IDIOTYPE-ANTIIDIOTYPE REGULATION

V. The Requirement for Immunization with Antigen or Monoclonal Antiidiotypic Antibodies for the Activation of $\beta 2 \rightarrow 6$ and $\beta 2 \rightarrow 1$ Polyfructosan-reactive Clones in BALB/c Mice Treated at Birth with Minute Amounts of Anti-A48 Idiotype Antibodies*

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The regulation of lymphocyte function by antibodies specific for the antigenic determinants of immunoglobulin molecules is well documented. In utero exposure to antiallotypic or antiidiotypic antibodies induces a chronic suppression of the corresponding allotype (1) or idiotype (Id)[1] (2, 3). Similarly, the parenteral administration after birth of antiisotypic, (μ or δ), antiallotypic, or antiidiotypic antibodies can cause the suppression of synthesis of immunoglobulins (4, 5), corresponding allotype (6), or Id (7), respectively. The parenteral administration of antiidiotypic antibodies in adult animals has many effects. It can cause idiotypic suppression, elicit the production of anti-(antiidiotypic) antibodies, or prime, in the absence of antigen, the Id-bearing B cell clones, the Id-bearing helper T cells or suppressor T cells, and T cells that mediate delayed-type hypersensitivity or contact sensitivity reactions (for a review see reference 8).

In previous studies we have shown that the administration of antiidiotypic keyhole limpet hemocyanin (KLH) antibody conjugates, in complete Freund's adjuvant, into adult BALB/c mice led to a substantial activation of minor clones (i.e., M460 Id trinitrophenyl [TNP]-specific clones) (9), or silent clones (i.e., A48 Id $\beta 2 \rightarrow 6$ fructosan-specific clones) (10). The expression of these clones, which are associated with the Igh^a haplotype, can be obtained in various strains of mice. We have shown that M460 Id^+ clones can be activated in DBA/2 mice (Igh^c) (11) and that the A48 Id^+ clones can be activated in CAL.20 (Igh^d), BAB.14 ($IghV^aC^b$), or CCB.R4 ($IghV^bC^a$) mouse strains (12).

* Supported in part by grants PCN1105788 from the National Science Foundation and IM-275A from the American Cancer Society.

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[1] *Abbreviations used in this paper:* BL, bacterial levan; BSA, bovine serum albumin; Id, idiotype; IdX, cross-reacive idiotype; IEF, isoelectric focusing; In, inulin; KLH, keyhold limpet hemocyanin; NP, (4-hydroxy-3-nitropenyl)acetyl; PFC, plaque-forming cell; SRBC, sheep erythrocytes; TNP, trinitrophenyl.

Recently Kelsoe et al. (13) have reported that the administration, in saline, of minute amounts of a monoclonal anti-(4-hydroxy-3-nitrophenyl) acetyl (NP) idiotypic antibody (AC146) into C57BL/6 mice induced a 20-fold enhancement of synthesis of the B1-8 idiotope borne by an anti-NP monoclonal antibody. Since we did not succeed in activating the A48 Id^+ $\beta 2 \rightarrow 6$ fructosan-reactive clones by the injection of syngeneic anti-A48 Id antibodies in saline into adult mice, we studied the effect of the administration of these antibodies after birth (14).

In this communication we present data that show that the administration at birth of minute amounts of anti-A48 Id antibodies causes a long-lasting activation of A48 Id^+ $\beta 2 \rightarrow 6$ fructosan-specific clones. This activation requires antigenic challenge (i.e., immunization with bacterial levan [BL]), and antigenic challenge can be replaced by a monoclonal anti-A48 Id antibody. This activation is related to a T-independent expansion of anti-$\beta 2 \rightarrow 6$ fructosan B cell clones expressing the A48 Id.

Materials and Methods

Animals. Normal BALB/c mice, 4–12-wk-old, were purchased from the Charles River Breeding Laboratory, Wilmington, MA. 1-d-old BALB/c mice were bred in our colony at the Mount Sinai School of Medicine. The recombinant inbred strain CXBJ was obtained from The Jackson Laboratory, Bar Harbor, ME.

Antigens. BL was isolated by alcohol precipitation from culture supernates of *Aerobacter laevenicum* (ATCC15552) grown at 23°C in nutrient broth supplemented with 5–10% sucrose. TNP-aminoethylcarbamylmethyl-Ficoll (TNP-Ficoll) was prepared as described previously (15). Inulin (In) was obtained from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA.

Myeloma Proteins. The BALB/c myeloma proteins used in this study were ABPC48 (A48), UPC10 (U10), W3082, and MOPC460 (M460); all are IgA_κ immunoglobulins except U10 which is $IgG2a_\kappa$. A48 and U10 possess $\beta 2 \rightarrow 6$ fructosan-binding activity and lack the cross-reactive idiotype (IdX) of myeloma proteins that bind both BL and In, such as W3082. M460 is a TNP-binding myeloma protein. These myeloma proteins were kindly provided by Dr. Michael Potter, National Cancer Institute.

Preparation of Anti-Id Antibody. Anti-A48 Id antisera were prepared in BALB/c mice by immunization with an A48-KLH conjugate. Anti-M460 Id, anti-U10 Id, and anti-W3082 Id antisera were prepared in the same way. The method used for coupling myeloma proteins to KLH as well as the schedule adopted for immunization, have been described elsewhere (10). Several syngeneic monoclonal anti-A48 Id antibodies were obtained from a fusion of immune lymphocytes from BALB/c mice immunized with A48 Id and the SP2/0 myeloma cell line.

Labeling of Sheep Erythrocytes (SRBC) with BL, In, and TNP. An *O*-stearoyl derivative of BL and In were prepared according to the method of Hämmerling and Westphal (16). Briefly, 1 ml of 10% SRBC, 3 ml saline, and 10 μl of the stearoyl derivative were added together and incubated for 30 min at 37°C. This was followed by extensive washing with saline before use in the hemolytic plaque assay. TNP was coupled to SRBC by the method of Rittenberg and Pratt (17).

