

THE COLLABORATIVE PHENOTYPE OF SECONDARY  
B CELLS IS DETERMINED BY T LYMPHOCYTES  
DURING IN VIVO IMMUNIZATION\*

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It is well established that the gene products of the major histocompatibility complex (MHC)<sup>1</sup> are important in allowing effective collaborative interactions between T and B cells in humoral immune responses. Results from several experimental systems have demonstrated that T cells are restricted to collaborating with B cells or macrophages derived from inbred murine strains that share genes within the I region of the MHC (1-2). Subsequent studies using bone marrow radiation chimeras have demonstrated that it is not the MHC identity between collaborating T cells and B cells per se that is crucial, but rather the ability of T cells to recognize antigen in the context of the MHC gene products of B cells or macrophages (3-7). These studies implied that T cells that are exported to the periphery have been selected to collaborate with B cells or macrophages of the thymus MHC type. However, several recent experimental results provide evidence that each T cell population is composed of a mixture of self- and allo-MHC-restricted immunocompetent T cells (8-13) and that the MHC restriction of T cells is a consequence of antigen priming (3, 14). If antigen selection does play a role in establishing a self-MHC-restricted T cell population, this process may be reflected in the immune B cell population. Previous studies have demonstrated that nonimmune (primary) B cells and immune (secondary) B cells have different requirements for MHC recognition by antigen-specific collaborating T cells (8-10, 15). Primary B cells are able to interact with MHC nonidentical T cells, whereas secondary B lymphocytes require some form of MHC recognition by collaborating T cells. Thus, antigen-driven events appear to influence the MHC collaborative phenotype of B cell populations. In this report, experiments were conducted to determine if the acquisition of the observed MHC collaborative phenotype of secondary B cells is dependent upon the presence and participation of T cells during immunization. The B cell populations in congenitally athymic and conventional thymic mice were analyzed after immunization with the predominantly T-dependent antigen 2,4-dinitrophenylhemocyanin (DNP-Hy) and the predominantly T-independent antigen DNP-Ficoll. Results of these studies demonstrate that the characteristic MHC collaborative phenotype of secondary B cells is only acquired after T-dependent antigen immunization in the presence of T cells. This is despite the fact that T-dependent and

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<sup>1</sup> *Abbreviations used in this paper:* B lymphocyte, bone marrow-derived lymphocyte; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DNP, 2,4-dinitrophenyl; Fl, fluorescein; Hy, hemocyanin; MHC, major histocompatibility complex.

T-independent stimulation of athymic nude mice induces an absolute increase in the number of antigen-specific B cells and, as will be shown in this and subsequent reports, that these B cells acquire certain functional characteristics of secondary B cells. Thus, T cells appear to play an essential role in determining the MHC preference of secondary B cells. The implications of these findings with regard to the antigen-driven acquisition of MHC preferences in collaborating T and B cell populations are discussed.

### Materials and Methods

*Antigens and Immunoabsorbents.* *Limulus polyphemus* hemocyanin (Hy) was purchased from Worthington Biochemical Corp., Freehold, N. J. Bovine serum albumin (BSA) was purchased from Sigma Chemical Co., St. Louis, Mo. 2,4-dinitrophenylated-Hy, 10–20 mol DNP per 100,000 mol wt Hy (DNP<sub>10–20</sub>-Hy), fluorescein-Hy (Fl<sub>10</sub>-Hy), Fl<sub>10</sub>-BSA, and DNP<sub>10</sub>-BSA, were prepared as described elsewhere, and the coupling ratios were determined by methods described for other protein antigens (17, 18). Aminoethylcarboxymethyl 70 Ficoll (aem-Ficoll) was obtained from Biosearch, San Rafael, Calif., and was dinitrophenylated by standard methods (17).

*Animals and Immunizations.* 6–8-wk-old male BALB/c mice were obtained from Carworth Farms, Wilmington, Mass. 6–8-wk-old A/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c *nu/nu* mice were bred at Northwestern University's Center for Experimental Animal Care, or purchased from ARS/Sprague-Dawley, Madison, Wis. Mice to be used as Hy-immunized recipients received two intraperitoneal injections of 0.1 mg Hy each; the first in complete Freund's adjuvant (CFA) 8 wk before use, the second in saline 4 wk after the first injection. BALB/c and BALB/c *nu/nu* B cell donors were immunized with 0.1 mg sterile DNP-Hy or DNP-Ficoll in CFA 1–8 wk before use.

