PHYSIOLOGY OF IgD

I. Compensatory Phenomena in B Lymphocyte Activation in Mice Treated with Anti-IgD Antibodies*

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Classic studies by Rowe et al. (1) in the human and by Vitetta and coworkers (2) in the mouse have established that IgD is a major membrane immunoglobulin. This has led to a variety of studies and speculation regarding its biological function. Based upon the effect of anti-IgD on the in vitro primary response, Cambier et al. (3) suggested that the presence of IgD on the cell surface was critical for responses to thymus-dependent but not thymus-independent antigens. Prolonged in vivo treatment of mice with anti-IgD, starting at birth, was reported by Layton et al. (4) to result in a decrease in the incidence of IgD positive cells, accompanied by a depression in the secondary indirect plaque-forming cell, (PFC)[1] response. In contrast, Bazin et al. (5) found that treatment with antiserum to IgD from birth led to little or no decrease in any of the immunoglobulin classes except for IgE, which was markedly reduced. IgE antibody responses were also depressed.

As in the case of the other immunoglobulin classes, allelic exclusion has been shown to exist for IgD (6, 7). The availability of monoclonal allotype-specific antibody to IgD allows the investigation of the effects of anti-IgD in allotype heterozygous mice, with restriction of the effects limited to one half of the B cell population. Comparison with the alternative allelic products permits a sensitive study of the role of IgD in immune responses with an ideal internal control. Furthermore, anti-IgD treatment of heterozygous or of homozygous mice might provide a clue to phenomena of internal balance and compensation (regulation) within an immune system. We therefore decided to re-investigate several aspects of the effects of anti-IgD on the immune system: (*a*) to compare the effect of anti-delta suppression in homozygous and heterozygous animals; (*b*) to determine whether IgD suppression is linked to the suppression of the corresponding IgG allotypes; and (*c*) to determine the effect of functional inactivation of all delta-positive cells on the development of germinal centers and on memory cell generation.

* Supported in part by grants AI-11694, AI-14398, AI-03076, and AI-14829 from the National Institutes of Health, Bethesda, Md.

‡ Supported by training grant 5T32 CA-09161 from the National Institutes of Health.

[1] *Abbreviations used in this paper:* BA, *Brucella abortus*; BGG, bovine gammaglobulin; CFA, complete Freund's adjuvant; KLH, keyhole limpet hemocyanin; PBS, phosphate-buffered saline; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TBS, Tris-buffered saline; TNP, trinitrophenyl.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/81/08/0318/15 $1.00
Volume 154 August 1981 318–332

Materials and Methods

Mice. BALB/c mice, homozygous for a allotypes, and SJL mice, homozygous for b allotypes, were bred in the animal facility at New York University Medical School, New York. (BALB × SJL)F_1 allotype heterozygotes were bred in the animal facility of the Department of Immunology, Merck and Co., Rahway, New Jersey. BALB/cAN mice used for breeding homozygotes were obtained from Charles River Laboratories, Inc., Wilmington, Mass. BALB/cAN females used for breeding heterozygotes were obtained from Simonsen Laboratories, Gilroy, Calif. All SJL breeding mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Antigens. Sheep erythrocytes (SRBC) were purchased from Colorado Serum Co., Denver, Colo.; keyhole limpet hemocyanin (KLH) was purchased from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; Ficoll, 40,000 mol wt, was obtained from Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.; and trinitrophenyl sulfonic acid was purchased from Sigma Chemical Co., St. Louis, Mo. *Brucella abortus* (BA) was provided by the U. S. Department of Agriculture, Ames, Iowa. TNP-BA and TNP-KLH were prepared as described by Little and Eisen (8) and TNP-Ficoll was prepared according to the method of Mitchell et al. (9).

Antibodies. Anti-IgD hybridoma 10-4.22, producing molecules of the IgG_{2a} class directed against specificity four of the Ig-5a allele (10) was obtained from Dr. Virgil Wood, National Institutes of Health. The cells were grown in RPMI-1640 with 10% fetal calf serum, glutamine (2 mM), and sodium pyruvate (1 mM) in the presence of penicillin, streptomycin, and fungizone (100 U, 100 μg, and 0.25 μg/ml). Cells were then allowed to secrete for 1 wk without feeding, and the supernate was absorbed on a Staph A-Sepharose 4B column. The antibodies were eluted with 0.1 M sodium citrate, pH 3.0, dialyzed against phosphate-buffered saline (PBS), and sterilized by filtration. Monoclonal antibodies of the IgM class directed against specificity three of allele Ig-5b from line H6-31 (11) were purchased from Accurate Chemical and Scientific Corporation (Hicksville, N. Y.), distributors for Sera Labs (London, England). A rabbit anti-mouse IgD antiserum, the preparation of which is described elsewhere (12) was kindly donated by Dr. F. Finkelman, National Institutes of Health. Anti-mouse IgM antiserum was prepared by immunizing a goat with the IgM lambda myeloma MOPC104E. The antiserum was absorbed with insolubilized IgA lambda myeloma protein J558 and with polyclonal mouse IgG. Anti-mouse Ig $F(ab')_2$ was prepared by immunizing a rabbit with normal mouse Ig in complete Freund's adjuvant (CFA). The antiserum was precipitated with 30% $(NH_4)_2SO_4$ and pepsin digested as described by Nisonoff et al. (13). The digest was passed over a Sepharose 4B column conjugated with a mixture of mouse IgG, IgA, and IgM, and eluted at acid pH. Allotype-specific antisera were prepared by immunizing mice as previously described (14). Antibodies specific for IgG_{2a} allotypes (anti-Ig-1a and anti-Ig-1b) were prepared by passing the relevant anti-allotype serum over Sepharose 4B columns to which purified myeloma proteins, RPC5 (Ig-1a) and CBPC101 (Ig-1b) were coupled. The specific antibodies were eluted with 3.5 M $MgCl_2$, neutralized, dialyzed, and concentrated by vacuum dialysis.

