MONOCLONAL ANTIBODY TO AN IgD ALLOTYPE INDUCES A NEW TYPE OF ALLOTYPE SUPPRESSION IN THE MOUSE*

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Studies of the various kinds of immunoglobulin (Ig) allotype and isotype suppressions induced by perinatal exposure to anti-Ig antibodies have yielded many valuable insights into the normal processes that control B cell differentiation and expression. For example, the demonstration that exposure to antibodies to an IgG isotype specifically suppresses production of that isotype, whereas the exposure to antibodies to IgM suppresses production of IgM and all IgG isotypes provided the first outlines of the precursor-progeny relationships in the B cell development pathway (1, 2). Similarly, the proof that suppressor T cells regulate antibody production rests in part on the highly specific suppression of allotype-marked adoptive secondary antibody responses demonstrable with suppressor T cells induced by perinatal exposure to antiallotype antibodies (3, 4). Thus, although the mechanisms responsible for suppression induction during the neonatal period are of interest per se, the properties of the suppressions induced by different kinds of antibody exposures frequently offer more broadly applicable information.

At least three characteristic patterns of allotype suppression have been described in allotype heterozygotes exposed to antibodies reactive with paternal Ig allotypes. In rabbits, the antibodies commonly used for suppression induction react with Ig light chain or Ig heavy chain variable-region determinants present on most of the Ig in serum in homozygotes and a little less than one-half the serum Ig in heterozygotes. After perinatal antiallotype exposure, B cells bearing the target allotype are depleted and gradually recover. Paternal allotype levels in serum remain low well after the allotype-bearing B cells return; however, older suppressed animals (>6-mo-old) usually initiate and maintain substantial serum allotype production. In general, the suppression of paternal allotype production is accompanied by a compensatory increase in maternal serum-allotype levels that return to normal when suppression is relieved (5-8).

In the mouse, antiallotype antibodies almost exclusively detect Ig heavy chain constant-region determinants and thus are restricted to reaction with allotypes represented on one or occasionally two Ig isotypes (9). The suppression induced by these antiallotype antibodies appears similar to the suppression induced by heterologous anti-isotype antibodies except that production of only one of the two allotypes in the heterozygote is affected (1, 2, 10-12).

Anti-Ig-induced suppression persists for a much shorter time in most mouse strains than it does in rabbits (8–12 wk); however, a long-term ("chronic") allotype suppression that differs from the usual allotype suppression obtained in either mouse or rabbit can be induced in genetically susceptible mice such as the (BALB/c × SJL)F$_1$ hybrid (BALB/c mother, SJL father). This suppression shows a characteristic biphasic pattern, persisting initially for about as long as the "short-term" suppression in other mouse strains and hybrids, abating for several weeks, and then returning to full strength when the animals reach 20–24 wk of age to completely suppress paternal allotype production thereafter. Maternal allotype production in the suppressed animals (of either type) remains comparable to controls, i.e., compensatory increases in maternal allotype serum levels do not occur (10, 13–15).

Short-term and chronic suppression are also distinguished by the breadth of their affect on IgG production. Short-term suppression can be induced for the paternal allotype represented in any IgG isotype. Chronic suppression, in contrast, has been induced thus far only for IgG2a allotypes and is more effective when induced for the paternal Igh-1b (SJL-derived) allotype in BALB/c × SJL than for the Igh-1a (BALB/ c-derived) paternal allotype in reciprocal hybrids (10). The allotype suppressor T cells used in the studies mentioned above (3, 4) were derived from 6-mo-old BALB/c × SJL hybrids in which Igh-1b (1b) allotype production is chronically suppressed because of perinatal exposure to maternal anti-1b antibodies.

Studies presented here describe a new kind of allotype suppression that occurs in mice but behaves more like the suppression described in rabbits. This suppression, induced by injecting neonatal BALB/c × SJL mice with monoclonal antibody to the paternal IgD allotype (Igh-5b), differs markedly from either chronic or short-term suppression. Igh-5b-bearing (5b+) cells are depleted from the neonate and gradually recover over the first several months of the animal's life. Production of paternal IgG allotypes is drastically (but not completely) reduced until the animals reach ~24 wk of age, after which it accelerates and the animals soon achieve and maintain normal serum levels of the paternal allotypes; and, in contrast with other forms of allotype suppression in the mouse, maternal IgG allotype serum levels show a clear-cut compensatory increase during the period of paternal allotype suppression induced by anti-5b exposure.

We have shown previously (11) that perinatal exposure to antibodies to IgM (6b) allotypic determinants usually present on the same cells as the IgD (5b) determinants induces suppression for IgG allotype production in BALB/c × SJL animals; however, in contrast with anti-5b, the anti-6b antibodies induce a suppression whose characteristics essentially correspond to the chronic form of allotype suppression induced by perinatal exposure to conventional or monoclonal anti-1b antibodies (injected or maternally transmitted). For example, anti-6b-exposed animals show the typical failure to produce 1b after 6 mo of age (11). This chronic suppression can be induced for either parental IgG2a allotype by exposure of reciprocal hybrid mice to maternal antibodies to the paternal IgM allotype and can be induced for both parental IgG2a allotypes in either hybrid by exposure to heterologous (goat) anti-IgM isotype antibodies. Thus, although the allotype specificity of the suppression induced by either anti-5b or anti-6b suggests that suppression results from reaction of the inducing antibody with Ig on the "haplotype-committed" precursors of 1b-producing cells, these two antibodies induce qualitatively different types of suppression.

