CELL-TO-CELL INTERACTION CONTROLLED BY IMMUNOGLOBULIN GENES

Role of Thy-1^-, Lyt-1^+, Ig^+ (B′) Cell in Allotype-restricted Antibody Production

By KO OKUMURA, KYOKO HAYAKAWA, and TOMIO TADA

From the Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo, Japan

A number of recent reports indicate the existence of more than one type of helper T cell (1–6). A previous publication from our laboratory (3) demonstrated two types of carrier-specific helper T cells that independently and synergistically exert helper effects on the hapten-specific antibody response. One type (Th_1)[1] is a more conventional helper T cell that helps hapten-specific B cells only in the presence of the relevant hapten-carrier conjugate (cognate help), whereas the other (Th_2) can help B cells stimulated with hapten on a different carrier (polyclonal help). A notable difference is that Th_2 possesses determinants coded for by a gene in I-J subregion of H-2 major histocompatibility complex, whereas Th_1 has not detectable Ia antigens. Both belonged to the Lyt-1^+, 2^-, 3^- subset. Similar findings have been reported by others using different experimental systems (4, 5).

Another criterion for distinguishing different types of helper T cells is whether their helper effect is restricted to B cells expressing certain allotypes and idiotypes (7–11). It has been noted (7) that allotype-specific suppressor T cells (Ts) inhibit the production of one allotype but not the other in F_1 animals through the neutralization of helper T cells. Because production of the second allotype is unaffected, it was presumed that there exists a helper T cell population recognizing allotype-related structures on B cells. Two kinds of helper cells, one being a conventional antigen-specific helper T cell and the other recognizing immunoglobulin (Ig) structures on responding B cells, were reported by Janeway et al. (2, 12–14). These two helper subsets act synergistically to promote a higher antibody response than either one alone. In addition, recent studies by many investigators demonstrate a specialized T cell population that preferentially help idiotype-positive responses (5, 8, 10, 11).

To determine whether our Th_1 or Th_2 are identical to the Ig-restricted helper T cell reported by other systems (7), we co-cultured B cells and Th_1 or Th_2 derived from Ig congenic strains and measured antibody production. No Ig restriction between B cells and either type of helper T cells was detected (K. Okumura et al., unpublished observation). Thus, we set up a series of experiments to identify Ig-restricted helper T cells. By the addition of different fractionated cells into cultures of B cells with limited

[1] *Abbreviations used in this paper:* Ars, arsonic acid; B′, Thy-1^-, Lyt-1^+, Ig^+ nylon-adherent cells; BAT, brain-associated T cell antigen; BSA, bovine serum albumin; C, complement; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; KLH, keyhole limpet hemocyanin; MEM, Eagle's minimal essential medium; MIg, mouse immunoglobulin; Th_1, nylon-nonadherent helper T cells; Th_2, nylon-adherent helper T cells.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/81/08/0443/11 $1.00
Volume 156 August 1982 443–453

numbers of conventional helper T cells, we found a novel cell type with a characteristic membrane phenotype that greatly augmented the antibody response only when B cells and this new cell type share the same Igh gene allotype. This cell type, designated B′ cell, is characterized by the surface expression of Ig and Lyt-1 antigen. The significance of the B′ cell in the Ig-restricted regulation of the antibody response is reported.

Materials and Methods

Animals. C3H/HeN and BALB/cAnN mice were obtained from Ohmura Laboratory Animals, Sagamihara, Kanagawa, Japan. C3H.SW/Hz (CSW), CWB (C3H.SW-Ig-1^b/Hz), and BAB/14 (BALB/c C57BL/Ka-Ig-1^b/Hz) were produced from breeding pairs provided by Dr. L. A. Herzenberg (Department of Genetics, Stanford University, Stanford, CA) and maintained in our animal facilities. CB-20 mice were a generous gift from Dr. S. Migita (Cancer Research Institute, Kanazawa University, Kanazawa, Japan). C3H *nu/nu*, C3H *nu/+* were supplied by Central Institute for Experimental Animals, Kawasaki, Japan. All mice were used at 6–12 wk of age.

Antigens and Immunization. Dinitrophenylated keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA) with eight groups per 10^5 daltons 2,4-dinitrophenyl$_8$ (DNP$_8$)-KLH) and DNP$_{31}$-bovine serum albumin (BSA) were prepared by the method of Eisen et al. (15). Mice were immunized with 100 μg of DNP-KLH or KLH with 1×10^9 of *Bordetella pertussis* vaccine. They were killed to obtain DNP-primed B cells and KLH-primed helper T cells 6–8 wk after immunization.

