Interleukin 4 Induces the Maturation of Idiotype-Specific Regulatory B Cell Populations (43446)

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Abstract. **CD5$^+$ B cells have been shown to be disproportionately associated with autoimmune diseases and transformation. In many cases, their apparent ability to bypass self-tolerance is manifested by the production of autoantibodies. These observations, plus the hypothesis that CD5$^+$ B cells represent a distinct B cell lineage, encourage studies into the conditions and factors that influence their development. In the present study, we employed a well-established assay for murine CD5$^+$ B cell function, i.e., their ability to augment the responses of IgH^b-linked idiotypic determinants on anti-(4-hydroxy-3-nitrophenyl) acetyl (NP) antibody (Nb^b) idiotype-bearing CD5$^-$ B cells to a T-independent antigen, together with multiple methods of cell surface phenotyping, to evaluate the potential for interleukin (IL) 4 to effect maturation of CD5$^+$ B cells, CD5$^+$, IgM$^+$, Thy-1$^-$, and NP^b idiotype-specific cells panned on antibody-coated petri dishes or sorted by flow cytometry from spleen were capable of augmenting NP^b idiotypic responses of NP-KLH-primed responder cells to NP-Ficoll. Splenic B cell populations depleted of CD5$^+$ B cells failed to affect idiotype expression even after 2 days in culture, a time when a small percentage of CD5$^+$ B cells appeared to be regenerated. However, idiotype-specific regulatory activity could be restored in CD5$^-$ splenic B cell populations by culture for 2 days with recombinant IL-4. Cells responsible for idiotype-specific regulatory activity after culture with IL-4 were in fact CD5$^+$, IgM$^+$, and Thy-1.2$^-$ B cells, demonstrating that IL-4 can drive the functional, if not the phenotypic, maturation of splenic B cells associated with the CD5$^+$ B cell lineage. The results illustrate one possible mechanism by which T cells could control the maturation of cells belonging to the CD5$^+$ B cell lineage.** [P.S.E.B.M. 1992, Vol 200]

B cells that express CD5 (Ly-1, Leu-1) have received considerable attention in the last 10 years because of their frequent association with lymphoproliferation (1), lymphocyte transformation (2–6), and autoimmunity (7–12). Although they represent 1–3% of the peripheral lymphoid pool in adult mice, CD5$^+$ B cells are frequently detected as murine B cell lymphomas and rapidly growing B lymphoblastoid cells (4, 6, 13). Originally thought to be rare in the human blood, this "minor" B cell subset may comprise 15% of normal peripheral B cell populations (8, 9, 14) and is responsible for 94% of all malignant human B cell chronic lymphocytic leukemias (3). A role for CD5$^+$ B cells in autoimmunity has been suggested by their substantially increased frequency in autoimmune New Zealand black and "motheaten viable" mice (10, 15) and their expansion in rheumatoid arthritis (8, 11), Sjogren's syndrome (16), and graves' disease (17) patients. CD5$^+$ B cells tend to secrete autoantibodies, including rheumatoid factors (8, 11, 18, 19). It has also been suggested that an expanded population of CD5$^+$ B cells plays a role in immunity to *Trypanosoma cruzi* (20).

In a number of autoimmune diseases and disease models, an increase in B cell activity and/or autoantibody production has been associated with hyperactivation of T cells (21–24). These observations, together with an increase in CD5$^+$ B cell frequency in autoimmunity, suggest that T cells play a role in CD5$^+$ B cell development. The purpose of the present study was to determine the potential for T cell-mediated development of CD5$^+$ B cells.

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Received September 26, 1991. [P.S.E.B.M. 1992, Vol 200]
Accepted January 31, 1992.

0037-9727/92/2003-0383$3.00/0

To approach this question, the maturational state of this B cell subset was defined by the ability of $CD5^+$ B cells to provide idiotype-specific activation signals to conventional, antigen-specific $CD5^-$ B cells (10, 25–30). We reported previously that $CD5^+$ B cell populations from normal or autoimmune mice or $CD5^+$ hybridomas can provide activation signals to (4-hydroxy-3-nitrophenyl acetyl hapten (NP)-specific responder B cell populations that express members of the IgH^b-linked idiotypic determinants on anti-NP antibody $(NP)^b$. $CD5^+$ B cell function is observed as an increase in the NP^b idiotype portion of B cell responses to NP-Ficoll in the absence of T helper cells. This activity resembles the idiotype-specific and/or IgH-restricted helper activity of B cells reported by other investigators (31–33). In the NP system, $CD5^+$, IgM^+ B cells perform this function by secreting (autoreactive) NP^b idiotype-specific antibody (27) and at least one nonspecific B cell-activating lymphokine (29). These results suggest that at least one nonpathologic function for autoantibody-producing $CD5^+$ B cells may be to regulate conventional $CD5^-$ B cell responses through idiotype networking.

Interleukin (IL) 4 was of particular interest for the present studies because of its documented stimulatory effects on several different cell types, including conventional B cells (34), peritoneal lymphocyte populations rich in $CD5^+$ B cells (35), and $CD5^+$ B lymphomas (36). By combining the assay for functionally mature $CD5^+$ B cells with multiple approaches to B cell surface antigen phenotyping, it was possible to investigate conditions for development of cells related to the $CD5^+$ B cell lineage. Data presented in this report provide support to our previous conclusion that idiotype-specific $CD5^+$ B cells affect idiotypic B cell responses. Furthermore, they demonstrate that IL-4 induces functionally mature $CD5^+$ B cells.

Materials and Methods

Mice. C57BL/6 male mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Responder mice were primed at 8–10 weeks of age. Animals were maintained according to the guidelines established by the Committee on Animals of the Harvard Medical School and by those initiated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS Publication [NIH] 85-23, revised in 1985.)

Antigens. NP-KLH and NP-Ficoll were prepared as described previously (26). These antigens had an average of 30 and 17 haptenic groups per 100,000 daltons, respectively. Mice were immunized intraperitoneally with 50 μg of NP-KLH in a 0.2-ml mixture containing 25% pertussis vaccine (Michigan Department of Public Health, Lansing, MI). Responder cells were challenged *in vitro* with 50 ng of NP-Ficoll.

Antibodies for Lysis/Blocking and Panning. Purified rat monoclonal IgG2a antibodies (anti-CD5, clone 53-7.8; anti-CD8, clone 53-6.7) were prepared by 50% saturated ammonium sulfate precipitation of serum-free supernatants of rat anti-CD5 and anti-CD8 hybridoma cultures followed by absorption of nonimmunoglobulin proteins with QAE-Sepharose (Pharmacia, Piscataway, NJ). These antibodies were used at a concentration of 200 μg/ml for panning studies. For lysis/blocking experiments, rat anti-CD5 or anti-CD8 supernatants were supplemented with commercial murine anti-CD5 or anti-CD8 ascites (Cedarlane, Hornby, CA). Supernatants from the murine HO 13-4 and 30 H-12 cell lines were used as a source of anti-Thy-1.2 antibody. For lysis of $Thy\text{-}1^+$ cells, these supernatants were mixed with a 1/20 dilution of AKR anti-C3H (anti-Thy-1.2) antisera followed by incubation with a 1/500 dilution of commercial anti-Thy-1.2 ascites (Cedarlane). Rabbit anti-mouse immunoglobulin antibody was affinity purified on mouse Ig-coupled Sepharose columns. Normal rabbit immunoglogulin was obtained from Jackson Immunoresearch (West Grove, PA). NP-specific antibodies were affinity purified from sera of NP-bovine γ-globulin-immunized C57BL/6 (IgH^b) or A.TH (IgH^e) mice. Supernatant from the 11B11 B cell hybridoma (37) was used as a source of anti-IL-4 antibody. Supernatants from all rat hybridoma cultures (anti-CD5, anti-CD8, anti-IL-4) contained between 5 and 10 μg of antibody/ml as assessed by enzyme-linked immunosorbent assay. The anti-CD5, anti-CD8, anti-Thy-1, anti-mouse Ig, and NP^b idiotypic antibodies have been used extensively in our laboratory (26, 38).