To verify that the serum antibody response of BALB/c *nu/nu* mice to T-dependent and T-independent antigens was consistent with previous reports (19), sera of immunized BALB/c and BALB/c *nu/nu* mice were assayed for DNP-specific antibodies 9 and 21 d after immunization, using the solid phase radioimmunoassay (15, 16, 20) with rabbit anti-mouse F(ab')<sub>2</sub> antisera as a detecting reagent. Conventional BALB/c mice produced appreciable amounts of DNP-specific antibody when immunized with the T-dependent antigen DNP-Hy, whereas BALB/c *nu/nu* mice produced no detectable levels of DNP-specific antibody under these conditions. Both BALB/c and BALB/c *nu/nu* mice produced appreciable amounts of DNP-specific antibody after DNP-Ficoll immunization.

*Cell Transfers and Fragment Cultures.* Monoclonal antibody responses were obtained in vitro using the splenic fragment culture assay, as described by Klinman (17, 21). Detection of antibody in culture fluids, and determination of heavy chain isotypes was accomplished using a solid phase radioimmunoassay, which was described previously (15, 16, 20).

*Antisera.* Rabbit anti-mouse IgG<sub>1</sub> antiserum was purchased from Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, Md., and purified by adsorption on DNP-binding hybridoma antibodies of the IgA, IgG<sub>2a</sub>, and IgM heavy chain isotypes, the kind gift of Dr. Judy Teale, Scripps Clinic and Research Foundation. Rabbit anti-mouse F(ab')<sub>2</sub> was prepared in this laboratory as described previously (17). Rabbit anti-mouse IgM was prepared in this laboratory. Briefly, serum was obtained from New Zealand White rabbits immunized with mouse IgM, isolated by gel filtration of a 42% ammonium sulfate precipitate of mouse serum on Sepharose 6B. A 42% ammonium sulfate fraction of this rabbit serum was adsorbed exhaustively on a column of mouse IgG coupled to CNBr-activated Sepharose 4B (Sigma Chemical Co.) and nonadsorbed fractions collected. The specificity of the antibodies prepared in this fashion were tested on the DNP-binding hybridoma antibodies of the IgA, IgM, IgG<sub>1</sub>, and IgG<sub>2a</sub> heavy chain isotypes.

### Results

*The Frequency of DNP-specific B Cells from DNP-Hy Immunized BALB/c and BALB/c Nude Mice Measured in Collaboration with Syngeneic T Cells.* Experiments were conducted

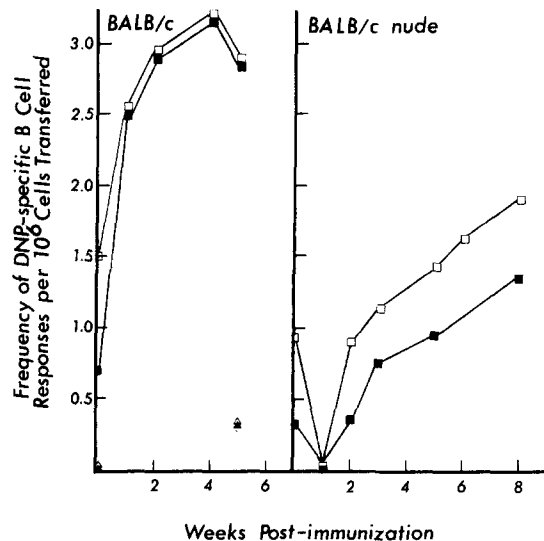


FIG. 1. The frequency of DNP-specific antibody-producing foci per  $10^6$  donor cells analyzed is plotted against the number of weeks the donor mice were immunized before cell transfer. BALB/c and BALB/c nude mice were immunized at various times before use. Donor spleen cells were transferred to Hy-primed irradiated (1,300 rad) BALB/c recipients (□, ■) and in control experiments to unimmunized irradiated (1,300 rad) recipients (Δ, ▲). Open characters (□, Δ) represent the total frequency of DNP-specific donor B cell responses determined using anti-F(ab)<sub>2</sub> antibodies as a detecting reagent in a radioimmunoassay; closed characters (■, ▲) represent the frequency of IgG<sub>1</sub> antibody-producing foci as determined using anti-γ antibodies as detecting reagents in a radioimmunoassay. Each point represents the result of an analysis of between  $50 \times 10^6$  and  $60 \times 10^6$  donor spleen cells, where between  $2 \times 10^6$  and  $5 \times 10^6$  donor cells were transferred to each irradiated recipient.

