this experiment show that administration of the weaker immunogen, BGG, alone fails to produce significant numbers of BGG helper cells in a short 4 day period. On the other hand, when soluble BGG is given together with allogeneic cells, there is a quantifiable increase in the level of BGG-specific helper function in the spleens of these animals at 4 days (group III).

The Enhancing and Suppressing Effects of Allogeneic Cells on BGG Helper Cell Priming.—This experiment was carried out to study the effect of the allogeneic cell dose and the time interval between allogeneic cell transfer and harvest of spleen cells on the development of BGG helper cells. In previous studies in both guinea pigs (1, 3) and inbred mice (4, 5, 10) the dose of allogeneic cells and time interval between cell transfer and challenge have been critical factors influencing the magnitude of the allogeneic effect on B cell function. Indeed, if the dose of cells given is too high or the interval between cell transfer and challenge is inappropriate, suppression rather than enhancement of the immune response occurs (4, 9).
Fig. 3. Preparation of BGG helper cells with a 4 day priming regimen. CAF1 mice were primed with BGG as indicated in the groups above. 10 × 10^6 spleen cells from these mice were transferred intravenously together with 25 × 10^6 DNP-KLH-primed CAF1 spleen cells and 100 μg of DNP-BGG into 550 R irradiated CAF1 mice. 7 days later recipient mice were bled and their sera assayed for anti-DNP antibody. The data are expressed as geometric means of groups of five mice. A comparison of various groups yielded the following P values:
(a) group I with group II, P = 0.00098; (b) group I with group III, P = 0.0142; (c) group I with group IV, P = 0.1818; (d) group I with group V, P = 0.1568.

In the present experiments either 5 × 10^6 (Fig. 4) or 25 × 10^6 (Fig. 5) parental A/St spleen cells were transferred to CAF1 recipients. On day 0, CAF1 mice either received an immunization of 100 μg of BGG in CFA or were not immunized and served as controls. On day 0 or on day 3, mice from both groups were given allogeneic spleen cells intravenously. Spleens were harvested on either day 7 or day 11 and assayed for BGG-specific helper cell activity by transferring a mixture of 10 × 10^6 helper cells, 10 × 10^6 DNP-KLH-primed CAF1 spleen cells, and 100 μg of DNP-BGG intravenously into 550 R irradiated CAF1 mice. Serum levels of anti-DNP antibody were determined 7 days later. A summary of the protocols and the results of these experiments are illustrated in Figs. 4 and 5.

Mice receiving DNP-KLH-primed spleen cells plus cells from unprimed donors made low levels of anti-DNP antibody when challenged with DNP-BGG (groups I, II, and III in Figs. 4 and 5). There is no evidence of carrying over allogeneic cells except in group III, Fig. 5, in which CAF1 mice were given 25 × 10^6 A/J spleen cells 4 days before cell transfer (day 3), and in which the level of response is somewhat higher, though not statistically significant, than control (group I). Spleen cells from donor mice primed with 100 μg of BGG in
ALLOGENEIC EFFECT ON T CELL FUNCTION

Fig. 4. The enhancing and suppressing effects of 5 × 10^6 allogeneic cells on BGG helper cell priming. CAF1 mice were either not primed (groups I-III) or were primed with 100 μg of BGG in CFA on day 0 as shown above (groups IV-VI). 5 × 10^6 parental allogeneic A/St spleen cells were then transferred to the groups as indicated on either day 0 or day 3. 10 × 10^6 spleen cells from these mice, removed on day 7 or day 11, were transferred intravenously together with 10 × 10^6 DNP-KLH-primed CAF1 spleen cells and 100 μg of DNP-BGG into 550 R irradiated syngeneic CAF1 recipients. 7 days later recipient mice were bled and sera assayed for anti-DNP antibody. The data are expressed as geometric means of groups of five mice. A comparison of various groups yielded the following P values: On day 7: (a) group I with group II, P = 0.2720; (b) group I with group III, P = 0.5038; (c) group I with group IV, P = 0.0023; (d) group I with group V, P = 0.0041; (e) group I with group VI, P = 0.0024; (f) group IV with group V, P = 0.8138; (g) group IV with group VI, P = 0.5955. On day 11: (a) group I with group II, P = 0.6744; (b) group I with group III, P = 0.4615; (c) group I with group IV, P = 0.008; (d) group I with group V, P = 0.0031; (e) group I with group VI, P = 0.0859; (f) group IV with group V, P = 0.0663; (g) group IV with group VI, P = 0.0179.

