

INDUCTION OF A PRIMARY ANTIHAPTEN RESPONSE IN VIVO BY A GRAFT-VS.-HOST REACTION*

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Katz et al. have tried unsuccessfully to enhance primary antidinitrophenyl (DNP) responses to immunogenic DNP conjugates by the administration of allogeneic cells (1). Such conditions usually resulted in suppression, rather than enhancement, of the primary response (1). However, Osborne and Katz (2) showed that the poorly immunogenic conjugate DNP-D-GL, when used in conjunction with an allogeneic stimulus, was best able to override the need for carrier-specific T cells in a secondary anti-DNP response. In view of these findings, the experiments described in this paper were designed to examine if the allogeneic effect could elicit a primary response to a hapten on a nonimmunogenic carrier.

Recently, 4-hydroxy-3-iodo-5-nitrophenylacetyl-conjugated syngeneic mouse erythrocytes (NIP-MRBC) were shown to be not only nonimmunogenic but also capable of rendering mice unresponsive at the level of the anti-NIP indirect plaque-forming cell (PFC) response when these mice were subsequently challenged with an immunogenic NIP-conjugate (3). It was reasoned that NIP-MRBC might be the type of hapten conjugate capable of eliciting a primary antihapten response as a result of allogeneic stimulation.

During preparation of this manuscript, Osborne and Katz (4) published data supporting the results presented in this paper. They have obtained a primary, direct and indirect anti-DNP PFC response to DNP-D-GL in the presence of an ongoing graft-vs.-host (GVH) reaction but failed to demonstrate enhanced primary responses to the immunogens DNP-KLH and DNP-OVA.

Materials and Methods

Animals.—Highly inbred CBA/J and (CBA/J × C57B1/6)F₁ hybrid mice were used (5).

Antigens.—NIP azide was prepared as described previously (3, 6). It was conjugated onto mouse erythrocytes (MRBC) or to chicken erythrocytes (CRBC) as described previously (3). Keyhole limpet hemocyanin (KLH) was supplied from Calbiochem, San Diego, Calif. NIP-KLH was prepared by coupling with azide (6). Assuming a molecular weight of 10⁶ daltons for KLH, the conjugate with an average of 180 hapten groups per molecule was used, as determined spectrophotometrically (6).

Cell Suspensions.—Spleen cell suspensions were prepared as described elsewhere (5).

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Detection of Plaque-Forming Cell (PFC).—Anti-NIP PFC responses in spleen cell suspensions were detected according to the method of Cunningham and Szenberg (7) and as described in ref. 3.

Statistical Analyses.—Performed as previously described (5).

RESULTS

Induction of a Primary Anti-NIP PFC Response to NIP-F₁·MRBC by Allogeneic Cell Transfer.—Groups of normal (CBA/J × C57Bl/6)F₁ mice were injected intravenously with 5×10^7 normal parental CBA/J spleen cells and with 2×10^9 NIP-coupled (CBA/J × C57Bl/6)F₁ erythrocytes (NIP-F₁·MRBC) on the same day, 2 days later, or 3 days later. A control group of normal (CBA/J × C57Bl/6)F₁ mice were given 2×10^9 NIP-F₁·MRBC only. As shown in Table I, the control mice (group 1) which did not receive the

TABLE I
Dependence of the Primary Response to NIP-F₁·MRBC on the Time of the Allogeneic Cell Transfer

Group	No. of CBA/J spleen cells transferred*	Primary challenge (cells)	Days between cell transfer and challenge	Anti-NIP PFC per spleen at 7 days after challenge	
				Direct	Indirect
1	none	2×10^9 NIP-F ₁ ·MRBC		0	0
1	5×10^7	2×10^9 NIP-F ₁ ·MRBC	0	4,000 (3,200–4,900)‡	16,000 (13,000–19,500)§
3	5×10^7	2×10^9 NIP-F ₁ ·MRBC	2	0	5,400 (4,900–6,000)
4	5×10^7	2×10^9 NIP-F ₁ ·MRBC	3	0	1,000 (800–1,200)

* For groups 2–4, normal (CBA/J × C57Bl/6)F₁ mice were injected intravenously with 5×10^7 parental CBA/J cells at various times before intravenous challenge with the NIP-F₁·MRBC; the mice in group 1, which received no allogeneic cells, served as controls.

‡ Geometric mean, upper and lower limits of SE, 5–10 mice per group.

§ P values between groups: Indirect PFC: 2 cf. 3, $P < 0.05$; 2 cf. 4, $P < 0.05$.

allogeneic cells, had no detectable anti-NIP PFC response when injected with 2×10^9 NIP-F₁·MRBC, a dose previously shown to be tolerogenic in mice (3). In contrast, all mice receiving allogeneic cells developed indirect anti-NIP PFC responses after challenge with 2×10^9 NIP-F₁·MRBC. The magnitude of the response obtained was dependent upon the time interval between administration of allogeneic cells and challenge. Thus, the group injected with NIP-F₁·MRBC on the same day as cell transfer (group 2) made the highest anti-NIP response, while an interval of 2 or 3 days between the administration of the allogeneic cells and challenge with NIP-F₁·MRBC (groups 3 and 4, respectively) resulted in lower PFC responses measured 7 days later. A small direct anti-NIP PFC response was also evident in group 2.

