ACTIVE SUPPRESSION OF IMMUNOGLOBULIN ALLOTYPE SYNTHESIS

I. Chronic Suppression after Perinatal Exposure to Maternal Antibody to Paternal Allotype in (SJL × BALB/c)F_1 Mice*

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Allotype suppression is a unique example in higher organisms of the regulation of gene expression by antibodies directed against the product of those genes. Both in rabbits and mice, prenatal and/or early postnatal exposure to anti-immunoglobulin allotype antibody suppresses production of the allotype in animals genetically capable of producing it. However, the long-lived or "chronic" suppression often observed in rabbits (1–4) contrasted sharply with the short-lived allotype suppression reported in mice (5).

In the mouse intercross previously studied (BALB/c × C57BL/10), allotype heterozygotes, sired by normal (Ig^b)[1] males mated with (Ig^a) females immune to the paternal (Ig-*1b*) allotype, exhibited an initial delay in the appearance of the Ig-*1b* immunoglobulins and continued to have less than normal levels of these immunoglobulins for some time. However, the delay was in the order of only a few weeks and the suppressed allotype reached near-normal levels by 12–14 wk after birth. The reasons for this major difference between mouse and rabbit have been difficult to understand.

We have now observed, using a different paternal strain (also Ig^b), that chronic suppression can be obtained in mice as well. The studies presented in this publication show that the suppression of Ig-*1b* production in Ig^a/Ig^b hybrids derived from the mating of SJL (Ig^b) males with BALB/c (Ig^a) females immunized to Ig-*1b* is much more long lasting. In about half the mice there is no paternal Ig-*1b* immunoglobulin detectable as late as 6–9 months of age, although transient production of Ig-*1b* occurs in a majority of these mice before this time.

The mechanism of allotype suppression is not known, but a commonly held view is that it is analogous to specific immunological tolerance, i. e., that cells committed to production of the suppressed allotype are eliminated or diverted from production of

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[1] The designations Ig^a and Ig^b without specification of locus means that all the Ig loci on that chromosome have the respective *a* or *b* alleles. Since the Ig loci are so closely linked that recombinants have not been observed in inbred strain matings, this is a useful shorthand. (For review see reference 6.)

these immunoglobulins by the maternal antibodies present at the time when these cells can differentiate from totipotent stem cells. The availability of chronically suppressed mice made it possible to test this hypothesis in several ways.

We report here the results of studies showing that chronic suppression cannot be "cured" (i.e. Ig-*1b* production cannot be reestablished permanently in chronically suppressed animals) by treatments which force the repopulation of the lymphoid system from primitive (stem) cells, such as total body irradiation with long-bone shielding. Further, we show that the injection of normal syngeneic spleen cells (from nonsuppressed animals) into lethally irradiated chronically suppressed mice fails to restore Ig-*1b* production.

The failure of these treatments to cure suppression leads us to conclude that suppression is maintained in these chronically suppressed animals by an active process and that nonproduction (suppression) dominates physiologically over production of Ig-*1b* globulins.

Materials and Methods

Mice.—The following strains, with their abbreviated names in parentheses, were used in these experiments: SJL/J, (SJL); BALB/cN (BALB/c); and C57BL/10 SnHz (C57). SJL and C57 have Ig^b allotypes. BALB/c has Ig^a allotypes.

Chronically suppressed mice: Derived from matings between normal SJL (Ig^b) males and BALB/c (Ig^a) females immunized against the paternal Ig-*1b* allotype. These mice, about half the progeny of the cross, although genotypically *a/b*, were not producing Ig-*1b* globulins (γG_{2a} of the *b* allotype) when tested at 6 months of age.

Normal (nonsuppressed) mice: The Ig^a/Ig^b progeny of SJL males and normal (nonimmune) BALB/c females.

Immunization of BALB/c Mothers.—BALB/c females (*H-2d*, Ig^a) were immunized against Ig-*1b* by injection of anti-*H-2d* serum (S-305A) produced in C57 (*H-2b*, Ig^b) mice. The initial injection consisted of 10 μl of S-305A in complete Freund's adjuvant given subcutaneously (s. c.)[2] and intraperitoneally (i.p.). This was followed 2 wk later by i.p. injection of 20 μl of S-305A in saline. A third i.p. injection of 20 μl was given 1 wk later. The mice were bled 1 wk after the last injection, and those which showed strong anti-Ig-*1b* levels in immunodiffusion analysis were mated to SJL males. Booster injections of C57 anti-*H-2d* were given once a month and the mothers tested periodically for continued high antibody production. This method of immunization results in the production of antibody directed against Ig-*1b* (γG_{2a}) but not Ig-*4b* (γG_1) or other immunoglobulin allotypes (7).

