

# REGULATION OF MURINE B CELLS THROUGH SURFACE IMMUNOGLOBULIN

## I. Monoclonal Anti- $\delta$ Antibody that Induces Allotype-Specific Proliferation\*

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Surface immunoglobulin (sIg)<sup>1</sup> molecules are the antigen-specific receptors on B lymphocytes (1). The precise role of sIg in regulating B lymphocyte function has been the subject of considerable investigation; a debate still exists over whether sIg acts as a signaling device per se (2), or whether it merely serves to focus antigen on the cell surface (3).

The majority of splenic B lymphocytes from adult mice bear both sIgM and sIgD (4). Whereas IgM is easily demonstrable as a secreted product, IgD has only recently been shown to be present in mouse serum (5). The presence of IgD as a common cell surface molecule, coupled with its paucity in the serum, has led to the suggestion that this isotype serves predominantly a receptor function. Inferential evidence in support of this suggestion has been obtained from experiments in which antisera to IgD, both allospecific (6) and heterologous (7), have been shown to block some, but not all, immune responses in vitro. The regulatory role of sIgM has been investigated in a number of studies and this isotype has been implicated in both tolerance (8) and triggering (9) of B lymphocytes. Direct evidence for a triggering function for sIgM has been shown by the demonstration that anti- $\mu$  antibodies induce proliferation in murine B lymphocytes (10, 11). Attempts to induce proliferation with antibodies directed against murine IgD have hitherto been unsuccessful (12).

The application of somatic cell hybridization to lymphocytes (13) provides a means of generating monoclonal antibodies against cell surface determinants. We report in this communication the derivation and characterization of a monoclonal alloanti- $\delta$  (designated H $\delta^a$ /1). The IgG<sub>2b</sub> product of this clone recognizes a specificity present on the  $\delta$  heavy chains of mice of the Ig<sup>a, c, d, f, g, h, n</sup> allotype groups. The H $\delta^a$ /1 Ig product is not only cytotoxic but also induces T-independent proliferation of B cells

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<sup>1</sup> *Abbreviations used in this paper:* C, complement, Con A, concanavalin A; DNP, 2,4-dinitrophenol; FcR, receptor for the Fc region of Ig heavy chain; FCS, fetal calf serum; <sup>3</sup>H-TdR, tritiated thymidine; IgCH, Ig heavy chain constant region; 2-ME, 2-mercaptoethanol; Ram $\kappa$ , rabbit anti-mouse  $\kappa$  light chain; SaCI, Cowan I strain of *Staphylococcus aureus*; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; sIg surface Ig.

from normal adult mice of the haplotypes that bear the target determinant. In contrast, no proliferation is induced by monoclonal anti- $\delta$  10-4.22 (14), a reagent that recognizes allotypic specificities expressed on cells from the same range of Ig heavy chain constant region (IgCH) haplotypes. Stimulation of proliferation by H $\delta^a$ /1 Ig thus represents the first direct evidence of triggering through sIgD, and is a further direct refutation of the one nonspecific signal hypothesis of B lymphocyte triggering.

### Materials and Methods

**Mice.** BALB/c $\pi$ , B.C8, C.B20, and C57BL/Ka mice were all bred at the Jewish Hospital of St. Louis, Missouri, from breeding nuclei originally obtained from Litton Bionetics, Kensington, Md. DBA/2J, B10.D2, and A/J mice were bred at Jewish Hospital from stock obtained from The Jackson Laboratory, Bar Harbor, Maine. NZB mice were the gift of Dr. Bevra Hahn, Washington University School of Medicine. AKR/J, CE/J, RIII/J, and SEA/Gn mice were all purchased from The Jackson Laboratory. BALB/c nu/nu mice and their euthymic littermates were the kind gift of Dr. Dale Isaak, Kirksville College of Osteopathic Medicine, Kirksville, Mo. All mice were at least 8 wk of age when used.

**Cultures.**  $2.5 \times 10^5$  viable spleen cells (erythrocyte-depleted with tris-buffered ammonium chloride) were cultured in vol of 200  $\mu$ l in 96-well flat-bottomed microtiter plates (Costar 3596; Costar, Data Packaging, Cambridge, Mass.) at 37°C, 5% CO<sub>2</sub> in air, 100% relative humidity. Cultures were pulsed with 1  $\mu$ Ci tritiated thymidine (<sup>3</sup>H-TdR) (6.7 Ci/mmol) (Research Products International Corp., Elk Grove Village, Ill.) 48 h after initiation, and 16 h later harvested onto glass-fiber filters, and the incorporated radioactivity counted by liquid scintillation spectrometer. Both RPMI 1640 and a modified Mishell-Dutton medium were used; the medium used in a given experiment and the fetal calf serum (FCS) supplementation is indicated. Data are expressed as the geometric mean ( $\times$ /+ relative SE) of triplicates.

