

AN IDIOTYPE-SPECIFIC HELPER POPULATION THAT BEARS IMMUNOGLOBULIN, Ia, AND Lyt-1 DETERMINANTS

BY DAVID H. SHERR AND MARTIN E. DORF

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

Most helper populations that specifically regulate immune responses are Lyt-1⁺ T lymphocytes specific for carrier or idiotypic determinants (1-5). These T lymphocytes perform a major role in the regulation of antigen-specific immune responses. In contrast, the role of B cells as regulators of the immune response has been less well established. There has been one report demonstrating that an Lyt-1⁺, Ig⁺ B cell subpopulation can augment the plaque-forming cell (PFC)¹ response in an Igh-restricted fashion, suggesting a regulatory role for such unique B cell subsets (6).

This report presents evidence supporting the existence of a similar regulatory B cell-like population in the (4-hydroxy-3-nitrophenyl)acetyl hapten (NP)-specific PFC response. This Ig⁺, Lyt-1⁺ helper population, termed B_H, is present in normal unprimed mice and specifically augments the PFC response of NP^b idiotype-bearing B cells in the absence of T helper populations. The B_H population functions in an Igh-restricted fashion and specifically binds to NP^b-idiotypic determinants, suggesting that B_H-mediated regulatory interactions are dependent on idiotype-antiidiotype complementation.

Materials and Methods

Mice. C57BL/6 male mice were purchased from the Jackson Laboratory, Bar Harbor ME. B.C-8 and B6.Ig1^c mice were reared in the Harvard Medical School Animal Facility. All experimental animals were between 8 and 12 wk of age at the beginning of immunization. Animals were age-matched in each experiment.

Keyhole limpet hemocyanin NP-(KLH), NP-Ficoll, and trinitrophenyl hapten (TNP)-KLH were prepared as described previously (7). They had an average of 30, 17, and 20 haptenic groups per 100,000 molecular weight, respectively. Mice were immunized intraperitoneally with 50 μg NP-KLH or 200 μg TNP-KLH in a 0.2-ml mixture containing 25% pertussis vaccine (Michigan Department of Public Health, Lansing, MI) or 20 μg NP-Ficoll in saline.

Preparation of B_H Cells. Spleen cells from normal C57BL/6, B.C-8, or B6.Ig1^c mice were fractionated on nylon wool columns as described by Julius et al. (8). The nylon-adherent cells were recovered by incubation at 4°C for 45 min followed by agitation of

This work was supported by grants AI-16677 from the National Institutes of Health and a grant from the March of Dimes, Inc. D. H. Sherr was the recipient of a Medical Foundation Inc., King Trust Fellowship.

¹ *Abbreviations used in this paper:* AECM, N-(2-aminoethyl) carbonylmethylacetyl; BA, Brucella abortus; B_H, Ig⁺, Ia⁺, Lyb-3⁺, Lyt-1⁺ helper cells; C, complement; Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; MEM, minimal essential medium; MHC, major histocompatibility complex; NMS, normal mouse serum; NP, (4-hydroxy-3-nitrophenyl) acetyl hapten; NP^b, Igh^b-linked idiotypic determinants on anti-NP antibody; PBS, phosphate-buffered saline; PFC, plaque-forming cell(s); RAMG, rabbit anti-mouse immunoglobulin; SRBC, sheep erythrocytes; TNP, trinitrophenyl hapten.

the nylon wool. The nylon-adherent cells recovered by this procedure were then treated with a mixture of two monoclonal anti-Thy-1.2 antibodies and AKR anti-C3H thymocyte (anti-Thy-1.2) antiserum for 30 min at room temperature, washed, and treated with a 1:5 dilution of rabbit serum as a complement source for 30 min at 37°C.

Antisera Treatment of B_H Cells. Nylon-adherent, Thy-1.2⁻ B_H cells were characterized by treatment with specific antisera or monoclonal antibodies as follows. 10⁷ B_H cells were incubated for 30 min at room temperature with 0.5 ml normal mouse serum (NMS) or affinity-purified rabbit anti-mouse Ig antiserum (RAMG) diluted 1:5 minimal essential medium (MEM), 0.5 ml supernatant from M5/114 hybridoma cultures (anti-I-A^b), 0.5 ml anti-Lyt-1.2 or anti-Lyt-2.2 monoclonal antibody containing ascites diluted 1:30 or 0.5 ml (CBA/N × BALB/c)F₁ anti-BALB/c antiserum (anti-Lyb-3) diluted 1:10. Cells were then washed and treated for 30 min at 37°C with rabbit complement diluted 1:5 in MEM. All cell preparations were washed extensively before addition to responder cultures. The affinity-purified RAMG has been used extensively in our laboratory to positively select B lymphocytes. The M5/114 monoclonal antibody, provided by Dr. K. Rock (Harvard University, Boston, MA) has been shown to specifically detect and lyse cells expressing I-A^b, I-A^d, I-E^d, or I-E^k molecules (9). The monoclonal anti-Lyt-1.2 (clone 2.2-1) and anti-Lyt-2.2 (clone 41-3) antibodies obtained from Dr. D. Eardley and Dr. H. Cantor (Harvard University, Boston, MA), respectively, have been used extensively in our laboratory and elsewhere to characterize helper and suppressor T cell populations (10, 11). The anti-Lyb-3 antiserum obtained from Dr. B. Huber (Tufts University, Boston, MA) has been shown to block tolerization of B lymphocytes (12) and to trigger B cells to produce specific antibody after immunization with suboptimal doses of sheep erythrocytes (SRBC) (13). It should be noted that the possible reactivity of the latter antiserum with other determinants not expressed in CBA/N defective mice cannot be formally excluded.