Flow Cytometry. Fresh splenic or cultured B cells were centrifuged through lymphocyte separation medium (Organon Teknika, Durham, NC) and pelleted in microfuge tubes. For triple-color stains, cells were treated first with 0.5 μg of biotin-goat F(ab′)2 anti-mouse IgM antibody (Southern Biotechnology Associates, Birmingham, AL), then washed and incubated in 1 μl of allophycocyanin-streptavidin (Phycochrome Corp., Wellesley, MA). Cells were washed and incubated for 20 min in 2 μl of normal mouse serum. Five microliters of phycoerythrin (PE)-anti-Thy-1.2 (IgG2b, clone 5a-8) murine antibody (Coulter Corp., Hialeah, FL) were then added directly to the mixture of normal mouse serum and cells. After incubation, cells were washed and 1 μl (0.1 μg) of isotype-matched fluorescein isothiocyanate (FITC)-anti-Ly-1.2 (IgG2b; clone CG16) murine antibody (Coulter) was added.

For quantitation of $CD5^+$ ($Ly\text{-}1^+$) cells, 1 μg of purified monoclonal rat anti-CD5 (clone 53-7.8; Becton-Dickinson, Sunnyvale, CA) was added immediately prior to the murine FITC-anti-Ly-1.2 antibody. Quadrants on dot plots were arbitrarily drawn such that 5–

10% of the lymphocyte population fell into the Thy-1.2$^-$, "CD5$^+$" (bottom right) quadrant of populations stained in the absence of blocking rat anti-CD5 antibody. Blocking with rat anti-CD5 antibody was specific since staining with the isotype-matched PE-anti-Thy-1.2 antibody was not affected.

CD3 expression was determined by incubation of cells with 1 ml of hamster anti-mouse CD3 monoclonal antibody supernatant or an irrelevant hamster antibody supernatant followed by FITC-goat F(ab')2 anti-hamster antibody (rat and mouse Ig absorbed; Caltag, San Francisco, CA).

Cells for analysis only were fixed in 1% paraformaldehyde. All antibodies were diluted in phosphate-buffered saline containing 5% fetal calf serum and 0.02 *M* sodium azide and, unless otherwise indicated, all incubations were performed for 40 min on ice. Analysis and sorting were performed with a dual-laser Coulter Epics V flow cytometer. Macrophages, dead cells, and remaining red blood cells were gated out using forward and 90° light scatter parameters.

To assess specificity and activity, all antibodies were initially titered and tested on unfractionated spleen cells, splenic B cells, splenic T cells, and peritoneal B cells. The murine allele-specific FITC-anti-Ly-1.2 antibody was absorbed with C57BL/6.Ly1.1 spleen cells (2×10^8 cells/0.5 ml of antibody) prior to use and did not stain C57BL/6.Ly1.1 T or B cells. In our hands, T cells and peritoneal B cells were more brightly stained with this reagent than with a rat PE-anti-CD5 antibody (clone 57.8; Becton-Dickinson).

IL-4 Induction Precultures. Spleen cells were fractionated over nylon wool (39). The adherent population was recovered and treated with anti-Thy-1.2 antibodies at room temperature followed by complement (Pel-Freeze, Rogers, AK) for 30 min at 37°C. Cells were washed and depleted of CD5$^+$ B cells by panning on polystyrene petri dishes (Fisherbrand) coated with purified rat monoclonal anti-CD5 or, as a specificity control, with anti-CD8 antibody (10). In approximately half the experiments, anti-CD5 antibody nonadherent cells were further treated with a 1/10 dilution of murine anti-Ly-1.2 ascites (Cedarlane) followed by complement.

CD5$^-$ B cells were recovered, washed, and resuspended at 10^7 cells/ml in minimal essential medium containing L-glutamine, sodium pyruvate, nonessential amino acids, Hepes buffer, and 10% fetal calf serum. Cells were plated at a concentration of 8×10^6 cells/0.75 ml in 24-well Costar plates with 20 μl of conalbumin-stimulated D10.G4.1 cell supernatant (40) or with 0.1–20 units of murine rIL-4 (Genzyme, Boston, MA) and incubated 20–66 hr in a 10% CO_2 rocking box. Approximately 40–60% of the starting population was recovered after culture in medium alone or medium supplemented with rIL-4.

Treatment of Precultured Cells. *Cell lysis/blockade.* CD5$^-$ B cells were cultured and washed three times with media. Viable cells ($1–3 \times 10^6$) were treated with anti-CD5, anti-CD8, anti-Thy-1.2, rabbit anti-mouse Ig antibodies, and NP^b id$^+$ or id$^-$ antibodies for 35 min at room temperature, followed by complement for 30 min at 37°C. The remaining cells were washed and counted, and $1–5 \times 10^5$ cells were added to responder cultures. Approximately 70–80% of the starting population was recovered after treatment with CD5$^-$, CD8$^-$, Thy-1.2-specific, NP^b id$^+$, or NP^b id$^-$ antibodies plus complement. Approximately 20% was recovered after treatment with rabbit anti-mouse Ig antibody plus complement.

Cell panning. B cells were panned on petri dishes coated for 2 hr at room temperature with purified monoclonal antibodies. Viable cells (10^6) were incubated on antibody-coated petri dishes for 45 min at room temperature. The nonadherent cells were removed and plates were washed extensively. Cold Versene (Gibco, Grand Island, NY) was added for 1 min to detach adherent cells. As in previous studies, anti-CD5 antibody adherent populations represented less than 3% of the starting population. Adherent and nonadherent cells were washed extensively and, in some experiments, further subjected to treatment with antibody plus complement prior to addition to responder cultures.

Responder Cultures. Preparation of NP-specific responder B cell cultures has been described (26). Spleen cells were obtained from mice primed 3–4 weeks earlier with NP-KLH. Responder spleen cells were treated with anti-Thy-1.2 antibodies plus complement prior to culture. Cells treated in this fashion were shown to be functionally (concanavalin A responsiveness) and phenotypically (Thy-1.2 expression) depleted of T cells (data not shown). Remaining CD5$^+$ cells were removed by passage of the Thy-1.2$^-$ responder populations over petri dishes coated with purified rat anti-CD5 antibody and/or treatment with anti-Ly-1.2 ascites plus complement.

Responder populations were washed extensively and 7.5×10^6 viable cells were added to duplicate wells in Costar culture plates in 0.75 ml of Mishell-Dutton medium. Cell cultures were challenged with 50 ng of NP-Ficoll and assayed 4 days later for the NP^b idiotype content of the NP-specific plaque-forming cell (PFC) response. The NP-specific PFC responses ranged from 500 to 1400 PFC/culture, with no significant differences between experimental groups. The relatively constant magnitude of NP-specific PFC response is a well-documented characteristic of this and other *in vitro* model systems (27, 28, 41, 42). This result appears to be due to the ability of B cells bearing minor idiotypic determinants to reconstitute the NP-specific response during the 4-day culture period.