CFA, manifested a good degree of helper activity when transferred either on day 7 or day 11 (group IV, Figs. 4 and 5). The administration of parental A/J spleen cells resulted in a significant enhancement of BGG helper activity of donor spleen cells, provided: (a) the allogeneic cells were given on day 3 after priming; (b) the spleens were harvested 4 days later (day 7); and (c) an appropriate amount of allogeneic cells (25 × 10^6) were employed. This is demonstrated by part of group VI, Fig. 5. In contrast, transfer of 5 × 10^6 allogeneic
DAVID P. OSBORNE, JR. AND DAVID H. KATZ

| Group | Day 0 BGG Priming | Day 0 Allogeneic AST Spleen Cells | Adoptive Cell Transfer* |
|-------|-------------------|----------------------------------|-------------------------|
| I     | NONE              | NONE                             | DAY 7                   |
|       |                   |                                  | DAY 11                  |
| II    | NONE              | DAY 0                            | DAY 7                   |
|       |                   |                                  | DAY 11                  |
| III   | NONE              | DAY 3                            | DAY 7                   |
|       |                   |                                  | DAY 11                  |
| IV    | 100 μg (CFA) IP   | NONE                             | DAY 7                   |
|       |                   |                                  | DAY 11                  |
| V     | 100 μg (CFA) IP   | DAY 0                            | DAY 7                   |
|       |                   |                                  | DAY 11                  |
| VI    | 100 μg (CFA) IP   | DAY 3                            | DAY 7                   |
|       |                   |                                  | DAY 11                  |

* Transfer 10 x 10⁶ Cells + 10 x 10⁶ DNP-KLH-Primed Cells: Boost with 100 μg DNP-BGG

Fig. 5. The enhancing and suppressing effects of 25 x 10⁶ allogeneic cells on BGG helper cell priming. The protocol is as described in Fig. 4, employing 25 x 10⁶ allogeneic cells. The data are expressed as geometric means of groups of five mice. A comparison of various groups yielded the following P values: On day 7: (a) group I with group II, P = 0.2806; (b) group I with group III, P = 0.0805; (c) group I with group IV, P = 0.0023; (d) group I with group V, P = 0.0107; (e) group I with group VI, P = 0.0004; (f) group IV with group V, P = 0.0457; (g) group IV with group VI, P = 0.0257. On day 11: (a) group I with group II, P = 0.7696; (b) group I with group III, P = 0.1622; (c) group I with group IV, P = 0.0008; (d) group I with group V, P = 0.0373; (e) group I with group VI, P = 0.1052; (f) group IV with group V, P = 0.0042; (g) group IV with group VI, P = 0.0004.

Cells failed to enhance BGG helper function when donor spleens were harvested on day 7, irrespective of when during the priming regimen parental cells were administered (groups V and VI, Fig. 4), indicating that this is a suboptimal dose of allogeneic cells for expression of the allogeneic effect in an enhancing fashion on T cell function. This is consistent with findings previously reported for the allogeneic effect on primed and unprimed mouse B lymphocytes (4, 10) in which the optimal dose of allogeneic cells was 25 x 10⁶.

Perhaps most striking, however, are the results obtained when the helper spleen cells were harvested on day 11. BGG-specific helper cells prepared by immunization with 100 μg of BGG in CFA (group IV) and harvested on day 11 cooperated as well as those harvested on day 7. In contrast, BGG-primed helper cells prepared in the presence of allogeneic cells and harvested on day 11...
(groups V and VI, Figs. 4 and 5) were totally incapable of supplying any helper activity. It appears that, by day 11, the allogeneic cells have had a strong suppressive effect on the function of BGG-specific helper T cells, and have greatly diminished their ability to cooperate in this transfer system. Moreover, whereas $5 \times 10^6$ allogeneic cells failed to enhance the priming of BGG helper cells (Fig. 4), they were as potent as $25 \times 10^6$ allogeneic cells in suppressing BGG helper cell function by day 11 (groups V and VI). Some suppression of BGG helper cell function was even somewhat evident when donor spleen cells from recipients of $25 \times 10^6$ parental cells on day 0 were harvested on day 7 (group V, Fig. 5). These allogeneic effects on the priming of T lymphocytes for helper function parallel our previous findings noted for the allogeneic effect on primed B lymphocytes (1, 3, 4, 8, 9) in which the transfer of allogeneic cells was found to be enhancing or suppressive depending on the time interval employed between the transfer of allogeneic cells and administration of secondary challenge.