NP^b Idiotype Fractionation of B_H Cells. B_H cells were fractionated as described previously for suppressor T cell populations (7). 5 ml of a 1 mg/ml solution of affinity-purified anti-NP antibodies from B10.S (7R) or A.TH mice were incubated on 100 × 15-mm polystyrene dishes for 1 h at 20°C. The dishes were washed extensively. 2–3 × 10⁷ B_H cells obtained from spleens of normal mice by nylon adherence followed by treatment with anti-Thy-1.2 antibodies plus complement (C) were added to each dish and incubated at 20°C for 1 h. Nonadherent cells were removed and the dishes washed extensively with warm (20°C) media. 5.0 ml cold media was added and the dishes were incubated on ice for 30 min. Adherent cells were removed with vigorous pipetting.

Responder Cultures. The use of the Mishell-Dutton culture system to produce NP-specific responses has been described previously (7). Briefly, spleen cells from mice primed 4 wk previously with NP-KLH in pertussis vaccine were used as the source of responder cells. Cultures were challenged with 200 ng NP-Ficoll. To eliminate Thy-1.2-bearing cells, 100 × 10⁶ responder cells were incubated for 30 min at 20°C with 0.5 ml AKR anti-CBA (anti-Thy-1.2) antiserum diluted 1:5 in supernatant from cultures of HO3.14 hybridoma cells, monoclonal anti-Thy-1.2 antibody, provided by Dr. K. Rock (Harvard University, Boston, MA) together with 20 μl HO3.14 ascites fluid. Cells were washed and incubated for 30 min at 37°C in a 1:5 dilution of rabbit C. Responder cells treated in this fashion were shown to be responsive to T-independent, but not T-dependent antigens. To eliminate Lyt-1.2-bearing cells, 10⁸ Thy-1.2⁻ viable responder cells were incubated for 30 min at 20°C in 0.5 ml anti-Lyt-1.2 ascites diluted 1:30 in MEM. Cells were washed and treated for 30 min at 37°C with a 1:5 dilution of rabbit C. All responder populations were washed extensively before addition of 7.5 × 10⁶ viable cells to duplicate wells in Costar culture plates (Cambridge, MA). Cell cultures were challenged in vitro following addition of B_H cells with 200 ng NP-Ficoll and assayed 5 d later for the magnitude and NP^b idiotype content of the PFC response.

Enumeration of NP^b Idiotype-bearing PFC. A modification of the Jerne plaque assay (14) was used to determine direct (IgM), NP-specific PFC responses in triplicate slides. The number of direct PFC expressing NP^b idiotype-related determinants was obtained by calculating the average percent inhibition of plaque formation relative to controls following the addition of 1–2 μl guinea pig anti-NP^b antiserum or 1 μl monoclonal anti-NP^b

antibody-containing culture supernatant to the plaquing mixture at the beginning of the assay. The percent inhibition of PFC was calculated according to the formula: percentage of inhibition = experimental PFC/control PFC \times 100.

Four preparations of antiidiotypic reagents specific for NP^b-related idiotypic determinants were used in this study. Two antisera were obtained by hyperimmunization of a guinea pig with purified, primary C57BL/6 anti-NP antibodies as detailed elsewhere (15). A third antiserum, kindly provided by Dr. S.-T. Ju (Harvard University, Boston, MA) was made by hyperimmunization of a guinea pig with an NP-specific B cell hybridoma (μ , λ_2) derived from SJL(Igh-1^b) mice (termed N hybridoma) (16). These reagents were adsorbed with normal (C57BL/6 \times DBA/2) immunoglobulin and MOPC-104E and MOPC 315 before use. All of the adsorbed antisera were shown to inhibit NP-specific responses only in mice of the Igh-1^b allotype and failed to inhibit TNP- and/or SRBC-specific responses of C57BL/6 (Igh^b) mice. In addition, all antisera lacked anti- λ activity since they failed to inhibit dextran-specific PFC responses in BALB/c mice. Thus, these antisera were specific for the Igh-V^b-linked NP^b idiotype-related determinants and not for constant region determinants on heavy or light immunoglobulin chains. A fourth reagent was obtained from Dr. T. Imanishi-Kari of the Massachusetts Institute of Technology. This rat monoclonal antibody was induced with monoclonal B-1-8 anti-NP hybridoma antibody (17). The specificity of the latter reagent was demonstrated by the ability of anti-NP^b idiotope to inhibit *in vivo* response of C57BL/6 mice to NP-Ficoll but not to SRBC, and its failure to affect the PFC responses of C3H (Ighj) mice to NP-Ficoll. All of these reagents gave identical results throughout these studies. Two reagents were used independently in each experiment to determine NP^b idiotype content.