to determine the effect of immunization with DNP-Hy on the frequency of DNP-specific B cells in BALB/c and BALB/c nude mice that were able to collaborate with a syngeneic carrier-primed T cell population. The analysis was conducted to establish a base line to which the response of the same B cell population in collaboration with allogeneic carrier-primed T cells could be compared. 1–5 wk after immunization, donor spleen cells were transferred to Hy-immunized irradiated recipient mice, recipient spleens were removed, and fragment cultures were prepared and stimulated with DNP-Hy *in vitro*. As shown in Fig. 1, after immunization with DNP-Hy, the frequency of DNP-specific B cells in BALB/c mice increased twofold during the first week and remained at approximately that frequency for the 6-wk period observed. The vast majority of antibody-producing clones of B cells derived from immunized BALB/c mice synthesized antibody of the IgG<sub>1</sub> heavy chain isotype *in vitro* as compared with the antibody-producing clones of nonimmune B cells, of which only ~50% secreted IgG<sub>1</sub> antibody *in vitro*. Thus, DNP-Hy immunization of BALB/c mice induced an increase in the frequency of DNP-specific B cells and a preference of IgG<sub>1</sub> antibody expression upon secondary stimulation *in vitro*.

As shown in Fig. 1, after DNP-Hy immunization of BALB/c nude mice, the frequency of DNP-specific B cells decreased during the first week, then by 8 wk after immunization increased to a level approximately twofold higher than that of the nonimmune nude. The increase in the frequency of DNP-specific B cells from nude mice observed 8 wk after immunization, indicated that the nude DNP-specific B cell

population was induced to expand following immunization with a T-dependent antigen. As shown in Fig. 1, ~40% of the B cells from nonimmune BALB/c nudes yielded clones that synthesized IgG<sub>1</sub> antibody upon secondary stimulation in vitro, and this proportion increased to ~70% after immunization in vivo. Thus, immunization with the T-dependent antigen, DNP-Hy, in the absence of mature functional T cells, resulted in an increase in the absolute number of DNP-specific B cells, and a shift to predominantly IgG<sub>1</sub> antibody synthesis in vitro.

Both the observed decrease and increase in B cell frequency in the nude mouse and the increase in B cell frequency in BALB/c mice were antigen specific, because the frequency of Fl-specific B cells in nude mice did not change following DNP-Hy immunization (data not shown). The observation of an increase in the frequency of BALB/c B cell antibody responses from 1.5 to ~3.0 was dependent on the appropriate carrier-priming of the recipient mice. When B cells from 5-wk-immune BALB/c mice were transferred to nonimmune recipients, the frequency of B cell responses was 0.35 (Fig. 1). Previously published results (16) have demonstrated that whereas nonimmune B cell responses are absolutely dependent on the carrier priming of recipient mice, ~10% of secondary B cells can be stimulated in the absence of carrier primed T cells. Removal of T cells from the BALB/c or BALB/c nude donor cell population by anti-Thy-1 and complement treatment did not effect the results shown. The increase in the number of antigen-specific B cells in nude mice occurred in the absence of any measurable serum antibody response as detailed in the Materials and Methods section. Thus, immunization in the absence of T cells appeared to induce an expansion of the antigen-specific nude B cell population without concomitant differentiation in vivo of antibody secreting cells.