Irradiation.—Mice used as recipients in the cell transfer experiment were given 600 R total body irradiation 1 day (approximately 18 hr) before cell transfer. Mice in which endogenous repopulation was studied were given 600 R irradiation with long-bone protection. This was done by anesthetizing the mice with Diabutol (5 mg/ml, 0.0135 ml/g body weight) positioning the unconscious mice by means of tape, and placing a double layer of lead, on wooden blocks, to cover the hind legs. Irradiation was carried out using a Siemens X-ray machine (Siemens Corp., Medical Industrial Div., Iselin, N. J.) operating under the following conditions: 250 kv, 15 ma, 0.25 mm of Cu + 1.0 mm of Al and a half-value layer (HVL) of 1.10 mm of Cu. The dose rate was 80 rads/min and the focal skin distance was 60 cm.

[2] *Abbreviations used in this paper:* i.v., intravenously; i.p., intraperitoneally; MEM-PM, Eagle's minimal essential medium without $NaHCO_3$ (see footnote 3); s.c., subcutaneously.

Spleen Cell Suspensions.—Mice were killed by cervical dislocation and their spleens removed. Single cell suspensions were obtained by gently pressing the tissue through a 50-mesh stainless steel screen into cold Eagle's minimal essential medium without HCO_3 (MEM-PM)[3]. Clumps were broken up by repeated aspiration using a Pasteur pipette, and the remaining fragments were allowed to settle. The supernatants were transferred, centrifuged for 10 min at 1000 rpm at 4°C, and the cells resuspended in MEM-PM. Cell counts were obtained using a Coulter counter, Model B (Coulter Electronics, Inc., Industrial Div., Hialeah, Fla.), fitted with a 100-μ aperture and using optimum window settings previously calibrated with hemacytometer counts of nucleated spleen cells.

Cell Transfers.—(SJL × BALB/c)F_1 chronically suppressed mice, age 6–9 months, were injected intravenously (i.v.) approximately 18 hr after irradiation, with 1.2–1.5 × 10^7 spleen cells. Spleen cell donors were normal (SJL × BALB/c)F_1 (Ig^a/Ig^b) mice with the exception of one experiment in which the donor was either an (SJL × BALB/c)F_2 or an F_1 × SJL offspring found to be Ig^b homozygous.

Antisera.—Anti-allotype antisera were used in this study for estimation of Ig-*1b* (γG_{2a} of SJL type) and Ig-*4b* (γG_1 of SJL type) levels in sera. Sera specific for Ig-*1b* were prepared in BALB/c female mice immunized by the protocol described in the section on immunization of mothers. Sera detecting Ig-*4b* were prepared by immunizing BALB/c mice with a conjugate of C57 anti-*Bordetella pertussis* and killed *B. pertussis*, as previously described (7). Such anti-allotype sera react with both Ig-*1b* and Ig-*4b*, (as well as Ig-*3b* [γG_{2b}] in some cases), however, under appropriate assay conditions (see below) they can be used for specific detection of Ig-*4b*.

Collection and Storage of Sera.—Animals were bled from the tail artery after warming. Antisera were tested, pooled, and stored in small aliquots at −20°C. Test sera were stored individually at −20°C.

Isotope-Labeled Antigens.—Antigens used in the quantitative estimation of Ig-*1b* and Ig-*4b* were prepared and labeled with ^{125}I as previously described (7). In the Ig-*4b* assay, MOPC-245, a γG_1 myeloma protein[4] originating in an Ig^a/Ig^b heterozygote and carrying Ig-*4b* (SJL) determinants, was used as the labeled antigen. For the Ig-*1b* assay, a protein peak (4–32) shown to be rich in γG_{2a} (the class carrying Ig-*1b*) was isolated from the serum of an aging SJL mouse and labeled. Although the protein in this peak was not necessarily as homogeneous as a myeloma protein, more than 60% of the label was precipitable by specific anti-Ig-*1b*. This same material (4-32) was used for isotope tracer half-life studies.