TABLE I

Monoclonal Antibodies and Myeloma Proteins Used in These Studies

Use	Antibody specificity	Name	Characterization* species/isotype/allotype	Label
Suppression induction	Igh-5b (IgD allo)	Igh(5b)6.3	Mouse IgG$_1$ Igh-4a	—
Suppression induction	Igh-1b (IgG$_{2a}$ allo)	Igh(1b)2.9	Mouse IgG$_{2a}$ Igh-1a	—
RIA	Igh-1a (IgG$_{2a}$ allo)	Igh(1a)8.3	Mouse IgG$_{2a}$ Igh-1b	^{125}I
RIA	Igh-1b (IgG$_{2a}$ allo)	Igh(1b)5.7	Mouse IgG$_3$	^{125}I
RIA	Igh-4a (IgG$_1$ allo)	Igh(4a)10.9	Mouse IgG$_{2a}$ Igh-1b	^{125}I
RIA	Igh-4b (IgG$_1$ allo)	Igh(4b)12.8	Mouse IgG$_1$ Igh-4a	^{125}I
RIA standard	Myeloma protein	GPC-8	Mouse IgG$_{2a}$ Igh-1a	—
RIA standard	Myeloma protein	C.BPC-101	Mouse IgG$_{2a}$ Igh-1b	—
RIA standard	Myeloma protein	MOPC-21	Mouse IgG$_1$ Igh-4a	—
RIA standard	Myeloma protein	MOPC-245T	Mouse IgG$_1$ Igh-4b	—
FACS analysis	Igh-5a (IgD allo)	Igh(5a)7.2	Mouse IgG$_{2a}$ Igh-1b	FITC
FACS analysis	Igh-5b (IgD allo)	H 6/31	Mouse IgM Igh-6a	FITC
FACS analysis	ThB (splenic B)	53-9.2	Rat IgG$_{2c}$	FITC
FACS analysis	IgM	151-119	Rat IgG$_1$	FITC
Depletion of T cells from spleen cell suspensions	Thy-1.2	30-H12	Rat IgG$_{2b}$	—

* Isotype/allotype correspondence: IgM, Igh-6; IgD, Igh-5; IgG$_3$, no known allotypes; IgG$_1$, Igh-4; IgG$_{2b}$, Igh-3; IgG$_{2a}$, Igh-1. BALB/c carries Igh^a allotypes, e.g., Igh-1a; C57BL/10 carries Igh^b allotypes, e.g., Igh-1b.

This distinction, which introduces a fascinating complexity into the mechanisms responsible for suppression induction, raises some intriguing questions about precursor-progeny relationships among the neonatal B cell populations that carry only IgM and those that carry both IgM and IgD and the potential role(s) that such B cell populations play in establishing favorable conditions for persistent IgG production in adult animals.

Materials and Methods

Mice. (BALB/c × SJL)F$_1$ mice raised in our colony (Genetics Department, Stanford University Stanford, Calif.) were used for these studies.

Antibodies. A wide variety of monoclonal antibodies and myeloma proteins were used (Table I). With one exception, H6/31, (kindly supplied by Dr. Terry Pearson and Dr. Cesar Milstein, Medical Research Council, Cambridge, England) all of the monoclonal antibodies were produced and characterized in our laboratory by Dr. J. Ledbetter, Dr. V. Oi, Dr. L. A. Herzenberg, and co-workers, Stanford University (16, 17).

Neonatal Antibody Exposures. Anti-5b-exposed mice were injected intraperitoneally with 250 µg of purified monoclonal antibody between 4 and 7 d of age. Anti-1b-exposed mice were exposed to maternally transmitted antibody, as previously described (10), or were injected with 250 µg of purified monoclonal anti-1b (2.9) intraperitoneally between 4 and 7 d of age (18).

Antigenic Exposures. For *in situ* responses and memory B cell priming, mice were injected intraperitoneally with 100 µg of DNP-KLH (2,4-dinitrophenyl hapten conjugated to keyhole limpet hemocyanin;[1] Pacific Bio-Marine Laboratories Inc., Venice, Calif.). For "carrier"

[1] *Abbreviations used in this paper:* DNP-KLH, 2,4-dinitrophenyl hapten conjugated to keyhole limpet hemocyanin; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; RIA, radioimmunoassay.

priming, mice were injected with 100 μg of KLH. All antigens were injected intraperitoneally as alum precipitates together with 2.2×10^{10} cells/ml of killed *Bordetella pertussis* organisms (Department of Public Health, Boston, Mass.).