*The MHC Collaborative Phenotype of BALB/c and BALB/c Nude B Cells after Immunization with DNP-Hy.* A similar analysis was conducted to determine the frequency and heavy chain isotype of the response of DNP-specific B cells from DNP-Hy immunized BALB/c and BALB/c nude mice (H-2<sup>d</sup>) that were able to collaborate with Hy-primed, irradiated allogeneic A/J (H-2<sup>a</sup>) recipient T cells. 1-6 wk after immunization, spleen cells from nonimmune mice, and DNP-Hy immunized mice, were transferred to irradiated Hy-primed recipient A/J mice. Fragment cultures were prepared from recipient spleens and stimulated in vitro with DNP-Hy. Culture fluids were analyzed for the presence of DNP-specific antibody, and the heavy chain isotype of the antibody was determined. The results of this analysis are summarized in Fig. 2. As shown, ~80% of nonimmune BALB/c B cells were stimulated in collaboration with MHC-dissimilar carrier-primed T cells and, as previously reported (15), these B cells yielded IgM or IgM + IgA antibody-producing clones. 1 wk after immunization there was a drop in the frequency of B cells able to be stimulated in vitro in collaboration with carrier-primed allogeneic T cells, followed during the next 3 wk by a linear increase in the number of B cells stimulated under these conditions. By 6 wk after immunization ~35% of BALB/c B cells were able to collaborate with allogeneic carrier-primed T cells. As observed in transfers of BALB/c B cells to nonimmune syngeneic recipients, the majority of secondary B cell responses (80%) and all primary B cell responses were dependent on the carrier priming of the recipient mice (Fig. 2). The vast majority (>90%) of these B cells yielded IgG<sub>1</sub> antibody-producing clones. Further, there was no recovery of an IgM-secreting population in the immunized BALB/c. Previously published results (8, 9) from this laboratory demonstrated that the IgG<sub>1</sub> responses of

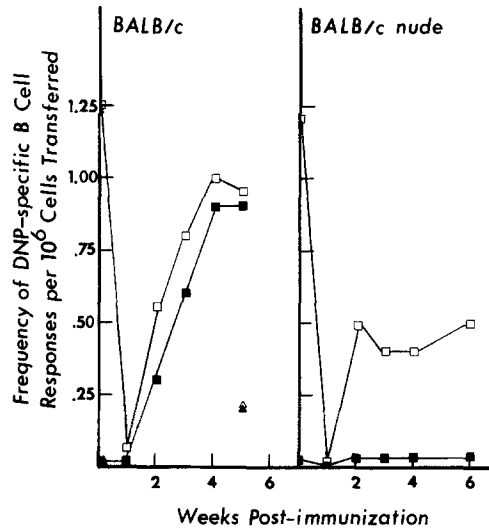


FIG. 2. The frequency of DNP-specific antibody-producing foci per  $10^6$  donor cells analyzed is plotted against the number of weeks the donor mice were immunized before cell transfer. BALB/c ( $H-2^d$ ) and BALB/c nude ( $H-2^g$ ) donor cells were transferred at various times after immunization with DNP-Hy to Hy-primed A/J ( $H-2^k$ ) irradiated (1,300 rad) recipients (□, ■), and in control experiments to unimmunized irradiated recipients (△, ▲). Open characters (□, △) represent the total frequency of DNP-specific B cell responses determined using anti-F(ab)<sub>2</sub> antibodies as a detecting reagent in a radioimmunoassay; closed characters (■, ▲) represent the frequency of DNP-specific IgG<sub>1</sub> antibody responses. Foci not producing IgG<sub>1</sub> antibody were demonstrated to synthesize IgM or IgM + IgA antibody. Each point represents an analysis of between  $50 \times 10^6$  and  $60 \times 10^6$  donor cells, where each carrier-primed irradiated recipient received between  $2 \times 10^6$  and  $6 \times 10^6$  donor cells.

secondary B cells in collaboration with MHC dissimilar T cell populations were, in fact, dependent on the interaction with "syngeneic-like" T cells within an allogeneic T cell population. This T cell population has been previously referred to as isologous T cells. In contrast, the IgM primary B cell responses did not require syngeneic-like interactions, but may have interacted with T cells that did not recognize antigen in the context of their MHC gene products (8, 9). This point will be elaborated upon in the discussion section. The results summarized in Fig. 2 demonstrated that immunization with a T-dependent antigen in the presence of T cells resulted in a B cell population that had the potential to interact with isologous T cells. Furthermore, there appeared to be very few, if any, primary B-like cells in the immune BALB/c mouse that collaborated with T cells from MHC-dissimilar murine strains in IgM antibody responses.

