ANTIGEN REQUIREMENTS FOR INDUCTION OF B-MEMORY CELLS

Studies with Dinitrophenyl Coupled to T-Dependent and T-Independent Carriers*

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A cooperative interaction between thymus-derived (T) helper cells (T_H)[1] and antibody-producing (B) cells is required for an optimal antibody response to most antigens, i.e. T-dependent (TD) antigens. (1). By contrast, another group of antigens, the so-called T-independent (TI) antigens, apparently does not require T_H to optimally activate B cells (1, 2). However antibody responses induced by TI antigens differ markedly from those induced by TD antigens. In particular, most TI antigens elicit antibody which is solely or primarily of the IgM class (1–5) and multiple injections of TI antigens do not elicit IgG memory responses (1, 2, 6–8).

The general inability of TI antigens to induce memory in IgG-producing B cells (B_γ) has several possible explanations. For example, the population of B cells which responds to TI antigens (9) may be inherently incapable of undergoing differentiation to memory B_γ cells. This may be due to the fact that TI antigens do not activate T_H which might be required for such differentiation to occur (10–12) or because TI antigens preferentially activate suppressor T cells (T_s) (4, 13) which might actively suppress B-cell differentiation. On the other hand, TI antigens may be able to induce B-cell differentiation but may be unable to activate T_H which are required for expression of the IgG memory response (14–16). The present study was undertaken to differentiate among these possibilities by determining whether B cells from mice primed with TI antigens could produce IgG memory responses when T_H (primed by a TD antigen) were provided at the time of secondary challenge.

Materials and Methods

Mice. CAF_1 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Female mice, 8–12 wk old, were used for all experiments.

Antigens. The thymus-dependent antigens used for this study included dinitrophenylated ovalbumin (DNP_{50}-OVA) and dinitrophenylated keyhole limpet hemocyanin (DNP-KLH) contain-

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[1] *Abbreviations used in this paper:* DNP-KLH, dinitrophenylated keyhole limpet hemocyanin; DNP-OVA, dinitrophenylated ovalbumin; LPS, lipopolysaccharide; PFC, plaque-forming cells; TD, T-dependent; T_H, thymus-derived helper cells; TI, T-independent; TNP, trinitrophenyl.

ing 31 DNP groups per 10^5 molecular weight of KLH. KLH (Pacific Bio-Marine Supply Co., Venice, Calif.) was used to stimulate carrier reactive T cells. The trinitrophenyl (TNP) or DNP haptens were coupled to TI carriers to provide the TI antigens used here. TNP was coupled to lipopolysaccharide (LPS) (Difco Laboratories, Detroit, Mich.) as described by Jacobs and Morrison (17). DNP was coupled to type III pneumococcal polysaccharide (S3) as described by Mitchell et al. (18) and DNP-Ficoll was a gift of Dr. Joseph Davie, Washington University, St. Louis, Mo. The preparations of TNP-LPS, DNP-S3, and DNP-Ficoll used here were considered to be TI since they elicited similar responses in both athymic nude and thymus-bearing littermate control mice (H. Braley-Mullen, unpublished results). Moreover, these antigens have been shown to be TI in other studies (2, 7, 8, 13).

Priming. Mice to be used as B-cell donors were primed with the above DNP- or TNP-conjugated antigens 1–2 mo before use in adoptive transfer experiments. Antigens were injected intraperitoneally, with 10^9 *Bordetella pertussis* organisms. Unless indicated otherwise, the amounts of antigen used for priming were: 100 μg DNP-OVA, 100 μg DNP-KLH, 10 μg TNP-LPS, 0.6 μg DNP-S3, and 2 μg DNP-Ficoll. Preliminary experiments established that these doses elicited optimal DNP-specific primary responses in CAF_1 mice. Mice used as T-cell donors were primed 1–2 wk before use with 100 μg KLH plus 10^9 pertussis organisms.

Depletion of T Cells from Donor Spleen Cells. Spleen cells from mice primed with the above DNP (TNP) conjugates were depleted of T cells by treatment with anti-Thy 1 serum and complement as previously described (10, 19). The specificity of the anti-Thy 1 serum was established previously (10). For each experiment B cells were obtained from a pool of at least three spleens from nonimmunized (normal) or primed donors.

Preparation of T Cells. Spleen cells from KLH primed mice were enriched for T cells by passage over nylon wool columns (20) as previously described (19).