Immunization. Homozygotes were injected intravenously at 3–4 wk of age (day 0) with either 10 μg TNP-Ficoll, 100 μg TNP-KLH, or 800 μg TNP-BA. Primary PFC responses to TNP-Ficoll and TNP-KLH were determined 4 d after immunization. Mice injected with TNP-BA were boosted 10 d later, and their splenic secondary PFC responses were determined 4 d after the last injection. Serum titers, determined in a few mice, were tested on day 7 of the primary or secondary response. In two experiments, TNP-BA was injected into the front footpads on days 0 and 10, using 0.8 μg for priming and either 0.8 or 8 μg for the boost. Cells from the draining lymph nodes were assayed for antibody producing cells 4 d after the boost. Heterozygotes were primed at 6 wk of age and boosted at 11 wk of age with 4×10^8 SRBC, administered intravenously. The antigen was given 1 d after an injection of anti-IgD. The mice were used for assay 6 d after the second antigen injection (7 or 42 d after the last anti-IgD injection).

Schedule of Anti-IgD Injections. BALB/c and SJL/J homozygotes were injected with anti-5a or anti-5b, respectively, three times weekly, using the following doses: 5 μg per injection from day 0 to day 10; 10 μg per injection from day 10 to day 20, and 50 μg per injection from day 21 until assay. Littermate controls were either not injected or were given the same amounts of

nonspecific myeloma protein (CBPC101 or LPC1). (BALB × SJL)F_1 heterozygotes were injected once a week with anti-5a and/or anti-5b. Four different injection schedules, designed to coincide with different stages of the immunization period, were used as follows: (*a*) from birth through week 11, covering the entire immunization period; (*b*) birth to week 6, the time of primary immunization; (*c*) week 6 to week 11, from priming to boost; and (*d*) a single injection on week 6, the time of priming only. Mice injected from birth (day 0) were given 10 μg of antibody on days 0, 7, 14, and 21. Beyond this age, mice were injected, on the appropriate weeks, with 2 μg of antibody per gram body weight. Littermate controls were not injected.

Immunofluorescence. Membrane immunofluorescence was performed by staining the cells at 4°C in the presence of sodium azide as previously described (15). Staining for Ig positive cells was done using rhodamine-conjugated rabbit anti-Ig $F(ab')_2$. The staining for IgD allotypes was done in some experiments by reacting the cells with purified monoclonal anti-IgD followed by fluorochrome-conjugated, pepsin-digested rabbit antibody against mouse IgG. This particular antiserum, absorbed with mouse $F(ab')_2$, reacted only with determinants of the mouse gamma chains not accessible on membrane-bound IgG endogenous to the lymphocytes. Thus, staining with this antiserum in the absence of previously added anti-IgD antibodies gave no background. Most of the staining was performed with purified anti-delta allotype monoclonal antibodies conjugated with biotin hydroxysuccinimide, followed by fluorochrome-conjugated avidin. For conjugation with biotin, proteins at concentrations of 0.1–1.0 mg/ml were dialyzed against 0.9% NaCl, and 1 M $NaHCO_3$ was added to this solution to bring the final concentration to 0.1 M. Biotin hydroxysuccinimide, synthesized as described by Becker et al. (16), or purchased from Pierce Chemical Co., Rockford, Ill., was dissolved in dimethyl sulfoxide at a concentration of 10 mg/ml and added to the protein solution at a 1:5 ratio. The mixture was left at room temperature for 90 min and then dialyzed against PBS. Fluorescein or rhodamine-conjugated avidin was purchased from Vector Laboratories (Burlingame, Calif.) and was routinely used at 100 μg/ml. The stained cells were viewed with a Leitz Dialux microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with a vertical illuminator and Ploem optics, allowing selective visualization of rhodamine or fluorescein.

Histology and Immunoperoxidase Staining. For routine histology, tissues were fixed in Carnoy's solution and stained with methyl green pyronin. For IgD staining, lymph nodes were fixed in cold ethanol, infiltrated, and embedded in ethanol soluble polyester wax (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.), as described by Sordat et al. (17). Four micron sections were placed on glass slides previously coated with amylopectin (Chemalog, Plainfield, N. J.). The sections were incubated in 0.5% H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase activity and washed with 0.05 M Tris-buffered saline (TBS). All sections were incubated with normal goat serum (1:10) for 10 min. The excess serum was wiped away and the sections were overlayed with rabbit anti-mouse IgD, diluted to 5 μg/ml, for 60 min. Control sections were incubated with 1% normal rabbit serum in TBS. All sections were subsequently incubated with goat anti-rabbit serum (N. L. Cappel Laboratories, Inc., Cochranville, Pa.), diluted 1:100, and peroxidase rabbit anti-peroxidase (N. L. Cappel Laboratories), diluted 1:1,500, each for 30 min. Between incubations, the sections were washed for 10 min in three changes of TBS. The sections were stained with 3, 3′ diaminobenzidine tetrahydrochloride (Sigma Chemical Co.), 0.02% and hydrogen peroxide, 0.003% in TBS, pH 7.6, for 7 min. The reaction was terminated by washing in TBS. The sections were counterstained with methyl green.

PFC Assay. Anti-SRBC and anti-TNP PFC were assayed by the slide modification (18) of the Jerne technique (19). TNP-SRBC were prepared using the method described by Rittenberg et al. (20). IgG-producing cells from homozygotes were developed with rabbit anti-mouse Ig and, from heterozygotes, with allotype-specific alloantisera.

Quantitation of Serum IgG_{2a}. Serum levels of IgG_{2a} were determined by means of the solid-phase radioimmune blocking assay described by Tsu and Herzenberg (21). Purified myeloma proteins RPC5 (Ig-1a) and CBPC101 (Ig-1b) were used to coat the wells and, in graded concentrations, to provide the standard curve against which the test samples were read. The purified antibodies, anti-Ig-1a and anti-Ig-1b, were radioiodinated by the chloramine T method (22).