In contrast, as shown in Fig. 2, B cells from athymic mice did not acquire the ability to collaborate with isologous T cells to synthesize IgG<sub>1</sub> antibody following DNP-Hy immunization. Approximately 80% of nonimmune nude B cells were stimulated in collaboration with allogeneic T cells, and these B cells yielded IgM- or IgM + IgA-secreting clones. 1 wk after immunization, the number of donor B cells available for collaboration with allogeneic carrier-primed T cells decreased, followed by an increase to a frequency of 0.5. However, the B cells from DNP-Hy immunized nude mice synthesized only IgM or IgA antibody, and by this criterion resembled primary B cells. Thus, immunization with the T-dependent antigen, DNP-Hy, in the

absence of mature functional T cells, was not sufficient to induce a B cell population with the potential to interact with isologous T cells. However, DNP-Hy immunization did affect the collaborative potential of the nude B cells, in that <30% of B cells from DNP-Hy immunized nude mice were able to collaborate with allogeneic T cell populations as opposed to 80% of nonimmune nude B cells.

*The MHC Collaborative Phenotype of BALB/c and BALB/c Nude B Cells after Immunization with DNP-Ficoll.* The frequencies and heavy chain isotypes expressed by DNP-specific B cells from DNP-Ficoll immunized BALB/c and BALB/c nude mice were determined in fragment cultures in vitro. Spleen cells from DNP-Ficoll-immunized donors were transferred to Hy-primed syngeneic and allogeneic recipients, and fragment cultures were stimulated with DNP-Hy in vitro. Thus, the effect of T-independent immunization in vivo was measured in a T-dependent assay in vitro. As shown in Table I, the DNP-specific B cell frequency measured in collaboration with syngeneic Hy-primed T cell populations increased only slightly in DNP-Ficoll-immunized BALB/c mice, and there was no increase in the percentage of cells which synthesized IgG<sub>1</sub> antibody. In contrast, the frequency of DNP-specific B cells in DNP-Ficoll-immunized nude mice increased fivefold, and >90% of the resultant clones synthesized IgG<sub>1</sub> antibody. It appeared that the observed potential of antigen-specific nude B cell clones to expand following T-independent immunization was limited in the euythmic BALB/c mouse. This limitation was presumably due to the presence of T cells. Thus, while antigen alone was sufficient to trigger the expansion of antigen-specific B cell clones and to induce a preference for the synthesis of IgG<sub>1</sub> antibody, T cells may have played an important regulatory role in limiting this expansion and differentiation.

The response of B cells from DNP-Ficoll-immunized euythmic BALB/c and BALB/c nude mice in collaboration with allogeneic Hy-primed T cells is shown in Table II. Approximately 60% of the B cells from DNP-Ficoll-immunized BALB/c mice were stimulated in allogeneic carrier-primed recipients as compared with syngeneic recipients. This percentage was intermediate to those observed for nonimmune B cells (~80%) and secondary B cells (~35%). Upon stimulation in allogeneic

TABLE I  
*Frequency of DNP-specific B Cell Responses in BALB/c and BALB/c Nude Mice after Immunization with DNP-Ficoll*

Donor cells*	Donor immunization‡	Total number of cells analyzed × 10 <sup>-6</sup>	Number DNP-specific foci per 10 <sup>6</sup> cells transferred	Percent response IgG <sub>1</sub> §
BALB/c	—	96	1.60	60
BALB/c	DNP-Ficoll	44	1.81	50
BALB/c nude	—	96	0.90	38
BALB/c nude	DNP-Ficoll	30	4.76	84

\* Between  $2 \times 10^6$  and  $6 \times 10^6$  donor spleen cells were transferred to each Hy-primed irradiated (1,300 rad) recipient.

‡ Donor mice were immunized with 0.1 mg DNP<sup>2</sup>Ficoll in CFA intraperitoneally 4–6 wk before use.

§ The percent of antibody producing clones synthesizing antibody of the IgG<sub>1</sub> heavy chain isotype is shown. These clones may also synthesize IgM antibody. Clones not synthesizing IgG<sub>1</sub> antibody were demonstrated to synthesize IgM or IgA antibody.