Adoptive Secondary Response. Donor T and B cells were injected i.v. into 650 rads irradiated (19) CAF_1 mice. All recipient mice received 5-10 $\times$ 10^6 T cells and 10 $\times$ 10^6 B cells. Preliminary control experiments established that mice repopulated with B cells alone produced very few DNP-specific PFC (<1,000 PFC/spleen for B cells from mice primed with DNP-KLH and <200 PFC/spleen for B cells from mice primed with any of the other antigens). Mice repopulated with KLH primed T cells alone always produced <1,000 PFC/spleen. Mice were challenged i.v. with 10 μg DNP-KLH immediately after cell transfer. DNP-specific plaque-forming cells (PFC) were enumerated 7 days later (shown by preliminary experiments to be the time of the optimal DNP-specific IgG response).

PFC Assay. The number of DNP-specific PFC in recipient spleens was determined by the slide modification of the Jerne plaque assay (21) by using TNP-coupled horse erythrocytes (TNP-HRBC) as indicator cells (22). The number of indirect (IgG) PFC was determined by subtracting the number of PFC developed with a rabbit antiserum to mouse IgG_1 and IgG_2 (19). The developing antiserum was used at a concentration (1/300) determined to develop optimal numbers of IgG PFC in sheep erythrocyte immunized mice. The antiserum did not inhibit direct PFC at this concentration. All data are expressed as PFC/spleen but the conclusions would not differ if data were expressed as PFC/10^6 cells.

Results

TI Antigens are Unable to Prime B_γ Memory Cells. The first experiment was designed to determine whether DNP-specific B_γ memory cells could be primed by DNP coupled to TI carriers if primed T_H were provided at the time of secondary challenge. CAF_1 mice were primed with the TD antigen DNP-KLH or with the TI antigens DNP-Ficoll, TNP-LPS, and DNP-S3. 1 mo later splenic B cells from these mice and from normal unprimed mice were transferred to irradiated recipients with T_H cells from KLH primed mice. All mice were challenged with DNP-KLH (Fig. 1). Clearly, only those mice which were primed with the TD antigen DNP-KLH had B cells which could produce significant DNP-specific IgG antibody. B cells from mice primed with the TI antigens produced no more IgG than B cells from normal unprimed mice. This was true whether or not the pertussis adjuvant was given at the time of priming

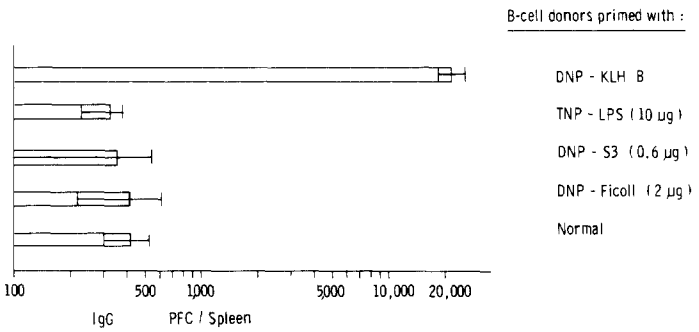

FIG. 1. Secondary IgG PFC responses of B cells from mice primed with TI or TD antigens. Irradiated recipients were repopulated with 10×10^6 T cells from mice primed with KLH and 10×10^6 B cells from mice primed with the antigens indicated in figure. B cells designated as normal were from nonimmunized mice in this and all subsequent figures. Recipient mice were challenged with 10 μg DNP-KLH. Values shown are mean DNP-specific indirect (IgG PFC/spleen ± SEM (5–10 mice/group).

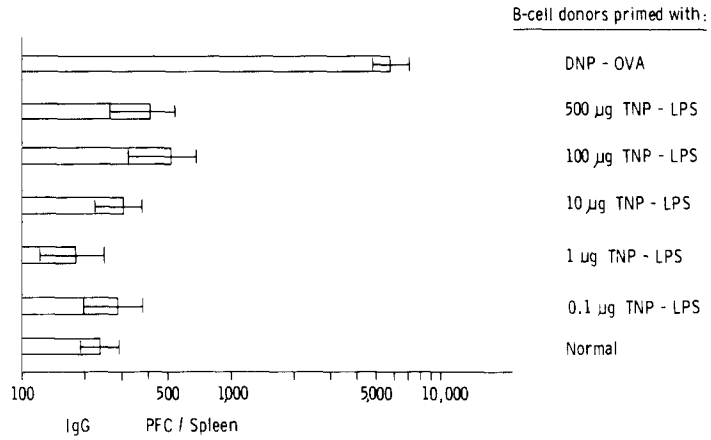

FIG. 2. Effect of dose of TNP-LPS on B-memory cell induction. Irradiated recipients were repopulated with 5×10^6 T cells from mice primed with KLH and 10×10^6 B cells from mice primed with the amounts of antigen indicated in the figure. Recipient mice were challenged with 10 μg DNP-KLH. Values shown are mean DNP-specific indirect (IgG) PFC/spleen ± SEM (6–12 mice group).