Serum Titers. Antibody titers were determined against TNP-sensitized SRBC (20) or BA, using standard agglutination techniques (23).

Results

In Vivo Suppression of IgD-bearing Cells and Their Recovery in Heterozygous and Homozygous Mice. BALB/c mice, injected with anti-Ig-5a starting at birth, were examined at various times for the incidence of splenic IgD- and Ig-bearing cells. As indicated in Table I, this treatment resulted in a profound suppression of IgD-positive cells and a reduction in the total number of Ig-bearing cells to ~30% of normal. Staining with rabbit anti-delta also failed to reveal any IgD-bearing cells (data not shown). After termination of treatment with anti-IgD, recovery of IgD-bearing cells was relatively slow, being minimal at 5 d and only partial at 11 d. There was a roughly parallel increase in total Ig-bearing cells.

Heterozygous (BALB × SJL)F_1 mice, treated from birth with anti-IgD directed against one allotype, exhibited a profound suppression of IgD-bearing cells of that allotype when examined 7 d after the last injection of anti-IgD (Table II). 6 wk after the last injection, the allotype distribution of the IgD-bearing cells was still abnormal and recovery was only partial. The serum concentration of IgG_{2a} of the linked (suppressed) allotype was also markedly depressed 7 d after the last injection of anti-IgD and was only approaching 50% of normal 6 wk later. Thus, the recovery of the serum IgG_{2a} concentration mirrored that of IgD-positive cells in the spleen.

Heterozygous mice injected simultaneously with anti-IgD antibodies directed against each of the IgD allotype specificities showed a reduction in the incidence of IgD-bearing cells of both allotypes at 7 d after the last injection. However, in contrast to the situations with heterozygous mice treated with antibody to only one allotype, the serum levels were only slightly reduced for both IgG_{2a} allotypes. In fact, five of the six animals studied had levels falling within the normal range and only one animal had low levels (17.8 and 36.3% of normal for the a and b allotypes, respectively).

TABLE I

Suppression and Recovery of IgD-bearing Cells after Treatment of BALB/c Mice with Anti-Delta from Birth

Treatment	Day after last anti-delta injection	Age of mice	Incidence of spleen cells bearing*	
			IgD	Ig
	d	*d*	%	%
None	—	33–35	20.4 ± 2.7	22.1 ± 4.6 (8)
None	—	38–42	34.6 ± 2.5	37.3 ± 1.3 (9)
Anti-delta‡	1–2	27–35	0.8 ± 0.5	7.5 ± 0.6 (5)
Anti-delta	2	39–42	1.2 ± 0.6	8.3 ± 0.8 (11)
Anti-delta	4	35	1.6	11.9 (2)
Anti-delta	5	35–38	3.3 ± 1.0	9.4 ± 1.6 (7)
Anti-delta	9	42	10.2 ± 2.6	18.8 ± 1.7 (5)
Anti-delta	11	41	19.7 ± 0.6	19.7 ± 1.8 (3)

* Determined by immunofluorescence. Expressed as arithmetic mean ± SE. Numbers of mice are in parentheses.

‡ Intraperitonal injections of 4.22 anti-5a given three times a week: 5 μg in the first 10 d, 10 μg in the next 10 d, and 50 μg thereafter.

TABLE II

Suppression and Recovery of IgD-bearing Cells after Treatment of (BALB/c × SJL)F_1 Mice with Anti-Delta from Birth

Treatment*	Time after last anti-delta injection	Allotype assayed	Percentage of control: Incidence of spleen cells bearing IgD of suppressed allotype‡	Percentage of control: Serum concentration of IgG_{2a} of suppressed allotype§
	d		%	%
Anti-5a (4.22)	7	a	17.9	13.6
	42		64.1	48.2
Anti-5b (H6/31 HL)	7	b	16.3	12.6
	42		62.8	41.8
Anti-5a + anti-5b	7	a	21.4	82.9
		b	30.7	64.6

* Days 0, 7, 14, and 21: 10 μg injected intraperitoneally. Thereafter, weekly injections of 2 μg/g body weight were given. All mice were assayed at 84 d of age.

‡ Determined by immunofluorescence.

§ Determined by radioimmune blocking assay.

Histological Observations on the Effect of Anti-IgD Treatment of Homozygous Mice. Spleen, lymph nodes, and Peyer's patches of suppressed and control BALB/c mice, 5–7 wk old, were examined histologically. In agreement with observations of others (24), germinal centers were present in apparently normal numbers in all tissues of suppressed mice that were examined (Fig. 1). The morphology of the germinal centers was also normal except for a distinct decrease in the numbers of small lymphocytes in the mantle zones. Primary follicles (Fig. 1A and B) also appeared less dense than in control mice. Staining with rabbit anti-IgD revealed a complete absence of IgD-bearing cells in the spleen, lymph nodes, and Peyer's patches (Fig. 1C and D).

Effect of Anti-IgD Suppression on the Immune Responses of Heterozygous Mice. Heterozygous (BALB × SJL)F_1 mice, treated from birth with antibody directed against a single IgD allotype specificity, were primed with SRBC at 6 wk of age. They were given a second injection of antigen 5 wk after priming and were assayed for splenic anti-SRBC IgG PFC of both a and b allotypes 6 d later. The number of IgG antibody secreting cells of linked allotype specificities was markedly depressed in these mice (Tables III and IV, protocols 1 and 2). When the anti-IgD antibody injections were terminated at the time of the primary antigen injection (protocol 2), there was little or no recovery in the number of anti-SRBC PFC of the suppressed allotype produced after boosting (compare protocols 1 and 2). Weekly injections of anti-IgD from priming until boosting (protocol 3), or a single injection of anti-IgD just before priming (protocol 4), caused only a moderate suppression of the PFC response of the relevant allotype. Unlike the results with mice treated from birth (protocols 1 and 2), where each animal in the group showed a marked suppression, the suppression observed with protocols 3 and 4 was variable in that some animals produced normal PFC responses, whereas others showed a significant depression. It is interesting to note that in most cases there was a moderate enhancement of the