**Microcytotoxicity.** This was determined by trypan blue exclusion using the method described by Sachs et al. (15). Erythrocyte-depleted spleen cells were used as the target population. Serum from rabbits 3–4 wk of age was used as the source of complement (C). The medium employed was Liebowitz L-15 that contained gelatin (0.1%) and sodium azide (0.02%).

**T Lymphocyte Depletion of Spleen Cells.** Spleen cells were suspended in a 1:500 dilution of monoclonal anti-Thy-1.2 (New England Nuclear, Boston, Mass.) at a cell density of  $10 \times 10^6$  ml. After 30 min on ice, the cells were recovered by centrifugation, resuspended in diluted rabbit C at the same density, and incubated at 37°C for 45 min. The cells were then washed twice in culture medium, counted, and cell density adjusted to  $2.5 \times 10^6$  viable cells/ml.

**Radioiodination of Spleen Cells and Identification of Lymphocyte Surface Molecules by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).** Lactoperoxidase-catalysed iodination of cell surface molecules was performed as described by Kessler (16). Extraction of cells with Nonidet NP-40 (0.5% vol:vol) and immunoprecipitation were performed as described by Cullen et al. (17), and SDS-PAGE was carried out in the discontinuous system described by Laemmli (18). Sequential precipitations were performed as described by Cullen et al. (17).

**Generation of Monoclonal Anti- $\delta$  H $\delta^a$ /1.** C57BL/6 mice were hyperimmunized with spleen cells from nonimmune adult DBA/2 donors. 4 d after the sixth immunization, spleen cell suspensions were made from individual animals and fused with SP2/0-Ag14 cells (19) using polyethylene glycol (20). After positive cultures had been identified by microcytotoxicity, these were cloned on mouse embryo fibroblasts as described by Coffino et al. (21). Monoclonality and the stability of H $\delta^a$ /1 have been confirmed by isoelectric focusing in polyacrylamide of Ig purified from both culture supernates and ascitic fluids.

**Ig Preparation.** This was by chromatography on protein A-Sepharose CL4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) with acid elution, as described by Ey et al. (22).

**Testing for Endotoxin Contamination of Ig Preparations.** We employed the limulus Amoebocyte assay (Microbiological Associates, Walkersville, Md.). Preparations were routinely tested at Ig concentrations at least 10-fold greater than the maximum employed in any experiment; only those found to be negative (assay sensitivity 0.25 ng/ml) were used.

## Results

The reactivity of the  $H\delta^a/1$  primary culture was initially identified by C-dependent microcytotoxicity. The culture fluid killed ~50% of DBA/2 spleen cells, with no detectable activity on cells from C57BL/6 or B10.D2 mice. Because the percentage of DBA/2 cells killed was about that expected for B lymphocytes, and the failure to kill B10.D2 cells excluded anti-MHC activity, it seemed possible that the secreted product exhibited anti-Ig activity. After cloning, both the culture supernatant and ascitic fluid from mice injected with  $H\delta^a/1$  cells retained the same pattern of cytotoxic activity on spleen cells. To test whether the target determinant was encoded in, or closely linked to, the IgCH region, the  $H\delta^a/1$  ascitic fluid was tested by cytotoxicity on the four strains: BALB/c $\pi$ , B.C8, C57BL/Ka, and C.B20. The data are shown in Table I. Spleen cells from both strains that bore the  $Ig^a$  haplotype (BALB/c $\pi$  and B.C8) were killed; cells from neither of the  $Ig^b$  haplotype strains (C57BL/Ka and C.B20) were affected. Thus, the target molecule appeared to be either an IgCH product or a molecule whose structural or regulatory gene was very closely linked to IgCH.

To test directly the proposition that the target molecule was an IgCH product, we employed lactoperoxidase-catalysed radioiodination of spleen cells, followed by detergent lysis, immunoprecipitation, and SDS-PAGE.