Fluorescent Staining. 1×10^6 lymphocytes in "staining" medium (RPMI 1640, 0.1% NaN$_3$, 10 mM Hepes, 1% fetal calf serum) were placed in wells in a 96-well microtiter plate, and 1 μl of fluorescein isothiocyanate (FITC)-conjugated antibody was added per well. After a 30-min incubation on ice, cells were washed once with staining medium and analyzed with a fluorescence-activated cell sorter (FACS-II, Becton, Dickinson FACS Systems, Mountain View, Calif.) (19). For monoclonal rat anti-IgM and anti-ThB, a two-step staining protocol was used in which cells were incubated first with unconjugated antibody and the bound antibody then revealed by incubation with affinity-purified "conventional" FITC-conjugated mouse anti-rat Ig (17). All staining reagents were titrated and used at concentrations slightly above the minimum amount of antibody required to reach "plateau" staining (where an increase in the antibody concentration does not increase the number of or the brightness of the stained cells).

Peripheral blood lymphocytes were prepared by taking arterial blood (tail artery) into medium (RPMI 1640) containing heparin (10 U/ml). Live lymphocytes were separated by centrifuging away dead cells and erythrocytes through a Ficoll layer (Lympholyte M; Accurate Chemical & Scientific Corp., Hicksville, N. Y.) and recovering the cells that remained at the interface between the Ficoll and the original suspending medium.

Quantitative Measurement of Serum Allotype Ig Levels.   Serum allotype levels were measured by a solid-phase "antibody consumption" radioimmunoassay (RIA), as previously described (20). Briefly, dilutions of a standard Ig (purified myeloma protein of the appropriate allotype) or serum samples from test mice were mixed with ^{125}I-labeled antiallotype antibody and then placed in a microtiter plate well precoated with the myeloma protein used as the standard. The amount of allotype Ig in the sample is calculated by comparing the amount of ^{125}I-labeled antibody whose binding is blocked by the sample with the amount of binding blocked by the purified myeloma protein standard.

Measurement of Memory B Cell Activity (Adoptive Secondary Assay).   Memory B cell activity generated in individual DNP-KLH-primed donors was measured by co-transferring T depleted B cells (spleen cells treated with monoclonal rat anti-Thy-1.2 and guinea pig complement) from these donors with nylon-passed T cells from carrier (KLH)-primed donors and stimulating the irradiated recipient with 1 μg aqueous DNP-KLH. Each recipient was injected intravenously with (T cell-depleted) B cells recovered from 10^7 spleen cells and 2.5×10^6 carrier-primed T cells. Cell populations and DNP-KLH were mixed just before injection into X-irradiated (650 rad, 18 h previously) BALB/c recipients. Anti-DNP antibody responses of sera from recipients at 7 d after transfer were measured by solid-phase RIA.

Measurement of IgG Anti-DNP Responses.   The solid-phase RIA used here to measure the amounts of anti-DNP antibody carrying each of four IgG allotypes (1a, 1b, 4a, and 4b) have been described previously (20, 21).

Results

IgD-bearing Cells Are Depleted in Neonates after Exposure to Anti-IgD Antibody.   Perinatal exposure to anti-Igh-5b (the paternal IgD allotype in BALB/c × SJL mice) depletes B cells bearing the Igh-5b allotype (5b$^+$ cells) from 3- to 5-wk-old mice (Fig. 1). The absolute frequency of IgD-bearing B cells in these animals varies considerably, as it does in untreated (control) mice; therefore, the ratio of 5a$^+$/5b$^+$ B cells in spleen or in peripheral blood lymphocytes provides a better index of disturbances in the 5b$^+$ population. This ratio always approximates 1 in control animals, regardless of the size of the B cell population or the age of the animals. In young anti-5b-exposed animals (3–5 wk of age), however, the 5a$^+$/5b$^+$ ratio rises substantially above 1. Because the frequency of 5a$^+$ cells is similar in control and anti-5b-exposed animals, this increased ratio indicates a severe and specific depletion of the 5b$^+$ population.

The failure to detect 5b$^+$ B cells in the young anti-5b-exposed animals represents a
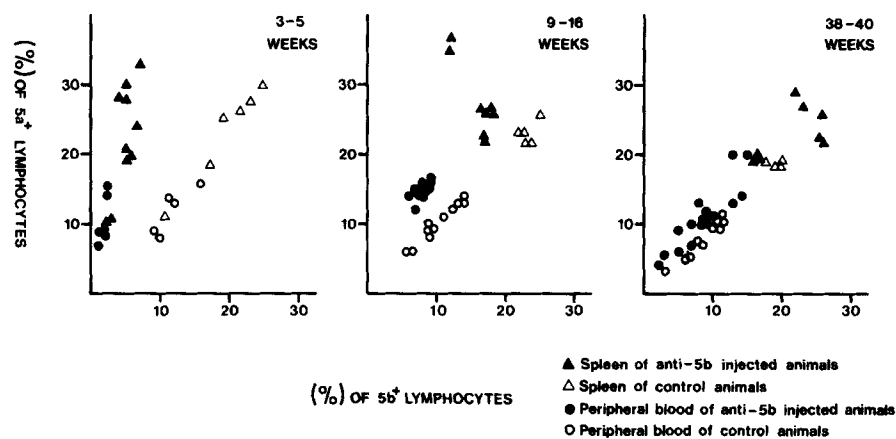
FIG. 1. Perinatal exposure to anti-5b specifically deletes 5b⁺ cells for a prolonged period. Percentage of 5a⁺ and percentage of 5b⁺ cells in spleen cell preparations from individual animals injected with 250 μg anti-5b (▲) or from littermate controls (△); Percentages in peripheral blood lymphocytes from anti-5b-injected mice (●) and littermate (○) controls.