Enumeration of NP^b Idiotype-Bearing PFC. A modification of the Jerne plaque assay was used to determine direct (IgM), NP-specific PFC responses in triplicate slides. Background PFC produced in the absence of NP-Ficoll were subtracted from all responses to obtain NP-specific PFC/culture. NP-specific PFC were completely inhibitable with NP hapten conjugates. The number of direct NP-specific PFC expressing NP^b idiotype-related determinants was obtained by calculating the average percentage of inhibition of plaque formation relative to controls after the addition of 1.0 μl of guinea pig anti-NP^b antiserum or 3.0 μl of monoclonal anti-NP^b antibody to the plaquing mixture. Both anti-NP^b idiotypic reagents were used independently in each experiment. The percentage of inhibition of PFC was calculated according to the formula:

$$\% \text{ Inhibition} = 1 - \frac{\text{experimental PFC}}{\text{control PFC}} \times 100\%$$

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

Results

Depletion of $CD5^+$ B Cells that Regulate NP^b Idiotypic PFC Responses. Previous studies have demonstrated that a nylon wool-adherent NP^b idiotype-specific B cell population from normal mice is capable of providing a selective advantage to NP^b idiotypic, NP-specific B cells responding to low doses of NP-Ficoll *in vitro* (10, 26–30). Treatment of B cell populations with CD5-specific antibodies plus complement abrogated this activity. To confirm these results by positive selection techniques and to generate a $CD5^-$ B cell population, nylon wool adherent spleen cells from normal C57BL/6 mice were depleted of T cells by treatment with anti-Thy-1 reagents plus complement and then panned on petri dishes coated with purified rat monoclonal anti-CD5 (IgG2a) or, as a specificity control, anti-CD8 (IgG2a) antibody. Adherent and nonadherent populations were then added to $Thy\text{-}1^-$, $CD5^-$ responder cells and the cultures were challenged with low doses of NP-Ficoll. Four days later, cultures were assayed for the NP^b idiotype content of NP-specific PFC responses.

The data presented in Table I demonstrate that $Thy\text{-}1^-$, $CD5^-$ responder cells produced significant NP-specific PFC responses to the T-independent antigen NP-Ficoll (1175 PFC/culture). However, NP^b idiotype-bearing B cells did not contribute to this response. The NP^b idiotypic PFC response was significantly increased to 81% when $1\text{–}5 \times 10^5$ anti-CD5 antibody adherent cells were added to the responder cultures. In contrast, as many as 2.5×10^6 anti-CD5 antibody nonadherent cells failed to affect idiotype expression (−10% NP^b idiotype). The failure of "anti-CD8 antibody adherent" cells to augment NP^b PFC responses (13% NP^b idiotype) and the recovery of this activity in the anti-CD8 nonadherent fraction demonstrated the specificity of this selection method. As in all previous studies (10, 25–30), the magnitudes of the PFC responses were not significantly altered by idiotype-specific helper activity. These data support the hypothesis that a $CD5^+$ B cell population can provide NP^b idiotype-specific activation signals to responding B cell subsets. Furthermore, they suggest the complete depletion of functional $CD5^+$ B cells by panning on anti-CD5-antibody-coated petri dishes.

Induction of $CD5^+$ B Helper Activity with IL-4. To investigate the role of IL-4 in the induction of $CD5^+$ B cells, $CD5^-$ B cell populations from normal mice were incubated (precultured) for 40 to 60 hr with or without IL-4 containing D10.G4.1 supernatant. After extensive washing, 2×10^5 precultured cells were added to NP-Ficoll challenged responder cultures. $CD5^-$ cells precultured in media without D10.G4.1 supernatant were unable to provide NP^b idiotype-specific activation signals to responder cultures (Table II). In contrast, cells cultured in D10.G4.1 supernatant significantly affected NP^b idiotype expression (81% NP^b idiotype). This effect was reversed when anti-IL-4 was added to the preculture.

To determine whether IL-4 was sufficient for the induction of idiotype-specific activation signals, titered amounts of recombinant IL-4 were added at the initiation of the 2-day preculture. Cells cultured with 10 units of rIL-4 significantly augmented NP^b idiotype expression in responder cultures (65% NP^b idiotype). Transfer of cells cultured with 1 unit of rIL-4 to responder cultures resulted in an intermediary (32%), but statistically insignificant level of NP^b idiotype expression. Addition of cells precultured with 0.1 unit of rIL-4 failed to affect NP^b idiotype expression. These data are consistent with the hypothesis that IL-4 induces B cells capable of augmenting NP^b idiotype expression of antigen-specific responder B cells.

Cells cultured with rIL-4 were titered to determine the minimum number required to augment NP^b idiotype expression on transfer to responder cultures. As few as 2×10^4 precultured cells were capable of significantly affecting NP^b idiotype expression (63%, Table III). The high frequency of IL-4 cultured B cells mediating this increase in NP^b helper cells is reminiscent of the high frequency of fresh (NP^b-specific) splenic B cells capable of augmenting NP^b idiotypic responses (26). Both observations parallel the high frequency of NP-specific and/or NP^b idiotypic B cells in IgH^b mice (43–45).

Table I. Anti-CD5 Antibody Adherent Cells Augment NP^b Idiotypic PFC Responses[a]

Cells added	Fraction	PFC/culture[b]	Percentage of NP^b idiotypic PFC on transfer to responder cultures[c]
—	—	1175 ⁂ 1.1	−16 ± 10 (4)
$1.0–5.0 \times 10^5$	Anti-CD5 Adherent	1150 ⁂ 1.2	81 ± 7 (4)[d]
2.5×10^6	Anti-CD5 Nonadherent	1050 ⁂ 1.2	−10 ± 22 (3)
$1.0–5.0 \times 10^5$	Anti-CD8 Adherent	1125 ⁂ 1.1	13 ± 28 (3)
5.0×10^5	Anti-CD8 Nonadherent	1000 ⁂ 1.1	95 ± 14 (4)[d]

[a] NP-KLH-primed C57BL/6 responder spleen cells were treated with anti-Thy-1.2 antibodies plus complement and then panned on anti-CD5-antibody-coated petri dishes to remove T and $CD5^+$ B cells. B cell populations for preculture were prepared by passage of spleen cells from unprimed C57BL/6 mice over nylon wool columns. Nylon adherent cells were treated with anti-Thy-1.2 antibodies plus complement to ensure the absence of T cells. B populations were then fractionated by panning on anti-CD5- or anti-CD8-antibody-coated petri dishes. Adherent or nonadherent populations were added to responder cells at the time of challenge with NP-Ficoll. Cultures were assayed 4 days later for the NP^b idiotype content of direct NP-specific PFC responses. Numbers in parentheses indicate the number of experiments.
[b] Geometric mean ⁂ log SE.
[c] Arithmetic mean ± SE.
[d] Significant increase in NP^b idiotypic PFC relative to the no-cell-added control; $P < 0.001$.

Table II. IL-4 Induces Cells that Augment NP^b Idiotypic PFC Responses[a]

Added to preculture	Percentage of NP^b idiotypic PFC on transfer to responder cultures[b]
Media	9 ± 10 (13)
D10.G4.1 supernatant	81 ± 9 (7)[c]
D10.G4.1 supernatant + anti-IL-4	12 ± 26 (5)
10 units rIL-4	65 ± 20 (3)[c]
1.0 units rIL-4	32 ± 34 (3)
0.1 units rIL-4	10 ± 32 (3)

[a] Responder cells were prepared as in Table I. Spleen cells for precultures were depleted of T and $CD5^+$ B cells by adherence on nylon wool columns, treatment with anti-Thy-1.2 antibody plus complement, panning on anti-CD5-antibody-coated petri dishes, and treatment with anti-CD5 antibody plus complement. D10.G4.1 culture supernatants (20 μl) or 0.1–10 units of rIL-4 were added on the first day of culture. Supernatant (10 μl) from cultures of 11B11 cells (anti-IL-4) were added where indicated. Two days later, precultured cells were washed and 2×10^5 cells were added to responder B cell cultures challenged with NP-Ficoll. NP^b idiotypic PFC responses were assayed 4 days later.
[b] Arithmetic mean ± SE. The number of experiments is indicated in parentheses.
[c] Significant level of NP^b idiotypic PFC; $P < 0.04$.