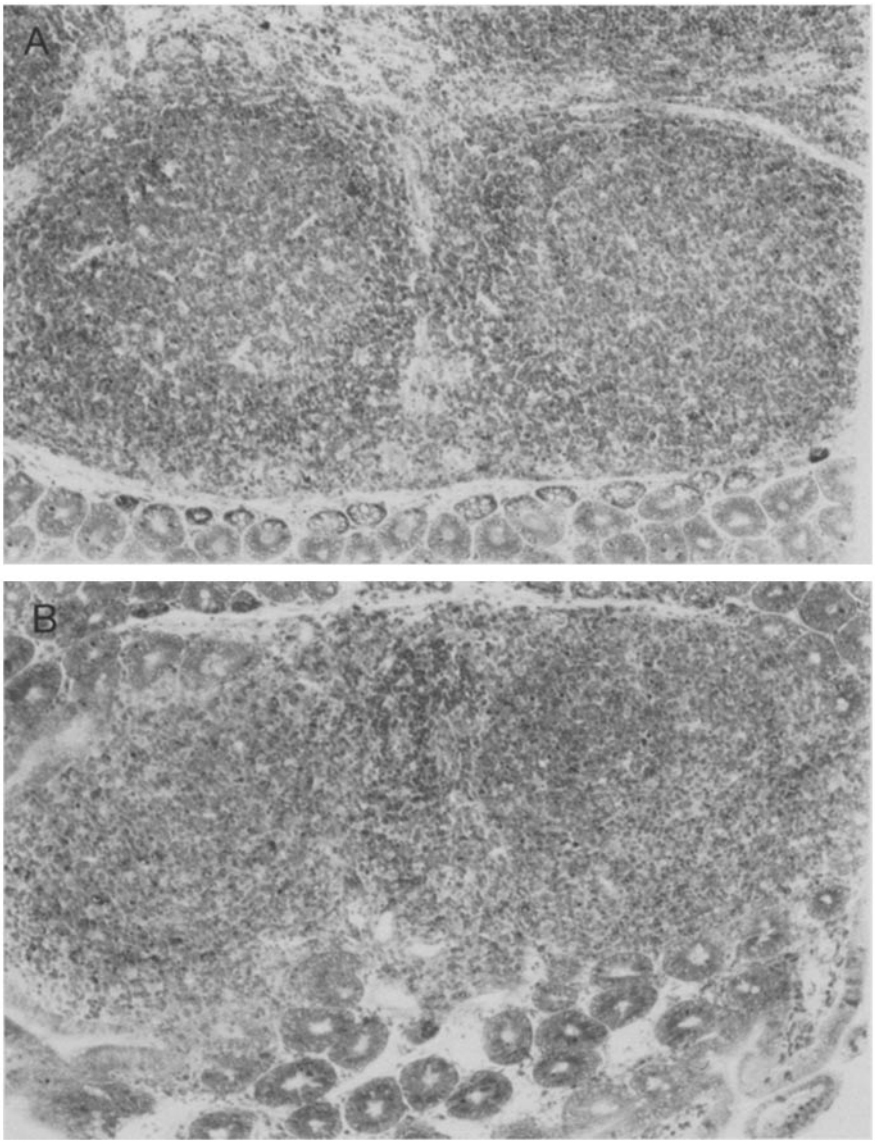

FIG. 1. Photomicrographs of Peyer's patches in the small intestine of control (A and C) and anti-IgD-treated (B and D) BALB/c mice. Staining in A and B was with methyl green pyronine, and in C and D staining was with rabbit anti-IgD followed by goat anti-rabbit Ig and rabbit anti-peroxidase-peroxidase complexes (PAP technique).

response of the nonsuppressed allotype. This trend was most consistently seen in animals treated with anti-5b.

A comparison between IgD-bearing cells of a and b allotypes and the numbers of anti-SRBC PFC of the linked IgG allotypes revealed some interesting points. Although there was a significant degree of recovery of IgD-bearing cells of the suppressed allotype over a 6-wk period after the last injection of anti-IgD (Tables III and IV, protocol 2), the anti-SRBC PFC response of the linked IgG allotypes was still