Antisera and Monoclonal Antibodies. A rabbit anti-mouse brain-associated T cell antigen (anti-BAT) was prepared as described previously (3). A polyvalent anti-mouse Ig antiserum (anti-MIg) was obtained by immunizing a rabbit twice with 100 μg of mouse Ig precipitated by 45% ammonium sulfate from normal mouse serum. Antibodies were purified by acid elution from Sepharose beads conjugated with mouse Ig that were purified from a protein A column. Fab and F(ab′)$_2$ fragments of purified anti-MIg were prepared according to the method of Nisonoff et al. (16). Rabbit anti-goat Ig was obtained by immunizing rabbits with purified goat Ig. An anti-ThB antiserum was raised in a goat by repeated immunization of MOPC 104E myeloma cells as described by Yutoku et al. (17). This antiserum was absorbed with Ehrlich tumor cells to remove cytotoxicity against mouse common lymphocyte antigens and were absorbed twice with an equal volume of pelleted thymocytes until no cytotoxicity against splenic T cells was detected. Monoclonal rat antibodies to mouse Thy-1.2, Lyt-2, and their arsonic acid (Ars) derivatives (Ars-anti-Lyt-1, Ars-anti-Lyt-2) were generously donated by Dr. L. A. Herzenberg. Rabbit anti-Ars antiserum prepared by immunizing rabbits with azobenzenarsonate coupled to BSA was kindly supplied by Dr. T. Hamaoka, Department of Oncogenesis, Institute of Cancer Research, Osaka University, Osaka, Japan. For indirect fluorescence staining of cells with monoclonal rat antibodies, a fluoresceinated mouse (SJL) antiserum against rat IgG was used. Anti-Ia^k (A.TH anti-A.TL) was prepared in our laboratory by repeated immunization of A.TH mice with A.TL spleen cells.

Preparation of ^{125}I-labeled Anti-Allotype Antibody for the Estimation of Antibody. Anti-allotype antibodies (anti-Igh-1^a, anti-Igh-4^a, anti-Igh-1^b, anti-Igh-4^b) were labeled with ^{125}I by the method of solid-phase labeling as described by Tsu and Herzenberg (18). The sources of these antibodies were C57BL/6 anti-CSW (anti-Igh-1^a and anti-Igh-4^a), CSW anti-C57BL/6 (anti-Igh-1^b), and SJA anti-SJL (anti-Igh-4^b). Purified myeloma protein from X5563 (Igh-1^j), MOPC31C (Igh-4^a), and CBPC101 (Igh-1^b) and Igh-4^b protein purified from normal serum of SJL were coupled to Sepharose to obtain allotype-specific reagents. The specificity of each reagent was confirmed by the radioimmunoinhibition assay (18).

Preparation of Hapten-primed B Cells Devoid of B′ Cells. A pure B cell fraction was prepared from DNP-KLH-primed spleen cells (3×10^7 cells/ml) by treatment with a mixture of 1:50 diluted anti-BAT and monoclonal arsonic acid (Ars)-conjugated anti-Lyt-1 (10 μg/3×10^7 cells) at room temperature for 20 min, followed by further incubation with 1:20 diluted rabbit anti-Ars (3×10^7 cells/ml) and guinea pig complement (C) at 37°C for 45 min. This procedure was adopted to eliminate Lyt-1$^+$, Ig$^+$ (B′) cells from the B cell fraction (see below).

Preparation of Th-1 and Th-2 Specific for KLH. KLH-primed spleen cells were fractionated into nylon-wool-adherent and -nonadherent populations as described previously (3). In brief, 1 g of nylon wool (Leuko-Pak, Fenwal Laboratories, Morton Grove, IL) was tightly packed in a 20-ml syringe to the 10-ml level and washed with warm (37°C) Eagle's minimal essential medium (MEM) supplemented with 3% fetal calf serum (FCS) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY). 1×10^8 spleen cells were applied to the column and incubated at 37°C for 45 min. Nylon-nonadherent cells (Th_1) were eluted with 10 ml of warm medium. After washing the column with warm medium, the adherent cells (Th_2) were obtained by teasing the nylon wool in chilled MEM. As this fraction contained a large number of B cells, the nylon-adherent cells were further incubated in a rabbit anti-MIg coated petri dish (3008; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) to remove all Ig^+ cells, including $Lyt\text{-}1^+$, Ig^+ cells (B′ cell).

Preparation of a Normal B Cell Fraction Containing $Lyt\text{-}1^+$, $Thy\text{-}1^-$ Cells (B′ Cell). The cell type designated as B′ cell was obtained from the nylon wool-adherent cell fraction from nonprimed spleen cells by treatment of the cell suspension with monoclonal rat anti-Thy-1.2 ($5\ \mu g/2 \times 10^7$) and guinea pig C. This cell population containing ~3–5% of $Lyt\text{-}1^+$, $Thy\text{-}1^-$, Ig^+ cells and <0.5% of $Thy\text{-}1^+$ cells was used as B′ cell fraction.