Fig. 1 shows an autoradiograph of a slab gel that compares immune precipitates from BALB/c $\pi$  (tracks A–D) and C.B20 (tracks E–H). Samples were reduced with 2 mercaptoethanol (2-ME) before electrophoresis. Precipitation with rabbit anti-mouse  $\kappa$  light chain (Ram $\kappa$ ) (tracks D and E) showed labeled  $\mu$ ,  $\delta$ , and light chains precipitated in each strain. Goat anti-mouse  $\mu$  (tracks B and G) showed  $\mu$  and light chains.  $H\delta^a/1$  protein purified from ascitic fluid precipitated radioactive material only from the BALB/c $\pi$  extract; the two bands showed comigration with  $\delta$  and light chains. A control Ig, 3-30-E (2,4-dinitrophenol [DNP]-binding  $IgG_{2b}$ ) (tracks A and H) shows the level of background binding. Therefore, by this analysis,  $H\delta^a/1$  Ig showed specificity for the  $Ig^a$  haplotype, consistent with the cytotoxicity data, and appeared to be an alloanti- $\delta$  antibody.

The Ig nature of the target molecule and specificity for the  $\delta$  chain were confirmed in two series of sequential immunoprecipitations. In the first, extracts from iodinated B.C8 spleen cells were exposed to excess amounts of Ram $\kappa$ , anti- $\mu$ ,  $H\delta^a/1$  ascites, or control ascites, followed by SaCl. The supernate from each clearing precipitation was then divided into four aliquots, and analytical precipitations performed with each of the four reagents, followed by Cowan I strain of *Staphylococcus aureus* (SaCl). Complexes were dissociated by boiling in the presence of 2-ME, and the supernates analyzed on

TABLE I  
Target Determinant of  $H\delta^a/1$  Ig Is Linked to IgCH

Spleen cell donor	Background	IgCH	Percentage specific kill*
BALB/c $\pi$	BALB/c $\pi$	a	48.9
B.C8	C57BL/Ka	a	53.2
C.B20	BALB/c $\pi$	b	4.4
C57BL/Ka	C57BL/Ka	b	1.8

\* Percentage specific kill =  $100 \times \frac{(\% \text{ dead Ab} + C) - (\% \text{ dead C control})}{100 - (\% \text{ dead C control})}$

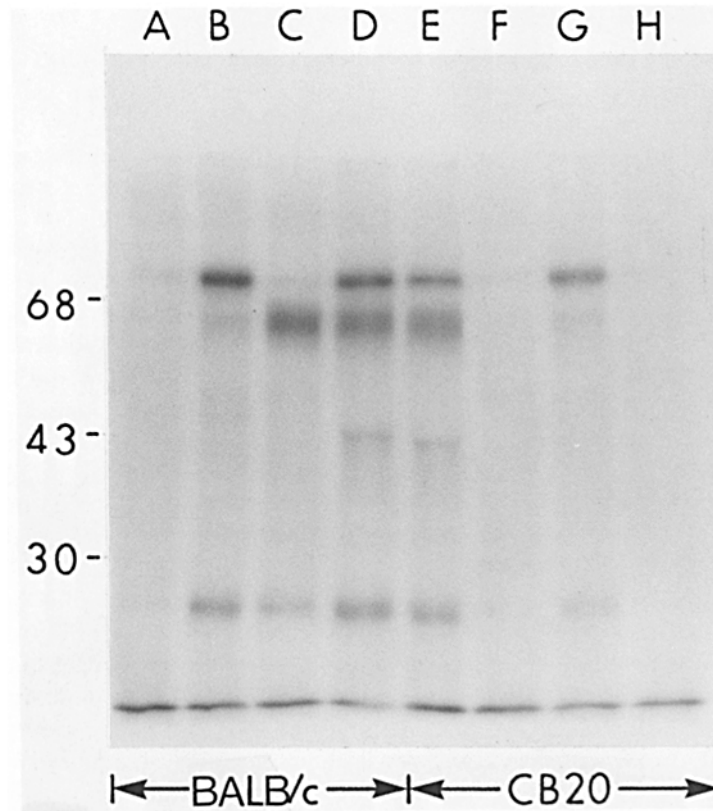


FIG. 1. Identification of the  $H\delta^a/1$  Ig target molecule and its linkage to IgCH.  $^{125}\text{I}$ -labeled spleen cell extracts from BALB/ $c\pi$  (tracks A-D) and C.B20 (tracks E-H) mice were incubated with Ram $\kappa$  (tracks D and E),  $H\delta^a/1$  Ig (tracks C and F), goat anti-mouse  $\mu$  (tracks B and G), or a control IgG $_{2b}$  of DNP-binding specificity (tracks A and H). Complexes were dissociated by boiling in the presence of 2-ME. Samples were analyzed on a 9.5% acrylamide gel. Each track contains material from  $10 \times 10^6$  cell-equivalents of labeled extract. Mobilities of molecular weight markers are shown: bovine serum albumin (68,000), ovalbumin (43,000), and carbonic anhydrase (30,000).