Immunodiffusion.—Sera obtained from experimental mice at 1 or 2 wk intervals were tested for Ig-*1b* by double diffusion in agar on microscope slides (8, 9). Positive reactions were scored as w+ to 4+, depending on the strength of the reaction. The approximate corresponding values in milligrams per milliliter, obtained by quantitative determination, are as follows: − = <0.025, w+ = 0.025–0.1, + = 0.1–0.15, 2+ = 0.15–0.5, 3+ = 0.5–1, 4+ = > 1. The antisera used for immunodiffusion were specific for Ig-*1b*.

Quantitative Estimation of Immunoglobulin Level.—Immunoglobulin levels were estimated by inhibition of precipitation of ^{125}I-labeled antigens (7). Values, expressed as milligrams per milliliter serum, were determined with reference to a standard C57 serum. The antiserum used for precipitation of Ig-*1b*–^{125}I was specific for Ig-*1b* and did not precipitate other Ig^b globulins. The antiserum used for Ig-*4b* precipitation reacted with Ig-*1b* as well, but the precipitation of Ig-*4b*–^{125}I was not inhibited by the presence of Ig-*1b*.

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[3] Eagle's minimal essential medium catalogue No. F-12 instant tissue culture powder medium without $NaHCO_3$ (Grand Island Biological Company, Grand Island, N. Y.), made up with $Na_2HPO_4 \cdot 12H_2O$ (358 mg/liter) and $MgCl_2 \cdot 6H_2O$ (200 mg/liter) instead of bicarbonate, in distilled, deionized water.

[4] Isolated from a tumor kindly provided by Dr. M. Potter.

Immune Elimination.—Six chronically suppressed and four normal (SJL × BALB/c)F_1 mice were each injected i.v. with a quantity of ^{125}I-labeled Ig-*1b* (having approximately 10^6 cpm). The mice were then bled at intervals to determine the rate of removal of the radioactive protein. 20 μl of blood was obtained from the tail vein, lysed in 0.6 ml of distilled water, and counted for 2 min in a well-type gamma scintillation counter.

RESULTS

Kinetics.—Fig. 1 shows a comparison between the short-lived allotype suppression obtained in (C57 × BALB/c)F_1 mice (5) and the long-term, or chronic, suppression seen in (SJL × BALB/c)F_1 mice. The percentage of suppressed animals which become positive for Ig-*1b* with time is indistinguishable in the two strain combinations up to about 8 wk of age. The number of (C57 ×

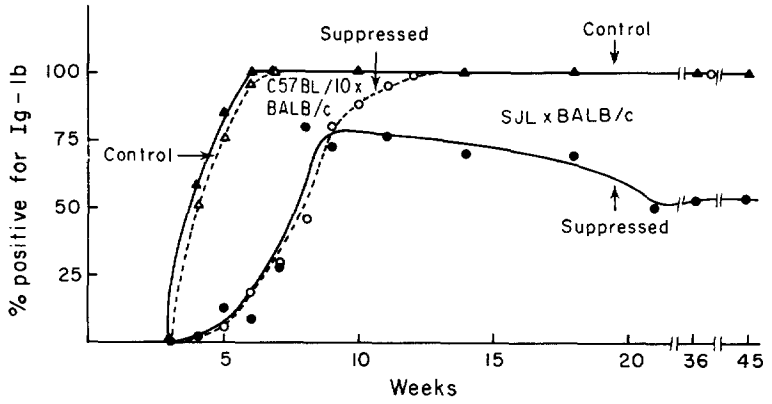

FIG. 1. Percentage of suppressed mice which become positive for Ig-*1b* with time after birth, in both short-term suppressed (C57 × BALB/c)F_1 mice (○) and long-term suppressed (SJL × BALB/c)F_1 mice (●). The appearance of Ig-*1b* production in normal (C57BL/10 × BALB/c)F_1 (△) and normal (SJL × BALB/c)F_1 (▲) mice is shown for comparison.

BALB/c)F_1 mice which develop the capability of producing Ig-*1b* then continues to increase until, eventually, all have broken suppression, although their Ig-*1b* levels, as shown by quantitative tests, are lower then those found in normal control mice. On the other hand, the number of (SJL × BALB/c)F_1 mice which produce Ig-*1b* does not increase after 8 wk of age, and, in fact, decreases slightly with time. It is important to note that the flat portion of the curve represents more or less a dynamic steady state, resulting from permanent suppression in some mice, permanent recovery in others, and fluctuating levels in a majority of the mice. Thus, in the extreme, chronic suppression manifests itself as the complete absence of detectable paternal allotype throughout life, although most frequently it takes a milder form in which levels of Ig-*1b* either fluctuate or are maintained over a period of several weeks, but eventually fall permanently below detectability.