Table III. Titration of IL-4-Induced Cells that Augment NP^b Idiotypic PFC Responses[a]

Added to induction precultures	Cells added to responder cultures	Percentage of NP^b idiotypic PFC on transfer to responder cultures[b]
–	None	6 ± 14 (3)
Media	2×10^5	11 ± 12 (5)
rIL-4	2×10^5	70 ± 14 (3)[c]
rIL-4	2×10^4	63 ± 18 (3)[c]
rIL-4	2×10^3	18 ± 21 (4)
rIL-4	2×10^2	−10 ± 20 (3)

[a] Responder cells were prepared as in Table I. $CD5^-$ B cell populations for induction precultures were prepared as described in Table II. Cells were cultured for 42 hr in media with or without 20 units of rIL-4 as indicated. Precultured cells were washed and added to responder cultures at the time of antigenic challenge with NP-Ficoll. NP^b idiotypic PFC responses were assayed 4 days later.
[b] Arithmetic mean ± SE.
[c] Significant level of NP^b idiotypic PFC; $P < 0.01$.

Phenotype and Specificity of IL-4-Induced B Cells. The expression of NP^b idiotypic PFC responses has been shown in this system to be dependent upon NP^b idiotype-specific $CD5^+$ B cells (10, 26–30). Three methods for phenotyping and fractionating lymphocytes were employed to test the hypothesis that a similar $CD5^+$ B cell population was induced by preculture of $CD5^-$ B cells with rIL-4: (i) lysis/blocking with antibodies plus complement, (ii) panning on antibody-coated petri dishes, and (iii) fluorescence-activated cell sorting.

Antibody-dependent lysis/blocking. $CD5^-$ spleen cells were cultured for 42 hr with rIL-4 and treated with the antibodies listed in Table IV plus complement. The results demonstrate that treatment with $CD5^-$ or Ig^-, but not $Thy\text{-}1.2^-$ or CD8-specific antibodies plus complement, ablated the ability of rIL-4-precultured B cells to augment NP^b idiotype expression. This result could be due either to complement-mediated cell lysis or to blocking of cell function through antibody-bound surface antigens. Our inability to kill greater than 80% of

Table IV. Phenotype of IL-4-Induced Cells that Augment NP^b Idiotypic PFC Responses[a]

Fraction	Antibody treatment	Percentage of NP^b idiotypic PFC on transfer to responder cultures[b]
No cells	—	8 ± 12 (7)
Unfractionated	Anti-CD5 + C	4 ± 16 (5)
Unfractionated	Anti-CD8 + C	75 ± 22 (3)[c]
Unfractionated	Anti-Thy-1.2 + C	80 ± 16 (3)[c]
Unfractionated	RAMIg + C	6 ± 14 (2)
Anti-CD5 Adherent	—	87 ± 8 (3)[c]
Anti-CD5 Nonadherent	—	−2 ± 21 (3)
Anti-CD8 Adherent	—	−2 ± 37 (3)
Anti-CD8 Adherent	—	−2 ± 37 (3)
Anti-CD8 Nonadherent	—	63 ± 24 (3)[c]
Anti-CD5 Adherent	NP^b id^- + C	−3 ± 24 (3)
Anti-CD5 Adherent	NP^b id^- + C	100 ± 0 (3)[c]
Anti-CD5 Adherent	RAMIg + C	−12 ± 17 (3)
Anti-CD5 Adherent	Normal Ig + C	69 ± 9 (3)[c]

[a] $CD5^-$ B cells were prepared as in Table II. Cells (7.5×10^6) were incubated with 20 units of rIL-4 for 42 hr, washed extensively, and either treated with antibody plus complement (C) or panned on petri dishes coated with purified anti-CD5 or anti-CD8 antibody. Antibody plus complement-treated (2×10^5), petri dish-adherent (2×10^4), or nonadherent (2×10^5) cells were added to responder cultures at the time of antigenic challenge with NP-Ficoll. Where indicated, anti-CD5 antibody adherent cells were further treated with C57BL/6 (NP^b id^+), A.TH (NP^b id^-) NP-specific, rabbit anti-mouse Ig-specific, or normal rabbit antibody plus complement prior to addition to responder cultures. NP^b idiotypic PFC responses were assayed 4 days later.
[b] Arithmetic mean ± SE. The number of experiments is indicated in parentheses.
[c] Significant level of NP^b idiotype; $P < 0.003$.

this IgM^+ B cell population (see below) with rabbit anti-mouse Ig antibody plus complement while completely ablating their ability to affect NP^b idiotype expression is consistent with the latter possibility. As in previous studies, Thy-1^+ cells appeared not to be involved in NP^b idiotype regulation.

Panning. $CD5^-$ B cells cultured in rIL-4 and positively selected by adherence to petri dishes coated with purified rat anti-CD5 antibody significantly augmented idiotype expression on transfer to responder cultures (Table IV: 87% NP^b idiotype). Anti-CD5 antibody nonadherent cells from IL-4 cultures failed to affect idiotype expression.

In contrast, limiting numbers of cells (nonspecifically) adhering to the isotype control anti-CD8-antibody-coated plates failed to augment NP^b idiotype expression in responder cultures. Significant activity was recovered in the anti-CD8 antibody nonadherent fraction.

The ability of IL-4-precultured, anti-CD5-antibody-adherent cells to affect NP^b idiotype expression was completely abrogated after treatment with NP^b id^+ antibody or rabbit anti-mouse Ig antibody plus complement. Significant activity was observed after treatment of anti-CD5 antibody adherent cells with control NP^b id^- antibodies or normal rabbit immunoglobulin.

These data are consistent with the hypothesis that IL-4 induces the development of NP^b idiotype-specific $CD5^+$ B cells which in turn are capable of augmenting the NP^b idiotype expression of NP-specific responder B cell populations.

Flow cytometry. Because of the low percentage of $CD5^+$ B cells in the spleen (7), a system for accurately quantitating small numbers of splenic $CD5^+$ B cells was developed. $CD5^+$ B cells were quantitated on the basis of the ability of unlabeled anti-CD5 antibody to specifically block staining of IgM^+, Thy-1.2^- lymphocytes with FITC-anti-CD5 (Ly-1.2). Murine monoclonal FITC-anti-CD5 (IgG2b) antibody was employed for these studies because staining with it could be specifically inhibited with a heterologous rat anti-CD5 antibody and because an isotype-matched PE-anti-Thy-1.2 antibody could be used simultaneously to control for nonspecific or Fc receptor-mediated staining of B cell populations and for nonspecific blocking of staining by rat anti-CD5 antibody.

Spleen cell populations were triple-color stained for IgM, Thy-1.2, and CD5 expression. In a representative experiment, 13.8% of the lymphocyte population stained for Thy-1.2, with 85% of those staining for CD5 (Fig. 1B). Staining of essentially all of those $CD5^+$ T cells was affected by the unlabeled anti-CD5 antibody (Fig. 1A). Blocking was CD5 specific, since Thy-1.2 staining was not affected (9.8 ± 4.0% or 2.1% ± 11.7% = 13.8% of the lymphocyte populations in Fig. 1, A and B). By arbitrarily drawing quadrants such that 5–10% fell into the Thy-1.2^-, $CD5^+$ B cell quadrant (lower right quadrant in each contour map), it was possible to observe blocking of 9.8 − 7.9% = 1.9% of the Thy-1.2^-