Detection of Plaque-forming Cells (PFC). The number of PFC secreting antibody specific for BL, In, or TNP was determined by a technique in which spleen cells (50 μl) were mixed at 45°C with 50 μl of 10% SRBC coated with BL, In, or TNP in 0.3 ml 0.5% agarose and placed on slides. The slides were then incubated for 2 h at 37°C in the presence of RPMI 1640 media; this was followed by a 1-h incubation at 37°C in the presence of guinea pig complement diluted 1:20 with Dulbecco's phosphate-buffered saline. To determine the number of anti-BL PFC secreting antibody carrying A48, U10, and W3082 idiotypes, anti-In PFC carrying the W3082 Id, and anti-TNP PFC carrying the M460 idiotype, anti-A48, anti-U10, anti-W3082, and anti-M460 antiserum in a final

dilution of 1:10,000, 1:5,000, 1:100, and 1:500, respectively, were added to the agarose at the time of plating. The number of PFC observed in the presence of anti-Id was subtracted from the number observed if no inhibitor was present. The difference was considered to be the number of PFC secreting anti-BL, anti-In, or anti-TNP antibody expressing the corresponding idiotypes.

Purification of T and B Cells. Purified splenic B lymphocytes were obtained from BALB/c mice injected intraperitoneally with 0.1 ml rabbit anti-mouse thymocyte serum (M.A. Bioproducts, Walkersville, MD) 2 d before being sacrificed. The splenic lymphocytes were harvested and treated with anti-Thy-1.2 antisera plus complement to remove any residual T cells. Purified splenic T lymphocytes were obtained in the following manner. Plastic petri dishes (100 × 15 mm) were coated with 3 ml of rabbit anti-mouse immunoglobulin serum (ammonium sulphate fraction, 1 mg/ml) for 1 h at 37°C. The plates were then washed twice with phosphate-buffered saline, and 3 ml of a spleen cell suspension (5 × 10^7 cells) was added. Plates remained at room temperature for 1 h with gentle agitation every 15 min. After 1 h, the nonadherent spleen cells were harvested from the plates.

Isoelectric Focusing (IEF). IEF was performed according to a method described in detail previously (18).

Results

Requirement of Antigenic Challenge for the Activation of the A48 Id$^+$ Response in BALB/c Mice Treated at Birth with Anti-A48 Id Antibodies. The A48 Id of a β2 → 6 fructosan-binding myeloma protein, ABPC48, from the BALB/c mouse is not expressed on antibodies of various mouse strains during a conventional immune response after immunization with BL. However, the study of the anti-β2 → 6 fructosan response in 1-mo-old BALB/c mice treated at birth with 0.01–10 μg of anti-A48 Id antibodies demonstrated the appearance of A48 Id$^+$ anti-β2 → 6 fructosan PFC (Fig. 1). The percentage of A48 Id$^+$ PFC was significantly higher in mice treated at birth with anti-A48 Id antibodies than in mice not so treated.

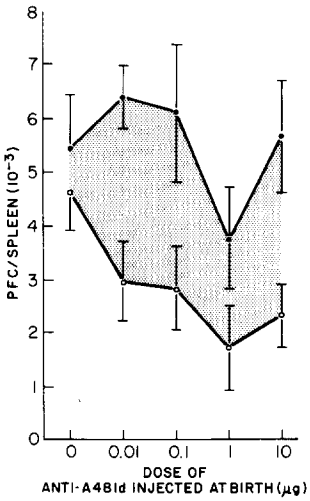

FIGURE 1. Dose-effect relationship of treatment of newborn mice with various doses of anti-A48 Id polyclonal antibody. Mice were 1-d-old at the time of treatment and challenged 1 mo later with 20 μg BL. (●) Anti-BL PFC/spleen; (○) anti-BL PFC/spleen detected when syngeneic anti-A48 Id antisera had been added to the agarose. Shaded area represents the number of PFC bearing the A48 Id. Each point represents the mean ± SEM of determinations performed on five mice.

The immunization with BL was critical for the activation, since mice treated at birth with anti-A48 Id antibodies and not immunized with BL did not develop a $\beta 2 \rightarrow 6$ fructosan antibody response. Therefore, it appears that the administration at birth of anti-A48 Id antibodies was not sufficient by itself to activate A48 Id^+ anti-$\beta 2 \rightarrow 6$ fructosan-specific clones, but must be supplemented with antigenic challenge. It was previously demonstrated (14) that this activation is Id-specific since the treatment with anti-A48 Id, but not the treatment with anti-M384 Id, followed by immunization with BL 1 mo later, led to the activation of A48 Id^+ clones (14). The M384 Id is borne on the MOPC384 myeloma protein that binds α-methyl-D-galactoside, the immunodominant sugar shared on the lipopolysaccharide of *Salmonella tranaroa*, *Salmonella telaviv*, and *Proteus mirabilis* (19). Furthermore, BALB/c mice treated at birth with anti-A48 Id antibody and immunized 1 mo later with TNP-Ficoll did not show a significant increase of the A48 Id^+ component of the anti-$\beta 2 \rightarrow 6$ fructosan response, nor of the M460 Id^+ component of the anti-TNP response (Table I). Therefore, the effect produced by anti-A48 Id antibodies is specific, since treatment with anti-A48 Id did not alter the M460 Id^+ component of the anti-TNP response.