Statistics. Results were analyzed with a two-tailed Student's *t* test. The data presented in each table represent the averages of all independent determinations of inhibition of plaque formation with two anti-NP^b idiotypic reagents. For example, data from three experiments represent statistical analyses performed on six measurements of NP^b idiotype content.

Results

Loss of NP^b Idiotype Expression After Anti-Lyt-1.2 Antibody Treatment. Spleen cells from C57BL/6 mice primed 4 wk previously were treated with anti-Thy-1.2 reagents plus C to eliminate T lymphocytes. When these T cell-depleted cultures were challenged *in vitro* with NP-Ficoll the responder cells produced a significant NP-specific PFC response, 41% of which was inhibitable with anti-NP^b idiotype reagents (Table I). If the Thy-1.2⁻ responder cells were further treated with anti-Lyt-1.2 antibody plus C the expression of NP^b-bearing PFC was significantly reduced to 7%. The magnitude of the response was similarly reduced from 2,850 to 1,550 PFC/culture. One explanation for the preferential loss of detectable NP^b idiotype-bearing PFC after anti-Lyt-1.2 antibody treatment was that a previously undefined Lyt-1.2-bearing non-T cell population was required for the expression of this B cell subset. To identify this hypothetical helper population, spleen cells from normal mice were fractionated on nylon wool columns. The adherent fraction (<3% Thy-1.2 positive by fluorescence analysis) was then treated with a combination of anti-Thy-1.2 antibodies and antiserum plus C to ensure the absence of Thy-1.2-bearing T cells. Graded numbers of nylon-adherent (B) cells or 2×10^6 nylon-nonadherent (T) cells (<1% Ig positive by fluorescence analysis) were added to NP-KLH-primed responder cells that were treated with anti-Thy-1.2 antibodies plus C and then with anti-Lyt-1.2 antibody plus C. Cultures were then challenged with NP-Ficoll and assayed 5 d later for the magnitude and NP^b idiotype content of the PFC

TABLE I
Nylon-adherent, Thy-1.2⁻ Cells (B_H) Are Required for Expression of
NP^b Idiotyp^e*

Responder cells	Cells added	Direct NP-specific PFC/culture \times log SE [‡]	Percent inhibition with anti-NP ^b idi- otypic re- agents \pm SE [§]
Thy-1.2 ⁻	0	2,850 \times 1.3 [†] (7)	41 \pm 6*
Thy-1.2 ⁻ , Lyt-1.2 ⁻	0	1,550 \times 1.1 (22)	7 \pm 3
Thy-1.2 ⁻ , Lyt-1.2 ⁻	2 \times 10 ⁶ B _H	2,720 \times 1.2 [†] (9)	41 \pm 4*
Thy-1.2 ⁻ , Lyt-1.2 ⁻	1 \times 10 ⁶ B _H	1,530 \times 1.2 (3)	56 \pm 13*
Thy-1.2 ⁻ , Lyt-1.2 ⁻	5 \times 10 ⁵ B _H	1,590 \times 1.1 (3)	28 \pm 6*
Thy-1.2 ⁻ , Lyt-1.2 ⁻	1 \times 10 ⁵ B _H	1,600 \times 1.2 (4)	33 \pm 12*
Thy-1.2 ⁻ , Lyt-1.2 ⁻	2 \times 10 ⁶ T	4,570 \times 1.7 [†] (3)	-9 \pm 5

* Responder spleen cells from C57BL/6 mice immunized 4 wk previously with NP-KLH were treated with a mixture of anti-Thy-1.2 reagents plus C. Where indicated the remaining responder cells were treated with anti-Lyt-1.2 monoclonal antibody containing ascites plus C. B_H cells were obtained from unprimed mice by passage over nylon wool columns. The nylon wool adherent cells were treated with anti-Thy-1.2 reagents plus C. Graded numbers of B_H or nylon-nonadherent T cells were added to 7.5 \times 10⁶ responder cells, which were then challenged with NP-Ficoll. The magnitude and NP^b idiotype content of the direct NP-specific PFC response were assayed 5 d later.

[†] Geometric mean \times log standard error. A cross indicates a significant increase in PFC relative to Thy-1.2⁻, Lyt-1.2⁻ responder cells to which no cells were added, $P < 0.02$. The number of experiments is indicated in parentheses.

[§] Arithmetic mean \pm standard error. An asterisk indicates a significant increase in the percent PFC inhibited with two anti-NP^b idiotype reagents relative to Thy-1.2⁻, Lyt-1.2⁻ responder cells to which no cells were added, $P < 0.05$.

response. Following addition of 2 \times 10⁶ unprimed nylon adherent, Thy-1.2⁻ B cells (B_H) the NP^b idiotype content of the response significantly increased to 41% while the magnitude similarly increased (Table I). Addition of 10⁶, 5 \times 10⁵, or 10⁵ B cells to Thy-1.2⁻, Lyt-1.2⁻ responder cultures significantly increased the NP^b idiotype content, while not affecting the magnitude of the PFC response (Table I). Addition of 2 \times 10⁶ nylon nonadherent T cells significantly increased the magnitude of the response to NP-Ficoll, but the NP^b idiotype content of the PFC response was not affected. Several conclusions can be drawn from these results. First, a nylon-adherent, Thy-1⁻ splenic population obtained from normal mice is capable of preferentially helping in the expression of NP^b idiotype-bearing PFC. Second, nylon-nonadherent T cells do not mediate the selective increase in NP^b idiotype expression. Finally, the unprimed B cells added to the responder cultures do not significantly contribute to the number of NP^b idiotype-

secreting B cells detected, since there is no direct relationship between the number of B cells added and the percent NP^b idiotype expression.