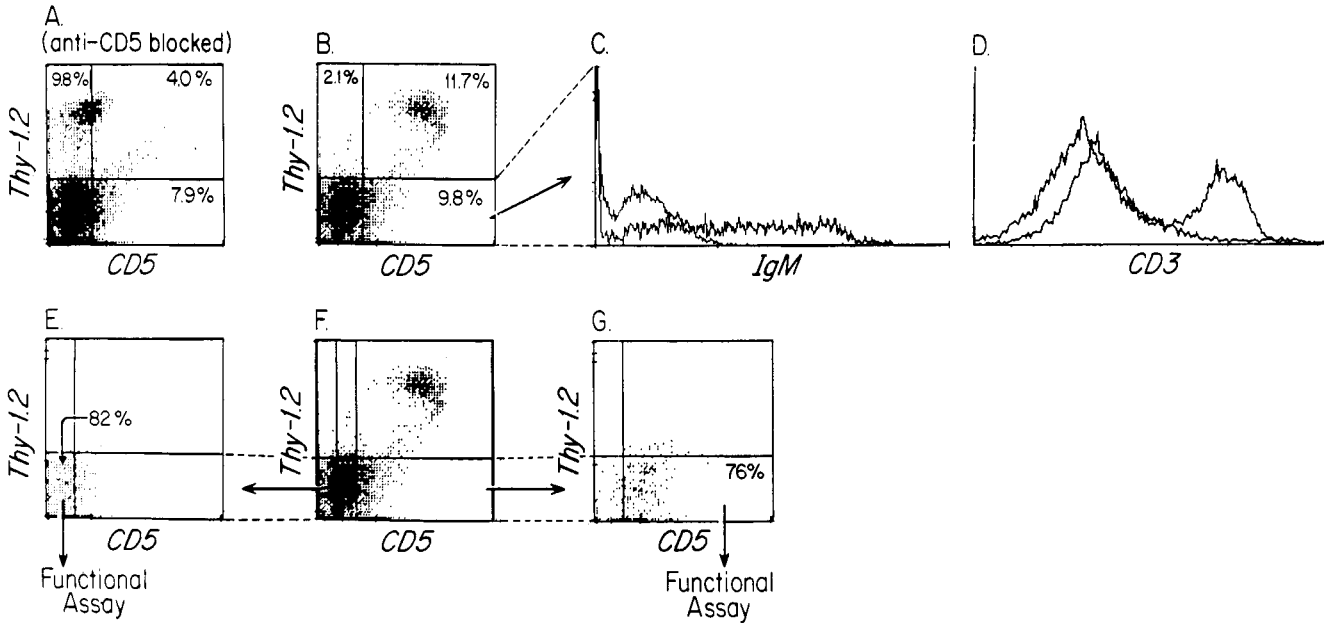

Figure 1. Flow cytometric analysis, sorting, and reanalysis of fresh splenic CD5$^+$ B cells. Spleen cells from naive C57BL/6 mice were treated in the presence or absence of 1 μg of rat anti-CD5 antibody with murine FITC-anti-CD5 (Ly-1.2) antibody, murine PE-anti-Thy-1.2 antibody, and biotin-anti-F(ab')2-anti-IgM antibody + allophycocyanin-streptavidin, as described in Materials and Methods. Samples were analyzed and sorted, with monocytes excluded on the basis of forward and 90° light scatter parameters. Each axis spans 3 logs of relative fluorescence. (Contours A and B) Thy-1.2 and CD5 staining in the presence or absence of unlabeled rat anti-CD5 antibody, respectively. (Histogram C) IgM profile of cells falling into the Thy-1.2$^-$, CD5$^+$ quadrant. (Histogram D) CD3 profile of whole spleen. (Contour F) Sorting gates. (Contours E and G) Reanalysis of Thy-1.2$^-$, CD5$^-$- and Thy-1.2$^-$, CD5$^+$-sorted populations used in functional studies (Table VI). Data from one representative experiment (eight total) are presented.

lymphocyte population in the presence of unlabeled anti-CD5 antibody. In eight experiments, this blocking averaged 2.6 ± 0.6% ($P < 0.001$; Table V), a percentage consistent with previously published estimates of CD5$^+$ B cell frequency in the spleen (7).

Gating on cells falling into the Thy-1.2$^-$, CD5$^+$ quadrant and analyzing for IgM expression indicated that most if not all of these Thy-1.2$^-$ cells expressed surface IgM (Fig. 1C). Approximately 30% of the splenic lymphocyte population expressed CD3 (Fig. 1D).

To correlate function with phenotype, cells were sorted into CD5$^-$, Thy-1.2$^-$ or CD5$^+$, Thy-1.2$^-$ populations as depicted in Figure 1F. Approximately 10% of the total lymphocyte population was recovered in each fraction. Sorted cells were then reanalyzed (Fig. 1, E and G) and equal numbers of cells were transferred to responder cultures. The ability of only the CD5$^+$ B cells to augment idiotype expression on transfer to responder cultures (67 ± 12% vs −9 ± 10%, Table VI) confirms our previous conclusion that CD5$^+$ B cells can regulate the idiotypic composition of a B cell response.

To evaluate IL-4-induced CD5$^+$ B cell maturation by flow cytometry, CD5$^-$ B cells were cultured 42–66 hr in media alone or media supplemented with rIL-4 prior to triple-color staining for CD5, Thy-1.2, and IgM expression and single-color staining for CD3 expres-

Table V. Re-Expression of CD5 in B Cell Cultures[a]

Population	Percentage inhibited with rat anti-CD5 antibody[b]
CD5$^-$, Thy-1.2$^-$, IgM$^+$ starting population	−0.3 ± 0.4 (4)
Media cultured	1.6 ± 0.7 (8)
IL-4 cultured	2.9 ± 0.9 (7)[c]
Fresh Thy-1.2$^-$ spleen cells	2.6 ± 0.6 (8)[c]

[a] CD5$^-$ B cells, prepared as in Table II, were cultured for 40 to 66 hr in media alone or in media supplemented with 20 units of rIL-4. After culture, B cells were triple color-stained with FITC-anti-CD5 (Ly-1.2), PE-anti-Thy-1.2, and allophycocyanin-streptavidin + biotin-F(ab')2-anti-IgM in the presence or absence of unlabeled rat anti-CD5 antibody. As a positive control fresh spleen cells were similarly triple-color stained. Thy-1.2$^-$ cells were analyzed for inhibition of FITC-anti-CD5 staining with unlabeled rat anti-CD5 antibody as described in the legends to Figures 1 and 2.

[b] Percentage of inhibition of staining with FITC-anti-CD5 (murine anti-Ly-1.2) antibody in the presence of rat anti-CD5 antibody ± SE. Inhibition of cells cultured in media alone was not statistically significant. The number of experiments is in parentheses.

[c] Significant level of inhibition relative to the starting CD5$^-$ population; $P < 0.03$.

Table VI. Functional Analysis of Splenic or IL-4-Induced $CD5^+$ B Cells Fractionated by Flow Cytometry[a]

Population sorted	Percentage of NP^b idiotypic PFC on transfer to responder cultures[b]
Fresh spleen, $CD5^-$, Thy-1.2^-, Ig^-	-9 ± 10 (4)
Fresh spleen, $CD5^+$, Thy-1.2^-, Ig^+	67 ± 12 (4)[c]
Media cultured, $CD5^-$, Thy-1.2^-, Ig^+	-16 ± 8 (4)
Media cultured, "$CD5^+$", Thy-1.2^-, Ig^+	18 ± 9 (3)
IL-4 cultured, $CD5^-$, Thy-1.2^-, Ig^+	9 ± 14 (4)
IL-4 cultured, $CD5^+$, Thy-1.2^-, Ig^+	43 ± 3 (3)[c]

[a] $CD5^-$ B cells, prepared as in Table II, were cultured for 40 to 66 hr in media alone or in media supplemented with 20 units of rIL-4. After culture, cells were washed extensively and dead cells were removed on lymphocyte separation medium. Cultured or fresh spleen cells were triple-color stained with FITC-anti-CD5 (Ly-1.2), PE-anti-Thy-1.2, and allophycocyanin-streptavidin + biotin-F(ab')2-anti-Ig. Lymphocytes cultured with or without rIL-4 were sorted for CD5 expression and reanalyzed as exemplified in Figure 2. Equal numbers of cells (from 3×10^3 to 2×10^4) were transferred in each experiment to responder B cell cultures and tested for their ability to augment NP^b idiotype expression.

[b] Arithmetic mean ± SE. Numbers in parentheses indicate the number of experiments.

[c] Significant increase in NP^b idiotypic PFC as compared with negative controls; $P < 0.002$.

sion. Representative fluorescence data are presented in Figure 2, average inhibitions of FITC-anti-CD5 antibody staining in the presence of unlabeled rat anti-CD5 antibody in four to eight experiments are presented in Table V, and data on the ability of sorted cell populations to augment NP^b idiotype expression are provided in Table VI.