(not shown). Mice primed with DNP on a TD carrier (DNP-OVA) different from that used for challenge also produced good secondary IgG responses (see below).

Effect of Various Doses of TI Antigens. In the experiment described above mice were primed with doses of TI antigens which elicited optimal primary DNP-specific IgM responses. Since a different amount of antigen might be optimal for priming B_γ memory cells, groups of CAF_1 mice were primed with 0.1–500 μg TNP-LPS or with the TD antigen, DNP-OVA. 1 mo later splenic B cells from these mice and from normal unprimed mice were transferred to irradiated recipients with KLH primed T_H and DNP-KLH (Fig. 2). Only the mice which were primed with DNP-OVA had B cells which could produce significant IgG antibody. B cells from mice primed with either low or high doses of TNP-LPS produced only slightly more IgG than B cells from normal unprimed

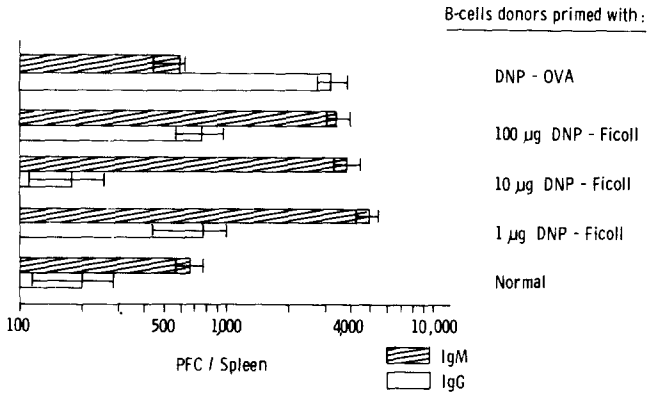

FIG. 3. Effect of dose of DNP-Ficoll on B-memory cell induction. Irradiated recipients were repopulated with 5×10^6 T cells from mice primed with KLH and 10×10^6 B cells from mice primed with the amounts of antigen indicated in the figure. Recipient mice were challenged with 10 μg DNP-KLH. Values shown are mean DNP-specific direct (IgM) and indirect (IgG) PFC/spleen ± SEM (five mice/group).

mice. Similar results were obtained when B-cell donors were primed with 1, 10, or 100 μg DNP-Ficoll, i.e. none of these doses of antigen could optimally prime B_γ memory cells (Fig. 3).

IgM Memory Responses. In the experiments described above we were concerned primarily with the effects of TI antigens on B_γ memory cells. The secondary IgM responses of B cells of mice primed with the TD antigens DNP-OVA and DNP-KLH or with the TI antigens TNP-LPS and DNP-S3 were no different from the IgM responses of B cells from normal mice i.e. these antigens apparently induced no IgM memory (data not shown). However, DNP-Ficoll, although incapable of inducing IgG memory, did induce a substantial IgM memory response in all experiments (Fig. 3).

TI Antigens Do Not Suppress the Induction of B_γ Memory Cells. Since TI antigens are unable to prime B_γ memory cells (Figs. 1–3) it is possible that they activate T_s which could suppress the differentiation of B cells to B_γ memory cells. To test this possibility, CAF_1 mice were injected simultaneously with a TI antigen (TNP-LPS) and a TD antigen (DNP-OVA). 1 mo later B cells from these mice, from normal mice, or from mice primed with only one of the antigens were transferred to irradiated recipients with KLH primed T_H and DNP-KLH (Fig. 4). Neither 10 or 100 μg of TNP-LPS suppressed the induction of B_γ memory cells by DNP-OVA. In other experiments 2 μg DNP-Ficoll also did not suppress the induction of B-memory cells by DNP-OVA (not shown).

Discussion

The results presented here show that B cells from mice primed with TI antigens do not produce significant IgG memory responses when memory T_H cells (induced by a TD antigen) are added at the time of secondary challenge. Thus the general inability of TI antigens to induce IgG memory responses (2, 6–8) is not due solely to the inability of these antigens to activate T_H which are required for the expression of memory (12, 14, 15). TI antigens also must be unable to activate a particular cell which is needed for the induction of IgG memory. This cell may be the B_γ precursor cell itself or the T_H cell.