Dependence of the Primary Anti-NIP PFC Response on the Dose of Allogeneic Cells.—Groups of normal (CBA/J × C57Bl/6)F₁ mice were injected intravenously with varying numbers of normal parental CBA/J spleen cells ranging from 10^7 – 10^8 cells per recipient. Normal (CBA/J × C57Bl/6)F₁ mice which received no allogeneic cells served as controls. All groups were injected intravenously with 2×10^9 NIP-F₁·MRBC shortly after cell transfer. Anti-NIP PFC responses were assayed 7 days later.

The results are presented in Table II. Allogeneic cell recipients manifested primary responses in terms of both direct and indirect anti-NIP PFC responses. The magnitude of the effect was clearly related to the number of allogeneic cells injected. The magnitude of the direct and indirect PFC responses on day 7 was highest either at a dose of 5×10^7 or 10^8 parental cells. However, it is possible that peak PFC responses with other doses of parental cells may have been reached at different times after the antigen injections.

Dependence of the Primary Anti-NIP PFC Response on the Dose of NIP-F₁·MRBC.—Groups of normal (CBA/J \times C57B1/6)F₁ mice were either injected intravenously with 5×10^7 normal parental CBA/J spleen cells, or received no allogeneic cells as controls. Immediately after transfer, mice from each group were injected intravenously with doses of NIP-F₁·MRBC varying from 4×10^7 to 8×10^9 cells. Direct and indirect anti-NIP PFC responses were determined 7 days later.

The results are summarized in Table III. Control mice displayed no detect-

TABLE II
Dependence of the Primary Response to NIP-F₁·MRBC on the Dose of Allogeneic Cells

Group	No. of CBA/J spleen cells transferred*	Primary challenge (cells)	Anti-NIP PFC per spleen at 7 days after challenge	
			Direct	Indirect
1	none	2×10^9 NIP-F ₁ ·MRBC	0	0
2	10^7	2×10^9 NIP-F ₁ ·MRBC	1,700 (1,200-2,500)‡	6,000 (3,100-12,000)§
3	2.5×10^7	2×10^9 NIP-F ₁ ·MRBC	1,600 (940-2,700)	10,000 (6,800-15,000)
4	5×10^7	2×10^9 NIP-F ₁ ·MRBC	13,000 (11,000-16,000)	50,000 (38,000-65,000)
5	10^8	2×10^9 NIP-F ₁ ·MRBC	27,000 (20,000-36,000)	31,000 (27,000-35,000)

* Groups of (CBA/J \times C57B1/6)F₁ mice were injected intravenously with varying numbers of parental CBA/J spleen cells; (CBA/J \times C57B1/6)F₁ mice which received no allogeneic cells served as controls. All mice were injected intravenously with the NIP-F₁·MRBC at the same time as the cell transfer.

‡ Geometric mean, upper and lower limits of SE, 5-10 mice per group.

§ P values between groups: Direct PFC: 2cf.4, $P < 0.05$; 3cf.4, $P < 0.05$; 5cf.4, $P < 0.05$. Indirect PFC: 2cf.4, $P < 0.05$; 3cf.4, $P < 0.05$; 5cf.4, NS.

TABLE III
Effect of Varying the Dose of NIP-F₁·MRBC on the Expression of the Allogeneic Effect

Group	No. of CBA/J spleen cells transferred*	Dose of NIP-F ₁ ·MRBC (cells)	Anti-NIP PFC per spleen at 7 days after primary NIP-F ₁ ·MRBC challenge	
			Direct	Indirect
1	5×10^7	4×10^7	130 (12-1,400)‡	100 (10-1,200)
2	5×10^7	4×10^8	620 (160-2,300)§	1,400 (300-6,600)
3	5×10^7	2×10^9	4,600 (2,300-9,000)	36,000 (25,000-53,000)
4	5×10^7	8×10^9	3,100 (1,900-5,000)	27,000 (23,000-31,000)

* Groups of (CBA/J \times C57B1/6)F₁ mice were injected intravenously with 5×10^7 parental CBA/J spleen cells. All mice were injected intravenously with varying doses of NIP-F₁·MRBC at the same time as the cell transfer.

‡ Geometric mean, upper and lower limits of SE, 5-10 mice per group.

§ P values between groups: Direct PFC: 1cf.3, $P < 0.05$; 2cf.3, NS; 4cf.3, NS. Indirect PFC: 1cf.3, $P < 0.05$; 2cf.3, $P < 0.05$; 4cf.3, NS.

able anti-NIP PFC at any dose of NIP-F₁·MRBC employed. The highest doses were shown previously to be tolerogenic if the indirect anti-NIP response to challenge with an immunogenic NIP-conjugate was measured (3). In contrast, allogeneic cell recipients developed increasing primary anti-NIP responses with increasing doses of NIP-F₁·MRBC up to 2-8 × 10⁹ cells.