TABLE II  
*Response of T Independently Immunized DNP-specific BALB/c (H-2<sup>d</sup>) and BALB/c Nude (H-2<sup>d</sup>) B Cells in Hy-primed Allogeneic (H-2<sup>a</sup>) Recipients*

Donor cells*	Immuniza- tion of do- nors‡	Number do- nor cells ana- lyzed $\times 10^{-6}$	Number foci per $10^6$ cells transferred	Percent re- sponse IgG <sub>1</sub> §
BALB/c	—	30	1.25	<3
BALB/c	DNP-Ficoll	42	0.74	16
BALB/c nude	—	48	0.82	<2
BALB/c nude	DNP-Ficoll	36	0.28	<1

\* Between  $2 \times 10^6$  and  $8 \times 10^6$  cells were transferred to each recipient.

‡ Donor mice were immunized 4–6 wk before cell transfer with 0.1 mg DNP-Ficoll in CFA intraperitoneally.

§ The percent of clones that synthesized IgG<sub>1</sub> antibody is shown. When no IgG<sub>1</sub>-producing clones were detected the percent is shown as <1 per the total number of clones tested. Clones not synthesizing IgG<sub>1</sub> antibody synthesized either IgM or IgA antibody.

recipients in vitro, >84% of the responding B cells synthesized IgM or IgM + IgA antibody, whereas 16% synthesized IgG<sub>1</sub> antibody. Thus, T-independent immunization of BALB/c mice in vivo induced only a very small population of B cells able to collaborate with isologous T cells.

The response of B cells from DNP-Ficoll immunized nudes in collaboration with allogeneic carrier-primed T cells is shown in Table II. A small proportion (~10%) of B cells from DNP-Ficoll-immunized nude mice were able to collaborate with allogeneic as compared with syngeneic carrier-primed T cells, and these yielded exclusively IgM or IgM + IgA antibody responses. Thus, T-independent stimulation in the absence of T cells was not adequate to induce a B cell population with the potential to interact with isologous T cells in IgG<sub>1</sub> antibody responses. Furthermore, DNP-Ficoll immunization does not eliminate the IgM antibody response in either BALB/c or BALB/c nude mice. The small portion of B cells in DNP-Ficoll-immunized BALB/c mice that acquired the collaborative phenotype of secondary B cells may have been induced to do so by T cells present during immunization which were not involved in the antigen-triggering process itself. Although DNP-Ficoll immunization was not sufficient to induce a B cell population with the potential to interact with isologous T cells in nude mice, it affected the collaborative potential of the B cell population. This was evidenced by the observation that the number of B cells from DNP-Ficoll immunized nudes able to collaborate with allogeneic T cells ( $0.25/10^6$  cells) was less than the number of nonimmune BALB/c nude B cells that have this potential ( $0.82/10^6$  cells).

### Discussion

Previously published studies have shown that B cells in nonimmune and immune mice manifest different MHC collaborative phenotypes with antigen-specific T cells (8, 9, 15, 16). These studies indicated that the restriction imposed on T cell-B cell collaborative interactions in humoral immune responses may be, in part, a result of antigen-driven events. The studies presented in this report were carried out to determine if T cells played a crucial role in the acquisition of the MHC collaborative

phenotype of secondary B cells. To do this, B cells were analyzed in BALB/c and BALB/c nude mice after immunization with the predominantly T-dependent antigen DNP-Hy and predominantly T-independent antigen DNP-Ficoll. While the nude mouse is not absolutely T deficient (22, 23), it is felt that, as a first approximation, it is adequate for studying the effects of antigen stimulation on a B cell population when the mature functional T cell population is greatly depleted. Results of the studies presented here demonstrate that the induction or selection of B cells with the MHC collaborative phenotype of secondary B cells, in particular the potential to interact with isologous T cells, is dependent on the presence and participation of T cells during immunization *in vivo*.

It is crucial to the interpretation of these studies that the observed MHC-restriction phenotype is not the result of nonspecific positive or negative allogeneic effects. Previous attempts to induce responses in the fragment culture system via allogeneic effects have invariably failed. All stimulatory interactions require that both the T and B cells recognize the stimulating antigen, and that both determinants be on the same antigen molecule. In addition, the extensive irradiation is likely to eliminate most allogeneic stimulatory interactions (8).