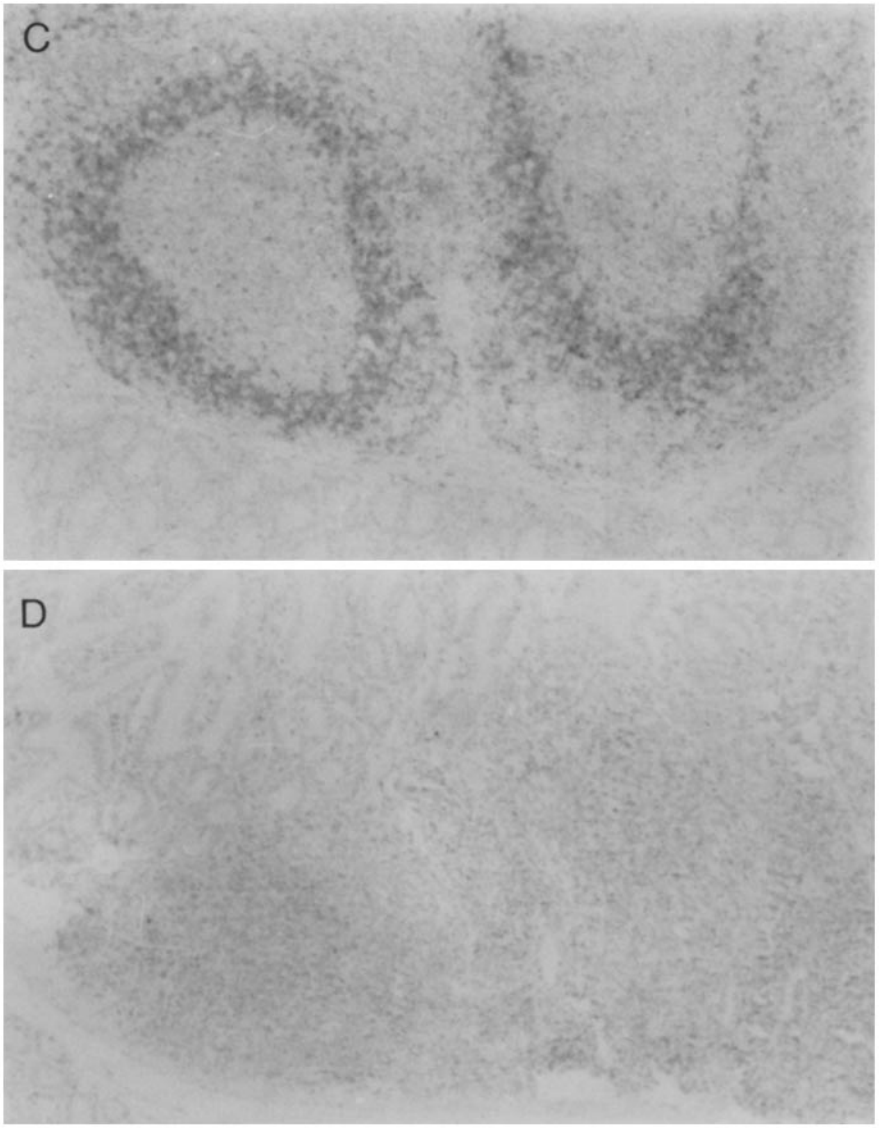

FIG. 1. C and D.

depressed. In fact, it was as depressed as in mice injected with anti-IgD throughout the entire immunization period (protocol 1). The reverse relationship could be seen with protocol 3 where, although the number of IgD-bearing cells of the suppressed allotype was low at the time of assay, the secondary PFC response was near normal.

Of particular interest are the observations on heterozygous mice injected simultaneously with both anti-Ig-5a and anti-Ig-5b starting at birth (Table V). These animals, although they showed a marked depression in the frequency of total IgD-bearing cells, produced normal or perhaps somewhat enhanced numbers of anti-SRBC IgG PFC of both linked allotypes.

TABLE III

Effect of Anti-Delta[b] Treatment on Secondary Immune Responses of Allotype Heterozygous Mice

Protocol*	Duration of anti-Ig-5b injections (0 — 42↓ — 77↓, d)	Number of mice	Anti-SRBC PFC per spleen $\times 10^{-3}$ for allotype‡		Frequency of spleen cells bearing IgD of allotype§	
			a	b	a	b
1	|·····|	9	153.7 ×÷ 0.62	11.5 ×÷ 1.20	18.8 ± 1.20	2.5 ± 0.83
2	|····· |	7	121.3 ×÷ 0.82	8.9 ×÷ 1.20	16.7 ± 1.40	9.6 ± 1.20
3	| ·····|	10	154.4 ×÷ 0.53	45.8 ×÷ 0.56	21.4 ± 1.10	1.4 ± 0.26
4	| · |	9	143.9 ×÷ 0.49	50.3 ×÷ 0.50	21.4 ± 0.77	6.6 ± 0.71
5	| |	31	79.6 ×÷ 0.34	70.7 ×÷ 0.41	14.5 ± 0.71	15.3 ± 0.68

* Days 0, 7, 14, and 21: 10 μg injected intraperitoneally. Thereafter, 2 μg/g body weight was injected weekly, according to the schedule indicated by dots. 4×10^8 SRBC were injected intravenously on days 43 and 78, as indicated by arrows. The assay was done on day 84.

‡ PFC developed with allotype-specific antisera. 19S PFC subtracted from total. Expressed as geometric mean ×÷ SE.

§ Determined by immunofluorescence. Expressed as arithmetic mean ± SE.

TABLE IV

Effect of Anti-Delta[a] Treatment on Secondary Immune Responses of Allotype Heterozygous Mice

Protocol*	Duration of anti-Ig-5a injections (0 — 42↓ — 77↓, d)	Number of mice	Anti-SRBC PFC/spleen $\times 10^{-3}$ for allotype‡		Frequency of spleen cells bearing IgD of allotype§	
			a	b	a	b
1	|·····|	12	15.4 ×÷ 1.14	120.3 ×÷ 0.61	2.6 ± 0.32	17.4 ± 1.20
2	|····· |	11	26.2 ×÷ 0.61	132.8 ×÷ 0.46	9.3 ± 1.50	15.6 ± 1.40
3	| ·····|	6	52.6 ×÷ 0.71	49.0 ×÷ 0.71	2.9 ± 0.65	13.7 ± 1.70
4	| · |	5	39.3 ×÷ 0.81	51.3 ×÷ 0.69	7.0 ± 1.10	11.5 ± 0.62
5	| |	31	79.6 ×÷ 0.34	70.7 ×÷ 0.41	14.5 ± 0.71	15.3 ± 0.68

* Days 0, 7, 14, and 21: 10 μg was injected intraperitoneally. Thereafter, 2 μg/g body weight was injected weekly, according to the schedule indicated by dots. 4×10^8 SRBC were injected intravenously, on days 43 and 78, as indicated by arrows. The assay was done on day 84.

‡ PFC developed with allotype-specific antisera. 19S PFC subtracted from total. Expressed as geometric mean ×÷ SE.

§ Determined by immunofluorescence. Expressed as arithmetic mean ± SE.