depletion of these cells rather than interference with detection of 5b determinants by the injected antibody or removal (stripping) of surface 5b from cells that nonetheless persist. The injected monoclonal antibody (11-6.3) reacts with different allotype determinants than those detected by the monoclonal reagent (H6/31) used to stain 5b-bearing B cells for FACS analysis and does not block staining by this reagent (data not shown). Furthermore, the anti-5b-exposed young animals do not have an excess of 5b⁻ cells carrying other B cell surface markers normally present on 5b⁺ cells. Instead, the splenic and peripheral blood lymphocytes taken from these animals show lower B cell frequencies consistent with depletion of the 5b⁺ population, i.e., fewer μ^+,ThB⁺ cells than the unexposed control animals. The depleted 5b⁺ population accounts for this reduction in the overall size of the B cell population because the non-5b⁺ B cell populations (5a⁺ plus μ^+,5a⁻) are equivalent in size in anti-5b-treated and control animals (Fig. 2).

Depleted IgD-bearing Populations Recover Gradually after Perinatal Exposure to Anti-IgD Antibodies. As anti-5b-exposed mice age, they gradually develop a 5b⁺ B cell population that reaches about one-half its normal size in 9- to 16-wk old animals and becomes very close to normal in size in mice >38 wk of age (Fig. 1). Because the size of the B cell population (μ^+,ThB⁺) increases concomitantly with the increase in the 5b-bearing population (Fig. 2), these data indicate the emergence of a distinct B cell population (5b⁺) that was absent in neonatal anti-5b-exposed mice.

This overall trend toward recovery is more pronounced in some mice than in others. Examination of the 5a/5b ratio in individual 38- to 40-wk-old mice, for example, reveals a few mice with apparently normal B cell populations (5a/5b ∼1) and a few mice in which B cell populations are more like the populations in 9- to 16-wk-old mice (5a/5b ∼2). These mice represent the upper and lower extremes of a group that generally shows 5a/5b ratios of ∼1.1–1.3 and thus come quite close to normality; however, sufficient numbers of mice fall towards the lower extreme to indicate a sizable variation in recovery rates.

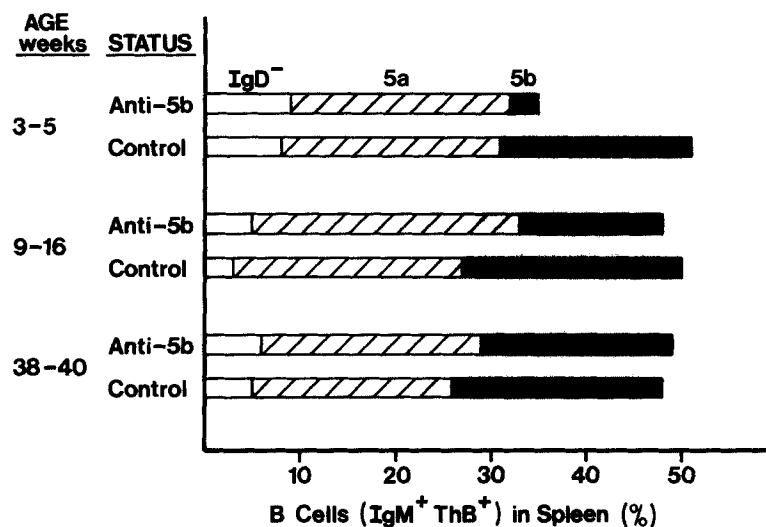The recovery of the 5b⁺ population does not signal a corresponding recovery of

Fig. 2. 5b⁺ B cells are missing (rather than masked) in young mice exposed perinatally to anti-IgD. Pooled data from FACS analyses of 10 anti-5b-injected mice (250 μg) and 8 littermate controls are plotted to indicate the overall percentage of B cells in spleen and the partition of the B cell population according to surface IgD allotype.

paternal haplotype IgG production. 12-wk-old suppressed mice that have more than one-half their normal complement of 5b⁺ cells frequently show maximally suppressed serum IgG allotype levels until 24 wk of age (see below). Thus, as in the allotype-suppressed rabbit (5-8), the reappearance of the depleted B cell population can substantially precede the onset of production of the suppressed serum Ig.

Anti-IgD-induced Allotype Suppression Differs from Chronic Allotype Suppression. Three properties distinguish the long-term suppression induced by anti-5b from the chronic allotype suppression induced by anti-1b or anti-IgM: (a) anti-5b-suppressed mice recover the ability to produce paternal haplotype serum IgG by 24 wk of age and subsequently maintain this ability, whereas anti-1b-suppressed mice recover transiently between 12 and 20 wk of age but typically develop a persistent suppression after 20-24 wk of age; (b) paternal allotype serum levels seldom fall below 10% of control levels in anti-5b-suppressed mice, whereas serum 1b levels in chronically suppressed mice are typically lower than 1a and then maintain <1% of control levels during the initial and terminal phases of suppression; and (c) maternal haplotype serum IgG levels rise to "compensate" for the reduced paternal haplotype IgG levels in anti-5b-suppressed mice but remain at control levels in anti-1b-suppressed animals (Figs. 3 and 4).