Long-lasting Activation of Precursors of A48 Id^+ Antibody-producing Cells After Treatment at Birth with Anti-A48 Id Antibodies. The persistence of activation of A48 Id^+-bearing clones after treatment of mice at birth with 0.01 and 10 μg of anti-A48 Id antibodies, was studied in BALB/c mice immunized at various ages. In these experiments the BALB/c mice after treatment at birth were immunized with 20 μg of BL at either 4, 6, 8, or 12 wk and their anti-BL PFC response was measured 5 d later. The data depicted in Table II shows that the A48 Id^+ component represents an insignificant fraction varying between 6 and 18% of the anti-BL response in 4–12-wk-old BALB/c mice. The A48 Id^+ component significantly increases (35–79%) in mice treated at birth with 0.01 or 10 μg of anti-A48 Id antibodies and challenged with the antigen, BL. Although the

TABLE I

Requirement of Antigen for the Activation of the A48 Id^+ Response in BALB/c Mice Treated at Birth with Anti-A48 Id Antibody

Mice treated at birth with:	Mice challenged with:	Anti-BL PFC/spleen*		Anti-TNP PFC/spleen‡	
		Total	A48 Id^+	Total	M460 Id^+
			%		%
Nil	20 μg BL	2,180 ± 513	6 ± 3	<100	ND§
Nil	20 μg TNP-Ficoll	<100	ND	44,800 ± 3,582	16 ± 7
0.01 μg anti-A48 Id	Nil	<100	ND	<100	ND
0.01 μg anti-A48 Id	20 μg BL	2,920 ± 718	52 ± 7	<100	ND
0.01 μg anti-A48 Id	20 μg TNP-Ficoll	<100	ND	39,700 ± 7,860	24 ± 6

* Total anti-BL PFC/spleen response was measured 5 d after immunization. The percentage of A48 Id^+ anti-BL PFC was determined by the addition of anti-A48 Id antisera to the agarose and scoring the difference in the number of PFC without antisera. Results represent the mean ± SEM for the data obtained from five mice.

‡ Total anti-TNP PFC/spleen response was measured 5 d after immunization. The percentage of M460 Id^+ anti-TNP PFC was determined by the addition of anti-M460 Id antisera to the agarose and scoring the difference in the number of PFC without antisera. Results represent the mean ± SEM for the data obtained from five mice.

§ Not determined, insufficient number of plaques.

TABLE II

Long-lasting Activation of the A48 Id$^+$ Response After Treatment at Birth with Anti-A48 Id Antibody

Age of mice when immunized with 20 μg BL	PFC/spleen after treatment at birth with:*					
	Nil		0.01 μg anti-A48 Id		10 μg anti-A48 Id	
	Total	A48 Id$^+$	Total	A48 Id$^+$	Total	A48 Id$^+$
wk		%		%		%
4	2,180 ± 513	6 ± 3	2,920 ± 718	52 ± 7	688 ± 85	39 ± 13
6	1,300 ± 120	14 ± 2	675 ± 196	79 ± 10	2,387 ± 950	53 ± 9
8	6,270 ± 480	18 ± 9	3,362 ± 2,234	46 ± 13	6,175 ± 1,561	49 ± 5
12	13,140 ± 5,125	11 ± 3	9,150 ± 2,850	47 ± 12	13,490 ± 3,054	35 ± 8

* Mean ± SEM for PFC/spleen detected for five mice, 5 d after immunization.

TABLE III

Ability of 17–38 Monoclonal Anti-A48 Id Antibody to Elicit an Anti-BL PFC Response in Mice Treated at Birth with 0.01 μg of Anti-A48 Id Antibody

Monoclonal anti-A48 Id antibody*	Isotype	Anti-BL PFC/spleen
26–125	IgG1	180
148–3	IgG1	140
100–105	IgG2b	180
23–113	IgM	120
14–114	IgM	40
17–38	IgM	2,120

* Each mouse received a 10 μg injection of the appropriate monoclonal anti-A48 Id antibody and the anti-BL PFC response was measured 5 d later. Results are expressed as the mean for two mice.

W3082 IdX anti-β2 → 6 and β2 → 1 fructosan-reactive clones become dominant in 6-wk-old mice (20) an A48 Id$^+$ anti-β2 → 6 fructosan response was still observed in 6–12-wk-old mice treated at birth with anti-A48 Id antibodies. Thus, the administration at birth of anti-A48 Id antibodies has a profound effect on B cell precursors because it causes a long-lasting activation during postnatal life.

A Monoclonal Anti-A48 Id Antibody Is Able to Replace the Antigenic Challenge Required for the Activation of an Anti-β2 → 6 Fructosan Response. The previous experiments demonstrated that antigenic challenge with BL was critical for the differentiation of A48 Id$^+$ anti-β2 → 6 fructosan precursors. In a pilot experiment we screened several syngeneic monoclonal anti-A48 Id antibodies for their ability to elicit an anti-β2 → 6 fructosan PFC response in mice treated at birth with polyclonal anti-A48 Id. These monoclonal antibodies recognize shared idiotopes on A48 and U10 monoclonal proteins (Goldberg et al., manuscript in preparation). The results depicted in Table III show that the injection of 10 μg of 17–38 monoclonal antibody instead of BL 1 mo after the treatment at birth with polyclonal anti-A48 Id antibodies induced a significant anti-BL PFC response. Monoclonal anti-A48 Id antibodies, including 17–38, did not inhibit any partic-