TABLE V

Effect of Simultaneous Anti-Delta[a] plus Anti-Delta[b] Treatment on Secondary Immune Responses of Allotype Heterozygous Mice

Protocol*	Number of mice	Anti-SRBC PFC/spleen $\times 10^{-3}$ for allotype		Frequency of spleen cells bearing IgD of allotype	
		a	b	a	b
1	6	122.9 ×÷ 0.65	95.0 ×÷ 0.68	3.1 ± 0.56	4.7 ± 0.80
5	31	79.6 ×÷ 0.34	70.7 ×÷ 0.41	14.5 ± 0.71	15.3 ± 0.68

* See footnotes to Tables III and IV.

In two experiments heterozygous mice suppressed with anti-5a, according to protocols 1 and 2, were injected with TNP-bovine gammaglobulin (BGG) in CFA instead of SRBC and were boosted with soluble TNP-BGG. As with SRBC, animals treated according to protocol 2 showed a similar degree of suppression of their anti-TNP antibody titers of the linked IgG_{2a} allotype (1:640 vs. 1:40,000 for controls) as did mice that had been injected, according to protocol 1, until boosting (mean titer 1:80). IgG_{2a} antibody titers of the nonsuppressed allotype were comparable in all groups (1:40,000).

Additional evaluation of the effect of anti-IgD on serum IgG levels was carried out at 12 wk of age (Table VI). When compared with the level of IgD-bearing cells, there was a concordant decrease in the serum IgG_{2a} concentration of the linked allotype. Thus, the serum IgG_{2a} allotype levels reflected the numbers of IgD-bearing cells of that allotype, whereas serum antibody levels reflected the numbers of PFC of that allotype.

Primary and Secondary Responses to TNP-BA in Homozygous Mice Suppressed with Anti-IgD from Birth. BALB/c mice injected from birth with anti-Ig-5a received a primary intravenous injection of TNP-BA at 21 d of age and a second intravenous injection 10 d later. Some mice were used 4 d after the second antigen injection for analysis of splenic anti-TNP PFC, whereas other animals were left for assay of serum anti-TNP titers at 7 d (Table VII). The anti-IgD treatment had no effect on serum antibody levels in either the primary or secondary responses or on the number of anti-TNP PFC in the secondary response, despite the absence of IgD-bearing cells and a marked depression in total Ig-bearing cells.

Additional groups of suppressed and control BALB/c mice were injected with TNP-BA in the front footpads and were boosted via the same route 10 d later. PFC in the brachial lymph nodes were assayed 4 d later (Table VIII). The observed suppression of the indirect anti-TNP PFC response was statistically significant in one of the two experiments.

TABLE VI

Concordant Effect of Anti-Delta Treatment on Serum IgG_{2a} Levels

Anti-delta injected	Protocol*	Time after last anti-delta injection	Serum concentration of IgG_{2a} immunoglobulin for allotype‡		Number of mice
			a	b	
		d	*mg/ml*		
Anti-5a (4.22)	1	7	0.3 ± 0.06	1.7 ± 0.18	15
	2	42	1.1 ± 0.15	2.2 ± 0.26	13
	3	7	1.4 ± 0.15	2.1 ± 0.16	6
	4	42	2.0 ± 0.17	1.7 ± 0.19	5
Anti-5b (H6/31HL)	1	7	2.2 ± 0.22	0.2 ± 0.08	8
	2	42	3.6 ± 0.43	0.7 ± 0.07	7
	3	7	2.7 ± 0.75	0.3 ± 0.09	13
	4	42	2.5 ± 0.25	0.7 ± 0.13	15
Anti-5a + anti-5b	1	7	1.9 ± 0.38	1.0 ± 0.11	6
None	5	—	2.3 ± 0.15	1.6 ± 0.09	31

* See footnotes to Tables III and IV.

‡ Determined by radioimmune blocking assay. Serum taken on day 84. Expressed as arithmetic mean ± SE.

TABLE VII

*Effect of Treatment with Anti-IgD from Birth on Immune Responses of BALB/c Mice to TNP-BA**

Parameter	Immune response studied‡	Mice treated with	
		Anti-Ig5a§	None§
Anti-TNP serum titers (log_2 ± SE)	Primary	8.5 ± 0.3 (10)	8.1 ± 0.4 (5)
	Secondary	14.3 ± 0.8 (5)	14.7 ± 0.5 (5)
Anti-BA serum titers (log_2 ± SE)	Primary	5.4 ± 0.4 (10)	6.4 ± 0.2 (5)
	Secondary	14.7 ± 0.2 (5)	14.5 ± 0.2 (5)
7S anti-BA serum titers (log_2 ± SE)	Secondary	11.8 ± 0.2 (5)	11.8 ± 0.9 (5)
19S anti-TNP PFC/spleen (geometric mean $\times/\div$ SE)	Secondary	213,796 $\times/\div$ 1.3 (5)	269,153 $\times/\div$ 1.4 (12)
7S anti-TNP PFC/spleen (geometric mean $\times/\div$ SE)	Secondary	478,630 $\times/\div$ 1.3 (5)	891,251 $\times/\div$ 1.5 (12)
Percentage of IgD-bearing cells/spleen ± SE‖		0 ± 0 (5)	18.4 ± 3.4 (4)
Percentage of Ig-bearing cells/spleen ± SE‖		6.6 ± 0.6 (5)	22.4 ± 4.0 (4)

* Intraperitoneal injections of 4.22 anti-5a were given three times a week as follows: 5 μg per injection the 1st 10 d, 10 μg per injection the next 10 d, and 50 μg per injection thereafter.

‡ 800 μg TNP-BA was injected intravenously on days 0 and 10. Sera were examined 7 d after primary or secondary antigen injection. 7S serum titers were determined in the presence of 0.1 M mercaptoethanol. Splenic PFC were determined on day 4 after the second injection; 7S PFC were developed with anti-Ig in the presence of anti-mu in the agar.

§ There were no statistically significant differences between anti-IgD-treated and control mice in any parameter of the immune response studied. Numbers of mice are in parentheses.