Anti-IgD-induced Suppression Interferes with Memory B Cell Development. The production of paternal allotype anti-DNP antibodies is suppressed when serum allotype production is suppressed in both anti-1b (19, 20) and anti-5b- (chronic) suppressed animals (Fig. 5). Furthermore, the ability to produce these allotype-marked antibody responses recovers in both types of animals whenever serum allotype production recovers, i.e., in older anti-5b-exposed animals and in anti-1b-exposed animals during the "remission" period that occurs between 12 and 20 wk of age. Thus, both types of suppression have similar effects on *in situ* antibody responses.

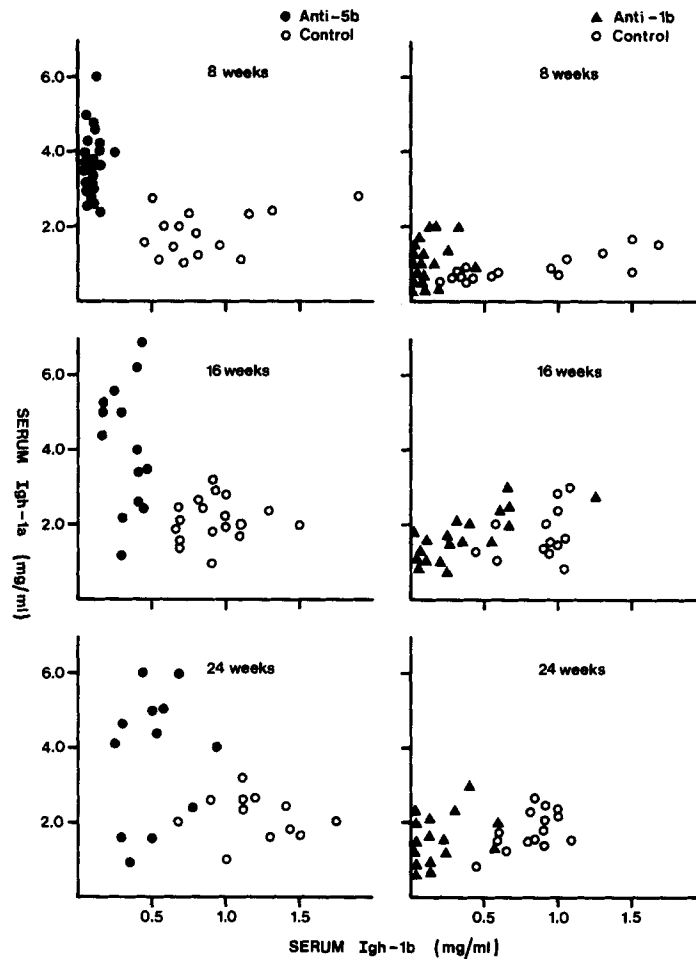Analysis of the memory B cell populations induced when animals are primed while

Fig. 3. Prolonged suppression but eventual recovery of 1b serum levels and compensatory increases in 1a serum levels distinguish anti-5b-induced suppression from "chronic allotype suppression" induced by anti-1b. Individual animals shown were injected at 5-7 d with 250 μg anti-5b (●), 250 μg anti-1b (▲), or not injected (○) (littermate controls). Serum allotype levels were measured by RIA.

suppressed, however, demonstrates a marked distinction between chronic and anti-5b-induced suppressions. Chronic suppression interferes with the later stages of B cell memory development (IgD^+ to IgD^- transition) but does not reduce the overall size of the memory population (21). Anti-5b-induced suppression, in contrast, clearly reduces the size (or effectiveness) of the paternal haplotype memory B cell population capable of generating adoptive secondary responses (Table II).