Surface Phenotype of the B_H Population. In order to further characterize this cell population, nylon-adherent spleen cells from naive C57BL/6 mice were treated with anti-Thy-1.2 reagents plus C and either NMS, RAMG, anti-Lyb-3 antiserum, anti-I-A^b, anti-Lyt-1.2 or anti-Lyt-2.2 monoclonal antibodies plus C. 2×10^6 antibody plus C treated nylon-adherent cells were added to syngeneic Thy-1.2⁻, Lyt-1.2⁻, NP-KLH-primed responder cells. The data presented in Table II confirm the significant increase in the NP^b idiotype content of the PFC response of Thy-1.2⁻, Lyt-1.2⁻ responder cells following reconstitution with NMS-plus-C-treated Thy-1.2⁻, nylon-adherent cells. Furthermore, treatment with RAMG, anti-Lyb-3 antisera, anti-I-A^b antibody, or anti-Lyt-1.2 antibody plus C completely abrogated the ability of the reconstituting population to selectively increase NP^b idiotype expression. Treatment with anti-Lyt-2.2 plus C did not affect the preferential increase in expression of NP^b idiotype-bearing clones. As in experiments presented in Table I, the increase in NP^b idiotype

TABLE II
*Surface Phenotype of Nylon-adherent, Thy-1.2⁻ (B_H) Helper Cells**

Treatment of B _H	Direct NP-specific PFC/Culture \times log SE [‡]	Percent inhibition with anti- NP ^b idiotype reagents \pm SE [§]
No cells added	1,400 \times \pm 1.1 [†] (6)	3 \pm 5*
NMS	2,450 \times \pm 1.2 (4)	41 \pm 7
Rabbit anti-mouse Ig antiserum	2,710 \times \pm 1.1 (3)	-15 \pm 4*
Anti-Lyb-3 antiserum	2,020 \times \pm 1.1 (4)	0 \pm 11*
Anti-I-A ^b antibody	1,790 \times \pm 1.0 (3)	-15 \pm 12*
Anti-Lyt-1.2 antibody	2,200 \times \pm 1.1 (3)	-14 \pm 7*
Anti-Lyt-2.2 antibody	3,010 \times \pm 1.0 (3)	54 \pm 5

* NP-KLH-primed C57BL/6 spleen cells were treated with anti-Thy-1.2 reagents plus C and then with anti-Lyt-1.2 antibody plus C. B_H were obtained by passage of spleen cells from unprimed C57BL/6 mice over nylon wool columns. Nylon-adherent cells were then treated with anti-Thy-1.2 reagents plus C. Where indicated these B_H cells were then treated with NMS, RAMG, anti-Lyb-3, anti-I-A^b, anti-Lyt-1.2, or anti-Lyt-2.2 reagents plus C. 2×10^6 viable treated B_H cells were then added to 7.5×10^6 Thy-1.2⁻, Lyt-1.2⁻ responder cells and challenged with NP-Ficoll. Cultures were assayed 5 d later for the magnitude and NP^b idiotype content of the direct NP-specific PFC responses.

[†] Geometric mean \times log standard error. A cross indicates a significant difference relative to addition of NMS plus C treated B_H, $P < 0.03$. The number of experiments is indicated in parentheses.

[§] Arithmetic mean \pm standard error. An asterisk indicates a significant decrease in the percent PFC inhibited with anti-NP^b reagents relative to addition of NMS plus C treated B_H, $P < 0.01$.

expression did not necessarily correlate with an increase in the magnitude of the response (Table II). From these data we conclude that a population containing Lyt-1⁺, Ig⁺, Lyb-3⁺, Thy-1⁻, Lyt-2⁻ cells preferentially helps NP^b idio-
type-bearing B cells in the PFC response.

Igh Restriction of B_H Population. Since the B_H population preferentially augments the response of NP^b idio-
type-bearing antibody-secreting B cells, it was postulated that this cell population was specific for the NP^b idio-
type-related determinants and its activity might be restricted by Igh-V^b genes. To test this prediction unprimed nylon-adherent, anti-Thy-1.2 antibody-plus-C-treated B_H cells from C57BL/6 (Igh^b), B.C-8 (Igh^a), or B6.Ig1^c (Ighⁿ) mice were added to Thy-1.2⁻, Lyt-1.2⁻ NP-KLH-primed responder cells. As shown in Table III, addition of C57BL/6 (Igh^b) B_H cells significantly increased the NP^b idio-
type expression, from 4% to 42%. In contrast, addition of B_H cells from B.C-8 (Igh^a) or B6.Ig1^c (Ighⁿ) congenic mice repeatedly failed to alter the NP^b idio-
type expression of C57BL/6 responder cells, demonstrating an Igh restriction on the interaction of B_H cells and B cells bearing the NP^b idio-
type.