This variability in the pattern of suppression seen in (SJL × BALB/c)F_1 mice is shown more clearly in Table I, in which weekly allotype testing data are presented for a large litter containing all three types of suppressed animals. The first three mice never showed any Ig-*1b* detectable by agar-gel diffusion analysis, indicating that their serum levels were less than 0.025 mg/ml. Animals 4–9 showed transient production of Ig-*1b*, in some cases only briefly and at barely detectable levels, while in other animals of this group the levels of Ig-*1b* frequently rose to relatively high values and persisted for several weeks. The last four mice recovered from suppression and remained positive for Ig-*1b* throughout their life, although animals 12 and 13 show temporary cessation of production at around 13 wk of age. Progeny testing of mice 1–4 verified that

TABLE I

Ig-1b Production in a Litter of (SJL × BALB/c)F_1 Offspring Born to a BALB/c Female Immune to Ig-1b

	Age (*wk*)												
Mouse	3	4	5	6	7	8	9	10	13	19	22	40	48
1*	−‡	−	−	−	−	−	−	−	−	−	−	−	−
2*	−	−	−	−	−	−	−	−	−	−	−	−	−
3*		−	−	−	−	−	−	−	−	−	−	−	−
4*	−	−	−	−	−	−	−	w+	−	w+	−	−	−
5	−	−	+	−	−	−	−	−	−	N.T.	N.T.	−	N.T.
6	−	−	−	−	−	−	−	−	−	3+	Dead		
7	−	−	−	−	−	−	4+	3+	+	−	−	−	−
8	−	−	−	−	−	4+	+	+	+	4+	−	−	−
9	−	+	+	w+	−	3+	3+	3+	+	−	+	−	−
10	−	w+	w+	w+	w+	4+	3+	2+	2+	N.T.	4+	4+	4+
11	−	−	−	−	−	3+	4+	2+	3+	3+	3+	2+	3+
12	−	2+	3+	2+	+	4+	3+	2+	w+	3+	4+	4+	4+
13	−	−	−	−	−	3+	3+	3+	−	+	3+	4+	4+

* These mice, phenotypically negative for the paternal allotype (Ig-*1b*), were backcrossed to the maternal (Ig^a) strain, and the presence of the paternal (Ig^b) allele was confirmed.

‡ Levels determined by immunodiffusion. N.T. = not tested.

these mice were heterozygotes, carrying both the Ig^a and Ig^b allele. A litter of 14 control mice (not shown) included in this experiment, showed the normal pattern of paternal allotype development. Ig-*1b* production was first detectable at 3–4 wk of age and was maintained at a high level after 5 wk of age.

In Table II the pattern of Ig-*1b* production in a large number of chronically suppressed mice is summarized. Two experimental groups (I and II) were bled at weekly intervals (the litter shown in Table I is included in the first group). Group III, containing a much larger number of animals, was tested only once, when the mice were approximately 6 months of age. In groups I and II, 23 of the 62 mice tested were negative for Ig-*1b* at 6 months of age. Of these, 7 of 23 never showed any paternal allotype. In group III, 77 of the 137 mice tested were chronically suppressed. Many of the animals used in the following experiments were taken from this group.

Specificity of Suppression.—While chronically suppressed animals have extremely low or undetectable levels of Ig-*1b*, there is no impairment of their capacity to maintain normal levels of Ig-*4b*, the paternal allotype found on γG_1 immunoglobulins. Studies on 19 chronically suppressed mice (see Table III) show that the one mouse with detectable Ig-*1b* had only 0.028 mg/ml, which is less than 1% of the average Ig-*1b* level in normal mice (3.3 mg/ml). In contrast, there is no difference between the average Ig-*4b* levels in suppressed and normal mice.

The restriction of chronic suppression to Ig-*1b* (γG_{2a} globulins) as opposed to Ig-*4b* (γG_1 globulins) suggests that the specificity of the maternal antibody determines the specificity of suppression, since the immunizing protocol used

TABLE II

Chronic Suppression of Ig-1b Production in (SJL × BALB/c)F_1 Mice

Group	Mothers immune to Ig-*1b*				Mothers not immune to Ig-*1b*			
	Positive*	Transient‡	Negative§	Total	Positive*	Transient‡	Negative§	Total
1	19	12	6	37	39	0	0	39
2	20	4	1	25	14	0	0	14
3	60	77		137	99	0		99
Total	99	100		199	152	0		152

* Mice which show Ig-*1b* production at 6 months are classed as positive.