This reduction can be seen clearly by comparing the a and b allotype responses obtained from cells derived from individual, suppressed donors of each type. Adoptive secondary anti-DNP responses from T cell-depleted spleen cell populations taken from individual DNP-primed donors and supplemented with the same carrier-primed T cell population often vary over a three- or fourfold range; however, the ratio of the allotypes represented in adoptive responses from allotype heterozygous donors tends
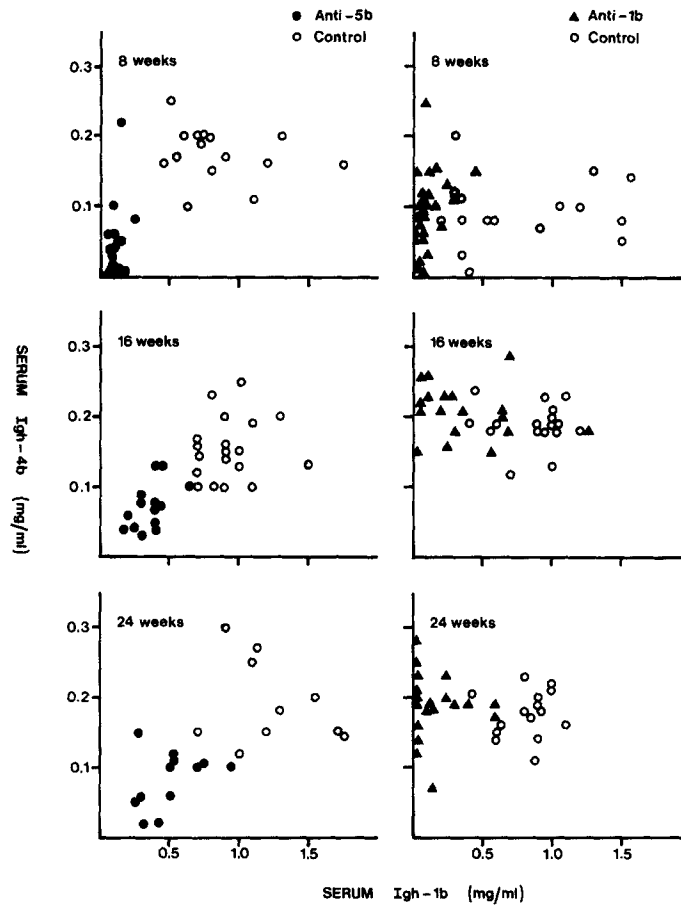
Fig. 4.   Anti-5b induces suppression for both 1b and 4b production, whereas anti-1b specifically induces suppression for 1b production. Individual animals shown were injected at 5–7 d of age with 250 μg anti-5b (●), 250 μg anti-1b (▲), or not injected (○) (littermate controls). Allotype levels were measured by RIA.

to deviate only slightly from 1 (± 20–30%) regardless of the overall magnitude of the adoptive response. Thus, it provides a reliable index of perturbations in memory development independent of factors that influence the overall size or activity of the memory pool.

This ratio reveals a striking difference when memory B cell responses from normal or chronically suppressed donors primed at 12 wk of age are contrasted with memory B cell responses from similarly primed anti-5b-suppressed animals. The B cells from normal and chronically suppressed donors give rise to comparable a and b haplotype adoptive responses indicative of the absence of interference with overall memory development in the chronically suppressed mice. The B cells from anti-5b-suppressed mice, however, give rise to responses that are heavily weighted in favor of the a allotype.

The increased a:b allotype ratios in these animals appear mainly to be due to a reduction in the size of the b allotype memory B cell population because the a allotype responses fall within normal range, whereas the b allotype responses are clearly lower
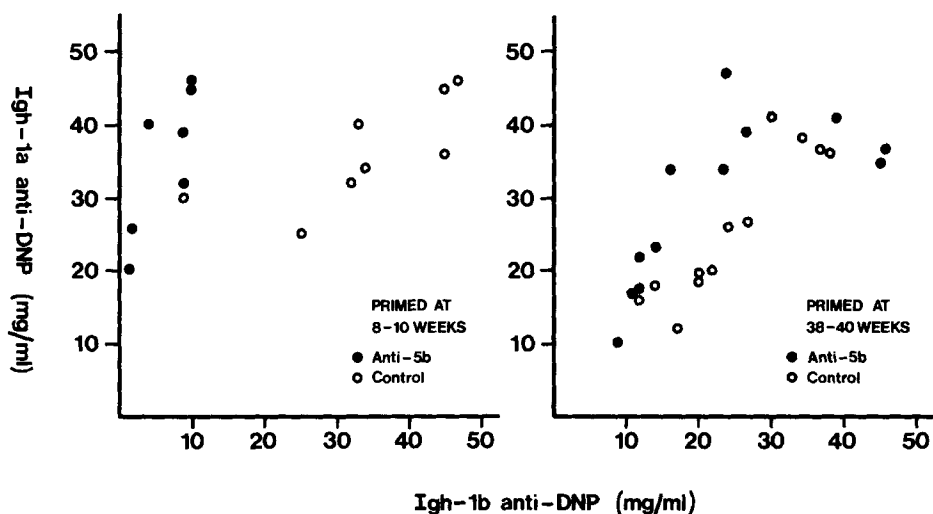
FIG. 5. Suppression of 1b antibody production induced by perinatal exposure to anti-5b. Anti-5b-exposed (●) and littermate control (○) animals were primed with 100 μg DNP-KLH on alum at the indicated time; serum antibody levels in individual mice, measured by RIA 2 wk later, are representative of levels measured 1–6 wk after priming. (See Figs. 3 and 4 for time-course of anti-5b-induced suppression)

TABLE II

Perinatal Anti-5b Exposure Interferes with b Haplotype Memory B Cell Development in Adults

Anti-DNP* memory B cell donors	Donor Number	Anti-DNP response‡					
		IgG$_{2a}$			IgG$_1$		
		Igh-1a	Igh-1b	1a/1b	Igh-4a	Igh-4b	4a/4b
		μg/ml					
Anti-5b injected	1	36	17	(2.1)	105	15	(7.0)
	2	72	22	(3.3)	135	15	(9.0)
	3	126	30	(4.2)	210	80	(2.6)
Anti-1b§ exposed	4	89	73	(1.2)	320	275	(1.2)
	5	78	58	(1.3)	225	250	(0.9)
Uninjected control	6	47	46	(1.0)	238	250	(1.0)
	7	108	99	(1.1)	230	255	(0.9)
	8	83	94	(0.9)	150	175	(0.9)