ular property with respect to their ability to bind labeled A48 or anti-(anti-A48 Id) antibodies. In addition, the binding of labeled A48 to these monoclonal Ab_2 was inhibited by BL (0.01–0.1 μg/ml) (data not shown). This "antigen-like" effect was obtained with various concentrations (1, 10, 100 μg) of 17–38 monoclonal antibodies (Fig. 2). In further experiments we studied the activation of both β2 → 6 and β2 → 1 fructosan-reactive clones in mice treated at birth with anti-A48 Id antibodies or BL and challenged 1 and 3 mo later with 1 μg of 17–38 monoclonal antibody. The rationale of this experiment followed our previous observation, that there was a substantial ontogenic delay of the anti-β2 → 1 fructosan antibody response (20). The results depicted in Table IV show that BALB/c mice treated at birth with anti-A48 Id antibodies or BL and challenged 1 mo later with 17–38 monoclonal antibody develop only an A48 Id^- anti-BL response. It should be noted that only a U10 Id^+ anti-BL response was observed in mice treated at birth with BL and injected 1 mo later with 17–38. By contrast, in mice challenged with BL, ~50% of the anti-β2 → 6 fructosan PFC expressed the A48 Id. Furthermore, the mice treated at birth with anti-A48 Id antibodies and challenged 3 mo later with 17–38 monoclonal antibody developed an anti-β2 → 6 and anti-β2 → 1 fructosan response expressing the dominant W3082 IdX. W3082 monoclonal protein shares IdX G, B, and A of E109 and J606 In-binding monoclonal proteins (3, 21). 1-mo- and 3-mo-old BALB/c mice not treated at birth with anti-A48 Id antibodies and injected with 1 μg 17–38 monoclonal antibody did not develop an anti-β2 → 6 or anti-β2 → 1 fructosan PFC response (data not shown). In addition, in both groups of mice the anti-TNP PFC response and M460 Id^+ component were not augmented in the mice treated at birth with anti-A48 Id antibodies and challenged 1 or 3 mo later with 17–38 monoclonal antibodies.

Analysis of IgG Antibodies by IEF. Sera from 1- and 3-mo-old mice treated or not at birth with anti-A48 Id antibodies and challenged with BL or 17–38 were analyzed by IEF with ^{125}I-In-bovine serum albumin (BSA) and ^{125}I-BL. In the sera of three individual 1-mo-old BALB/c mice immunized with BL, a set of faint bands that bound BL and focused between pH 7 and 8.2 were observed

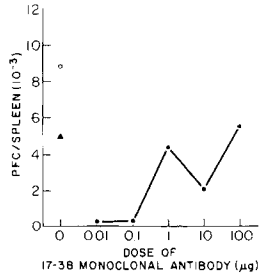

FIGURE 2. Dose-effect relationship of the challenge with various doses of 17–38 monoclonal anti-A48 Id antibodies. Mice were 1-d-old at the time of treatment with 10 ng polyclonal anti-A48 Id antibody and were challenged 1 mo later with various doses of 17–38 monoclonal anti-A48 Id antibody. (●) Anti-BL PFC/spleen; (○) anti-BL PFC/spleen of mice treated at birth with anti-A48 Id antibody and challenged 1 mo later with 20 μg BL; (▲) anti-BL PFC/spleen of normal mice challenged with 20 μg BL at 1 mo of age. Each point represents the mean of determinations performed on two mice.

TABLE IV

Age Dependence and the Specificity of Activation of Anti-β2 → 6 and Anti-β2 → 1 Fructosan Clones in BALB/c Mice Treated at Birth with 0.01 μg Anti-A48 Id Antibody and Challenged with 17–38 Monoclonal Antibody

Mice treated at birth with:	Challenged with:*	Age of mice	Anti-BL PFC/spleen‡				Anti-In PFC/spleen		Anti-TNP PFC/spleen	
			Total	A48 Id+	U10 Id+	W3082 IdX+	Total	W3082 IdX+	Total	M460 Id+
		mo		%	%	%		%		%
Nil	BL	1	2,850 ± 824	14 ± 7	29 ± 16	76 ± 9	<100	ND§	<100	ND
Nil	TNP-Ficoll	1	<100	ND	ND	ND	<100	ND	48,600 ± 6,713	1 ± 1
BL‖	Nil	1	<100	ND	ND	ND	<100	ND	<100	ND
Anti-A48 Id	Nil	1	<100	ND	ND	ND	<100	ND	<100	ND
BL	BL	1	4,100 ± 2,800	5 ± 5	14 ± 14	23 ± 23	<100	ND	<100	ND
Anti-A48 Id	BL	1	2,920 ± 718	52 ± 7	NP	NP¶	<100	ND	<100	ND
BL	17–38	1	1,750 ± 310	0	33 ± 1	30 ± 3	310 ± 70	0	<100	ND
Anti-A48 Id	17–38	1	1,815 ± 225	7 ± 7	8 ± 6	49 ± 13	<100	ND	<100	ND
Nil	BL	3	15,417 ± 5,140	15 ± 10	34 ± 6	57 ± 24	3,400 ± 1,588	85 ± 9	<100	ND
Nil	TNP-Ficoll	3	<100	ND	ND	ND	<100	ND	97,300 ± 18,631	0
Anti-A48 Id	Nil	3	<100	ND	ND	ND	<100	ND	<100	ND
Anti-A48 Id	BL	3	9,150 ± 2,850	47 ± 12	NP	NP	NP	NP	<100	ND
Anti-A48 Id	17–38	3	6,750 ± 3,384	0	14 ± 7	78 ± 9	5,250 ± 2,905	76 ± 5	925 ± 364	28 ± 24

* Mice were challenged with either 20 μg BL, 20 μg TNP-Ficoll, or 1 μg 17–38.

‡ All PFC results are expressed as the mean ± SEM. The percentage of Id+ PFC in each group were detected by plaque inhibition experiments incorporating the appropriate antisera and scoring the difference in the number of plaques vs. the total.

§ Not determined, insufficient number of plaques.

‖ Mice were immunized at birth with 10 μg BL.

¶ Experiment not performed.