tube gels that were then sliced and counted to identify radioactive species. The data from five of the sixteen gels are shown in Fig. 2. The vertical arrows in all four panels of Fig. 2 show the positions of the  $\mu$  and  $\delta$  molecules precipitated by Ram $\kappa$  after clearance with control (non-anti-Ig) ascites. The electropherograms shown in panels A and C were obtained after clearance with excess  $H\delta^a/1$ ; analytical precipitations were with Ram $\kappa$  (Fig. 2A) and anti- $\mu$  (Fig. 2C). In both panels, only the  $\mu$  chain peak was retained. Panels B and D show the effect of clearance with excess anti- $\mu$ ; subsequent precipitation with Ram $\kappa$  (Fig. 2B) or  $H\delta^a/1$  (Fig. 2D) revealed only residual  $\delta$  molecules. Clearance with Ram $\kappa$  left no labeled species precipitable by Ram $\kappa$ , anti- $\mu$ , or  $H\delta^a/1$  (data not shown). Control ascites plus SaCl in the second precipitation showed no peaks of radioactive material (data not shown). And, finally, clearance with excess control ascites plus SaCl followed by precipitation with anti- $\mu$  or  $H\delta^a/1$  showed single  $\mu$  and  $\delta$  peaks, respectively.

The second series of sequential precipitations provided the final evidence that  $H\delta^a/1$  Ig recognized a determinant on the  $\delta$  chain.  $^{125}\text{I}$ -labeled extracts of BALB/ $c\pi$

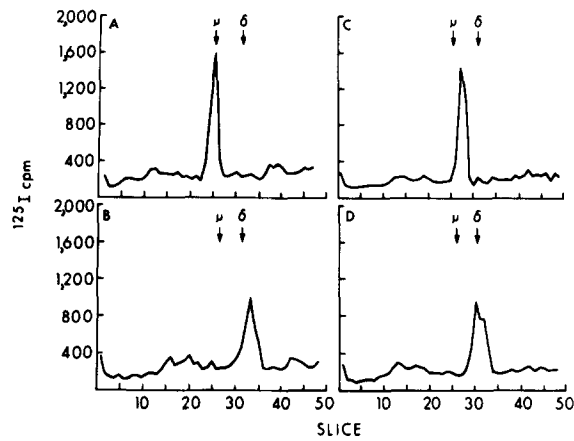


FIG. 2.  $H\delta^8/1$  Ig recognizes a determinant present on  $\delta$  molecules but absent from  $\mu$ .  $^{125}\text{I}$ -labeled spleen cell extracts from B.C8 mice were incubated with  $H\delta^8/1$  ascites (A and C) or goat anti-mouse  $\mu$  (B and D), each in excess, followed by SaCl. The supernates were then divided and reincubated with Ram $\kappa$  (A and B), goat anti-mouse  $\mu$  (C), or  $H\delta^8/1$  ascites (D), again followed by SaCl. Complexes were dissociated as described and analyzed on 7.5% acrylamide tube gels, which were then cut into 2-mm slices and counted for radioactivity. The vertical arrows in each panel show the positions of the  $\mu$  and  $\delta$  peaks identified in a fifth gel (of the 16 in this experiment) in which molecules precipitated by Ram $\kappa$  after initial exposure to control, non-anti-Ig ascites, were analyzed (see Results). Each panel contained material equivalent to  $7.5 \times 10^6$  cells.