‡ Mice which show Ig-*1b* production at any time before 6 months, but which were negative at 6 months, are classed as transient.

§ Mice which never produced detectable amounts of Ig-*1b* by 6 months are classed as negative. Where weekly testing was not done (group 3), the distinction between transient and negative could not be made. All of these mice are referred to as chronically suppressed.

for the BALB/c mothers in these experiments evokes antibody reacting only with Ig-*1b* (see Materials and Methods).

Clearance of Ig-1b Globulins.—In order to test whether chronic suppression might be due to the continued presence (or production) of anti-Ig-*1b* antibody, studies were carried out to determine the rate of clearance of labeled exogenous circulating Ig-*1b* in normal and suppressed mice. The data in Fig. 2 show that the rate at which the labeled Ig-*1b* was removed from the circulation was the same in chronically suppressed mice as in normal mice, demonstrating that the presence of circulating anti-paternal allotype antibody is not a factor in the maintenance of chronic suppression.

Effect of Repeated Blood Loss.—Since the data in the following experiments were determined from serum samples obtained by weekly bleeding, a preliminary experiment was set up to determine whether repeated blood loss, by forcing continuous rapid replacement of serum proteins, might result in sufficient stimulus to break suppression. 20 chronically suppressed mice, age 3–5 months,

were chosen at random from several litters. One group of seven mice was bled extensively (approximately 1 ml of blood taken each week from each mouse). In this group, one mouse became positive for Ig-*1b* after the first week and remained positive for the duration of the experiment (9 wk), while two other mice

TABLE III

Levels of Ig-1b (γG_{2a}) and Ig-4b (γG_1) in Chronically Suppressed and Nonsuppressed (SJL × BALB/c)F_1 Mice

(SJL × BALB/c)F_1	No. positive for Ig-*1b**/total	Mean‡ level of Ig-*1b* in positive mice	No. positive for Ig-*4b*/total	Mean level of Ig-*4b*
		mg/ml		*mg/ml*
Chronically suppressed	1/19	0.028§	19/19	0.9
Nonsuppressed	18/18	3.3	18/18	0.9

* Positive in ^{125}I inhibition assay (see Materials and Methods).

‡ Arithmetic mean of positive mice only. All mice positive for Ig-*4b*.

§ 18/19 mice tested had <0.025 mg Ig-*1b*/ml serum.

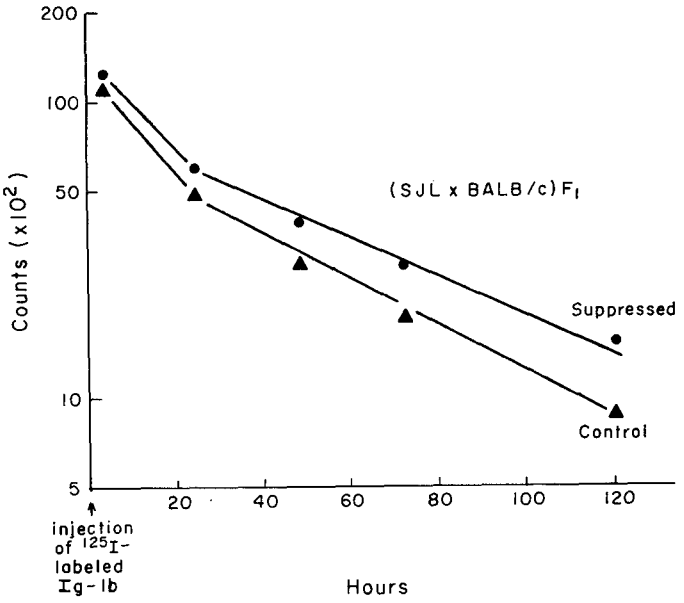

FIG. 2. Elimination of exogenously administered ^{125}I-labeled Ig-*1b*. Counts (× 10^2) per 2 min in 20 μl samples of whole blood, lysed in distilled water, from suppressed (●) and normal control (▲) (SJL × BALB/c)F_1 mice.

showed transient production, lasting for 4–6 wk. From a second group of seven mice only 40 μl of blood was taken each week. Two of these mice produced detectable Ig-*1b* by the second or third bleeding date and remained positive up to week 9. A third group of six mice was not bled until the end of the experiment,

at which time two had become positive for Ig-*1b*. These patterns of Ig-*1b* production are like those shown in Tables I and II, and the similarity in the resuts obtained with the three groups indicates that supression is not affected by repeated heavy blood loss.