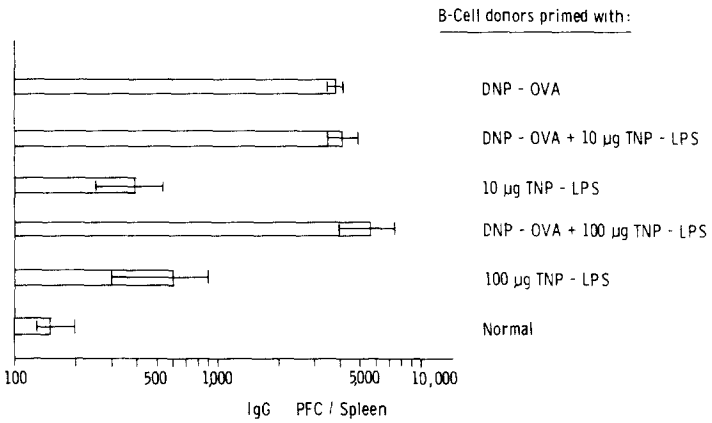

FIG. 4. Effect of simultaneous injection of TI and TD antigens on B-memory cell induction. Irradiated recipients were repopulated with 8×10^6 T cells from mice primed with KLH and 10×10^6 B cells from mice primed with the antigens indicated in the figure. Recipient mice were challenged with 10 μg DNP-KLH. Values shown are mean DNP-specific indirect (IgG) PFC/spleen ± SEM (five mice/group).

It is also possible that TI antigens are unable to prime B_γ memory cells because they preferentially activate T_s rather than T_H. These T_s could prevent memory cell priming by suppressing T_H (23) or by directly suppressing the B_γ cell (1, 4). The results presented here suggest, but do not prove, that this is an unlikely explanation, since the induction of B_γ memory cells was not suppressed when B-cell donors were primed simultaneously with a TI and a TD antigen (Fig. 4). Moreover since T cells were eliminated from the DNP (TNP)-primed B cells before transfer, T_s could not have prevented the expression of memory in this system. It should be emphasized, however, that T_s have not been shown to markedly influence the antibody response to the two TI antigens used here, TNP-LPS and DNP-Ficoll (24). Possibly other TI antigens such as S3 or polyvinylpyrrolidone which are known to activate T_s (13, 25, 26) would suppress B_γ memory cells induced by a TD form of those antigens. Studies are in progress to investigate this possibility by using the S3 antigen.

Thus, TI antigens are presumably unable to activate T_H and/or B_γ precursor cells which are required for the induction of IgG memory responses. Although the present results do not provide any information as to which of these two cell types fails to be activated by TI antigens, it is known from other studies that TI antigens do have the ability to activate B_γ cells. For example, S3 can elicit good IgG responses when T_H are nonspecifically activated by allogeneic cells (27), when B-memory cells are induced by a TD form of the antigen, i.e. S3 coupled to erythrocytes (S3-RBC) (19, 28) or when T_s are eliminated (4, 27). Moreover at least some preparations of DNP-Ficoll elicit significant IgG responses (8) particularly when T_H are nonspecifically activated by allogeneic cells (29). However in none of these cases will a second injection of these antigens elicit an IgG memory response (8 and H. Braley-Mullen, unpublished results).

Since TI antigens do have the ability to activate B_γ cells the inability of TI antigens to prime B_γ memory cells is more likely to be due to the fact that TI antigens cannot activate T_H. We and others have previously shown that T_H are required to induce differentiation of B_γ cell precursors to memory cells after

priming with TD antigens (10–12) although the evidence on this point is controversial (14–16). Moreover there is no evidence as yet that any TI antigen can directly activate conventional antigen-specific T_H. If T_H could be activated by a TI antigen under an appropriate experimental condition we would predict that such an immunization procedure should result in the induction of B_γ memory cells for that antigen. Studies are now in progress to test this hypothesis.

Summary

Mice were primed with dinitrophenyl (DNP) (trinitrophenyl, TNP) coupled to thymus-independent (TI) or thymus-dependent (TD) carriers. B cells from these mice were transferred to irradiated recipients with T cells from keyhole limpet hemocyanin (KLH)-primed mice. After secondary immunization with DNP-KLH a significant DNP-specific IgG memory response was produced only by mice which received B cells which had been primed with TD antigens. TI antigens were unable to induce differentiation of B-cell precursors to IgG producing memory B cells but they did not suppress the induction of B-memory cells by TD antigens. The results indicate that TI antigens fail to activate a cell type which is required for the induction of memory B cells.

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