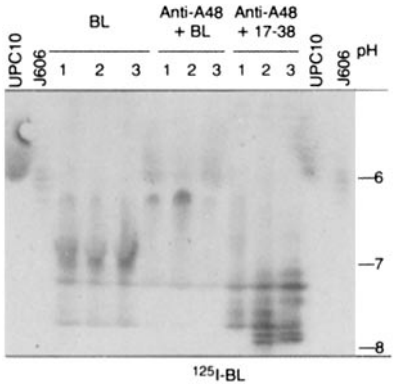

FIGURE 3. 10 μl of serum from individual, 1-mo-old BALB/c mice were analyzed by IEF autoradiography using ^{125}I-BL antigen overlay. The groups are indicated above the autoradiogram and the individual sera are indicated by the numbers within each group. 10 μl of a 1 mg/ml solution of U10 and J606 purified myeloma proteins were applied to the gel for reference. Autoradiograms were obtained by exposing the film for 8–10 d.

(Fig. 3). The dark curved bands seen in this group and in two of the three sera in the anti-A48 plus -BL group that focus between pH 6.8 and 7.2, are nonspecific and are due to the fact that these sera were highly hemolyzed. The bands focusing between pH 7 and 8.2 correspond to the major anti-BL spectrotype described previously in adult BALB/c mice (18). An additional spectrotype, also described, can be seen in serum 1 in this group. These spectrotypes were not observed in sera of mice treated at birth with anti-A48 Id antibodies and immunized 1 mo later with BL. Some BL binding was seen, however, in the region between pH 5.7 and 6.2. These antibodies and U10, which also focused in this region and was poorly resolved, were specific for BL. All the mice treated at birth with anti-A48 Id antibodies and challenged 1 mo later with 17–38 produced an IgG anti-BL response consisting of five spectrotypes focusing between pH 7 and 8.5 (Fig. 3). Again, these spectrotypes were the same as those previously shown in the sera of adult BALB/c mice immunized with BL. No IgG anti-In antibodies were detected in the sera of 1-mo-old mice in all three groups (data not shown). The sera of 3-mo-old normal mice immunized with BL showed the characteristic BALB/c anti-In spectrotype shown previously (18) (Fig. 4). This spectrotype has the same focusing pattern as J606 (i.e., an In-binding myeloma protein of the IgG3 subclass). This spectrotype was also observed (Fig. 4) in three mice treated at birth with anti-A48 Id antibodies and immunized 3 mo later with BL. However, BL binding showed that this spectrotype in the sera from anti-A48 Id-treated mice bound better to BL than to In (Fig. 5).

We have previously reported (18) that the recombinant inbred strain, CXBJ, also has the characteristic BALB/c anti-In spectrotype. We report here that this spectrotype in both CXBJ and BALB/c sera is indistinguishable from that of the J606 myeloma (Fig. 6). We have observed, however, that late in the response after a single immunization with BL, this spectrotype shows preferential binding to BL rather than In in sera from CXBJ mice (Fig. 7), but not in sera from BALB/c mice (18). We have also reported (22) that adult CXBJ mice, but not adult BALB/c mice, after treatment with anti-E109 (anti-IdX) and immunization

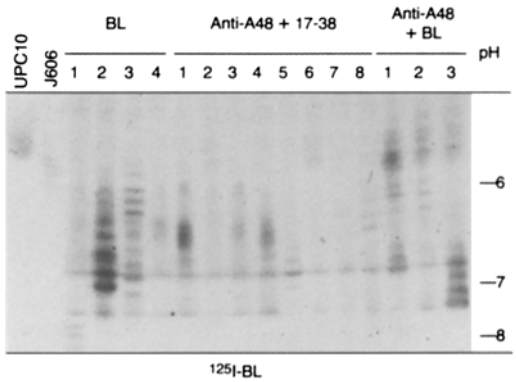

FIGURE 4. 10 μl of serum from individual, 3-mo-old BALB/c mice were analyzed by IEF autoradiography using a ^{125}I-BL antigen overlay. The groups are indicated above the autoradiogram and the individual sera are indicated by the numbers within each group. 10 μl of a 1 mg/ml solution of U10 and J606 purified myeloma proteins were applied to the gel for reference. Autoradiograms were obtained by exposing the film for 8–10 d.

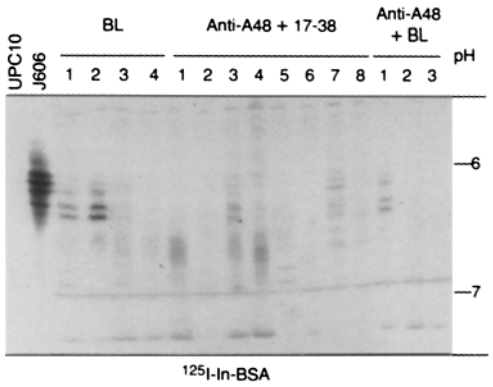

FIGURE 5. 10 μl of the same sera from the individual, 3-mo-old BALB/c mice, as were shown in Fig. 4, were analyzed using a ^{125}I-In-BSA overlay. The groups are indicated above the autoradiogram and the individual sera are indicated by the numbers within each group. 10 μl of a 1 mg/ml solution of U10 and J606 purified myeloma proteins were applied to the gel for reference. Autoradiograms were obtained by exposing the film for 8–10 d.

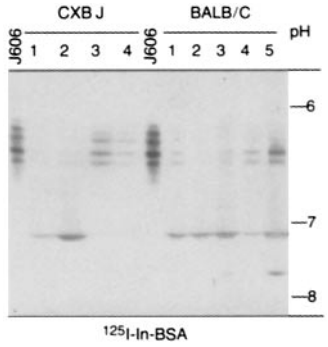

FIGURE 6. 10 μl of serum obtained from individual CXBJ and BALB/c mice 10 d after intravenous immunization with 20 μg BL were focused and overlaid with ^{125}I-In-BSA. 10 μl of a 1 mg/ml solution of J606 were applied for reference.