Effect of X-Irradiation.—In order to stimulate division of stem cells and regeneration of new immunoglobulin-producing cells, chronically suppressed mice

TABLE IV

Failure of Irradiation with Long-Bone Shielding to Break Suppression in Chronically Suppressed (SJL × BALB/c)F$_1$ Mice

Treatment*	Numbers positive‡ for Ig-*1b*/total (weeks after irradiation)				
	0	2	4	6	9
Irradiated and shielded	0/11	1/10§	0/10	0/10	0/10
Nonirradiated controls	0/11	1/11§	0/11	0/11	0/11

* Irradiated (600 R) and nonirradiated animals, 5–7 months of age, were caged together in groups of six.

‡ Positive in immunodiffusion (Ouchterlony) analysis.

§ The two transiently positive animals were from the same litter and were housed together

TABLE V

Failure of High Levels of Circulating Ig-1b to Promote Release from Suppression in Irradiated, Long-Bone-Shielded Mice

Serum recipient*	Mouse No.	Level of Ig-*1b*‡					
		Before serum injection	Weeks after irradiation				
			0	2	4	6	9
(SJL × BALB/c)F$_1$ chronically suppressed	1	–	3+	3+	+	–	–
	2	–	3+	3+	+	–	–
	3	–	3+	3+	+	–	–
Normal BALB/c	4	–	3+	2+	–	–	–
	5	–	3+	2+	–	–	–

* Mice were injected with a total of 1.5 ml normal Ig^b serum over a period of 1 wk and irradiated (600 R) 1 day after the last injection.

‡ Levels were determined by immunodiffusion in agar.

were exposed to 600 R irradiation with long-bone (hind-limb) shielding. These mice, together with nonirradiated, chronically suppressed control mice, were bled at regular intervals and the presence or absence of serum Ig-*1b* determined. Even after sublethal irradiation followed by endogenous repopulation, the mice were incapable of producing Ig-*1b* (see Table IV), despite the fact that immune clearance studies indicate that circulating anti-Ig-*1b* is no longer present.

Effect of High Circulating Levels of Ig-1b Before Irradiation.—In the event that maintenance of suppression is due to the presence of sequestered or of cel-

lular antibody to Ig-*1b*, it should be possible to block this antibody by injecting large amounts of Ig^b serum before irradiation.

In order to test this possibility a few chronically suppressed mice were injected with a total of 1.5 ml of Ig^b serum over a period of 1 wk and then irradiated (600 R) with long-bone protection. BALB/c mice, injected simultaneously

TABLE VI

Production of "b" Allotype after Transfer of Spleen Cells from Normal (SJL × BALB/c)F_1 into Irradiated Chronically Suppressed and Normal Mice

Recipient* mice	No. positive for Ig-*1b*/total‡ (weeks after irradiation)			
	1	3	5	7
Suppressed (SJL × BALB/c)F_1	1/14	1/14	3/14	2/14
Normal BALB/c (allotype *a/a*)	8/8	8/8	8/8	8/8

* Recipients were given 600 R total body irradiation approximately 18 hr before cell transfer.

‡ Ig-*1b* determined by immunodiffusion.

TABLE VII

Production of "b" Allotype after Injection of Normal Ig^b Homozygous Spleen Cells into Chronic Suppressed (SJL × BALB/c)F_1 and Normal BALB/c Mice

Recipient*	Ig-*1b* Levels‡ (weeks after transfer)				Level§ of Ig-*1b* on week 7
	1	3	5	7	
					mg/ml
(SJL × BALB/c)F_1 suppressed	−	−	−	−	<0.025
	−	−	w+	−	<0.025
	−	−	−	−	<0.025
	−	−	−	−	<0.025
	−	−	2+	2+	0.3
Normal BALB/c	3+	3+	3+	4+	1.5
	3+	3+	4+	4+	2
	3+	4+	4+	4+	1.1

* 600 R total body irradiation was given to recipient mice approximately 18 hr before i.v. cell transfer.

‡ Determined by immunodiffusion.