* BALB/c X SJL mice were primed with DNP-KLH at 8 wk of age. 4 wk later, T cell-depleted spleen cells (5 X 10⁶) were supplemented with 2.5 X 10⁶ KLH-primed nylon-purified T cells and transferred with 1 μg of DNP-KLH into irradiated BALB/c mice.
‡ Antibodies in serum measured by RIA at 6 d after transfer.
§ BALB/c X SJL mice exposed perinatally to maternal anti-Igh-1b.

than normal; however, we have not tested a large enough number of animals to exclude the occurrence of some compensatory development of maternal (a) allotype memory. Thus, although some memory development is permitted in anti-5b-suppressed mice, the suppression definitely interferes with the normal pattern of memory B cell development.

These mice (12 wk of age when primed) typically have about one-half the normal

number of 5b⁺ B cells, but this deficit cannot solely explain the deficit in b allotype memory. The ratios of 5a⁺:5b⁺ cells were not measured for the donors used in this experiment; however, these ratios are usually around 2, whereas the a:b allotype ratio in the IgG adoptive response generated by the memory B cells from the suppressed animals ranges from 2.1–9. This suggests either that the missing 5b⁺ cells constitute a subpopulation that selectively gives rise to memory B cells or that the mechanisms through which the suppression interferes with memory development are independent of the size of the 5b⁺ populations. The recovery of normal memory development in 38-wk-old anti-5b-exposed mice (data not shown) is consistent with both of these hypotheses and further documents the relatively complete recovery from suppression in these mice.

Different Mechanisms Are Involved in Chronic and Anti-IgD-induced Allotype Suppression.   The failure to induce chronic suppression by perinatal anti-5b exposure cannot be merely the result of exposure to suboptimum doses of anti-5b. Increasing the dose of anti-5b by injecting three sequential 250 μg doses of antibody at 4, 7, and 11 d of age (total 750 μg/mouse) or injecting anti-5b within a few hours of birth does not alter the typical suppression pattern obtained with this antibody (data not shown). Chronic suppression, however, can be induced by 125–250 μg of monoclonal anti-1b administered as late as 17 d after birth (18). In addition, although allotype suppressor T cells are readily detectable in young as well as in older anti-1b- or anti-IgM-suppressed mice (3, 4, 11, 18, 22), lymphocyte populations from anti-5b-suppressed mice show no suppressive activity when tested in the same kinds of assays (data not shown). Thus, the differences between chronic and anti-5b-induced allotype suppression must represent fundmental mechanistic differences occasioned by the induction of suppression with antibodies to different immunoglobulins.

We have suggested that anti-IgM or anti-1b exposures temporarily deplete neonatal B cell populations required to prevent the induction of allotype suppressor T cells and consequently to prevent the induction of chronic suppression (10). Data presented here showing that anti-5b depletes virtually all 5b⁺ cells from the neonate but does not induce chronic suppression indicates either that this suggestion is incorrect or that the relevant B cell population in newborn and neonatal animals does not carry a sufficient amount of surface 5b (IgD) to permit its depletion.

Discussion

The prolonged suppression of IgG production that occurs in BALB/c × SJL mice after exposure to anti-Ig antibodies during the first few weeks of life dramatizes the importance of the neonatal period in establishing the immunologic behavior of the animal. Perinatal exposure to anti-Igh-1b (anti-1b) or anti-IgM antibodies results in a lifelong suppression of 1b production because of the neonatal induction of a persistent population of allotype-suppressor T cells (4, 10, 11). Similarly, as we have shown here, perinatal exposure to anti-IgD (5b) allotype antibodies induces a long-term suppression of paternal allotype IgG production that compromises the animal vis-á-vis antibody production until ~6 mo of age. The mechanisms responsible for the induction and for the differences between these two types of suppression are still obscure; however, their existence clearly demonstrates that alterations in the neonatal immune system occasion immunoregulatory deviations manifested many months later.

The curious coincidence between the time the chronic suppression mechanism tends to reassert control over antibody production and the time that anti-5b suppression tends to be relieved suggests that the immune system undergoes a "pleiotropic" change late in the life of the animal (~5-6 mo of age in the mouse). Several other kinds of immunoregulatory abnormalities also show a tendency to initiate or exacerbate at this time. For example, high levels of autoantibodies tend to appear in certain mouse strains (23) and IgG paraproteins are frequently detected in others (particularly SJL) (24). Taken together, these findings raise the possibility that older adults normally develop a qualitatively different set of regulatory mechanisms whose behavior is determined in part by genetic elements and in part by individual perinatal experience.