Trypsin Treatment for the Removal of Cell Surface Ig. To strip off cell surface Ig from B′ cells, cells (2×10^7 cells/ml) were incubated in 2.5 mg/ml of trypsin- (Sigma Chemical Co., St. Louis, MO) containing medium for 30 min at 37°C. After washing with trypsin inhibitor- (Sigma Chemical Co.) containing medium, cells were cultured in RPMI 1640 medium supplemented with 10% FCS to regenerate endogenously synthesized cell surface Ig.

Cell Culture Technique. A mixture of B cells and graded numbers of T cells with or without B′ cells were cultured in a 24-multiwell plate (Falcon 3008) using RPMI 1640 medium supplemented with 10% FCS and 5×10^{-5} M 2-mercaptoethanol, as described previously (19). At the start of culture, 0.05 μg of DNP-KLH was added, then 3 d after the cultivation the culture supernatant was aspirated and replaced by an equal amount of fresh medium. The amount of antibody produced in culture supernatant was assessed by the radioimmunoassay 8 d after the initiation of culture. Schema of the experimental system are depicted in Fig. 1.

Estimation of Anti-DNP Antibody in Culture Supernatant. Quantitation of anti-DNP antibodies of different classes was done by the solid-phase radioimmunoassay using anti-allotype reagents according to the method described by Tsu and Herzenberg (18). The wells of polyvinyl plates (Tomy titer plate; Tomy-seiko, Tokyo, Japan) were precoated with DNP_{31}-BSA (5 μg in 50 μl), and 40 μl of culture supernatant was applied. The plate was incubated for 1 h at room temperature, washed three times with 1% BSA in phosphate-buffered saline, and ^{125}I-labeled anti-allotype antibodies were applied to each well. The radioactivity bound to each well was measured by a gamma scintillation counter, and the amount of antibodies of each class was extrapolated from a plot of the standard curve made by a secondary anti-DNP antiserum from (SJL × BALB/c)F_1 mice containing known levels of the four allotypes. The standard antiserum

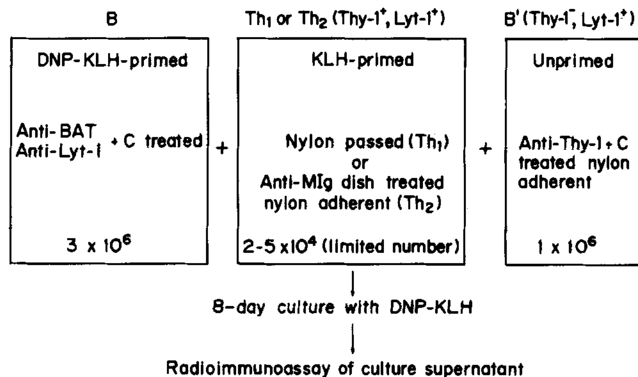

FIG. 1. Co-culture system to examine B′ cell function (see Materials and Methods).

was provided by Dr. L. A. Herzenberg. The amount of antibody produced was calculated from the mean of five culture wells.

Results

The Supplementation of Suboptimum Helper Effect by the Addition of B′ Cell (Lyt-1$^+$, Thy-1$^-$) from Normal Mice. DNP-primed B cells were obtained by treating DNP-KLH-primed spleen cells from CSW mice with anti-BAT and anti-Lyt-1 antisera. Such B cells were co-cultured in the presence of DNP-KLH with graded numbers of KLH-specific helper T cells of either Th_1 or Th_2 type (Fig. 1). Fig. 2 shows the helper T cell-dependent anti-DNP antibody response of DNP-primed B cells. To this system, 1×10^6 cells of a B′ cell fraction containing Thy-1$^-$, Lyt-1$^+$ nylon wool-adherent cells were added. As demonstrated in Fig. 2, the addition of this latter cell type greatly augmented the antibody response in the range where the number of helper T cells (Th_1 or Th_2) was suboptimal. Thus, B′ cells were found to supplement the suboptimum helper effect delivered from carrier-specific T cells to induce the maximum antibody production.

Table I shows the dose effect of B′ cells on the IgG_{2a} (Igh-1) and IgG_1 (Igh-4) anti-DNP antibody responses as determined by radioiodinated anti-allotype antibodies. The addition of the B′ cell fraction enhanced the production of both classes of anti-DNP antibodies, and this enhancement was primarily dependent on the dose of B′ cells.