**DISCUSSION**

The bulk of previous experimentation on the allogeneic effect has dealt primarily with enhancing and suppressive influences exerted directly on B lymphocytes (8, 9). Accordingly, relatively little is known about what various effects a transient GVH reaction might have on T cell-mediated immune responses in the host animal. There is some indirect evidence that the allogeneic effect may have an enhancing influence on cell-mediated immunity that derives from recent studies in which the highly fatal course of acute lymphocytic leukemia (L$\alpha$C) in inbred strain 2 guinea pigs was significantly altered after induction of a transient GVH reaction (17, 18). Since detailed studies of immunity induced to this particular leukemia have demonstrated that it is predominantly a cell-mediated immunity (19), it is possible that the protection observed during the allogeneic effect reflected enhancement of specific cell-mediated immune reactivity in such animals. Another line of evidence for the allogeneic effect influencing host T cell function stems from studies reported by McBride et al. several years ago on the transferability of GVH splenomegaly in chick embryos (20). These investigators found that the 17-day embryo recipient of adult immunocompetent lymphocytes undergoes an accelerated immunological maturation that in 7 days confers upon its lymphoid cells an unusually high degree of immunocompetence as reflected by their capacity to induce a GVH reaction in a new host embryo (20). Careful analysis of their data leaves little doubt that what they observed represented the allogeneic effect on a T cell-mediated immune response.

The studies presented herein were designed to directly evaluate the effects of a transient GVH reaction on T lymphocyte functions. To this end, we have shown that generation of carrier-specific helper cell function can be significantly influenced by the allogeneic effect. Thus, carrier-primed helper cells specific for
either KLH or BGG derived from CAF₁ donor mice were generally much more active in specifically cooperating with syngeneic DNP-primed B cells in adoptive recipients when parental A strain lymphocytes had been administered at some time during the priming regimen (Figs. 1–3). This was true when allogeneic cells were administered concomitantly with the initial priming dose of carrier protein as well as when the GVH was induced in animals that had been exposed to antigen several days previously. This indicates that the allogeneic enhancing effects can be manifested on either primed or unprimed T cell populations.

The ultimate effect of the GVH reaction on the development of helper T cell activity was found to be related to the number of allogeneic cells employed and the duration of the resultant GVH reaction in the carrier-primed host animal. Hence, allogeneic stimulation of slightly greater magnitude and/or longer duration resulted in marked suppression rather than enhancement of helper cell function in such donor mice. In these studies, prolonged allogeneic stimulation completely suppressed the capacity of carrier-primed donor cells to exert a helper effect (group VI, day 11, Figs. 4 and 5) when a dose of $25 \times 10^6$ allogeneic cells was employed in their preparation, while helper cells prepared in an identical manner but harvested from donor mice a few days earlier manifested enhanced helper cell function (group VI, day 7, Fig. 5). On the other hand, neither enhancement nor suppression of helper activity was noted when a low dose of allogeneic cells was employed ($5 \times 10^6$) and cells were harvested on day 7 after carrier priming, whereas by day 11 this dose of allogeneic cells was comparably suppressive to the higher ($25 \times 10^6$) dose (Fig. 4, groups V and VI). This time and allogeneic cell dose biphasic effect on T cell function is fully concordant with our previous studies demonstrating such contrasting influences of the allogeneic effect directly on B cell function (9). Whereas it has been clearly established by appropriate controls that the enhancing influences of the allogeneic effect on T cell priming are not due to carry-over and subsequent effects of allogeneic cells on DNP B cells, it has not been possible to devise similar definitive controls for the suppressive effects discussed above. We interpret these data to indicate suppressive effects of allogeneic cells on helper T cells; however, the possibility of suppression by allogeneic T cells of DNP-primed B cells has not been ruled out.

The allogeneic effect on T cell function may be mediated by either the direct interaction of donor T cells with host T lymphocytes, the interaction of donor T cells with host macrophages, or a combination of both of these, and represents the recognition of foreign histocompatibility antigens by the allogeneic T cells, and their subsequent activation. These results cannot be accounted for by the carry-over of allogeneic lymphocytes with carrier-primed spleen cells and their ensuing interaction with DNP-specific B lymphocytes, i.e. the allogeneic effect on B cells, since this possibility was ruled out by appropriate control groups (Figs. 2–5). The mechanism by which these activated allogeneic T lymphocytes
increase the priming and subsequent functional capacity of the carrier-specific host T cells is not clear, but the possibilities include: (a) the release of a mitogenic factor that would increase the number of antigen-specific cells in the host T lymphocyte pool, (b) some direct effect that lowers the threshold of antigen triggering in host T cells during carrier priming, or (c) an acceleration of maturation of T cell function. A possible role for the host macrophage in any of these postulated mechanisms cannot be ruled out by our studies, and may indeed be of crucial importance. The initiation of these mechanisms could require direct membrane-membrane interaction, the release of soluble mediators, or both, and as a function of activated alloantigen-specific T cells, are most likely non-specific with respect to the carrier molecule.