‖ Determined by immunofluorescence.

TABLE VIII

Secondary Immune Response in Lymph Nodes Draining Footpad Injection of TNP-BA of Anti-IgD-treated and Normal BALB/c Mice

Experiment	Days of TNP-BA injection*	Anti-IgD treatment‡	Geometric Mean $\times/\div$ SE (n) of anti-TNP PFC/lymph node on day 4		Frequency of spleen cells bearing§	
			Direct	Indirect	Delta	Ig
1	Day 0 and day 10	None	219 $\times/\div$ 1.6	1071 $\times/\div$ 1.6 (5)‖	22.3 ± 5.8	22.1 ± 4.6
	Day 0 and day 10	Anti-5a	135 $\times/\div$ 3.0	223 $\times/\div$ 2.2 (5)‖	1.6 ± 0.5	13.8 ± 1.0
	Day 10	None	17 $\times/\div$ 1.6	5 $\times/\div$ 4.7 (5)	ND	ND
2	Day 0 and day 10	None	1230 $\times/\div$ 2.3	3631 $\times/\div$ 1.5 (4)¶	37.1 ± 3.4	ND
	Day 0 and day 10	Anti-5a	257 $\times/\div$ 1.4	158 $\times/\div$ 1.5 (4)¶	3.8 ± 1.1	ND
	Day 10	None	68 $\times/\div$ 1.2	22 $\times/\div$ 1.8 (4)	ND	ND

* Dose of TNP-BA in each footpad was 0.8 μg on day 0 and 0.8 μg (experiment 1) or 8 μg (experiment 2) on day 10.

‡ Injections of 4.22 anti-5a were given three times a week as follows: 5 μg per injection the 1st 10 d, 10 μg per injection the next 10 d, and 50 μg per injection thereafter.

§ Determined by immunofluorescence.

‖ $P < 0.2$.

¶ $P < 0.01$.

Response to Other TNP Conjugates by Homozygous Mice Treated with Anti-IgD from Birth. In vitro experiments have suggested that responses to TNP-BA are much less dependent on IgD-bearing cells than are responses to thymus-dependent antigens (3). In view of the lack of suppression of the splenic PFC response to TNP-BA by mice chronically suppressed with anti-IgD, the effect of anti-IgD treatment on the in vivo response to a T dependent antigen (TNP-KLH) was determined (Table IX). Despite the usual depression of IgD-bearing cells, there was no significant inhibition of either

TABLE IX

Primary Responses in Homozygous Mice Treated with Anti-IgD from Birth

Mouse strain	Antigen on day 0	Anti-IgD treatment*	Geom mean ×÷ SE (n) of anti-TNP PFC/spleen on day 4		Frequency of spleen cells bearing§	
			19S	7S‡	delta	1g
					%	%
BALB/c	TNP-Ficoll (10 μg)	None	269,200 ×÷ 1.4	251,200 ×÷ 1.4 (5)	19.0 ± 2.1	24.6 ± 3.1
	TNP-Ficoll (10 μg)	Anti-Ig-5a	457,100 ×÷ 2.0	489,800 ×÷ 1.8 (5)	0.8 ± 0.5	8.2 ± 0.7
BALB/c	TNP-KLH (100 μg)	None	10,200 ×÷ 1.5	7,200 ×÷ 1.2 (6)	17.1 ± 3.4	18.1 ± 2.6
	TNP-KLH (100 μg)	Anti-Ig-5a	30,900 ×÷ 1.1	15,500 ×÷ 1.3 (3)	0	8.0 ± 0.8
SJL/J	TNP-KLH (100 μg)	None	1,700 ×÷ 1.1	11,000 ×÷ 1.1 (3)	28.9 ± 4.6	29.7 ± 4.0
	TNP-KLH (100 μg)	Anti-Ig-5b	5,200 ×÷ 1.1	5,600 ×÷ 1.3 (4)	2.5 ± 1.4	5.9 ± 1.6

* Intraperitoneal injections of 4.22 anti-5a (BALB/c) or of H6/31 HL anti-5b (SJL/J) three times a week: 5 μg per injection the 1st 10 d, 10 μg per injection the next 10 d, and 50 μg per injection thereafter.
‡ Indirect plaques were developed by anti-Ig with goat anti-μ added to agar.
§ Determined by immunofluorescence. Expressed as arithmetic mean ± SE.

the 19S or 7S responses to TNP-KLH in homozygous mice. If any effect occurred, it was in the direction of enhancement, especially in the 19S response.

It has been shown that a highly mature B cell subpopulation that is Lyb5 positive and has a high surface delta:mu ratio is, under normal conditions, responsible for the response to TNP-Ficoll, a class 2 thymus-independent antigen (25). The effect of treating homozygous mice with anti-IgD from birth on their response to TNP-Ficoll was therefore studied (Table IX). No depression of either the 19S or 7S splenic anti-TNP PFC responses was observed. The apparent increase in the PFC response of treated mice was not statistically significant.

Discussion

The results reported here indicate that it is possible to readily suppress the immune response of one allotype specificity in heterozygous mice by in vivo treatment with the appropriate anti-IgD antiserum. In contrast, even with fivefold higher doses of anti-IgD, the immune response of homozygous mice is not suppressed, despite a virtual absence of IgD-bearing cells and a marked reduction in total Ig-bearing cells. In agreement with results in homozygous mice, simultaneous treatment of heterozygotes with antisera directed against IgD of both allotypes causes no depression of the immune response.

In the present studies, recovery from anti-IgD suppression with respect to number of IgD-bearing cells was slow, particularly in the heterozygous mice, where a normal ratio of allotypes was not observed even 6 wk after termination of anti-IgD treatment. Suppressed mice, in some cases, showed a compensatory increase both in the PFC responses and in the serum IgG_{2a} levels of the alternate allotype. A corresponding compensatory increase in IgD-bearing cells of the nonsuppressed allotype was not seen.