Characterization of B′ Cell in Athymic Nude Mouse. Because a normal B cell fraction obtained by treatment with anti-Thy-1 and C was capable of supplementing the suboptimum helper effect, the question was asked whether such cells are present in athymic nude mouse. DNP-primed B cells and the limited number of KLH-specific Th_2 from C3H mice were co-cultured to obtain basal anti-DNP antibody response. As a source of B′ cells, nylon wool-adherent spleen cells of unprimed nude mice of C3H background were added to the culture. As depicted in Fig. 3, the addition of nude spleen cells showed a definite augmenting activity, indicating that B′ cells are present in athymic nude mouse.

The surface phenotype of B′ cells in nude mouse was studied by the treatment of the cells with various antibodies and C. As shown in Fig. 3, the treatment with anti-Thy-1.2, anti-Lyt-2, anti-Ia^k, or anti-ThB did not eliminate the augmenting activity, whereas the treatment with anti-Lyt-1 and C completely abolished the activity. This indicates that the observed augmenting activity is performed by a cell type expressing

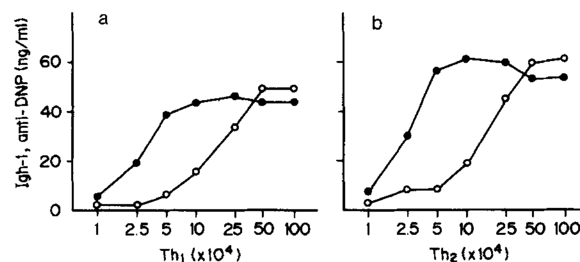

FIG. 2. Synergistic effect of Th_1, Th_2, and B′ cells. Graded numbers of KLH-primed Th_1 or Th_2 from CSW were co-cultured with DNP-primed B cells (3×10^6). 1×10^6 B′ cells were added to each culture. DNP-specific antibody response depicted was measured after 8 d of culture. a, Th_1 + B (○); Th_1 + B + B′ (1×10^6) (●). b, Th_2 + B (○); Th_2 + B + B′ (1×10^6) (●).

TABLE I

*Titration of B′ Cell Fraction in the Augmentation of Antibody Production Induced by B Cells and Limited Numbers of Th_1**

DNP-primed B cells (× 10^6)	KLH-primed Th_1 (× 10^4)	Unprimed B′ cells (× 10^5)	Anti-DNP‡	
			Igh-1	Igh-4
			ng/ml	
3	—	—	<5	<5
3	2.5	—	<5	<5
3	2.5	2.5	8.2 ± 2.2	10.1 ± 2.4
3	2.5	5	18.4 ± 3.3	24.3 ± 4.6
3	2.5	10	19.9 ± 4.2	23.9 ± 5.7
3	5	—	5.5 ± 2.6	6.1 ± 4.2
3	5	2.5	13.3 ± 3.1	18.5 ± 5.9
3	5	5	38.2 ± 6.1	44.4 ± 4.6
3	5	10	40.1 ± 4.3	42.1 ± 3.3

* Cells used for co-culture were prepared from CSW.
‡ Arithmetic means and SD were calculated from five cultures.

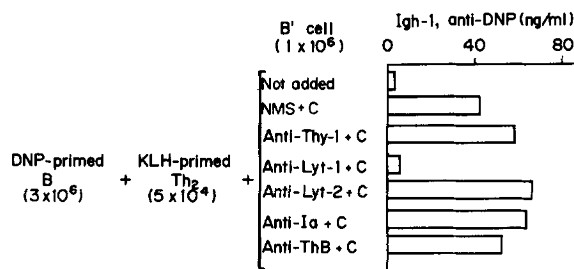

FIG. 3. Characterization of surface phenotype of B′ cell with various antisera. B and Th_2 cells were prepared from C3H. B′ cells from C3H *nu/nu* that were treated with antisera indicated and were co-cultured with B and Th_2.

Lyt-1 but not Thy-1 antigen. The frequency of Lyt-1^+ cells in the nude spleen cells determined by fluorescence analysis with a fluorescence-activated cell sorter (FACS) was 2–3%.

