NEONATAL TREATMENT WITH LOW DOSES OF ANTI-IDIOTYPIC ANTIBODY LEADS TO THE EXPRESSION OF A SILENT CLONE

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BALB/c mice immunized with bacterial levan (BL), a $\beta(2\rightarrow6)$ fructosan with $\beta(2\rightarrow1)$ branched points, produce antibodies of two general types (1). One group of molecules is specific for inulin, which is a $\beta(2\rightarrow1)$ fructosan, as well as for levan; such antibodies, which can first be detected in BALB/c mice at 28 d of age (2), express idiotypes (Id) that cross-react with those found on inulin-binding myeloma proteins (3). The second principal group of antibodies binds BL, but not inulin, and fails to express cross-reactive idiotypes (IdX) of anti-inulin antibodies (1); these can be elicited in 1-d-old BALB/c mice after immunization with BL (2). The Id found on myeloma proteins ABPC 48 (A48) and UPC 10 (U10), which bind BL but fail to bind inulin, are generally not detected on anti-BL antibodies of BALB/c mice (3). However, in nu/nu BALB/c mice, pretreatment with anti-E109 IdX antibodies before immunization with BL results in the occurrence of A48 Id^+ anti-BL antibodies (4). This observation indicates that the A48 Id^+ anti-BL response belongs to a normally silent fraction of the anti-BL repertoire. This observation prompted us to investigate the activation of A48 Id^+-bearing clones in neonates after the administration of anti-A48 Id antibodies. Our results indicate that the administration of anti-A48 Id antibodies to 1-d-old BALB/c mice causes a substantial increase in the A48 Id^+ component of the anti-BL antibody response.

Materials and Methods

Animals. 1-d-old BALB/c mice were purchased from the Charles River Breeding Laboratory, Wilmington, Mass.

Antigen. BL was isolated by alcohol precipitation from culture supernates of *Aerobacter laevenicum* (ATCC 15552) grown at 23°C in nutrient broth supplemented with 5-10% sucrose. Trinitrophenyl-aminoethylcarbamylmethyl-Ficoll (TNP-Ficoll) was prepared as described previously (5).

Myeloma Proteins. The BALB/c myeloma proteins used in this study were A48, MOPC 460 (M460), and MOPC 384 (M384); all are IgA,κ immunoglobulins. A48 possesses $\beta(2\rightarrow6)$ fructosan-binding activity and lacks the IdX of myeloma proteins that bind both BL and inulin. M460 is a TNP-binding myeloma protein, whereas M384 possesses *Salmonella tranaroa* lipopolysaccharide-binding activity. These myeloma proteins were kindly provided by Dr. Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md.

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Preparation of Anti-Id Antibody. Anti-A48 Id (Ab_2) antisera were prepared in BALB/c mice by immunization with an A48-keyhole limpet hemocyanin conjugate. Anti-M460 Id and anti-M384 Id antisera were prepared in the same way. The method used for coupling myeloma proteins to keyhole limpet hemocyanin, as well as the schedules employed for immunization, have been described elsewhere (6).

Sensitization of Sheep Erythrocytes (SRBC) with BL and TNP. A palmitoyl derivative of levan was prepared by the method of Tsumita and Ohashi (7) as modified by Leon et al. (8). However, we used 50 μl instead of 25 μl of palmitoyl chloride to make the derivative, which was lyophilized and stored in the dry state. Before use, the palmitoyl-BL was dissolved in phosphate-buffered saline (pH 7.4) at a concentration of 1 mg/ml; then SRBC were incubated with an optimal quantity of the solution of palmitoyl-BL (25 μl) at 37°C for 1 h. TNP-sensitized SRBC were prepared by the method of Rittenberg and Pratt (9).

Detection of Plaque-forming Cells (PFC). The number of cells secreting antibody specific for BL or TNP was determined by a slide version of the technique of localized hemolysis-in-gel (10). Here, 50 μl of a suspension of immune spleen cells was added to 0.4 ml of 0.6% agarose that contained 0.2 ml of sensitized erythrocytes. The slides were incubated for 2 h at 37°C and then in the presence of guinea pig complement (1:20) for an additional 1 h. For controls, additional slides were prepared using a mixture of the same immune spleen cells and unsensitized SRBC. Anti-BL PFC carrying the A48 Id were estimated by the addition to agarose of BALB/c anti-A48 antiserum at a final dilution of 1:100 and then by subtracting the number of PFC obtained from the number of those observed when no inhibitor (anti-A48) was present. Similarly, the number of anti-TNP PFC expressing the M460 Id was determined by the addition to agarose of BALB/c anti-M460 antiserum (1:100) as inhibitor.