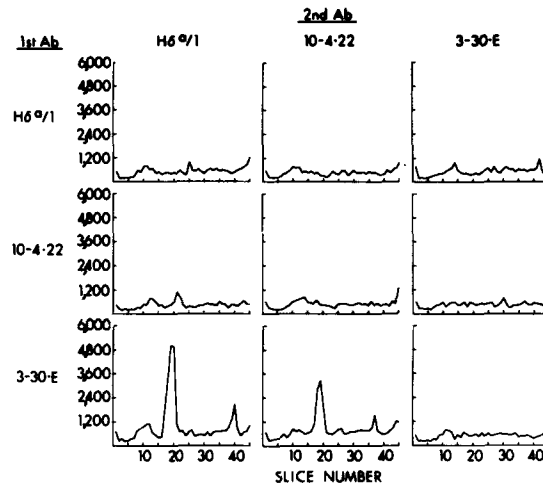


FIG. 3. Confirmation of the anti- $\delta$ -specificity of  $H\delta^8/1$  Ig by cross-precipitation with 10-4.22 Ig.  $^{125}\text{I}$ -labeled spleen cell extracts from BALB/ $c\pi$  mice were incubated with  $H\delta^8/1$ , 10-4.22, or 3-30-E Ig in excess (1st Ab), followed by SaCl. The supernates from each of these precipitations were divided into three aliquots, which were analyzed with the same three Ig preparations. Complexes were dissociated under reducing conditions and analyzed on 9% polyacrylamide tube gels that were subsequently sliced and counted for radioactivity. Each panel contains material equivalent to  $10 \times 10^6$  cells.

spleen were used for this experiment. The electropherograms are shown in Fig. 3. Clearing precipitation with the monoclonal anti- $\delta$  10-4.22 completely removed radioactive species precipitable by either 10-4.22 or  $H\delta^8/1$ ; the converse was also true. Therefore, the two antibodies recognize determinants on the same molecule. Peaks of

precipitable radioactivity were obtained only when the initial step employed 3-30-E Ig, followed by analysis with either H $\delta^a$ /1 Ig or 10-4.22 Ig.

In parallel with the identification of the target antigen for H $\delta^a$ /1 Ig, we also investigated its ability to trigger murine lymphocytes. This became particularly interesting when the direct precipitations suggested that the target determinant was present on  $\delta$  heavy chains. In spite of the reported failure of anti- $\delta$  antibodies to induce proliferation, we felt that this might have been the result of the heterogeneity of the antisera employed, and that a monoclonal reagent might yield a different result. Erythrocyte-depleted spleen cells from B.C8 and C57BL/Ka mice were cultured as described. Ig prepared from H $\delta^a$ /1 and 3-30-E ascitic fluids and 10-4.22 culture supernate were titrated into the cultures of cells from each strain. Proliferation was measured as described;  $^3\text{H}$ -TdR incorporation on day 3 is shown in Fig. 4. Comparable responses to lipopolysaccharide (LPS) were obtained in the two strains; H $\delta^a$ /1 Ig induced proliferation only in cells from B.C8 (Ig-5<sup>a</sup>) mice, thus showing its effect to be allotype-specific. In contrast, both 10-4.22 and 3-30-E Ig failed to induce B.C8 cells to proliferate; they also had no effect on C57BL/Ka cells (data not shown). Quite clearly, then, the use of H $\delta^a$ /1 Ig indicated that lymphocytes may be induced to proliferate by interaction of H $\delta^a$ /1 anti- $\delta$  antibody with sIgD. The experiment shown in Fig. 4 was carried out in medium supplemented with 2% FCS; however, H $\delta^a$ /1 Ig also induced allospecific proliferation in the absence of serum (I. M. Zitron. Unpublished data.).

The requirement for T lymphocytes was investigated in two ways: by depletion of splenic T cells with anti-Thy-1.2 antibody and C, and the use of congenitally athymic mice. Table II contains data from one representative experiment of each type. In part A, anti-Thy-1.2 depletion of BALB/c $\pi$  spleen cells enriched responses to both LPS and H $\delta^a$ /1 Ig, while completely eliminating the response to phytohemagglutinin (PHA) and depleting the concanavalin A (Con A) response by 98%. In part B, spleen cells from athymic BALB/c mice failed to respond to either PHA or Con A, yet were able to proliferate in response to both LPS and H $\delta^a$ /1 Ig. In both experiments, spleen cells from CB20 mice provided the allotype specificity control. These experiments show the T cell independence of the proliferative response. The data in Table II also make the point that the mechanism by which proliferation is induced is not solely a