Presence of Ig Determinant on B′ Cell. The presence of surface Ig on this cell type was confirmed by the following experiments. The nylon wool-adherent cells from C3H mice were incubated in plastic dishes coated with antibodies against Ig determinants. The cells that did not adhere to the dishes were examined for their augmenting activity by adding to the culture of B and Th_2 cells. As shown in Fig. 4, the incubation in the dishes coated with rabbit anti-MIg resulted in the loss of ability to supplement the helper T cell. The dishes coated with $F(ab')_2$ or Fab fragment of rabbit anti-MIg also removed the cells responsible for the augmentation, while the incubation in the rabbit anti-goat Ig antibody coated dishes failed to do so. The identical results were obtained with the nylon-adherent population of athymic nude mice. The cells responsible for the observed enhancing effect were unlikely to be monocytes or Fc receptor-bearing T cells. This was proven by the following experiment. Nylon wool-adherent cells of CSW mice were treated with trypsin to remove cell surface Ig (see Materials and Methods) and then cultured for 12 h to allow regeneration of surface Ig. The cells were further incubated in Fab anti-MIg-coated dishes to remove B′ cell

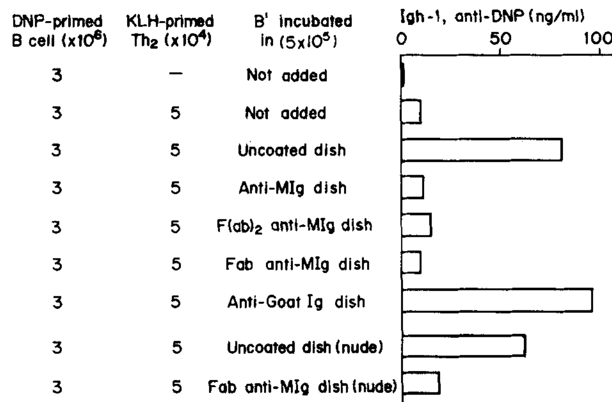

FIG. 4. Presence of Ig on B′ cell. Anti-Thy-1-treated spleen cells (B′ cell fraction) were further incubated in antibody-coated petri dishes. Nonadherent cells were tested for the enhancing activity. Note that the complete loss of B′ cell function occurred by incubating anti-MIg dishes.

TABLE II

*Evidence That B′ Cells Carry Endogenously Synthesized Surface Ig**

DNP-primed B cells (× 10^6)	KLH-primed Th_1 (× 10^4)	Trypsinized B′ cells (× 10^6)‡	Absorption of B′ cells with ‡	Anti-DNP Igh
				ng/ml
3	—	0	—	<5
3	2.5	0	—	6.2 ± 2.6
3	2.5	1.0	Uncoated dishes	52.3 ± 4.9
3	2.5	1.0	Fab anti-MIg dishes	8.6 ± 3.1

* Cells used for co-culture were prepared from CSW.

‡ B′ cell fraction was treated with trypsin (2.5 mg/ml) to digest surface Ig. After this treatment, <0.1% of lymphocytes were stained with fluoresceinated anti-MIg. Cells were cultured for 12 h for the *de novo* synthesize of surface Ig. More than 80% were positively stained at this time. Such cells were then applied to petri dishes with or without anti-MIg antibodies. The cells that did not adhere to the dishes were added to the culture of B and Th_1.

activity. As shown in Table II, the trypsin-treated cells after regeneration of surface Ig still had an augmenting activity that was removed by incubation in anti-MIg dishes. These results indicate that the B′ cells express endogenous Ig together with Lyt-1 antigen.

Igh-linked Restriction between B and B′ Cell for the Augmentation of Antibody Production. To delineate the significance of B′ cells in the regulatory cell interaction, co-culture experiments were performed with B cells, Th_1 or Th_2 cells, and normal B′ cells obtained from Igh-allotype congenic mice, i.e., CSW (Igh^a) and CWB (Igh^b). Cell types of different origins were co-cultured by the protocol depicted in Fig. 5. In short, DNP-primed B cells prepared from CSW or CWB were co-cultured with a limited number of Th_1 or Th_2 or syngeneic or reciprocal combinations. B′ cells from unprimed CSW or CWB mice were supplemented. As shown in Fig. 5, regardless of the origin (CSW or CWB) of KLH-primed helper T cells (both Th_1 and Th_2), the anti-DNP response was greatly augmented only when the Igh-allotype of B and B′ cells was identical. No augmentation was observed when the Igh-allotype of B and B′ cells differed. Such an effect was completely independent of the source of Th_1 or Th_2 cells.