Although T cells are required for the acquisition of the MHC collaborative phenotype of secondary B cells, antigen alone in the absence of T cells has a demonstrable effect on the antigen-specific B cell population. Immunization with DNP conjugated to either Hy or Ficoll induces an absolute increase in the number of DNP-specific precursors in nude mice, and an increased proportion of these precursors are stimulated *in vitro* to synthesize antibody of the IgG<sub>1</sub> isotype. However, whereas the nude B cell population is induced to expand and differentiate by T-independent and T-dependent antigens, it does not acquire the secondary B cells' MHC collaborative phenotype.

We have defined the MHC collaborative phenotype of secondary B cells as the ability to collaborate with syngeneic like T cells within an MHC-dissimilar individual (8). Evidence has been presented by this laboratory and others (8, 13) that the T cell repertoire is composed of a mixture of both self- and allo-MHC restricted immunocompetent helper T cells. Secondary B cells require some form of MHC recognition by collaborating T cells, and in its absence fail to be stimulated. When offered carrier-primed T cells from MHC dissimilar murine strains, secondary B cells are able to acquire syngeneic-like T cell help within that population (8, 16). These interactions, referred to as isologous interactions, yield IgG<sub>1</sub> antibody responses. In the absence of the potential for isologous interactions secondary B cells fail to be stimulated. In contrast, primary B cells manifest a less stringent requisite for MHC recognition by collaborating T cells and under conditions in which secondary B cells fail to be stimulated, primary B cells are stimulated to synthesize IgM antibody (8, 15, 16).

In this study it is demonstrated that ~30% of B cells immunized in the presence of T cells with a T-dependent antigen are able to be stimulated by isologous T cells *in vitro* upon transfer to an allogeneic individual. In contrast, 90% of the B cells immunized in the absence of T cells by T-independent antigens are not able to be stimulated by any T cells within an allogeneic population. Thus, these B cells, unlike primary B cells, require MHC recognition by collaborating T cells, yet are unable to obtain this help in an allogeneic population. Consequently, there appear to be two steps involved in the maturation of secondary B cells. The first step is T independent



and results in a B cell population which requires MHC syngeneic T cell recognition and is unable to be stimulated in collaboration with allogeneic T cell populations. The second step, which is apparently T cell dependent, results in a B cell population that requires syngeneic MHC recognition by collaborating T cells and can obtain this through isologous T cells in allogeneic populations. While the biology of the B cells dictating this behavior in allogeneic recipients remains to be elucidated, the differential behavior of B cells which have been immunized in the presence or absence of T cells has allowed the dissection of the process of memory B cell differentiation into a T-independent and T-dependent phase.

Although we have discussed the process of memory generation in terms of a single B cell population, there is nothing that argues against the alternative, which is that we are viewing two separate B cell populations that may manifest different T cell dependencies in their maturation. One B cell population may be T independently stimulated, resulting in a population unable to obtain T cell help in allogeneic recipients *in vitro*. A second B cell population may be strictly T dependent in its stimulation and result in a B cell population that can obtain T cell help in allogeneic recipients *in vitro*.

It will be important in future experiments to determine the gene products of secondary B cells that dictate their need for syngeneic or isologous T cell collaborative interactions. Independent evidence from several laboratories suggests that B cells may exist as defined subsets that require interaction with a reciprocal T cell subset. This has been best defined by Katz and co-workers (3, 24) for the MHC molecules in the theory of adaptive differentiation and more recently by Gorczynski et al. (25), but has also been suggested for immunoglobulin idiotype (26-28) or isotype (29). Currently, the existing data do not allow us to discriminate between these alternatives.

Several studies to date have investigated the role of T cells in the induction of secondary B cells. Although conflicting evidence exists (30, 31), in certain experimental systems T-dependent immunization of athymic mice has been demonstrated to result in an increase in the magnitude and rate of the antibody response (32-34), a shift in the secreted heavy chain isotype to IgG (32, 33), and an increase in the number of antigen-binding cells (34, 35), although these antigen-binding cells do not appear to bear IgG-cell surface receptors (35). Recent studies by Herzenberg et al. (36) and Black et al. (37) have demonstrated that late stages of memory development, correlated with the loss of IgD cell surface receptors, and involving the affinity maturation of immune responses, manifest a T cell dependence. It is possible that the observation that secondary B cells have more stringent requirements for MHC recognition by collaborating T cells may be correlated with the cell surface isotype of the B cells' receptor and the cells' affinity requirements for stimulation when expressing that receptor. In summary, evidence presented in this report supports the idea that immunologic memory in the B cell population is acquired in more than a single step, having different T cell requirements.