Radioimmunoassay. Purified Ab_2 (anti-A48 Id) antibodies were tritiated according to the method of Wilder et al. (11). Microtiter plates were coated with 50 μl of a 30 μg/ml solution of A48 Id^+ antibodies for 18 h at 4°C. Then the plates were washed three times with saline and incubated for 1 h with 50% fetal calf serum in saline at 4°C. Thereafter, the plates were washed three times with saline and used for inhibition by incubation of ^3H-Ab_2 antibodies (5,000 cpm/50 μl) with various dilutions of inhibitory sera for 3 h at 4°C. These plates were then washed extensively and the radioactivity was measured in a liquid-scintillation spectrometer.

Statistics. The Student's *t* test was used to evaluate the significance of the differences observed. Differences were considered to be significant when probability (P) values <0.05 were obtained.

Results

The anti-BL PFC response was studied in 1-mo-old BALB/c mice which were pretreated after birth (i.e., at 1 d of age) with various amounts of affinity-purified BALB/c anti-A48 Id antibody. As can be noted from the data of Table I, pretreatment with 0.01–10 μg of antibody leads to the activation of A48 Id^+ clones after immunization with BL; the percentage of A48 Id^+ PFC detected was significantly larger than that found in mice not treated with anti-A48 Id antibody ($P < 0.05$). Moreover, pretreatment with 0.1–10 μg of antibody induces a significant overall suppression of the anti-BL PFC response. Because there is a substantial ontogenic delay in the expression of the IdX^+ anti-inulin response, which is expressed maximally at 4–6 wk, we also studied the activation of A48 Id^+ clone in 7-wk-old mice. The data presented in Table II indicate that pretreatment with 0.01 μg of affinity-purified BALB/c anti-A48 Id antibody produces a similar effect with respect to activation of the A48 Id^+ clone in older mice, even though the anti-BL response is higher in magnitude at that age. These results indicate that the activation of anti-inulin clone by BL does not interfere with the activation of the A48 Id^+ clone. In other experiments, we investigated the specificity of the activation of the A48 Id^+ clone in neonatal mice pretreated with anti-A48 Id antibody. The data of Table III show that pretreatment with affinity-purified BALB/c anti-M384 Id antibody, followed by immunization with BL

TABLE I

Effect of Treating Newborn Mice with Various Doses of Anti-A48 Id Antibody on the Anti-BL PFC Response

Dose of anti-A48 Id given*	BL-specific PFC/spleen‡	A48 Id^+ PFC
μg		%
—	3.600 ± 0.125 (3,977)	6 ± 3
0.01	3.508 ± 0.123 (3,218)	46 ± 14
0.1	2.855 ± 0.218 (717)	65 ± 17
1.0	2.960 ± 0.127 (913)	73 ± 20
10.0	2.999 ± 0.183 (997)	73 ± 19

* Mice were 1 d old at the time of treatment.

‡ Mean ± SEM for $\log_{10}$ PFC/spleen detected for 5 mice, 5 d after i.v. immunization with 20 μg BL; the geometric mean is in parentheses. Mice were 5 wk of age at the time of immunization.

TABLE II

Anti-BL Response of Mice Pretreated With 0.01 μg of Anti-A48 Id Antibody at 1 d of Age

Age of mice when immunized with 20 μg BL	BL-specific PFC/spleen*	A48 Id^+ PFC
wk		%
4	3.431 ± 0.081 (2,700)	52 ± 7
5	3.508 ± 0.123 (3,218)	46 ± 14
7	4.025 ± 0.121 (10,586)	47 ± 9

* Mean ± SEM for $\log_{10}$ PFC/spleen detected for 5 mice, 5 d after immunization; the geometric mean is in parentheses.

TABLE III

Specificity of the Enhancement of the A48 Id^+ Component of the Anti-BL Response by Anti-Id Antibody

Mice primed with	BL-specific PFC/spleen*	A48 Id^+ PFC	A48 Id-bearing molecules‡
		%	*μg/ml*
—	3.290 ± 0.110 (1,950)	6 ± 3	0.004 ± 0.002
0.01 μg anti-A48 Id	3.431 ± 0.081 (2,700)	52 ± 7	0.430 ± 0.162
0.01 μg anti-M384 Id	3.150 ± 0.043 (1,411)	3 ± 1	0.048 ± 0.014

* See footnote of Table II. Mice were 4 wk of age at the time of immunization with BL.

‡ The results calculated are based on the capacity of various concentrations of A48 Id antibody to inhibit the binding of ^{3}H-anti-A48 Id antibody to plates coated with A48 Id antibody (30 μg/ml). The results are expressed as the mean ± SEM for five mice.