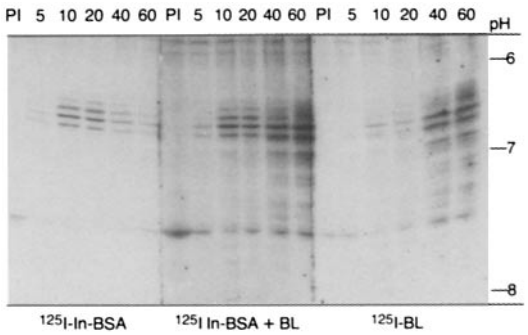

FIGURE 7. 10 μl of serum from an individual CXBJ mouse obtained before immunization or 5, 10, 20, 40, and 60 d after intravenous immunization with 20 μg BL, were focused in triplicate and each of the three panels was overlaid with radioactive antigen as indicated.

with BL, express A48 anti-BL. These data taken together suggest that the BALB/c anti-In spectrotype is comprised of two antibody clones, one of which preferentially binds BL rather than In and which may bear the A48 Id. In the sera of BALB/c mice treated at birth with anti-A48 Id and immunized at 3 mo of age with BL, we observed anti-BL antibody spectrotypes characteristic of normal BALB/c mice (Fig. 5). The sera of mice treated at birth with anti-A48 Id antibodies and challenged 3 mo later with 17–38, showed typical BALB/c In and BL spectrotypes when present. Therefore, the PFC and IEF data showed important alterations in the expression of anti-BL and -In clones in animals treated at birth with anti-A48 Id antibodies and immunized later with BL or 17–38.

T Independence of the Activation of A48 Id$^+$ Clones After Treatment at Birth with Anti-A48 Id Antibodies. In further experiments we investigated the requirement of T cells for the A48 Id$^+$ response. Thus, highly purified T and B cells from normal mice and mice treated at birth with anti-A48 Id antibodies were infused alone or in different combinations into lethally irradiated BALB/c mice. The mice were immunized with 20 μg BL at the time of cell transfer and the anti-BL PFC response was measured 5 d later. The data depicted in Table V show that the transfer of B cells from 1-mo-old animals treated at birth with anti-A48 Id antibodies into lethally irradiated BALB/c mice, followed by immunization with BL, was sufficient to obtain an A48 Id$^+$ PFC response. The infusion of B cells together with T cells from either normal animals or animals treated at birth with anti-A48 Id antibodies into lethally irradiated BALB/c mice, did not significantly alter the expression of A48 Id$^+$ clones. Furthermore, the infusion of B cells from normal animals along with T cells from animals treated at birth with anti-A48 Id antibodies, did not elicit an A48 Id$^+$ response. Therefore, these results suggest

TABLE V

Transfer of A48 Id$^+$ Response with B Cells from Mice Treated at Birth with 0.01 μg of Anti-A48 Id Antibody

Lethally irradiated BALB/c mice infused with:		Number of mice	Anti-BL PFC/spleen[§]	
B cells*	T cells[‡]		Total	A48 Id$^+$
				%
Nil	Nil	2	180 ± 170	7 ± 7
Normal	Nil	2	843 ± 375	0
Anti-A48	Nil	7	1,857 ± 964	46 ± 14
Normal	Anti-A48	3	940 ± 530	0
Anti-A48	Normal	7	1,094 ± 449	42 ± 11
Anti-A48	Anti-A48	2	650 ± 75	28 ± 6

* 40×10^6 B cells isolated from either normal BALB/c mice or BALB/c mice treated at birth with 0.01 μg anti-A48 Id.

‡ 20×10^6 T cells isolated from either normal BALB/c or BALB/c mice treated at birth with 0.01 μg anti-A48 Id.

§ Anti-BL PFC response was assayed 5 d after the infusion of B and/or T cells along with 20 μg BL. The percentage of A48 Id$^+$ PFC was determined by adding anti-A48 Id antisera into the agarose and scoring the difference in the number of plaques vs. the total response. Results are expressed as the mean ± SEM for the number of mice indicated.

that there is a direct interaction of anti-A48 Id antibodies with the immunoglobulin receptor of A48 Id^+ B cell precursors, which is responsible for their activation.

Discussion

Immunization of BALB/c mice with BL, a $\beta 2 \rightarrow 6$ polyfructosan with $\beta 2 \rightarrow 1$ branch points, leads to a vigorous T-independent antibody response (23). Two families of clones are involved in this response. The first family of clones is specific for the $\beta 2 \rightarrow 6$ fructosan linkage and does not express the Id of A48 and U10 myeloma proteins that are specific for the $\beta 2 \rightarrow 6$ linkage. An A48 Id^+ response was detected only CXBJ recombinant inbred strains of mice, among the various strains that were investigated (22). The synthesis of anti-$\beta 2 \rightarrow 6$ fructosan antibodies is independent of IghC genes and occurs early in ontogeny (20). The second family of clones produces antibodies specific for $\beta 2 \rightarrow 1$ fructosan linkages (21). These antibodies share the IdX G, B, and A of In-binding myeloma proteins. The expression of IdX is associated with the $IghC^a$ allotype. The clones producing these antibodies belong to the Lyb-5^+ subset and appear late in development (20).

In previous studies we have shown that A48 Id^+ $\beta 2 \rightarrow 6$ fructosan-specific clones can be activated in nude and recombinant inbred CXBJ mice (22), after administration at birth of A48 Id monoclonal protein (24), or in adult animals producing anti-(anti-A48 Id) antibodies (10). These results clearly show that antigen, idiotypic, or antiidiotypic antibodies can have a similar effect, leading to the expansion of cells bearing the corresponding Id. This suggests that the delicate balance between idiotypic and antiidiotypic clones that characterize the steady state network can be perturbed by antigen, idiotypic or antiidiotypic antibodies.