Binding Specificity of the B_H Cell Population. Since the B_H population preferentially helped NP^b idio-
type expression and since its activity was Igh restricted, it was hypothesized that receptors on this population could react with NP^b idio-
typic determinants. Nylon-adherent, Thy-1.2⁻ B_H cells were fractionated on culture dishes coated with affinity-purified B10.S (7R) (NP^b) or A.TH (NP^c) anti-NP antibody. Adherent and nonadherent fractions were then tested for NP^b idio-
type-specific helper activity by addition to Thy-1.2⁻, Lyt-1.2⁻ responder cul-

TABLE III
*Igh Restriction of B_H Activity**

Source of nylon-adherent, Thy-1.2 ⁻ B _H	Direct NP-specific PFC/culture \times log SE [‡]	Percent inhibition with anti-NP ^b idio- type reagents \pm SE [‡]
None	1,570 \times 1.5 (4)	4 \pm 9*
C57BL/6 (Igh ^b)	2,010 \times 1.5 (5)	42 \pm 8
B.C-8 (Igh ^a)	2,030 \times 1.4 (4)	-9 \pm 8*
B6.Ig1 ^c (Igh ⁿ)	2,450 \times 1.6 (3)	2 \pm 8*

* NP-KLH-primed C57BL/6 (Igh^b) spleen cells were treated with anti-Thy-1.2 reagents plus C and then with anti-Lyt-1.2 antibody plus C. B_H were obtained by passage of spleen cells from unprimed C57BL/6 (Igh^b), B.C-8 (Igh^a), or B6.Ig1^c (Ighⁿ) mice on nylon wool columns. Nylon-adherent cells were treated with anti-Thy-1.2 reagents plus C. 2×10^6 cells (B_H) were added to 7.5×10^6 Thy-1.2⁻, Lyt-1.2⁻ responder cells and challenged with NP-Ficoll. The magnitude and percent PFC inhibition with anti-NP^b idio-
type reagents was assayed 5 d later.

[‡] Arithmetic mean \pm standard error. An asterisk indicates a significant difference in the percent anti-NP^b inhibitable PFC relative to addition of C57BL/6-derived B_H cells, $P < 0.004$.

[‡] Geometric mean \times log standard error. The number of experiments is indicated in parentheses.

tures. The data presented in Table IV show that addition of as few as $1-5 \times 10^5$ NP^b adherent cells significantly increased the NP^b idiotype expression from 10% to 48%. The NP^b nonadherent fraction was depleted of this helper activity. In contrast, NP^c adherent cells were unable to effect an increase in the NP^b idiotype expression, while all of the helper activity was recovered in the NP^c antibody nonadherent fraction. These data indicate that a NP^b idiotype-specific B_H population present in unprimed mice preferentially increased the expression of NP^b idiotype-bearing, antibody-secreting B cell clones.

Discussion

This report characterizes a lymphocyte subset that carries both B and T cell markers and is capable of preferentially augmenting the response of NP^b idiotype-bearing antibody-secreting B cell subsets. This helper population was detected in a system in which NP-KLH-primed B cells were responding in the absence of Thy-1.2-bearing T cells to the relatively T-independent antigen NP-Ficoll. Following treatment of NP-primed Thy-1.2⁻ responder cells with anti-Lyt-1.2 antibody plus C the NP^b idiotype level, detected by PFC inhibition with specific anti-NP^b idiotype reagents, was shown to decrease significantly from 40-50% to <10%. The depletion of an Lyt-1.2-bearing helper cell population by this

TABLE IV
*Binding Specificity of B_H Population**

B _H cells added	NP-specific PFC/ culture \bar{x} log SE [‡]	Percent inhibition with anti-NP ^b reagents \pm SE [§]
None	2,230 \bar{x} 1.2 (5)	10 \pm 8
2×10^6 unfractionated	2,890 \bar{x} 1.3 (4)	35 \pm 6*
$1-5 \times 10^5$ NP ^b adherent	3,190 \bar{x} 1.4 (4)	48 \pm 10*
2×10^6 NP ^b nonadherent	2,450 \bar{x} 1.2 (5)	5 \pm 5
$1-5 \times 10^5$ NP ^c adherent	2,300 \bar{x} 1.3 (5)	-13 \pm 3
2×10^6 NP ^c nonadherent	2,350 \bar{x} 1.2 (5)	35 \pm 4*

* NP-KLH-primed C57BL/6 spleen cells were treated with anti-Thy-1.2 reagents plus C and then with anti-Lyt-1.2 antibody plus C. B_H cells were obtained by passage of spleen cells from unprimed C57BL/6 on nylon wool columns. Nylon-adherent cells were treated with anti-Thy-1.2 reagents plus C. 30×10^6 viable B_H cells were then added to culture dishes coated with affinity-purified B10.S (7R) or with A.TH (NP^c) anti-NP antibody. 2×10^6 nonadherent or $1-5 \times 10^6$ adherent cells were added to 7.5×10^6 Thy-1.2⁻, Lyt-1.2⁻ responder cells and challenged with NP-Ficoll. The magnitude and the anti-NP^b inhibitable direct PFC responses were assayed 5 d later.