In addition to the effect of T cells on the MHC collaborative phenotype of B cells, these studies demonstrate that two potential effects of antigen exposure on the B cell population may be modulated or regulated by the presence of T cells. These include the preference for the isotope expressed by B cells and the maintenance of a primary B cell population. After immunization with a T-dependent antigen *in vivo*, in the presence or absence of T cells, a B cell population is induced that has a greater

preference for IgG<sub>1</sub> antibody synthesis *in vitro* as compared with nonimmune B cells. T independently immunized nude B cells manifest this same preference for IgG<sub>1</sub> antibody expression *in vitro*. In contrast, DNP-Ficoll immunization of B cells in the presence of T cells in the BALB/c mouse does not induce an expansion of the DNP-specific B cell population, and the DNP-Ficoll immunized population does not manifest a preference for IgG<sub>1</sub> antibody synthesis *in vitro* as compared with the nonimmune B cell population. One interpretation of these results is that T cells in the BALB/c mouse, although not directly involved in the antigen-stimulation event, influence the potential expansion and isotype expression of B cells. Several independent lines of research suggest that T lymphocytes play a regulatory role in influencing the heavy chain isotype expressed by B cells (33-40). Mongini and collaborators (40) have recently demonstrated that the heavy chain isotype of antibody expressed *in vivo* to TNP-Ficoll was influenced by the presence of T cells during priming. It is possible that these observed T cell functions are related, and that T cells that exert regulatory influences on primary antibody responses *in vivo* also determine the further expansion and differentiation of B cell clones.

A second observation concerns the role of T cells in permitting expression of primary-like B cells in an immune animal. It appears that primary-like B cells not observed in euthymic BALB/c mice are present in T dependently immunized nudes. These primary-like B cells are identified by their ability to secrete IgM antibody upon transfer to allogeneic recipients. One interpretation of this finding is that T cells serve both to promote the generation of memory B cells and suppress the expression of primary B cells that arise from the generative pool. It has been previously demonstrated (41) that antibody-specific T cells are generated after *in vivo* immunization that function to suppress primary but not secondary B cell responses. In the absence of T cells in the nude mouse, T cell suppressor functions would not be generated, and consequently primary-like B cells would be allowed to persist in the repertoire. Thus, these findings present new evidence that T lymphocytes play an influential role in shaping the secondary B cell repertoire.

In summary, the findings presented in this report demonstrate that T cells play an essential role in the acquisition of the MHC collaborative phenotype of secondary B cells. It was shown that, although immunization in the absence of T cells has the potential to induce an expansion of antigen-specific precursors and an increased preference for IgG<sub>1</sub> antibody synthesis *in vitro*, the acquisition of the secondary B cells' collaborative phenotype is dependent on T cell collaboration during antigen priming. Thus, as a population, the B cells' MHC-dependent collaborative potential is influenced by T cells during antigen-driven events.

### Summary

Previous studies have demonstrated that the B cells in immune and nonimmune mice manifest different major histocompatibility complex (MHC) collaborative phenotypes with antigen-specific T cells. Immune, or secondary B cells require syngeneic-like MHC recognition by collaborating T cells, and in its absence fail to be stimulated. Primary B cells manifest a much less stringent requisite for MHC recognition by T cells, and under conditions in which secondary B cells fail to be stimulated, primary B cells are stimulated to secrete IgM antibody.

Experiments were conducted to determine whether the acquisition of the secondary

B cells' MHC collaborative phenotype was dependent on the presence of T cells during in vivo immunization. B cell populations from T dependently and T independently immunized conventional BALB/c and athymic BALB/c *nu/nu* mice were compared in their ability to collaborate with allogeneic T cells. Although antigen alone promotes the differentiation of several secondary B cell characteristics, including an increase in the frequency of antigen-specific B cells and a preference for IgG<sub>1</sub> antibody synthesis in vitro, the acquisition of the secondary B cells' MHC collaborative phenotype was found to be dependent on the presence of T cells during in vivo immunization. Thus, the restriction imposed on T cell-B cell-collaborative interactions in secondary humoral immune responses appears to be the result of T dependent antigen-driven events.

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