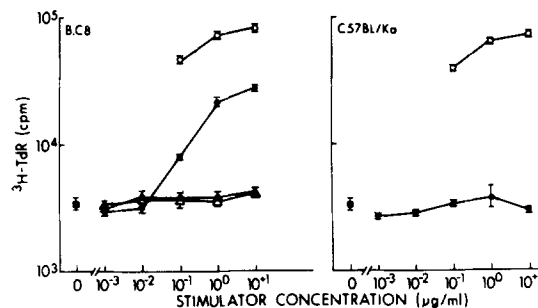


FIG. 4. Allotype-specific proliferation is induced by H $\delta^a$ /1 Ig. Erythrocyte-depleted spleen cells were cultured as described in Materials and Methods; RPMI 1640 supplemented with 2% FCS was employed in this experiment. The stimuli used were LPS *Escherichia coli* 0111:B4 (○), purified H $\delta^a$ /1 Ig (●), 10-4.22 Ig (Δ), and 3-30-E Ig (▲). The medium background in each strain is shown by the closed squares.

TABLE II  
Proliferative Response to  $H\delta^a/1$  Ig Is Independent of T Cells

	$^3\text{H-TdR}$ incorporation		
	BALB/c spleen cells		CB20 spleen cells*
	C alone	Anti-Thy-1.2 + C	
<b>A Anti-Thy-1.2 + C depletion</b>			
Medium‡	4,775 (1.07)	6,711 (1.08)	3,661 (1.09)
LPS 100 $\mu\text{g/ml}$	69,768 (1.01)	92,894 (1.02)	60,628 (1.04)
$H\delta^a/1$ Ig 10 $\mu\text{g/ml}$	27,816 (1.06)	35,228 (1.04)	3,229 (1.04)
3 $\mu\text{g/ml}$	18,462 (1.09)	22,415 (1.11)	3,544 (1.05)
1 $\mu\text{g/ml}$	11,677 (1.08)	15,354 (1.05)	4,771 (1.13)
3-30-E Ig 10 $\mu\text{g/ml}$	4,181 (1.04)	6,702 (1.05)	5,198 (1.10)
3 $\mu\text{g/ml}$	5,354 (1.05)	6,139 (1.03)	3,654 (1.06)
1 $\mu\text{g/ml}$	5,728 (1.06)	6,981 (1.01)	4,373 (1.10)
Con A 0.5 $\mu\text{g/ml}$	322,240 (1.01)	14,514 (1.01)	ND§
PHA 10 $\mu\text{g/ml}$	53,536 (1.01)	2,477 (1.12)	ND
<b>B</b>			
	Euthymic	Athymic	
Medium‡	7,190 (1.04)	7,420 (1.05)	6,847 (1.01)
LPS 10 $\mu\text{g/ml}$	81,390 (1.02)	59,704 (1.03)	78,623 (1.01)
$H\delta^a/1$ Ig 10 $\mu\text{g/ml}$	43,154 (1.04)	40,514 (1.04)	6,839 (1.10)
3 $\mu\text{g/ml}$	36,969 (1.05)	31,608 (1.04)	6,254 (1.11)
1 $\mu\text{g/ml}$	27,740 (1.06)	20,334 (1.08)	6,456 (1.09)
3-30-E Ig 10 $\mu\text{g/ml}$	6,463 (1.06)	8,172 (1.02)	ND
3 $\mu\text{g/ml}$	6,800 (1.10)	7,110 (1.03)	ND
1 $\mu\text{g/ml}$	8,233 (1.04)	7,599 (1.05)	ND
Con A 1 $\mu\text{g/ml}$	427,061 (1.04)	5,015 (1.06)	ND
PHA 10 $\mu\text{g/ml}$	43,144 (1.02)	1,828 (1.11)	ND

\* Only treatment erythrocyte depletion.

‡ Cells cultured in Mishel/Dutton medium supplemented with 2% FCS.

§ ND, not done.

function of the Fc region of the  $\text{IgG}_{2b}$ . The control reagent used in these experiments, 3-30-E, is an  $\text{IgG}_{2b}$  with DNP-binding specificity, and it does not induce proliferation.