In this communication we present further results of the effect of the administration at birth of minute amounts of anti-A48 Id antibodies on the activation of normally silent A48 Id^+ anti-BL B cells. The priming at birth with minute amounts of antiidiotypic antibodies has a long-lasting effect, since an A48 Id^+ response was observed even in 12-wk-old mice. The long-lasting effect is not related to the persistence of antiidiotypic antibodies. Radiolabelled monoclonal proteins injected at birth are completely cleared from the blood by 30 d (24). The transfer of an A48 Id^+ response with highly purified B cells from mice treated at birth with anti-A48 Id antibodies and then infused into lethally irradiated mice, suggests that the activation of precursors of A48 Id^+ secreting cells is T-independent under these conditions. It might be envisioned that the interaction of anti-A48 Id antibodies with cellular receptors bearing the A48 Id mimics the binding of a T-independent antigen to a B cell receptor, and that in the absence of T cell help leads to the activation of that B cell. In a general sense, globular proteins such as a mouse immunoglobulin bearing a combining site with antiidiotypic specificity is very likely to behave as a "thymus-independent" antigen.

Although the anti-A48 Id antibodies can prime, independently of T cell help, the A48 Id-bearing precursors, their effect is not related to a mitogen-like effect. No anti-$\beta 2 \rightarrow 6$ fructosan response was observed in BALB/c mice treated at

birth with anti-A48 Id antibodies that were not challenged with BL or that were challenged with TNP-Ficoll. In several experimental systems it was clearly shown that the enhancement of the Id response after the administration of Id (13, 24, 25) or anti-Id antibodies (9, 10, 13) requires antigenic challenge.

The most striking observation in our study was the ability of a monoclonal anti-A48 Id antibody to elicit an anti-BL response in mice treated at birth with either polyclonal anti-A48 Id antibodies or BL. Such mice do not develop an anti-BL response without antigen or anti-Id challenge. The results of PFC and IEF summarized in Table VI show that whereas BL-immunized 1-mo-old mice develop only an anti-$\beta 2 \rightarrow 6$ fructosan response lacking A48 and U10 Id and exhibiting typical BALB/c spectrotypes, the 3-mo-old mice developed a vigorous anti-$\beta 2 \rightarrow 1$ and $\beta 2 \rightarrow 6$ fructosan response, expressed the W3082 IdX, and exhibited a typical BALB/c anti-BL spectrotype and anti-In J606-like spectrotype. The administration at birth of minute amounts of anti-A48 Id antibodies profoundly altered the clonal expression. Thus, the challenge with BL of 1-mo-old mice led to the activation of A48 and U10 Id^+ anti-BL clones which correspond to a U10-like spectrotype. Furthermore, immunization with BL of 3-mo-old mice still led to the activation of A48 and U10 Id anti-BL clones, but the product of these clones corresponds to a spectrotype that was observed in CXBJ mice, the single strain that developed an A48 Id^+ response (22). CXBJ spectrotype is characterized by five bands focusing in the same region as J606 but binds BL rather than In.

The challenge with 17–38 monoclonal anti-A48 Id antibody of mice treated at birth with anti-A48 Id antibodies or BL presented a completely different picture. In 1-mo-old mice the anti-BL antibodies failed to express the A48 and U10 Id and the spectrotype is of the type found in adult BALB/c mice. In 3-mo-old mice, in addition to an A48 Id^- and U10 Id^- anti-BL response, a vigorous In W3082 Id^+ PFC response was observed. These responses correspond to a

TABLE VI

Summary Results of PFC and IEF in Mice Treated at Birth with Anti-A48 Id and Challenged with BL or 17–38

Treatment at birth	Challenged with:	Age of mice	PFC response					IEF pattern (spectrotype)	
			BL	A48 Id^+	U10 Id^+	In	W3082 Id^+	BL	In
		mo							
Nil	BL	1	+	−	−	−	−	BALB/c	—
Anti-A48 Id	BL	1	+	+	ND*	−	−	U10-like	—
Anti-A48 Id	17–38	1	+	−	−	−	−	BALB/c	—
Nil	BL	3	+	−	±	+	+	BALB/c	BALB/c J606
Anti-A48 Id	BL	3	+	+	ND	ND	ND	BALB/c and CXBJ	CXBJ
Anti-A48 Id	17–38	3	+	−	±	+	+	BALB/c	BALB/c J606

* Not determined.

BALB/c BL spectrotype and In J606 spectrotypes as seen in normal 3-mo-old mice. These findings indicate that:

(*a*) the challenge with antigen can be replaced by one out of six monoclonal anti-A48 Id antibodies, clearly demonstrating that not all anti-A48 Id antibodies exhibit antigen-like properties. The ability of 17–38 monoclonal antibody to stimulate anti-$\beta 2 \rightarrow 6$ and $\beta 2 \rightarrow 1$ clones can not be related to a particular isotype or its avidity since other IgM monoclonal anti-A48 Id antibodies lack this property. The pentameric structure of IgM confers a high avidity to the antibodies belonging to this class of immunoglobulin; and (*b*) there is a fundamental difference between the challenge with antigen and the challenge with 17–38 monoclonal antibody. Whereas the challenge with antigen led to the activation of A48 and U10 Id^+ component of the anti-BL response (~50% in 1- and 3-mo-old mice), the challenge with 17–38 monoclonal antibody led to the suppression of A48 and U10 Id^+ clones and to the activation of A48 Id^- anti-$\beta 2 \rightarrow 6$ fructosan-reactive clones in 1-mo-old mice. In 3-mo-old mice there was an activation of W3082 IdX^+ anti-$\beta 2 \rightarrow 6$ and $\beta 2 \rightarrow 1$ fructosan-reactive clones. One possible explanation of the "antigen-like" property of this monoclonal antibody, is that it actually represents a homobody (26), i.e., an anti-Id antibody carrying the internal image of the antigen.