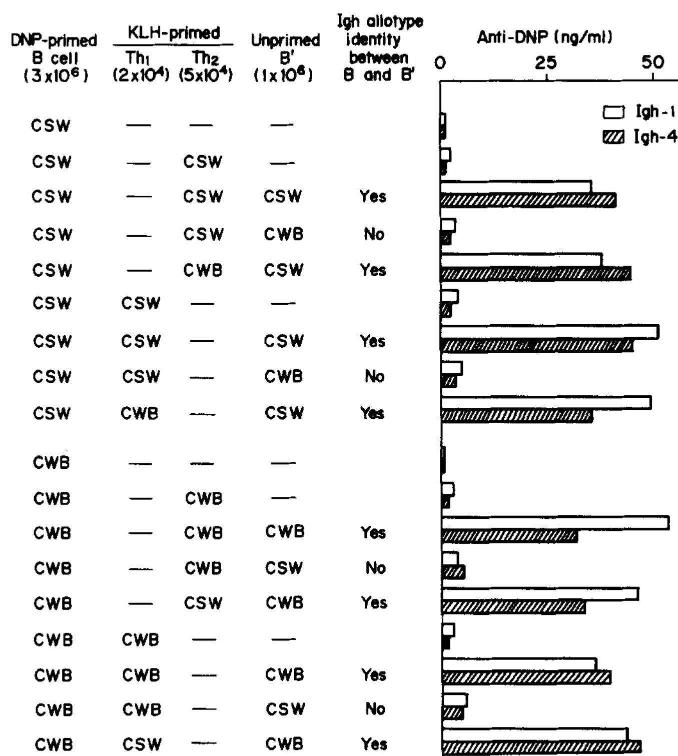

FIG. 5. Igh allotype restriction for synergistic interaction between B and B′ cells. Th_1, Th_2, B, and B′ cells were obtained from Ig congenic mice and were co-cultured to study the requirements of critical identity of genes between them. Note that only when B and B′ cell are an Ig-compatible combination was prominent augmentation observed.

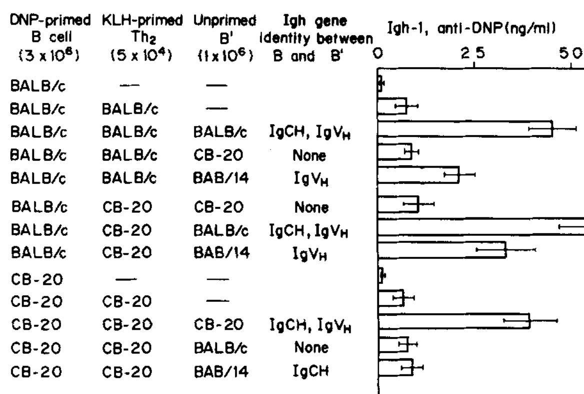

FIG. 6. IgV_H gene restriction for synergistic interaction between B and B′ cells. Th_2, B, and B′ cells were obtained from IgV_H or IgCH congenic strains. Note that IgV_H-matched combinations, i.e., BALB/c B and BAB/14 B′ cells, induce the effective augmentation, and IgCH identity is not required.

Having established that Igh genes are the restricting elements in the B and B′ cell interaction, we asked whether the identity of IgCH or IgV_H genes is required for the effective B–B′ cell collaboration. This was examined by the use of congenic mice that

share only IgV_H of IgCH genes. The combination of animals is shown in Fig. 6. The augmentation was observed in the syngeneic combination where both IgCH and IgV_H-compatible between B and B′ cell.

No enhancement was observed when the Ig genes of B cells were totally different from B′ cell, i.e., B cells from BALB/c and B′ cells from CB-20 and vice versa. However, when B and B′ cells shared the same IgV_H (i.e., the combination of B cells from BALB/c and BAB/14 B′ cells), a significant augmentation was observed. In contrast, no augmentation was observed when B cells from CB-20 (Igh^b) were co-cultured with B′ cells from BAB/14 (Igh^b), where B and B′ cells share only IgCH genes. Thus, the minimum requirement for the effective Ig restriction between B and B′ cell is the identity of IgV_H genes.

Discussion

The present communication described a novel lymphocyte subset characterized by the dual expression of surface Ig and Lyt-1 antigen, which augments the antibody response by B cells under the condition where conventional helper T cell is limited. The cell type designated as B′ cell differs from either conventional B or T cells by its coincidental expression of Ig and Lyt-1. Although the existence of such cells has been reported by Ledbetter et al. (21), no functional role associated with this cell type has been demonstrated.

We have shown that the addition of a small number of B′ cells from unprimed animals greatly enhanced the in vitro antibody response of animals sharing the same Ig allotype locus with the donor of B′ cells. No enhancement was observed when B′ cells and responding B cells differed in Ig genes. The requirement of Ig gene identity was found to exist between B′ and B cells, but no such requirement was observed in the collaboration between B or B′ cells and helper T cells. B′ cells are by themselves not helper cells, as they alone cannot induce the B cell response. Thus, B′ cells appear to deliver an additional signal other than the T cells' help to the responding B cells for their expansion. The nature of this cooperative signal is not antigen specific, as the normal unprimed B′ cells can deliver the same effect. Such a signal may, in fact, be delivered by the surface Ig, as the critical identity of Ig genes between B and B′ cells is needed. Because Lyt-1 antigen is in general associated with the inducer function (22, 23), Lyt-1^{+}, Ig^{+} (B′) cells may also be a type of inducer cell in the Ig-restricted cell interation.