We are interested in asking whether proliferation in response to  $H\delta^a/1$  Ig is a marker for a discrete B cell subpopulation. To begin to investigate this we have used animals bearing the X-linked immune defect of the CBA/N strain. Spleen cells from immune-defective male (CBA/N  $\times$  DBA/2) $F_1$  and phenotypically normal female littermates were cultured with LPS,  $H\delta^a/1$  Ig, or 3-30-E Ig. The data are shown in Table III, and indicate that cells from the male mice are incapable of response to  $H\delta^a/1$  Ig, although they do respond to LPS. The failure of response by male cells is not the result of a lack of the target determinant because a similar percentage, of  $\text{sIg}^+$  spleen cells, stain with fluorescein isothiocyanate-conjugated  $H\delta^a/1$  Ig from mice of each gender (data not shown).

Finally, a strain survey, which employed unfractionated spleen cells from mice that are the prototype strains for a range of allotype groups, has been performed. The analysis has been performed by both microcytotoxicity and induction of proliferation.  $H\delta^a/1$  and 10-4.22 Ig preparations have been compared. With the sole exception of

TABLE III  
Cells from Immune-Defective (CBA/N × DBA/2)F<sub>1</sub> Male Mice Fail to Proliferate in Response to Hδ<sup>a</sup>/1 Ig

	<sup>3</sup> H-TdR incorporation		CB20 cells
	(CBA/N × DBA/2)F <sub>1</sub> male cells	(CBA/N × DBA/2)F <sub>1</sub> female cells	
Medium*	1,287 (1.18)	11,478 (1.11)	4,492 (1.02)
LPS 10 μg/ml	29,707 (1.03)	68,775 (1.01)	37,174 (1.06)
Hδ <sup>a</sup> /1 Ig 30 μg/ml	1,359 (1.16)	53,604 (1.06)	2,535 (1.17)
10 μg/ml	1,540 (1.13)	52,306 (1.04)	2,484 (1.10)
3 μg/ml	1,410 (1.16)	58,737 (1.05)	2,829 (1.11)
3-30-E Ig 30 μg/ml	701 (1.08)	9,875 (1.04)	3,811 (1.11)
10 μg/ml	1,273 (1.06)	7,978 (1.02)	3,329 (1.09)
3 μg/ml	1,630 (1.06)	8,254 (1.31)	3,208 (1.13)

\* Medium used RPMI 1640 supplemented with 2% FCS.

TABLE IV  
Identical Strain Distributions of the Two Monoclonal Alloanti-δ Antibodies

Prototype strain	Allotype group*	Target specificity recognized by‡	
		Hδ <sup>a</sup> /1	10-4.22
BALB/cπ	a	+	+
C57BL/Ka	b	-	-
DBA/2	c	+	+
AKR/J	d	+	+
A/J	e	-	-
CE/J	f	+	+
RIII/J	g	+	+
SEA/Gn	h	+	+
NZB	n	+	+

\* Taken from Green (23).

‡ Presence (+) or absence (-) of target determinant identified by C-dependent cytotoxicity on spleen cells. For all strains except NZB presence of Hδ<sup>a</sup>/1 determinant confirmed by induction of proliferation.

mice that bore the CBA/N defect (vide supra), the presence of the target determinant for Hδ<sup>a</sup>/1 Ig was associated with the ability to be induced to proliferate. For simplicity, the data shown in Table IV have been reduced to +, denoting ability to be killed (and to proliferate in response to Hδ<sup>a</sup>/1 Ig), and -, denoting inability to be killed.

### Discussion

The data reported here provide the first direct evidence for triggering of murine B lymphocytes through sIgD. The function of sIg and existence of two isotypes on the majority of B lymphocytes in the adult spleen are incompletely understood. Moller (3) has proposed the one nonspecific signal model in which sIg, regardless of isotype, serves a passive role, merely serving to focus antigen on the B cell. Reports by a number of groups that anti-Ig and anti-μ antibodies (10, 11, 24) could stimulate B cell proliferation indicated that at least sIgM can directly deliver activating signals to B cells. The role of sIgD has been less clear. Experiments in which anti-δ reagents



have inhibited the antibody responses to some antigens are consistent with a number of models, including an active receptor function for sIgD. However, one might postulate the necessity for cross-linking of sIgM and sIgD by a multivalent antigen, such that either anti- $\mu$  or anti- $\delta$  alone would inhibit this. Alternatively, one might propose that sIgD plays a role in which it serves to bind antigen without any signal delivery but, by this, diverts antigen from interacting with sIgM. Thus, the latter isotype might be the only means of delivering Ig-mediated signals. The ability of the H $\delta^a$ /1 Ig product to induce proliferation clearly rules out the latter two models and indicates an active role for sIgD.