The "starting B cell population" was shown to be depleted of anti-CD5 antibody inhibitable cells (Fig. 2, A versus B). In four experiments, the percentage of inhibition with anti-CD5 antibody averaged $-0.3 \pm 0.4\%$ (Table V), supporting the conclusion reached by functional analyses of these cells (Table I) that mature $CD5^+$ B cells were completely depleted from this population. Most, if not all, of these Thy-1.2^- cells expressed IgM (Fig. 2C). None of them expressed CD3 (Fig. 2D).

After culture in media alone, a low level of inhibition of the CD5 stain with unlabeled anti-CD5 antibody was observed (Table V: $1.6 \pm 0.7\%$). Although this apparent increase in CD5 expression was not statistically significant, the results suggest that culture alone may result in the re-expression of CD5 on splenic B cell populations. IgM and CD3 expression in cultured populations was indistinguishable from that of starting populations (not shown).

In contrast, the percentage of $CD5^+$ B cells was significantly increased when B cells were cultured in the presence of rIL-4 (Table V: $2.9 \pm 0.9\%$; $P < 0.03$). Cells from the IL-4 preculture expressed IgM, but not CD3 (Fig. 2, G and H).

To correlate function with phenotype, cells cultured in the absence or presence of IL-4 were sorted for $CD5^+$, Thy-1.2^- or $CD5^-$, Thy-1.2^- populations as depicted in Figure 2J. Sorted populations were reanalyzed (Fig. 2, I and K) and limiting numbers of cells ($\leq 2 \times 10^4$) were transferred to responder cultures. Sorting efficiencies for cells cultured with or without rIL-4 were indistinguishable. Approximately 8% of the total lymphocyte population was recovered in each fraction.

Neither population sorted from B cell populations precultured with media alone significantly affected NP^b idiotype expression in responder cultures (-16% and 18%, respectively; Table VI). Similarly, B cells from IL-4 precultures sorted for their lack of CD5 failed to affect idiotype expression (9%).

In contrast, significant levels of idiotypic PFC responses were observed when $CD5^+$ B cells, sorted from rIL-4-supplemented cultures, were added to responder cultures ($43 \pm 3\%$). These data support our previous conclusion that $CD5^-$ B cells cultured for 2 to 3 days re-express CD5 and, when cultured with IL-4, reacquire the ability to affect idiotype expression of hapten-specific B cells populations.

Discussion

We reported previously that $CD5^+$ B cells were capable of regulating idiotype expression within an *in vitro* response to a T-independent antigen (reviewed in [25]). One advantage of this biologic system was its sensitivity to functional $CD5^+$ B cells. By combining this functional assay with several methods of cell surface phenotyping, it was possible to evaluate maturation of the $CD5^+$ B cell subset *in vitro*.

We were repeatedly unable to observe $CD5^+$ lymphocytes at the start of IL-4 precultures. In contrast, significant numbers of $CD5^+$, Thy-1.2^-, IgM^+ B cells were readily detected after 42–66 hr of culture in media supplemented with rIL-4. It should be noted that it is possible that B cell population re-expressed CD5 to some extent when cultured in medium without IL-4 (Table V). Nevertheless, cells sorted from media control cultures failed to augment NP^b idiotype expression (Table VI), which suggests a disassociation between CD5 expression and regulatory activity. Identical results were obtained with $CD5^-$ B cells from the peritoneal cavity (data not shown). The percentage of $CD5^+$ B cells did not increase with longer periods of incubation (up to 76 hr, data not shown).

Similarly, $CD5^+$ B cell function, as defined by the ability to augment NP^b idiotype expression on transfer to responder B cell cultures, was not detectable in the

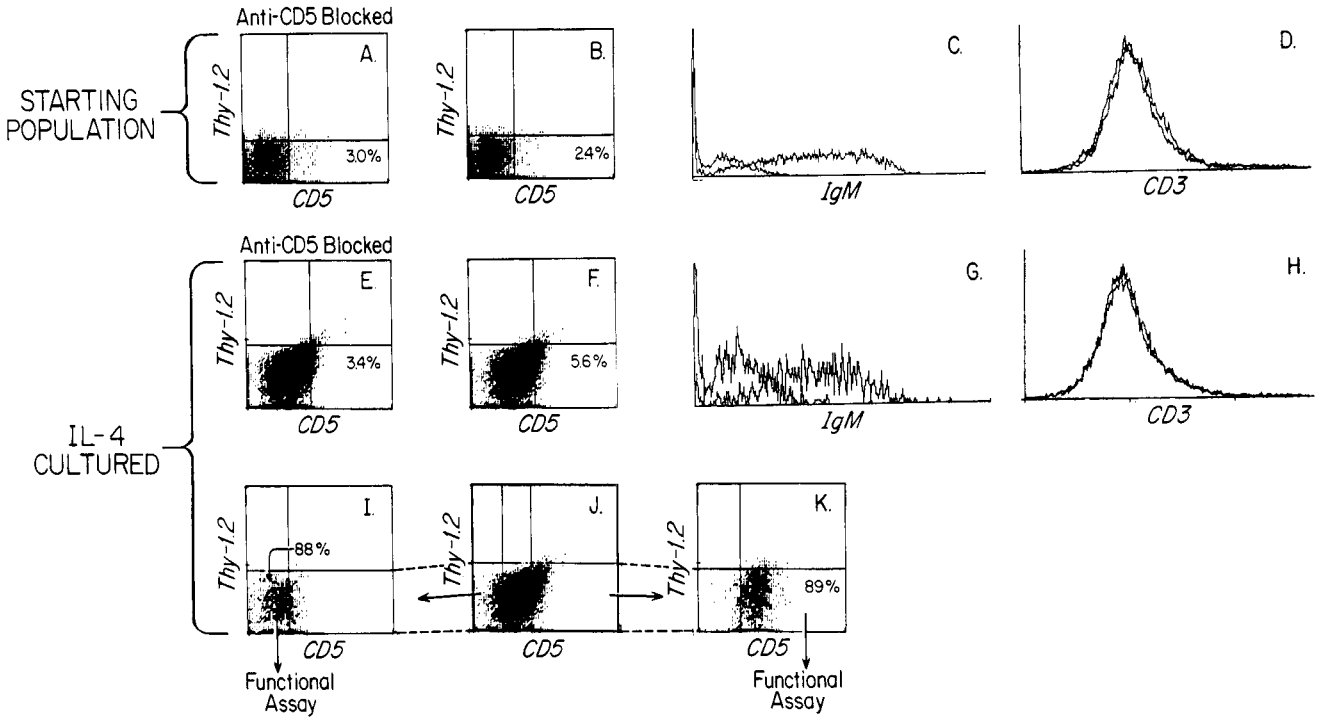

Figure 2. Flow cytometric analysis, sorting, and reanalysis of cultured B cells. $CD5^-$ B cells ("starting population") were cultured for 42 hr in media alone or in media supplemented with 20 units of rIL-4. Aliquots of the starting $CD5^-$ population and cultured populations were triple-color stained for CD5, Thy-1.2, and IgM expression in the presence or absence of 1 μg of rat anti-CD5 antibody. Aliquots were also tested in single-color stains for the expression of CD3. Samples were sorted with monocytes excluded on the basis of forward and 90° angle light scatter parameters. Each axis spans 3 logs of relative fluorescence. (Contours A and B) $CD5^-$ starting population stained in the presence or absence of unlabeled rat anti-CD5 antibody, respectively. (Histogram C) IgM profile of the starting population. (Histogram D) CD3 profile of the starting $CD5^-$ cell population. (Contours E and F) B cells cultured in the presence of rIL-4 and stained in the presence or absence of blocking anti-CD5 antibody, respectively. (Histogram G) IgM profile of IL-4 cultured B cells. (Histogram H) CD3 profile of rIL-4-cultured B cells. (Contour J) Sorting gates. (Contours I and K). Reanalyses of rIL-4-treated populations sorted for functional studies.

starting B cell population, even when a 250-fold excess of anti-CD5 nonadherent B cells (2.5×10^6, Table I, vs 10^4 unfractionated B cells, Refs. 26 and 27) was tested. However, as few as 2×10^4 B cells cultured in rIL-4 were able to affect idiotype expression. These results led to the working hypothesis that IL-4 promotes the functional maturation of $CD5^-$ or immature $CD5^+$ B cells. It is not known at this time whether IL-4 directly stimulates functionally immature B cells or whether it acts through intermediary molecules, produced perhaps by accessory cell populations (34).