The BALB/c × SJL mice used in many of our studies, including those presented here, have a genetic defect that renders them sensitive to chronic suppression induction when exposed perinatally to anti-1b or anti-IgM (10). We have shown earlier (11) that this suppression is induced in the presence of maternally derived anti-5b antibodies in animals exposed to a mixture of maternal antibodies reactive with paternal allotypes on IgM and IgD, and we have shown here that the suppression induced by anti-5b differs substantially from chronic suppression. Thus, because anti-5b neither induces nor interferes with the induction of chronic suppression, the genetic defect responsible for the induction and/or maintenance of this suppression apparently involves processes independent of the mechanisms that induce and maintain the anti-5b-induced suppression.

We have not investigated the effects of anti-5b exposure in other mouse strains. Studies from other laboratories (25), however, suggest that the suppression induced by this antibody in BALB/c × SJL is probably representative of anti-IgD-induced suppression in general. The similarities between the anti-5b-induced allotype suppression in mice and the typical allotype suppression observed in rabbits (5-8) also suggests a common mechanism. Further study, however, is clearly required to establish this point and to develop some ideas as to what the nature of such a mechanism could be.

In a sense, our findings are a new addition to the already puzzling array of data on immunoregulatory processes. We have shown clearly that perinatal exposure to antibodies reactive with paternal IgD (Igh-5b) allotypic determinants induces a unique form of allotype suppression characterized by (a) the initial depletion of the B cell population that carries this IgD allotype; (b) the gradual increase of this population with age; (c) the interference with paternal haplotype IgG memory B cell development; (d) the suppression of paternal allotype IgG production even after the depleted B cell populations have substantially recovered; (e) the compensatory production of maternal IgG allotype production; and (f) the eventual recovery of all affected functions late in life.

We wonder, for example, whether neonatal B cell populations may be more complex than were previously imagined. Is it possible that the small percentage of IgM^+,IgG^+ B cells seen shortly after birth (26) represents a different B cell lineage rather than early-emerging precursors of the IgM^+,IgD^+ population that appears a few days later? Could these cells perhaps be a regulatory population? Are they related to the Lyt-1-bearing B cells apparently present in splenic B cell areas in the adult?[2]

Does their depletion in the neonate permit the induction of chronic allotype suppression (if such suppression is due to B cell depletion)?

Considering the adult, one might ask why the $5b^+$ population takes so long to recover after perinatal depletion. The rapid turn-over of "virgin" B lymphocytes in young adults would predict that a depleted population would be rapidly replaced once the depleting agent disappears, yet anti-5b-treated mice take months to recover a normal-sized $5b^+$ population. Could there be homeostatic mechanisms that fix the size of this population in adults according to its size in the neonate? Do these homeostatic mechanisms lose force as the animal ages?

The reader can undoubtedly extend this list; however, for the moment it serves its purpose by pointing out the kinds of basic information still required before an understanding of the complex processes involved in suppression induction is likely to emerge. At a minimum, the findings presented here clearly indicate that current views of B cell development regulatory T cell interactions will have to be revised or expanded to account for the distinctive patterns of suppression induced by antibodies to the two cell surface immunoglobulins present on the majority of neonatal and adult B cells.

Summary

Studies presented here show that perinatal exposure to anti-IgD allotype antibodies induces a persistant IgG-allotype suppression in the mouse that differs markedly from either the short-term or chronic allotype suppressions induced by antibodies to IgG or IgM allotypes. This novel form of allotype suppression induced by injecting neonatal BALB/c × SJL mice with monoclonal antibody to the paternal Igh-5b (IgD) allotype drastically reduces paternal allotype production during the first 6 mo of the affected animal's life and simultaneously stimulates compensatory production of maternal allotype IgG. In addition, it interferes with the development of B cells carrying the paternal IgD allotype and impairs the development of memory B cells destined to give rise to paternal allotype IgG-producing cells. Thus, its properties make it more like allotype suppression as described in the rabbit than like the known forms of allotype suppression in the mouse.

As shown here, Igh-5b-bearing ($5b^+$) B cells are completely depleted from the neonate after anti-5b exposure and only gradually appear as the animal ages. The recovery of the $5b^+$ population to near normal size (by ~14 wk of age) substantially preceeds recovery of the ability to generate normal-sized memory B cell populations. Paternal allotype levels in serum remain well below normal until the anti-5b-exposed animals reach ~6 mo of age and then climb rapidly, finally stabilizing at levels comparable to levels in controls of the same age. The elevated maternal allotype levels characteristic of the suppression period begin falling somewhat earlier and are clearly stabilized within the normal range in 6-mo-old animals. Thus, perinatal exposure to anti-5b compromises B cell development and IgG production throughout early adulthood but has little apparent effect in older animals.

Perinatal exposure to antibody to the paternal IgG2a allotype (Igh-1b) or IgM allotype (Igh-6b), in contrast, induces a chronic allotype suppression that has relatively little affect on IgG production in young adults but severely suppresses allotype production in older animals. Furthermore, this type of (chronic) suppression does not influence maternal allotype production and does not interfere with memory B cell

development. These differences, illustrated here by data from parallel sets of animals exposed either to anti-5b or anti-1b, raise a series of intriguing questions concerning the mechanisms regulating B cell development and expression and the nature of the neonatal (B) cell populations with which the suppression-inducing antibodies react.

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