Effect of Allogeneic Cell Transfer on Primary Anti-NIP PFC Responses to Different NIP-Conjugates.—Groups of normal (CBA/J × C57B1/6)F₁ mice were injected intravenously with 5 × 10⁷ normal parental CBA/J spleen cells, while control (CBA/J × C57B1/6)F₁ mice received no allogeneic cell transfer. Immediately after cell transfer, both groups of mice were injected intraperitoneally with NIP-F₁·MRBC or NIP-CBA·MRBC, or with the immunogenic conjugates, NIP-CRBC or NIP-KLH.

The results are summarized in Table IV. Control mice (groups 1 and 3),

TABLE IV
Effect of Varying the Carrier on the Expression of the Allogeneic Effect

Group	No. of CBA/J spleen cells transferred*	Priming antigen	Anti-NIP PFC per spleen at 7 days after challenge	
			Direct	Indirect
1	none		0	0
2	5 × 10 ⁷	2 × 10 ⁹ NIP-F ₁ ·MRBC	110 (20-540)†	16,000 (14,000-19,000)
3	none		0	0
4	5 × 10 ⁷	2 × 10 ⁹ NIP-CBA·MRBC	3,000 (1,800-5,000)	13,000 (10,000-16,500)
5	none		0	2,200 (1,800-2,800)§
6	5 × 10 ⁷	2 × 10 ⁹ NIP-CRBC	0	2,800 (2,000-3,900)
7	none		240 (140-400)	1,800 (1,100-2,900)
8	5 × 10 ⁷	250 μg NIP-KLH	370 (220-630)	3,300 (3,000-3,500)

* Groups of (CBA/J × C57B1/6)F₁ mice were injected intravenously with 5 × 10⁷ parental CBA/J cells. Control (CBA/J × C57B1/6)F₁ mice received no allogeneic cells. All mice were injected intraperitoneally with either NIP-F₁·MRBC, NIP-CBA·MRBC, NIP-CRBC, or fluid NIP-KLH immediately after cell transfer.

† Geometric mean, upper and lower limits of SE, 5-10 mice per group.

§ P values between groups: Direct PFC: 7cf.8, NS. Indirect PFC: 5cf.6, NS; 7cf.8, NS.

which received no allogeneic cells and were challenged with either NIP-F₁·MRBC or with NIP-CBA·MRBC, produced no detectable anti-NIP PFC response while there was a small response on challenge with NIP-CRBC and with NIP-KLH (groups 5 and 7). The administration of allogeneic cells failed to increase the primary, indirect and indirect anti-NIP PFC response to NIP-CRBC and to NIP-KLH (groups 6 and 8). In marked contrast, the mice which were injected with NIP-F₁·MRBC or with NIP-CBA·MRBC (groups 2 and 4) developed primary, direct and indirect anti-NIP PFC responses. No time-course for the PFC responses was carried out.

DISCUSSION

Data presented here shows that NIP-F₁·MRBC, which is normally able to induce a state of hapten-specific tolerance (3), can be made to elicit a primary anti-NIP PFC response in the F₁ mouse provided an ongoing GVH reaction is established by the injection of parental spleen cells. There appears to be some

property of the parental or the syngeneic F_1 MRBC carrier which distinguishes it from a carrier such as CRBC or KLH. This difference in carriers possibly reflects the degree of T cell recognition by either the F_1 host or the parental donor and suggests that a "poor carrier," when attached to a hapten, can cause the induction of a primary response to the hapten in the presence of a GVH reaction. The work of Osborne and Katz with DNP-D-GL (see Introduction and reference 4) and that of Byfield et al. with pneumococcal polysaccharide (SIII) (quoted in reference 4), also demonstrate that the allogeneic effect can elicit primary antibody responses to substances for which little or no helper T cell function exists. Other common features of these antigens are their high degree of persistence in vivo and their ability to induce tolerance (discussed, for example, in reference 8).

The relationship of the production of the antihapten primary response described in this paper to the response obtained with "immunogenic" hapten-protein conjugates is uncertain. The antihapten primary PFC response produced by the allogeneic effect presumably results from the stimulation of the T cells in the parental spleen population by the F_1 antigens with subsequent release of nonspecific factors capable of triggering B cells in the F_1 host, either directly or indirectly. Experiments are in progress to determine the cell interactions involved, using an adoptive transfer system.

The nature of the allogeneic effect is consistent with the mechanism of the classical T-B collaboration being via "nonspecific factors" acting in conjunction with antigen for the triggering of B cells, as discussed by Katz and Benacerraf (1, 9).

SUMMARY

A primary anti-NIP PFC response can be elicited by a GVH reaction provided certain structural requirements of the NIP-carrier are met. NIP-coated F_1 erythrocytes and NIP-coated parental erythrocytes give rise to substantial anti-NIP PFC responses in F_1 mice after the injection of parental spleens. These conjugates do not of themselves give rise to an anti-NIP PFC response and, in fact, normally give rise to NIP-specific tolerance. In contrast the GVH reaction is not able to enhance the primary anti-NIP PFC response to immunogenic NIP-conjugates.

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