Modulation of CD5 on B cell populations is supported by work from other laboratories that document the induction of CD5 on $CD5^-$ B cell tumors (46), peripheral B cell populations (47–49), and bone-marrow-derived B cell lymphomas (50). It is interesting to note that in at least two instances, IL-4 was reported to decrease CD5 expression either on human B cell tonsillar populations (51) or on a pre-B cell line (52). The data collectively indicate that expression of CD5 by B cells is variable and subject to modulation by a number of as yet poorly defined factors.

B cells transferred from IL-4 precultures do not appear to be producing NP^b idiotypic, NP-specific antibodies. Had the added B cells been secreting NP-specific antibody expressing NP^b idiotypic determinants, it would have been expected that the percentage of NP^b PFC would decrease in proportion to the number of cells added to responder cultures. However, addition of either 2×10^4 or 2×10^5 IL-4-precultured B cells to responder cultures resulted in equivalent levels of NP^b expression (63–70%, Table III). Furthermore, activity could be ablated by treatment of IL-4-precultured cells with NP^b idiotype plus complement (Table IV), substantiating their anti-NP^b idiotype specificity.

Carry-over of IL-4 into responder cultures appears not to be responsible for the specific augmentation of NP^b PFC responses. Addition of anti-IL-4 antibody, shown to be effective when added to precultures (Table II), did not affect the ability of IL-4 precultured B cells to augment NP^b idiotype expression when the antibody was added to responder cultures along with ($CD5^+$) B cells (56% NP^b idiotype, six experiments). Furthermore, IL-4-precultured $CD5^-$ B cells failed to affect idiotype expression when transferred to responder cultures either alone (Tables IV and VI) or anti anti-NP^b antibody (8 ± 20% NP^b idiotype, four experiments). Addition of

this NP^b antibody with a $CD5^+$ B cell-derived lymphokine to responder cultures has been shown to mimic the activity of $CD5^+$ B cell populations (28, 29).

Another important consideration is the potential contribution of T cells to NP^b idiotype expression. For the following reasons, we have postulated in the past that the transfer of idiotype-specific activation signals to responder B cell populations is not dependent upon T cells: (1) the activity is transferred by small numbers of nylon wool-adherent Thy-1.2^- splenic populations, (ii) the activity is readily transferred with spleen cells from athymic mice, (iii) T cell populations either alone or in combination with $CD5^-$ B cell populations do not affect NP^b idiotype expression, and (iv) the activity can be highly enriched on ligand (NP^b idiotype)-coated petri dishes. This conclusion is supported in the present studies by: (1) the demonstration that $CD5^+$, IgM^+, Thy-1^- cells, sorted from fresh splenic populations, can affect the idiotypic repertoire of antigen-specific B cell populations (Table VI).

Data supporting a similar conclusion for IL-4 precultured B cells include: (i) an equivalent ability to augment NP^b idiotype expression, (ii) the resistance of cells acquiring this activity following culture with IL-4 to treatment with anti-Thy-1.2 antibody plus complement, (iii) the ablation of activity by treatment of IL-4-cultured cells with ligand (NP^b idiotype) plus complement, (iv) the transfer of activity exclusively with cells sorted on the basis of their $CD5^+$, IgM^+, Thy-1^- phenotype, and (v) the failure to detect Thy-1.2^+ or $CD3^+$ lymphocytes by flow cytometry at either the beginning or the end of the IL-4 culture period.

While experiments are in progress to resolve the mechanism of IL-4 action on $CD5^+$ B cell precursors and to determine whether lymphokines secreted by "Th1" cells are also capable of inducing functional $CD5^+$ B cells, the present study indicates that at least one T cell lymphokine is capable of contributing to the development of $CD5^+$ B cells. It should be acknowledged that other factors are likely to be at work in the normal development of $CD5^+$ B cells (27). It will be of interest to determine whether induction of $CD5^+$ B cells with lymphokines, perhaps in concert with their perpetual stimulation by autoantigen (idiotype), contributes to their association with autoimmunity and B cell hyperactivity.

This work was supported by Grants 1-810 from the March of Dimes, Inc., AI 23978-01 from the National Institute of Allergy and Infectious Diseases, and IM-570 from the American Cancer Society and a grant from The Elsa U. Pardee Foundation. J. A. H. is a fellow of the Leukemia Research Foundation.

1. Seldin MF, Conroy J, Steinberg AD, D'Hoosteleare LA, Raveche ES. Clonal expansion of abnormal B cells in old NZB mice. J Exp Med **166**:1585–1590, 1987.
2. Caligoris-Cappio F, Gobbi M, Janossy G. Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. J Exp Med **155**:623–628, 1982.
3. Anderson KC, Bates MP, Slaughenhoupt BL, Pinkus GS, Schlossman SF, Nadler LM. Expression of human B cell associated antigens on leukemias and lymphomas: A model of human B cell differentiation. Blood **63**:1424–1433, 1984.
4. Davidson WF, Fredrickson TN, Rudikoff EK, Coffman RL, Hartley JW, Morse HC. A unique series of lymphomas related to the Lyt-1^+ lineage of B lymphocyte differentiation. J Immunol **133**:744–753, 1984.
5. Boumsell LH, Coppin H, Pham D, Raynol B, Lemerle J, Dausett J, Bernard A. An antigen shared by a human T cell subset and B cell chronic lymphocytic leukemic cells. Distribution on normal and malignant lymphoid cells. J Exp Med **152**:229–234, 1980.
6. Lanier LL, Warner NL, Ledbetter JA, Herzenberg LA. Expression of Lyt-1 antigen on certain murine B cell lymphomas. J Exp Med **153**:998–1003, 1981.
7. Hayakawa K, Hardy RR, Parks DR, Herzenberg LA. The "Ly-1 B" cell subpopulation in normal, immunodefective, and autoimmune mice. J Exp Med **157**:202–218, 1983.
8. Hardy RR, Hayakawa K, Shimizu M, Yamasaki K, Kishimoto T. Rheumatoid factor secretion from human Leu-1^+ B cells. Science **236**:81–83, 1987.
9. Nakamura M, Burastero SE, Notkins AL, Casali P. Human monoclonal rheumatoid factor-like antibodies from $CD5^+$ (Leu-1^+) B cells are polyreactive. J Immunol **140**:4180–4186, 1988.
10. Sherr DH, Dorf ME, Gibson M, Sidman CL. Ly-1 B helper cells in autoimmune "viable motheaten" mice. J Immunol **139**:1811–1817, 1987.
11. Plater-Zyberk C, Maini TN, Lam K, Kennedy TD, Janossy G. A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. Arthritis Rheum **28**:971–976, 1985.
12. Lydyard PM, Youinou PY, Cooke A. CD5-positive B cells in rheumatoid arthritis and chronic lymphocytic leukemia. Immunol Today **8**:37–39, 1987.
13. Braun J. Spontaneous *in vitro* occurrence and long-term culture of murine B lymphoblast cell lines. J Immunol **130**:2113–2116, 1983.
14. Gadol N, Ault KA. Phenotypic and functional characterization of human LEU1 (CD5) B cells. Immunol Rev **93**:23–34, 1986.
15. Sidman CL, Schultz LD, Hardy RR, Hayakawa K, Herzenberg LA. Production of immunoglobulin isotypes by $CD5^+$ B cells in motheaten and normal mice. Science **232**:1423–1425, 1986.
16. Dauphinee M, Tovar Z, Talal N. B cells expressing CD5 are increased in Sjogren's Syndrome. Arthritis Rheum **31**:642–647, 1988.
17. Iwatani Y, Amino N, Kaneda T, Ichihara K, Tamaki H, Tachi J, Matsuzuka F. Marked increase of $CD5^+$ B cells in hyperthyroid Graves's disease. Clin Exp Immunol **78**:196–200, 1989.
18. Burastero SE, Casali P, Wilder RL, Notkins AL. Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by $CD5^+$ B cells from patients with rheumatoid arthritis. J Exp Med **168**:1979–1992, 1988.
19. Sthoeger.ZM, Wakai M, Tse DB, Vinciguerra VP, Allen SL, Budman DR, Lichtman SM, Schulman P, Weiselberg LR, Chiorazzi N. Production of antibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. J Exp Med **169**:255–268, 1989.
20. Minoprio P, Bandeira A, Pereira P, Mota Santos T, Coutinho A. Preferential expansion of Ly-1 B and $CD4^-$, $CD8^-$ T cells in the polyclonal lymphocyte responses to murine *T. cruzi* infection. Int Immunol **1**:176–183, 1989.
21. Michel C, Boitard C, Bach JF. Insulin autoantibodies in nonobese diabetic (NOD) mice. Clin Exp Immunol **75**:457–462, 1989.
22. Wofsy D, Seaman WE. Successful treatment of autoimmunity in