[‡] Geometric mean \bar{x} log standard error. The number of experiments is indicated in parentheses.

[§] Arithmetic mean \pm standard error. An asterisk indicates a significant increase in the percent PFC inhibitable relative to no B_H added, $P < 0.04$.

procedure was confirmed by experiments in which addition of unprimed nylon-adherent, Thy-1.2⁻ Lyt-1⁺ populations resulted in the expression of NP^b idiotype-bearing plaque-forming cells. Additional reconstitution experiments demonstrated that the helper population specifically reacted with monoclonal anti-I-A antibody and antiserum with activity for mouse immunoglobulin constant region and Lyb-3 determinants.

Despite the expression of Lyt-1.2 determinants on the helper population, there is strong evidence arguing against the involvement of a T cell population capable of preferentially increasing the expression of a predominant idiotype as described in other systems (4, 5, 18, 19). First, nylon-nonadherent T cells lacked NP^b idiotype-specific helper activity while nylon-adherent, Thy-1.2⁻ cells consistently increased the NP^b idiotypic response. While nylon-adherent helper T cells have been previously described, such cells had phenotypes distinct from the B_H cells; most notably, they expressed detectable levels of Thy-1.2 antigens (20). Secondly, the helper cell population in the present system was lysed by treatment with RAMG or (CBA/N × BALB/c)F₁ anti-BALB/c (anti-Lyb-3) antiserum plus C, suggesting the expression of Ig and Lyb-3 antigenic determinants. While it is formally possible that a T cell population low in Thy-1.2 determinants could passively acquire Ig or Lyb-3 determinants, this seems unlikely given the extreme difficulty of detecting IgC (21) or Lyb-3 (22) determinants on T cells. Finally, the helper population was shown to adhere to culture dishes coated with NP^b idiotype alone. Conventional helper T cells do not generally bind to substrates in the absence of major histocompatibility complex (MHC) determinants.

In a similar fashion it appears unlikely that nylon-adherent macrophages are responsible for the selective increase in NP^b idiotype expression, since the phenotyping studies would require that such a regulatory macrophage passively acquire Lyt-1.2, Lyb-3, Ig but not Lyt-2.2 determinants. Furthermore, in order to be consistent with binding specificity studies, the regulatory cell would have to selectively acquire NP^b idiotype-specific receptors *in situ* in the unprimed donor.

The conclusion most consistent with the data is that an Ig and Lyt-1.2-bearing cell population present in the nylon-adherent fraction is responsible for expression of NP^b idiotype-bearing B cell clones. Two experimental protocols indicate that this population is a regulatory cell subset that helps NP^b idiotype expression and is not a NP^b-bearing PFC population. First, the level of NP^b idiotype expressed can be independent of the number of nylon-adherent Thy-1.2⁻ cells added (Table I). In addition, binding studies demonstrate that the B_H cells in question are antiidiotypic (Table IV) and therefore would be unable to contribute to the response as NP-specific PFC. While the mechanism of the help provided by the B_H population is still unknown, the Igh restriction and the NP^b idiotype-binding data suggest that recognition of NP-specific B cells that express NP^b related idiotypic determinants is responsible for the selective augmentation of the NP^b-idiotypic PFC responses.

In the experiments presented an increase in the magnitude of the response did not correlate with an increase in NP^b idiotype expression. Thus, while addition of 2×10^6 B_H containing nylon-adherent Thy-1.2⁻ cells from C57BL/6 mice consistently increased the magnitude and NP^b idiotype content of the

Thy-1.2⁻, Lyt-1.2⁻ responder cells, reconstitution with nylon-nonadherent T cells, B_H-depleted nylon-adherent C57BL/6 cells, or B_H from Igh congenic mice only affected the magnitude. These data indicate that selection of NP^b idiotype-bearing B cells is not simply a function of increased B cell proliferation. The reason for the general increase in the size of the response after reconstitution with 2×10^6 cells that did not exhibit NP^b idiotype-specific helper activity is as yet unknown. However, this finding may have resulted from improved culture conditions following reconstitution with higher numbers of filler cells. Supporting this hypothesis is the finding that with the exception of $1-5 \times 10^5$ NP^b idiotype-adherent B_H cells (Table IV), fewer than 2×10^6 nylon-adherent Thy-1.2⁻ cells did not profoundly affect the magnitude of the PFC response (Tables I and IV). This nonspecific augmenting activity may account for the difficulty in observing an increase in the PFC response after selective expansion of the NP^b-idiotypic B cell subsets.

An important finding was that the B_H population was present in normal unprimed mice. The B_H population may be stimulated by germline gene products expressed on the few NP^b idiotype-bearing B cells that exist in unprimed mice. Indeed, the B_H population may select the NP^b-related idiotype-bearing clonotypes that predominate during an *in vivo* primary response.