§ Determined by inhibition of ^{125}I precipitation.

with Ig^b serum, were used as controls to determine the length of time that the exogenously administered serum could be detected. The results in Table V show that even in the presence of high Ig-*1b* levels irradiated, endogenously repopulating mice are unable to produce Ig-*1b*. The rate of disappearance of the injected Ig-*1b* in these mice is not appreciably different from that seen in irradiated BALB/c (Ig^a/Ig^a) control mice.

Transfer of Normal Spleen Cells into Suppressed Mice.—In view of the inabil-

ity to break suppression in intact mice by the means described, the question arose as to whether maintenance of suppression is due to the absence of Ig-*1b*–producing cells (or their progenitors) or to unfavorable condition(s) in the environment in which they must develop and function. To answer this question, spleen cells from normal (nonsuppressed) mice (Ig^a/Ig^b or Ig^b/Ig^b) were injected into irradiated suppressed recipients and, for controls, into irradiated BALB/c mice. As shown in Table VI, the controls all had detectable circulating Ig-*1b* 1 wk after transfer and continued to produce large amounts of globulin of this allotype. In contrast, only 1 of the 14 suppressed recipients had detectable Ig-*1b* by 1 wk and remained positive throughout the experiment. During the course of the experiment, two more mice became Ig-*1b* positive. One of these mice was weakly positive at week 5 only and the Ig-*1b* level of the other was considerably below the control levels at 7 wk. The number of Ig-*1b* producers in this group is comparable with the number of chronically suppressed mice which spontaneously break suppression.

It is particularly notable that Ig^b/Ig^b homozygous cells were suppressed when injected into chronically suppressed mice. These results are shown in Table VII.

DISCUSSION

Chronic (long-term) allotype suppression, long known to occur in rabbits as a result of prenatal or early postnatal exposure to antibody directed against that allotype (1–4), has now been shown to occur in at least one strain combination of mice as well. In contrast to earlier experiments using (C57BL/10 × BALB/c)F_1 mice (5), in which allotype suppression was relatively short-lived, the offspring of SJL (Ig^b) males and BALB/c (Ig^a) females hyperimmunized to the paternal allotype frequently show only sporadic production, or no production at all, of the paternal allotype throughout their life. This pattern of chronic suppression differs from that seen in rabbits, in which the paternal (suppressed) allotype is eventually produced but at a far lower level (less than 10%) throughout life than that found in normal controls. In addition, the compensatory increase in another immunoglobulin class found in suppressed rabbits (4), has not been detected in the chronically suppressed (SJL × BALB/c)F_1 mice used in these experiments (unpublished observation), nor was it observed by Anderson (10), who studied the immune response to sheep erythrocytes of adult mouse spleen cells, allotypically suppressed after transfer to irradiated syngeneic recipients.

One hypothesis able to account for suppression is that the interaction of circulating anti-allotype antibody with the progenitors of the cells destined to produce this allotype results in either elimination of these cells or in their diversion from production of immunoglobulin of this allotype (3, 5). The results of immune clearance studies, reported here, indicate that no circulating anti-paternal allotype antibody can be detected in chronically suppressed mice at 6 months of age or older. Therefore, it would seem reasonable that if the lymphoid population of these mice were destroyed, and endogenous repopulation forced

to occur, that new progenitor cells, now arising in the absence of circulating antibody, would give rise to cells which produce immunoglobulin carrying the paternal allotype. The results of such studies, reported here, show that this is not the case. Stimulation of hematopoiesis, lymphopoiesis, and gamma globulin production by repeated bleeding and by irradiation with long-bone shielding in chronically suppressed mice does not result in a release from the suppressed state. Even if mice are injected before irradiation with large amounts of serum containing Ig-*1b*, which should block any sequestered antibody that might have gone undetected or, if necessary, "prime" the newly developed stem cells to produce globulins carrying this allotype (11), no Ig-*1b* production results.

These results suggest that maintenance of the suppressed state may be due not to the inherent capabilities of the cells themselves, but to the "environment" in which these cells develop and function. In other words, even if there are cells in suppressed mice capable of producing Ig-*1b*, they are not detectable because the environment prohibits them from functioning (or developing) in a normal manner. Direct support for this hypothesis arises from the experiments in which chronically suppressed irradiated mice were injected with Ig-*1b*–producing cells obtained from normal (nonsuppressed) mice, either heterozygous (*a/b*) or homozygous (*b/b*) for the Ig^b allotypes. Such cells, when injected into irradiated normal BALB/c (*a/a*) control mice, continue to produce high levels of Ig-*1b* globulins, but, injected at the same time and under the same conditions into irradiated mice suppressed for the Ig-*1b* allotype, fail to produce Ig-*1b*. Thus, in suppressed animals, there is an active physiologic process capable of exerting its influence even on exogenously administered, normally functioning cells. This factor appears to be relatively radioresistant, since sublethal irradiation of 600 R was administered to the recipient suppressed mice before transfer of normal cells.