Herzenberg et al. (7) reported the presence of allotype-specific helper T cells (Ig-Th) based on the fact that the targets of allotype-specific suppressor T cells (allotype Ts) are neither B cells nor carrier-specific Th. They demonstrated a cell type that is required for the production of one of the parental allotypes in F_1 mice (24, 25). The nature of such Ig-Th has not yet been well established. The Ig-Th being affected by allotype-specific Ts was detectable so far in the experiments only with (SJA × BALB/c)F_1 and (SJL × BALB/c)F_1 (7, 24, 25), whereas Igh-gene-restricted T-B cell cooperation has not been definitively demonstrated in other systems, including ours. Whether the target of allotype Ts is, indeed, Ig-Th or the presently described B′ cells has to be re-examined. As yet, the B′ cell function in allotype or idiotype-suppressed animals has not been studied.

The existence of Th that preferentially help B cells with restricted IgCH or IgV_H (idiotype) has been reported by a number of investigators (7–14, 24, 25). Janeway et

al. (14) proposed that the Ig-Th is generated during the T cell differentiation by making contact with an Ig-related structure and that Ig-Th delivers the second signal to B cells expressing that Ig. Recent reports by Nutt et al. (26) and L'Age-Stehr (27) also indicated that Ig-Th was induced by priming of T cells with Ig-determinants of B cells. The relationship between these Ig-Th and B′ cells is also unclear.

Taking all available information about Ig-restricted cell interactions together, B′ cells seem to play a key role in the full-blown B cell response. As B′ cells are present in unprimed athymic nude mice, they have developed independently from a thymic environment and may actually be involved in the expansion and limitation of the B cell repertoire that constitutes the immune network. As the depletion of B′ cells limited the magnitude of B cell response, it is presumed that B′ cells induce a series of B–B cell interactions to magnify the B cell response triggered by antigen and a limited number of T cells. This may lead to a disturbance of network. Indeed, the cooperative interaction occurs only if B′ cell and B cells share IgV_H genes, which suggests that B′ cells are involved in the cascade reaction among the members in the network repertoire. Our subsequent studies demonstrate that the depletion of B′ cells, in fact, diminishes the production of an Igh-linked major cross-reactive idiotype in the antibody formation against 4-hydroxy-3-nitro-phenylacetyl (K. Okumura et al., manuscript in preparation).

The presence of such cells in unprimed animals also suggests that B′ cells may indeed constitute the internal image set of the Jerne's network formula (28), by which idiotype and isotype restricted Th may be generated. A crucial role of B′ cells in the idiotype regulation will be presented in the succeeding paper.

Summary

A novel lymphocyte subpopulation, designated "B′ cell" because of its characteristic dual expression of Ig and Lyt-1 antigen, is described in relation to its ability to augment the in vitro secondary antibody response. The cells are found in the spleens of normal unprimed mice as well as those of athymic nude mice and represent a small number (2–3%) of immunoglobulin-positive cells. No other distinguishing surface markers of conventional T and B cells, such as Thy-1, Lyt-2, Ia, and ThB antigens, are detected on the B′ cell. In the in vitro anti-hapten secondary antibody response, the addition of a small number of B′ cells from unprimed mice to the mixture of T and B cells greatly augmented the anti-hapten antibody formation when the number of carrier-specific helper T cells was limited. This augmentation was observed only when B and B′ cells shared the same set of IgV_H genes. The identity of the immunoglobulin gene between T cells and B or B′ cells was not necessary for optimum antibody production. The results indicate that the presence of B′ cells is necessary for the induction of an optimum antibody response when helper T cells are limited. It is suggested that B′ cells deliver an additional signal to the B cell network to magnify the antibody response.

We are grateful to Dr. J. Ledbetter for supplying monoclonal antibodies, Dr. L. A. Herzenberg for discussion and encouragement, Dr. R. Hardy, Dr. P. Nakajima, and Dr. H. H. Wortis for helpful suggestions, and Ms. Yoko Yamaguchi and Ms. Mariko Sato for their excellent secretarial work in preparing the manuscript.

Received for publication 7 April 1982.