Regardless of the final elucidation of the mechanism involved, these studies clearly indicate that the allogeneic effect may be a useful tool for regulating specific T cell function operating in various parameters of cell-mediated immunity. Bearing in mind the apparent balance that exists between enhancing and suppressive T cell influences on the immune system that is well exemplified by the allogeneic effect, the question of applying this potential tool for non-specific suppression of the immune system to certain problems of autoimmunity merits investigation. Perhaps more likely is the utilization of the enhancing influences of the allogeneic effect as a potent method for inducing and/or increasing specific T cell-mediated immunity against relatively weak tumor antigens. As mentioned above, promising results along these lines have already been obtained in guinea pigs inoculated with highly lethal L2C leukemia (17, 18). These findings together with the results of the present experiments demonstrating a clear influence of the allogeneic effect on T cell function support the feasibility of utilizing such an approach to provide considerable benefit to an individual harboring an actively growing tumor. Such studies are currently underway in our laboratory using a variety of spontaneous and induced tumors in mice and rats.

SUMMARY

The studies presented herein were designed to directly evaluate the effects of a transient GVH reaction on T lymphocyte functions. To this end, we have shown that generation of carrier-specific helper cell function can be significantly influenced by the allogeneic effect. Thus, carrier-primed helper cells derived from CAF1 donor mice were generally much more active in specifically cooperating with syngeneic 2,4-dinitrophenyl (DNP)-primed B cells in adoptive recipients when parental A strain lymphocytes had been administered at some time during the priming regimen. This was true when allogeneic cells were administered concomitantly with the initial priming dose of carrier protein as well as when the GVH was induced in animals that had been exposed to antigen several days previously. This indicates that the allogeneic enhancing effects can be manifested on either primed or unprimed T cell populations. The ultimate
The effect of the GVH reaction on the development of helper T cell activity was found to be related to the number of allogeneic cells employed and the duration of the resultant GVH reaction in the carrier-primed host animal. Hence, allogeneic stimulation of slightly greater magnitude and/or longer duration resulted in marked suppression rather than enhancement of helper cell function in such donor mice. These findings may have general relevance to problems in autoimmune diseases and tumor immunity.

The authors are deeply indebted to Professor Baruj Benacerraf for his advice and encouragement during the course of these studies. We thank Miss Mary Graves, Mr. Michael Moran, and Miss Melissa Varney for expert technical assistance and Miss Candace Maher for her excellent secretarial assistance in the preparation of the manuscript.

REFERENCES

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.

4. Osborne, D. P., Jr., and D. H. Katz. 1972. The allogeneic effect in inbred mice. I. Experimental conditions for the enhancement of hapten-specific secondary antibody responses by the graft-versus-host reaction. *J. Exp. Med.* 136:439.

5. 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.

6. Kreth, H. W., and A. R. Williamson. 1971. Cell surveillance model for lymphocyte cooperation. *Nature (Lond.).* 234:454.

7. Rajewskey, K., G. E. Roelants, and B. A. Askonas. 1972. Carrier specificity and the allogeneic effect in mice. *Eur. J. Immunol.* 2:592.

8. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T lymphocytes on B cell responses to antigen. *Adv. Immunol.* 15:1.

9. Katz, D. H. 1972. The allogeneic effect on immune responses: model for regulatory influences of T lymphocytes on the immune system. *Transplant. Rev.* 12:141.

10. Osborne, D. P., Jr., and D. H. Katz. 1973. The allogeneic effect in inbred mice. III. Unique antigenic structural requirements in the expression of the phenomenon in unprimed cell populations in vivo. *J. Exp. Med.* 137:991.

11. Ordal, J. C., and F. C. Grumet. 1972. Genetic control of the immune response to poly-L (Tyr, Glu)-poly-d, L-Ala--poly-L-Lys in nonresponder mice. *J. Exp. Med.* 136:1195.
12. Byfield, P., G. H. Christie, and J. G. Howard. 1973. Alternative potentiating and inhibitory effects of a GVH reaction on antibody formation against a thymus-independent polysaccharide (S III). J. Immunol. In press.

13. Katz, D. H., W. E. Paul, E. A. Goldl, 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.

14. Benacerraf, B., and B. B. Levine. 1962. Immunological specificity of delayed and immediate hypersensitivity reactions. J. Exp. Med. 115:1023.

15. Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. J. Infect. Dis. 103:329.

16. 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.

17. Katz, D. H., L. Ellman, W. E. Paul, I. Green, and B. Benacerraf. 1972. Resistance of guinea pigs to leukemia following transfer of immunocompetent allogeneic lymphoid cells. Cancer Res. 32:133.

18. Ellman, L., D. H. Katz, I. Green, W. E. Paul, and B. Benacerraf. 1972. Mechanisms involved in the antileukemic effect of immunocompetent allogeneic lymphoid cell transfer. Cancer Res. 32:141.

19. Ellman, L., and I. Green. 1971. LaC guinea pig leukemia: immunoprotection and immunotherapy. Cancer. 28:347.

20. McBride, R. A., L. W. Coppleson, N. W. Nisbet, M. Simonsen, A. Skowron-Cendrzak, and H. L. R. Wigzell. 1966. Accelerated immunological maturation in the chick. Immunology. 10:63.