The overall results suggest that IgD-bearing cells are not essential for an immune response in vivo. In homozygous suppressed animals, lacking IgD-bearing cells, the remaining B cells (~1/3–1/4 of control) can operate at a normal level. It is therefore clear that the immune system is able to employ the cell population that is present at the time of antigen exposure in an effective manner. Thus, it seems that the presence of IgD on the cell surface is not obligate for the in vivo activation of primary and secondary immune responses to intravenously injected antigens.

It is known from studies on the rabbit (26) and the chicken (27) that it is far more difficult to suppress homozygous than heterozygous animals. In homozygous rabbits, suppression of a V_H locus product leads to a compensatory increase in the products of alternative loci (28). If there were IgD subclasses in the mouse, suppression of homozygous mice with anti-allotypic antibodies might result in a comparable effect. This possibility was excluded by the finding that a heterologous anti-IgD also failed to reveal any IgD-bearing cells in the suppressed mice. Furthermore, studies at the DNA level have detected only a single IgD locus in the mouse (29). In heterozygous animals, in which essentially complete suppression can readily be obtained, a skewed representation of the allotype tends to persist for a long time after anti-allotype injection. In the chicken and the rabbit, it was shown that the stability of the altered allotype expression is due to what has been referred to as B cell clonal dominance (27, 30). In studies on idiotype suppression in mice, clonal dominance has also been observed (31). We would suggest that, in line with a network concept of immune function (32), IgD allotype suppression results in a perturbation of the B cell clone distribution. A certain distribution of idiotypic specificities occurs and, because of its stabilizing properties, clonal interactions tend to perpetuate this distribution and prevent the establishment of new clones. Therefore, the skewed allotype representation in responses to antigens presented during suppression would tend to persist long after the suppression wanes. Alternatively, allotype-specific regulatory cells could determine and stabilize the prevalence of the alternative allotype in antibody responses of suppressed heterozygous mice. Thus, in our studies, the effect on the allotype representation in the antibody response persists longer than does the suppression of IgD-bearing cells.

The lack of suppression of the in vivo immune response after chronic treatment with anti-IgD is not inconsistent with inhibition of in vitro responses. Treatment with anti-IgD from birth evidently permits an expansion of compensatory cell populations that probably could not occur under in vitro conditions. It is interesting that the operation of compensatory mechanisms is seen most dramatically after intravenous antigen presentation. In the present studies, after footpad injection of antigen, and in the results of Layton et al. (4), after intraperitoneal injection of antigen, significant depressions of the 7S secondary immune response were seen in homozygous mice suppressed with anti-IgD from birth. The variation in results obtained with different routes of antigen presentation suggests that the capacity for compensation for a lack of IgD-bearing cells differs in the compartments of the immune system. Presumably, this reflects differences in the distribution of cell populations in the various compartments. Whether these differences are qualitative or quantitative cannot be determined from the available data. It seems reasonable to suggest that the patterns of migration and recirculation of distinctive subsets of cells (e.g., IgD-positive vs. IgD-negative cells) could account for these differences. One must also take into account the possible

influence of antigen concentration and/or epitope density in antibody responses involving IgD (12, 33).

The critical conclusion of the present studies is that under some experimental conditions, an alternative pathway of response, not involving IgD-positive cells, can be visualized and can operate at a high level of efficiency with respect to primary responses to TNP-KLH, TNP-BA, and TNP-Ficoll, secondary responses to SRBC and TNP-BA, and germinal center generation. It should be pointed out that our hypothesis would lead to different predictions for the results of acute and chronic suppression. Time would be required for an imbalance of the B cell system to be established as a consequence of treatment with anti-IgD allotype in heterozygotes. Thus, effects such as we have been studying would probably only be seen in chronically suppressed animals. Recently Metcalf et al. (24) have described experiments using a heterologous anti-IgD antiserum, the results of which were interpreted along similar lines to those discussed here. It is interesting that we have found that the capacity to compensate for the absence of IgD-bearing cells extends even to the response to TNP-Ficoll, which is normally limited to a subset of B cells that have a high delta:mu ratio of surface Ig (25). It should be emphasized that the results reported here in no way contradict the view that the immune response predominantly proceeds via IgD-bearing cells under normal conditions.

Summary

The role of delta-positive cells in the immune response was studied by comparing the effects of treatment with allotype-specific IgD hybridoma antibody on homozygous BALB/c or SJL/J and heterozygous (BALB × SJL)F_1 mice.

Homozygous mice, injected from birth with the relevant anti-delta antibody, made primary or secondary immune responses to intravenously injected trinitrophenyl (TNP)-*Brucella abortus*, TNP-Ficoll, and TNP-keyhole limpet hemocyanin, which did not differ significantly from those of control mice, despite the fact that IgD^+ cells were depleted and Ig^+ cells were markedly reduced in the spleens of treated mice. Responses in nodes draining a local injection of TNP-*Brucella abortus* were, however, significantly suppressed.

Heterozygous mice, injected from birth with either anti-Ig-5a or anti-Ig-5b, showed a marked reduction in the number of cells producing IgG antibody of linked allotype specificity in the secondary response to intravenously injected sheep erythrocytes. A corresponding decrease in the amount of serum IgG_{2a} of that allotype specificity was also noted. However, in agreement with the results obtained in homozygotes, heterozygotes injected simultaneously with anti-IgD directed against each of the allotypes made normal, if not enhanced, plaque-forming cells responses of both allotype specificities. Similarly, serum IgG_{2a} levels were normal in all but one mouse treated in this fashion.

These results indicate that IgD^+ cells are not essential for an immune response in vivo. Although the delta-positive cell is used preferentially under normal conditions, it appears that an alternative mechanism exists by which, in the absence of these cells, the animal is able to make a normal immune response.

We would like to thank Dr. Fred Finkelman for his generous gift of rabbit anti-mouse IgD.

Received for publication 6 April 1981.

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