Several recent reports have demonstrated that some murine B lymphocytes can express Lyt-1 determinants (6, 23-26). Hayakawa et al. (23) and Manohar et al. (24) have shown through fluorescence analysis that an Ly-1 B cell population is present in normal and immunodeficient mice. Braun (25) has demonstrated that long-term B lymphoblast cell lines as well as a population of normal cells (23) express Ly-1 and Ia determinants (25). In addition, human B cell chronic lymphocytic leukemias may also express Leu-1, the human equivalent of Lyt-1 (26). In one system the functional significance of an Lyt-1⁺ B cell population was determined (6). In that system, as in the NP system, the Lyt-1⁺, Ig⁺, Thy-1.2⁻ B cell population was shown to affect a hapten-specific PFC response in an allotype-restricted fashion. Nevertheless, some differences between the B_H population described in this report and the B' population defined by Okumura et al. (6) should be noted. B_H cells are lysed with I region-specific antibody, while B' cells are not. While the activity of both can be shown to be Igh-restricted the B_H was detectable only by its ability to affect NP^b idiotype expression, whereas the B' increased the overall magnitude of the DNP-specific PFC response. Another functional difference between these populations is that the B_H, but not the B', population is capable of functioning in the apparent absence of T helper cells. Experiments are now underway to determine whether these functional differences are a result of the use of a T-dependent antigen (DNP-KLH) in the B' system, as opposed to a relatively T-independent antigen (NP-Ficoll) in the B_H system. Despite these differences, the data obtained in both systems substantiate the hypothesis that a B cell-like subset may serve a regulatory role in the immune response.

According to the Jerne network hypothesis, immune regulation is effected by a series of idiotype-antiidiotype complementary interactions (27). Previously it has been shown that these types of idiotype-dependent interactions are important in T cell interactions in a suppressor T cell pathway (16, 28). More recently the

presence of a distinct population of idiotypic-specific suppressor T cells that directly suppress NP^b idiotypic-bearing B cells was demonstrated.² In this report we extend the concept of idiotypic regulation to the NP^b idiotypic-specific B_H population. In addition to expanding the known boundaries of network regulatory mechanisms, the identification of the B_H population suggests the possibility that this population is itself the target of regulatory signals. For example, it is possible that suppression of NP-specific PFC responses by an effector suppressor T cell factor bearing NP^b-related determinants (TsF₃) may be a result of interaction of this factor with NP^b idiotypic-specific B_H cells. The potential role of the B_H population as a target or effector of immune regulation is currently under investigation.

Summary

A helper cell population with phenotypic characteristics of both B and T cells is described. This helper population, called B_H, is present in normal unprimed C57BL/6 mice and preferentially helps the expression of NP^b idiotypic-bearing plaque-forming B cells in the absence of T helper cells. Its surface phenotype is Lyt-1.2⁺, Ig⁺, Lyb-3⁺, Thy-1.2⁻, Lyt-2.2⁻. The helper activity of the B_H population is IgH restricted and B_H cells selectively bind NP^b idiotypic determinants. Collectively the data demonstrate that this unique subpopulation can regulate the response of antibody-secreting B cells through specific recognition of idiotypic determinants.

The authors would like to acknowledge the excellent technical work of Michael J. Onyon and the expert secretarial work of Nancy S. Axelrod.

Received for publication 29 November 1983.

Note added in proof: Subsequent to submission of this manuscript, a related report (29), which also implicated a B_H (or related) cell required for NP^b idiotypic expression, was noted.

References

1. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. *Eur. J. Immunol.* 1:18.
2. Lewis, G. K., and J. W. Goodman. 1978. Purification of functional determinant-specific idiotypic-bearing murine T cells. *J. Exp. Med.* 148:915.
3. Adorini, L., M. Harvey, and E. E. Sercarz. 1979. The fine specificity of regulatory T cells. IV. Idiotypic complementarity and antigen-bridging interactions in the anti-lysozyme response. *Eur. J. Immunol.* 9:906.
4. Bottomly, K., B. J. Mathieson, and D. E. Mosier. 1978. Anti-idiotypic induced regulation of helper cell function for the response to phosphorylcholine in adult BALB/c mice. *J. Exp. Med.* 148:1216.
5. Quintans, J., Z. S. Quan, and M. A. Arias. 1982. Mice with the Xid defect have helper cells for T15 idiotypic-dominant antiphosphorylcholine primary and secondary plaque-forming cell responses. *J. Exp. Med.* 155:1245.
6. Okumura, K., K. Hayakawa, and T. Tada. 1982. Cell-to-cell interaction controlled by immunoglobulin genes. Role of Thy-1-, Lyt-⁺, Ig⁺ (B') cell in allotype-restricted antibody production. *J. Exp. Med.* 156:443.

² Sherr, D. H., and M. E. Dorf. 1983. Suppression of B cell responses by antigen-induced anti-idiotypic T cells (Ts_{id}). Submitted for publication.