References

1. Marrack, P. C., and J. W. Kappler. 1974. Antigen-specific and non-specific mediators of T cell/B cell cooperation. I. Evidence for their production by different T cells. *J. Immunol.* **114:**1116.
2. Janeway, C. A. 1975. Cellular cooperation during in vivo anti-hapten antibody responses. I. The effect of cell number on the response. *J. Immunol.* **114:**1394.
3. 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.* **147:**446.
4. Janeway, C. A., D. L. Bert, and F.-W. Shen. 1979. Cell cooperation during in vivo anti-hapten antibody responses. V. Two synergistic $Ly\text{-}1^+23^-$ helper T cells with distinctive specificities. *Eur. J. Immunol.* **10:**231.
5. Bottomly, K., and D. E. Mosier. 1979. Mice whose B cells cannot produce the T15 idiotype also lack an antigen-specific helper T cell required for T15 expression. *J. Exp. Med.* **150:**1399.
6. Eichmann, K., I. Falk, I. Melchers, and M. M. Simon. 1980. Quantitative studies on T cell diversity. I. Determination of the precursor frequencies for two types of Streptococcus A-specific helper cells in nonimmune, polyclonally activated splenic T cells. *J. Exp. Med.* **152:**477.
7. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, J.-W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T cell regulation of antibody responses: demonstration of allotype specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.
8. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotypic selectivity in the cooperation between T and B lymphocytes. *Eur. J. Immunol.* **8:**846.
9. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes in lymphocyte interactions. II. Antigen-independent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. *Eur. J. Immunol.* **8:**853.
10. 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.
11. 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.
12. Janeway, C. A. 1975. Cellular cooperation during in vivo anti-hapten antibody responses. II. The effect of in vivo and in vitro X-irradiation on T and B cells. *J. Immunol.* **114:**1402.
13. Janeway, C. A. 1975. Cellular cooperation during in vivo anti-hapten antibody responses. III. The helper cell activity of activated thymocytes, of spleen cells treated with anti- serum, and of spleen cells from anti-thymocyte serum-treated or adult thymectomized donors. *J. Immunol.* **114:**1408.
14. Janeway, C. A., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. *Proc. Natl. Acad. Sci. U. S. A.* **74:**4582.
15. Eisen, H. W., S. Belman, and M. E. Carsten. 1953. The reaction of 2,4-dinitrobenzenesulfonic acid with free amino group of proteins. *J. Am. Chem. Soc.* **75:**4583.
16. Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley. 1960. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* **89:**230.
17. Yutoku, M., A. L. Grossberg, R. Stout, L. A. Herzenberg, and D. Pressman. 1976. Further studies on Th-B: a cell surface antigenic determinant present on mouse B cell, plasma cells and immature thymocytes. *Cell. Immunol.* **23:**140.
18. Tsu, T. T., and L. A. Herzenberg. 1980. Solid-phase radioimmunoassays. *In* Selected

Methods in Cellular Immunology. B. B. Mishell and S. M. Shiigi, editors. W. H. Freeman & Company Publishers, San Francisco, California. 337.
19. Okumura, K., T. Takemori, T. Tokuhisa, and T. Tada. 1979. Specific enrichment of the suppressor T cell bearing I-J determinants: parallel functional and serological characterizations. *J. Exp. Med.* **146:**1234.
20. Riblet, R., B. Blomberg, M. Weigert, R. Lieberman, B. A. Taylor, and M. Potter. 1975. Genetics of mouse antibodies. I. Linkage of the dextran response locus, V_H-DEX, to allotype. *Eur. J. Immunol.* **5:**775.
21. Ledbetter, J. A., R. V. Rouse, H. S. Micklem, and L. A. Herzenberg. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. *J. Exp. Med.* **152:**280.
22. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T cell subclasses as a differentiative process independent of antigen. *J. Exp. Med.* **141:**1376.
23. Cantor, H., and R. K. Gershon. 1979. Immunological circuits: cellular composition. *Fed. Proc.* **38:**2064.
24. Herzenberg, L. A., S. J. Black, and L. A. Herzenberg. 1980. Regulatory circuits and antibody response. *Eur. J. Immunol.* **10:**1.
25. Herzenberg, L. A., K. Okumura, and C. M. Metzler. 1975. Regulation of immunoglobulin and antibody production by allotype suppressor T cells in mice. *Transplant. Rev.* **27:**57.
26. Nutt, N., J. Haber, and H. V. Wortis. 1981. Influence of Igh-linked gene products on the generation of T helper cells in the response to sheep erythrocytes. *J. Exp. Med.* **153:**1225.
27. L'Age-Stehr, J. 1981. Priming of T helper cells by antigen-activated B cells. B cell-primed Lyt-1^+ helper cells are restricted to cooperate with B cells expressing the IgV_H phenotype of the priming B cells. *J. Exp. Med.* **153:**1236.
28. Jerne, N. K. 1974. Toward a network theory of the immune system. *Ann. Immunol. Inst. Pasteur.* **125C:**337.