Anti-Id antibodies carrying the internal images of the antigens (i.e., homobodies) were reported in several systems. The majority of the studies, carried out on homobodies, were focused on their ability to inhibit the binding of antigen to its corresponding receptor (e.g., insulin, 27; retinol, 28; catecholamine, 29; formyl peptide, 30; reovirus hemagglutinin, 31; murine antiidiotypic antibody displaying anti-human γ-globulin activity, 32) or to mimic the antigen effects after the binding of homobodies to the "antigen cell receptor" (e.g., insulin, 27; alprenolol, 29). However, it is obvious that homobodies like all anti-Id antibodies ($Ab_2 = p_2i_2$) exhibit two functions, because through their paratopes (p_2), they can bind to clones bearing the corresponding Id and through their Id (i_2), they can stimulate other clones. We were fortunate that in our system we were able to analyze these two functions of 17–38 anti-A48 Id monoclonal antibody, since the administration at birth of minute amounts of anti-A48 Id antibodies induces a long-lasting activation of A48 Id^+ anti-$\beta 2 \rightarrow 6$ fructosan-reactive clones and the natural ontogenic delay of the expression of W3082 IdX anti-$\beta 2 \rightarrow 6$ and $\beta 2 \rightarrow 1$ fructosan-reactive clones.

Results suggest that through its idiotype(s) (i_2), the 17–38 monoclonal antibody activated the proliferation of A48 Id^- or W3082 IdX^+ clones ($Ab_1 = p_1{}^a i_1{}^a$ [Ab, antibody; p, paratope; i, idiotope]) and through its paratope occluded the expression of A48 Id^+ clones ($Ab_1 = p_1{}^b i_1{}^b$) (Fig. 8). Recently Jerne et al. (33) classified anti-Id antibodies into two categories: $Ab_{2\alpha}$, the anti-Id antibodies directed against the conventional idiotopes of Ab_1, and $Ab_{2\beta}$, the anti-Id antibodies carrying the internal image of the antigen. Our results show that a monoclonal anti-Id antibody carrying the internal image of the antigen is bifunctional because, by its paratope, it interacts with the immunoglobulin receptor of clones bearing the corresponding Id and by its idiotope can interact with the immunoglobulin receptor of clones expressing unrelated idiotypes. No available data exists on the structural correlates of the homobodies. It is very likely that the

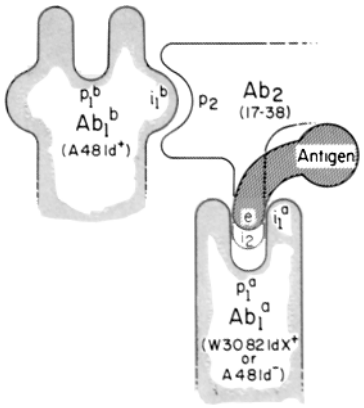

FIGURE 8. Functional dualism of a homobody. Ab, antibody; p, paratope; i, idiotope; e, epitope.

internal images are only putative copies (i.e., topochemical copies) of the antigen, a product of steric resemblance and not of identity in amino acid sequence. Crystallographic studies of Fab fragments of several anti-A48 Id monoclonal antibodies (including 17–38) will shed light on the shapes of variable regions of antibodies that mimic the antigen.

Summary

The anti-$\beta 2 \rightarrow 6$ fructosan antibodies sharing the idiotypes (Id) of ABPC48 (A48) monoclonal protein represent a silent fraction of the anti-$\beta 2 \rightarrow 6$ fructosan repertoire, since these antibodies cannot be detected during a conventional immune response elicited by bacterial levan (BL). However, the administration at birth of minute amounts of anti-A48 Id antibodies causes a long-lasting activation of A48 Id^+-bearing clones. This activation is related to direct interaction of anti-A48 Id antibodies with precursors bearing the A48 Id^+ immunoglobulin receptor, since an A48 Id^+ response can be transferred with highly purified B cells in lethally irradiated mice. The maturation of these precursors into A48 Id^+ anti-$\beta 2 \rightarrow 6$ fructosan antibody-secreting cells requires challenge by the antigen. Isoelectric focusing (IEF) data showed that in 1-mo-old mice an UPC10 (U10)-like spectrotype was observed, whereas in 3-mo-old mice, a new spectrotype binding BL rather than inulin (In) was identified. This spectrotype was observed only in CXBJ mice, the single strain in which an A48 Id^+ response was observed. The antigenic challenge can be replaced by a monoclonal anti-A48 Id antibody (i.e., 17–38). Interestingly, in 1-mo-old BALB/c mice treated with anti-A48 Id antibodies, the challenge with 17–38 monoclonal antibody led to the activation of A48 Id^- anti-$\beta 2 \rightarrow 6$ fructosan-reactive clones with BALB/c type IEF spectrotypes, whereas in 3-mo-old BALB/c mice treated with anti-A48 Id antibodies, the challenge with 17–38 monoclonal antibody led to the activation of W3082 IdX^+ anti-$\beta 2 \rightarrow 6$ and $\beta 2 \rightarrow 1$ fructosan-reactive clones. In these animals, inhibition of A48 Id^+ anti-$\beta 2 \rightarrow 6$ fructosan clones was observed. This antibody probably represents a homobody carrying the internal image of the antigen, which through its paratope suppresses the A48 Id^+ response and through

its Id activates an A48 Id$^-$ anti-β2 → 6 fructosan response in 1-mo-old mice and in 3-mo-old mice leads to an anti-β2 → 6 and β2 → 1 fructosan response dominated by the W3082 IdX. These differences are related to the natural ontogenic delay of the development of W3082 IdX anti-β2 → 6 and β2 → 1 fructosan clones previously described.

Received for publication 25 April 1983 and in revised form 20 June 1983.

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