NZB/NZW F1 mice with monoclonal antibody to L3T4. J Exp Med **161**:378–391, 1985.
23. Wofsy D. Administration of monoclonal anti-T cell antibodies retards murine lupus in BXSB mice. J Immunol **136**:4554–4560, 1986.
24. Marcos MAR, Toribio M-L, de la Hera A, Marquez C, Gaspar M-L, Martinez-A C. Mutual cell interactions and the selection of immune repertoires. Immunol Today **9**:204–206, 1988.
25. Hardin JA, Vos K, Kawano Y, Sherr DH. A function for Ly-1$^+$ B cells. Proc Soc Exp Biol Med **1952**:172–182, 1990.
26. Sherr DH, Dorf ME. An idiotype specific helper population which bears immunoglobulin, Ia and Lyt-1 determinants. J Exp Med **159**:1189–1200, 1984.
27. Sherr DH, Dorf ME. H-2 restricted helper activity mediated by immunoglobulin hearing lymphocytes. J Immunol **134**:2084–2089, 1985.
28. Sherr DH, Braun J, Dorf ME. B helper cell hybridomas. Idiotype specific Ly-1 B cell mediated helper activity. J Immunol **138**:2057–2062, 1987.
29. Hardin JA, Gibson M, Kawano Y, Spielberg S, Martin C, Collins M, Dorf ME, Sherr DH. Characterization of a B cell helper factor(s) derived from CD5$^+$ B cell hybridomas. Cell Immunol **126**:304–321, 1990.
30. Gibson M, Hardin JA, Sherr DH. CD5$^+$ B helper cell hybridomas produce a lymphokine which induces CD5$^+$ B helper cell populations. J Mol Cell Biol **4**:241–253, 1990.
31. Okumura K, Hayakawa K, Tada T. Cell to cell interaction controlled by immunoglobulin genes. Role of Thy-1$^-$, Lyt-1$^+$, Ig$^+$ (B′) cells in allotype-restricted antibody production. J Exp Med **156**:443–453, 1982.
32. Ono K, Kato F, Taniguchi M. Possible involvement of Ly-1 B cells in the effector phase of IgG suppression mediated by suppressor T cell factor. J Mol Cell Immunol **3**:167–175, 1987.
33. Bitoh S, Fujimoto S, Yamamoto H. Evidence for idiotypic and antiidiotypic B-B cellular interaction with the use of cloned antiidiotypic B cell line. J Immunol **144**:2046–2052, 1990.
34. Paul WE, Ohara J. B-cell stimulatory factor-1/interleukin 4. Annu Rev Immunol **5**:429–459, 1987.
35. Rothstein TL. Interleukin-4 enhances peritoneal B cell stimulation induced by phorbol ester. Cell Immunol **120**:450–459, 1989.
36. Scott DW, Chace JH, Warner GL, O'Garra A, Klauss GGB, Quill H. Role of T cell-derived lymphokines in two models of B-cell tolerance. Immunol Rev **99**:153–171, 1987.
37. Ohara J, Paul WE. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. Nature **315**:333–336, 1985.
38. O'Hara RM, Sherr DH, Dorf ME. *In vitro* generation of suppressor T cells: Induction of CD3$^+$, IgH restricted suppressor cells. J Immunol **141**:2935–2942, 1988.
39. Julius MH, Simpson E, Herzenberg LA. A rapid method for isolation of functional thymus-derived murine lymphocytes. Eur J Immunol **3**:645–649, 1973.
40. Kaye J, Gillis S, Mizel SB, Shevach EM, Malek TR, Dinarello CA, Lachman LB, Janeway CA. Growth of a cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: Interleukin 1 is required for the expression of receptors for Interleukin 2. J Immunol **133**:1339–1345, 1984.
41. Sherr DH, Dorf ME. Characterization of anti-idiotypic suppressor T cells (Tsid) induced after antigen priming. J Immunol **133**:1142–1150, 1984.
42. Hirai Y, Nisonoff A. Selective suppression of the major idiotypic component of an anti-hapten response by soluble T cell derived factors with idiotypic or anti-idiotypic receptors. J Exp Med **151**:1213–1218, 1980.
43. Andersson J, Coutinho A, Melchers F. Frequencies of mitogen-reactive B cells in the mouse. II: Frequencies of B cells producing antibodies which lyse sheep or horse erythrocytes, and trinitrophenylated or nitroiodophenylated sheep erythrocytes. J Exp Med **145**:1520–1530, 1977.
44. Nishikawa S, Takemori T, Rajewsky K. The expression of a set of antibody variable regions in lipopolysaccharide-reactive B cells at various stages of ontogeny and its control by anti-idiotypic antibody. Eur J Immunol **13**:318–325, 1983.
45. Stashenko P, Klinman NR. Analysis of the primary anti-(4-hydroxy-3-nitrophenyl) acetyl (NP) responsive B cells in Balb/c and B10.D2 mice. J Immunol **125**:531–537, 1980.
46. Kearney JF, Vakil M. Idiotype-directed interactions during ontogeny play a major role in the establishment of the adult B cell repertoire. Immunol Rev **94**:40–49, 1986.
47. Miller RA, Gralow J. The induction of Leu-1 antigen expression in human malignant and normal B cells by phorbol myristic acetate (PMA). J Immunol **133**:3408–3414, 1984.
48. Freedman AS, Freeman G, Whitman J, Segil J, Daley J, Nadler LM. Studies of *in vitro* activated CD5$^+$ B cells. Blood **73**:202–208, 1989.
49. Ying-Zi C, Rabin E, Wortis HH. Treatment of murine CD5$^-$ B cells with anti-Ig, but not LPS, induces surface CD5: Two B cell activation pathways. Int Immunol **3**:467–476, 1991.
50. Palacious R, Stuber S, Rolink A. The epigenetic influences of bone marrow and fetal liver stroma cells on the developmental potential of Ly-1$^+$ pro-B lymphocyte clones. Eur J Immunol **19**:347–356, 1989.
51. Defrance T, Vanbervliet B, Durand I, Banchereau J. Human interleukin down-regulates the surface expression of CD5 on normal and leukemic B cells. Eur J Immunol **19**:293–299, 1989.
52. Jyonouchi H, Voss RM, Good RA. Up-regulation and down-regulation of cell surface and mRNA expression of CD5 antigen by various humoral factors on murine 70Z/3 pre-B cell leukemia cell line: IL-4 down-regulates CD5 antigen expression. Cell Immunol **130**:66–78, 1990.