7. Sherr, D. H., and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. IX. Characterization of idiotype specific effector phase suppressor cells on plaque forming cell responses in vitro. *J. Exp. Med.* 153:1445.
8. Julius, M. H., Z. Simpson, and L. A. Herzenberg. 1973. A rapid method for isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
9. Bhattacharya, A., M. E. Dorf, and T. A. Springer. 1982. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J. Immunol.*
10. Reiss, C. S., and S. J. Burakoff. 1981. Specificity of the helper T cell for the cytolytic T lymphocyte response to influenza virus. *J. Exp. Med.* 154:541.
11. Fresno, M., G. Nabel, L. McVay-Boudreau, H. Furthmayer, and H. Cantor. 1981. Ag-specific T cell clones. I. Characterization of a T cell clone expressing Ag-specific suppressive activity. *J. Exp. Med.* 153:1246.
12. Sherr, D. H., B. T. Huber, R. K. Gershon, B. Benacerraf, and M. E. Dorf. 1981. Effect of anti-Lyb3 antiserum on poly(L-glutamic acid, L-lysine) induced B cell tolerance. *Eur. J. Immunol.* 11:241.
13. Cone, R. E., B. Huber, H. Cantor, and R. K. Gershon. 1978. Molecular identification of a surface structure on B cells (Lyb3) and its relationship to B cell triggering. *J. Immunol.* 120:1733.
14. Jerne, N. K., A. A. Nordin, and C. Henry. 1963. The agar plaque technique for recognizing antibody producing cells. In *Cell-bound Antibody*. B. Amos and H. Koprowski, editors. Wistar Institute Press, Philadelphia, PA. p. 109.
15. Weinberger, J. Z., R. N. Germain, S. T. Ju, M. I. Greene, B. Benacerraf, and M. E. Dorf. 1979. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. *J. Exp. Med.* 150:761.
16. Sherr, D. H., S. T. Ju, and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XII. Fine specificity of antiidiotypic suppressor T cells (Ts₂). *J. Exp. Med.* 154:1382.
17. Reth, M., T. Imanishi-Kari, and K. Rajewsky. 1979. Analysis of the repertoire of anti-(4-hydroxy-3-nitrophenyl) acetyl (NP) antibodies in C57BL/6 mice by cell fusion. II. Characterization of idiotopes by monoclonal anti-idiotope antibodies. *Eur. J. Immunol.* 9:1004.
18. Woodland, R., and H. Cantor. 1978. Idiotype-specific T helper cells are required to induce idiotype-positive B memory cells to secrete antibody. *Eur. J. Immunol.* 8:600.
19. Pierce, S. K., N. A. Speck, K. Gleason, P. J. Gearhart, and H. Kohler. 1981. BALB/c T cells have the potential to recognize the TEPC 15 prototype antibody and its somatic variants. *J. Exp. Med.* 154:1178.
20. Tada, T., T. Takemori, K. Okumura, M. Nonaka, and T. Tokuhisa. 1978. Two distinct types of helper T cells involved in the secondary antibody response: independent and synergistic effects of Ia⁻ and Ia⁺ helper T cells. *J. Exp. Med.* 145:446.
21. Krawinkel, U., M. Cramer, T. Imanishi-Kari, R. S. Jack, K. Rajewsky, and O. Makela. 1977. Isolated hapten-binding receptors of sensitized lymphocytes. I. Receptors from nylon wool-enriched mouse T lymphocytes lack serological markers of immunoglobulin constant domains but express heavy chain variable portions. *Eur. J. Immunol.* 7:556.
22. Huber, B., R. K. Gershon, and H. Cantor. 1977. Identification of a B-cell surface structure involved in antigen-dependent triggering: absence of this structure on B cells from CBA/N mutant mice. *J. Exp. Med.* 145:10.
23. Hayakawa, K., R. R. Hardy, D. R. Parks, and L. A. Herzenberg. 1983. The "Ly-1 B" cell subpopulation in normal, immunodeficient, and autoimmune mice. *J. Exp. Med.* 157:202.

24. Manohar, U., E. Brown, W. M. Leiserson, and T. M. Chused. 1982. Expression of Lyt-1 by a subset of B lymphocytes. *J. Immunol.* 129:532.
25. Braun, J. 1983. Spontaneous in vitro occurrence and long-term culture of murine B lymphoblast cell lines. *J. Immunol.* 130:2113.
26. Wang, C. Y., R. A. Good, P. Ammisato, G. Dymbort, and R. L. Evans. 1980. Identification of a p69/70 complex expressed on human T cells showing determinants with B type chronic lymphocytic leukemia cells. *J. Exp. Med.* 151:1539.
27. Jerne, N. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris)*. 373.
28. Sherr, D. H., M. J. Onyon, and M. E. Dorf. 1984. Role of idiotypes in the 4-hydroxy-3-nitrophenyl acetyl (NP) specific suppressor T cell pathway. *In* *Idiotypes—Structure and Function*. A. Nisonoff and M. I. Greene, editors. Plenum Publishing Corp., New York In press.
29. Tada, T., K. Okamura, K. Hayakawa, G. Suzuki, R. Abe, and Y. Kumagai. 1981. Immunological circuitry governed by MHC and V_H gene products. *In* *Immunoglobulin Idiotypes*. C. Janeway, E. Sercarz, and H. Wigzell, editors. Academic Press, New York. p. 563.