Alternative trivial explanations, such as endotoxin contamination or activation through an IgG<sub>2b</sub>-specific Fc receptor, have been ruled out by the allotypic specificity of the proliferation. The negative results on the limulus Amoebocyte assay also argue against endotoxin being responsible. The possible role of receptor for the Fc region of Ig heavy chain (FcR)-mediated activation still exists, though this would necessarily involve an sIgD-FcR cross-link.

The ability of H $\delta^a$ /1 Ig to induce proliferation gives rise to a number of questions. First, why does H $\delta^a$ /1 Ig induce vigorous proliferation, whereas 10-4.22 Ig fails to? Second, is the capacity to respond to an sIgD-induced proliferative signal a discrete subpopulation marker, distinct from the ability to respond (by proliferation) to anti- $\mu$ ? Third, if the anti- $\delta$  and anti- $\delta$ -responsive subpopulations are identical or significantly overlapping, are there different biological sequelae that result from stimulation through each of the two isotypes? And, finally, is sIgD capable of delivering the complete set of signals for both proliferation and differentiation to high-rate Ig secretion?

There are a number of alternative answers to the first question. The simplest and perhaps most attractive explanation is that H $\delta^a$ /1 and 10-4.22 Ig molecules recognize different alloantigenic determinants on the  $\delta$  chain and, consequently, each induces a different change in conformation, with only that induced by H $\delta^a$ /1 Ig accurately mimicking the change induced by antigen-binding. A preliminary strain-survey (Table IV) revealed no IgCH haplotype that permitted separation of the determinants recognized by H $\delta^a$ /1 and 10-4.22 Ig. One interesting possibility regarding the two determinant model is that the determinant recognized by H $\delta^a$ /1 Ig is in the CH<sub>1</sub>-hinge region, as Kessler et al. (25) have recently localized the 10-4.22 determinant in the Fc $\delta$  region, proximal to the trypsin-cleavage site.

An alternative explanation is that both monoclonal anti- $\delta$  antibodies recognize the same determinant, but that there is a marked difference in the affinities of their binding sites reflected in their biological function.

The third explanation is that we are observing an effect related to the Fc region of the anti- $\delta$ : the 10-4.22 product is IgG<sub>2a</sub>; the H $\delta^a$ /1 product is IgG<sub>2b</sub>. Stimulation of B cell proliferation by fragments of human IgG1 have been reported (26). Proliferation in this system is independent of T cells (26), as is the response which we report here (Table II). Generation of the Fc fragment requires macrophages (26), though the 14,000-dalton fragment once generated appears to be capable of direct stimulation of B cells. We have not vigorously investigated the macrophage-dependence of H $\delta^a$ /1-induced proliferation but, in preliminary experiments, proliferation appears resistant to macrophage depletion by Sephadex G-10 (27) passage. Again, however, strict allotypic specificity militates against a nonspecific mechanism of this type.

In terms of  $H\delta^a/1$ -induced proliferation being a subpopulation marker, spleen cells from immune-defective (CBA/N  $\times$  DBA/2) $F_1$  male mice fail to respond (Table III), though they bear the target determinant. This may indicate that the response is a characteristic of the  $Lyb3,5,7^+$  (28–30) subset of B cells lacking from defective animals. This, however, is dependent upon the assumption that those B cells present in immune-defective mice are completely normal.

Finally, we have examined the biological sequelae of proliferation induced through sIgD and have found a striking increase in response to thymic-independent antigens (I. M. Zitron. Unpublished observations.) when we employ a sequential culture system. Interestingly, no such increase is observed after proliferation induced through sIgM. These observations are being extended.

In conclusion, then, our data indicate an active signaling role for sIgD and, assuming that anti- $\delta$  and anti- $\mu$  induce proliferation in overlapping, or identical, subpopulations of  $\mu^+\delta^+$  B cells, a system exists to investigate further the relative contributions of each isotype to the process of B cell activation.

### Summary

We describe the identification of a monoclonal antibody that recognizes a determinant on the  $\delta$  chain of mice of the  $Ig^{a, c, d, f, g, h, n}$  allotype groups. The monoclonal Ig in soluble form induces allotype-specific proliferation by splenic B lymphocytes from normal animals of these haplotypes. Spleen cells from mice bearing the X-linked defect of CBA/N mice fail to respond, although they bear the determinant. Proliferation is independent of T lymphocytes. The data indicate a direct triggering function for sIgD.

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