TABLE IV

Specificity of the Enhancing Effect of Anti-A48 Id Antibody on the Anti-BL PFC Response

Mice primed with	Mice challenged with	BL-specific PFC/spleen*	A48 Id^+	A48 Id-bearing molecules‡	TNP-specific PFC/spleen*	M460 Id^+
			%	*μg/ml*		%
—	20 μg BL (i.v.)	3.290 ± 0.110 (1,950)	6 ± 3	0.004 ± 0.002	ND§	ND
0.01 μg anti-A48 Id		3.431 ± 0.081 (2,700)	52 ± 7	0.430 ± 0.162	ND	ND
—	20 μg TNP-Ficoll (i.v.)	1.280 ± 0.171 (19)	ND	ND	4.590 ± 0.067 (38,919)	16 ± 7
0.01 μg anti-A48 Id		1.309 ± 0.208 (20)	ND	0.002 ± 0.001	4.438 ± 0.206 (27,442)	24 ± 6

* See footnote of Table II. Mice were 4 wk of age at the time of immunization.

‡ See footnote ‡ of Table III.

§ Not determined.

4 wk later, does not lead to the activation of the A48 Id^+ clone. Furthermore, in BALB/c mice pretreated with anti-A48 Id antibody and then immunized 1 mo later with TNP-Ficoll, we did not observe a significant increase in the anti-BL response nor a significant alteration in the M460 Id^+ component of the anti-TNP response (see Table IV). It can be seen in Tables III and IV that the radioimmunoassay data confirm the PFC results. These experiments clearly indicate that the activation of A48 Id^+ clone by affinity-purified BALB/c anti-A48 Id antibody is a specific phenomenon that requires antigenic stimulation for its expression.

Discussion

The Id expressed on the $\beta(2\rightarrow6)$ polyfructosan-binding myeloma protein A48 cannot be detected on anti-BL antibody produced by BALB/c mice, IgC_H^a congenic mice, or recombinant inbred strains of mice after immunization with BL (3). However, A48 Id^+ anti-BL antibody can be detected in E109 IdX-suppressed nude BALB/c mice, as well as in adult BALB/c mice producing anti-(anti-A48 Id) antibody after immunization with BL (4, 12). This observation indicates that the A48 Id^+ anti-BL response belongs to a normally silent fraction of the anti-BL repertoire. In this communication, we present additional data that indicate that a substantial proportion of A48 Id^+ anti-BL response can be activated after a single injection of 0.01–10 μg of affinity-purified BALB/c anti-A48 Id antibody given near the time of birth. Such activation requires antigenic stimulation to be expressed, because no significant anti-BL PFC response was noted in mice pretreated with anti-A48 Id antibody but not immunized with BL. The effect produced by anti-A48 Id antibody is specific, because such treatment did not alter the M460 Id^+ component of anti-TNP response and pretreatment with anti-M384 antibody did not lead to the activation of the A48 Id^+ component of anti-BL response. Interestingly, neonatal pretreatment with 0.1–10 μg of antibody, which leads to a significant decrease in the magnitude of the total anti-BL antibody response, also activates the A48 Id^+ component. These data are in agreement with previous reports showing that small amounts of anti-Id antibody can prime (in adult animals) the precursors of Id-bearing, antibody-forming cells that belong to the expressible repertoire (13–15).

Studies now in progress are designed to investigate the cellular basis for the activation of the A48 Id^+ silent clone. However, the results of this work clearly indicate that low doses of anti-Id antibody preferentially and substantially activate precursors of cells capable of making A48 Id^+ antibody; this clone can be expanded by immunization with BL of either 1-mo-old mice that lack the ability to mount an IdX^+ anti-inulin response or of older mice that are able to make an anti-inulin antibody response after immunization with BL.

Summary

BALB/c mice immunized with bacterial levan (BL) produce an immune response that fails to generate antibody expressing the idiotype (Id) of the $\beta(2\rightarrow6)$ fructosan-binding myeloma protein ABPC 48 (A48). Pretreatment of newborn BALB/c mice (at 1 d of age) with 0.01–10 μg of affinity-purified BALB/c anti-A48 Id antibody followed by immunization with BL 1–2 mo later produces an anti-BL response that expresses the A48 Id. This shows that A48 Id^+ anti-BL clones belong to a normally silent fraction of the anti-BL repertoire. The activation of A48 Id^+ anti-BL clones by

anti-A48 Id antibody is specific because the pretreatment of newborn mice with anti-MOPC 384 Id antibody, followed by immunization with BL, does not lead to its activation. Moreover, pretreatment of mice with anti-A48 Id antibody does not alter the MOPC 460 Id^+ component of the anti-TNP response. It is also important to note that the activation of the A48 Id^+ clone in pretreated mice requires subsequent immunization with BL.

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