The publication which follows describes the results of cell transfer studies which further implicate an active process in chronic allotype suppression.

SUMMARY

Long-term (chronic) allotype suppression, previously reported only in rabbits, is shown here to occur in at least one strain combination of mice as well. Close to 50% of the offspring of SJL (Ig^b) males mated to BALB/c (Ig^a) females immunized against the paternal allotype were found to be suppressed for Ig-*1b* (γG_{2a}) at 6 months of age. These mice are called "chronically" suppressed.

The percentage of offspring in this strain combination suppressed for the paternal allotype at 8 wk of age is the same as that seen in an earlier strain combination tested, [(C57 × BALB/c)F_1], in which all mice recover from suppression by 10–12 wk. After 8 wk, two distinct patterns of long-term (chronic) suppression emerge in (SJL × BALB/c)F_1 mice: a small number of these mice never produce detectable amounts of Ig-*1b* throughout their lives, while the majority produce detectable Ig-*1b* sporadically, sometimes over a period of several weeks, the level of which eventually falls below detectability.

Attempts to "cure" suppression by destroying the existent lymphoid population and forcing endogenous repopulation in chronically suppressed animals were unsuccessful. Furthermore, attempts to restore Ig-*1b* production by injection of cells from syngeneic Ig^a/Ig^b donors into irradiated, chronically suppressed recipients were also unsuccessful, although the same cell inocula, when injected into irradiated BALB/c (Ig^a/Ig^a) mice produced high levels of gamma globulin carrying the allotype. These results suggest that long-term allotype suppression resulting from perinatal exposure of offspring to specific anti-allotype antibody (anti–Ig-*1b*), is not due merely to an absence of Ig-*1b*–producing cells or their progenitors, but appears to be an active process, which dominates physiologically over normal production.

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BIBLIOGRAPHY

1. Dray, S. 1962. Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling gamma globulin allotypic specificities. *Nature (London).* **195:**677.
2. Mage, R., and S. Dray. 1965. Persistent altered phenotypic expression of allelic γG-immunoglobulin allotypes in heterozygous rabbits exposed to isoantibodies in fetal and neonatal life. *J. Immunol.* **95:**525.
3. Mage, R. G. 1967. Quantitative studies on the regulation of expression of genes for immunoglobulin allotypes in heterozygous rabbits. *Cold Spring Harbor Symp. Quant. Biol.* **32:**203.
4. Dubiski, S. 1967. Suppression of synthesis of allotypically defined immunoglobulins and compensation by another subclass of immunoglobulins. *Nature (London).* **214:**1365.
5. Herzenberg, L., L. A. Herzenberg, R. C. Goodlin, and E. Rivera. 1967. Immunoglobulin synthesis in mice: suppression by anti-allotype antibody. *J. Exp. Med.* **126:**701.
6. Herzenberg, L. A., H. O. McDevitt, and L. A. Herzenberg. 1968. Genetics of antibodies. *Annu. Rev. Genet.* **2:**209.
7. Herzenberg, L. A., and N. L. Warner. 1967. Genetic control of mouse immunoglobulins. *In* Regulation of the Antibody Response. B. Cinader, editor. Charles C Thomas, Publisher, Springfield, Ill. 322.
8. Ouchterlony, O. 1949. Antigen-antibody reactions in gels. *Acta Pathol. Microbiol. Scand.* **26:**507.
9. Herzenberg, L. A., N. L. Warner, and L. A. Herzenberg. 1965. Immunoglobulin isoantigens (allotypes) in the mouse. I. Genetics and cross-reactions of the 7S γ_{2a} isoantigens controlled by alleles at the Ig-1 locus. *J. Exp. Med.* **121:**415.
10. Anderson, H. R. 1970. Allotypic suppression of adult mouse spleen cells. *Immunology.* **19:**169.
11. Mage, R. G. 1967. The quantitative expression of allelic allotypes in normal and "allotype suppressed" heterozygous rabbits. *In* Ontogeny of Immunity. R. A. Good, editor. University of Florida Press, Gainesville, Fla. 78.