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Chapter 2

Short-Term and Chronic Allotype Suppression in Mice

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INTRODUCTION

The phenomenon called allotype suppression, in which exposure of the neonate to antibody against its own immunoglobulins suppresses production of those immunoglobulins, offers fertile ground for studying the mechanisms of differentiation and regulation of the immune system. Frequently the suppression is short-lived, and is measurable in weeks post exposure rather than months. In other cases, however, the short exposure of the young animal to antibody to an allotypic antigen on immunoglobulins appears to permanently modify the immune system of the treated animal so that it never regains its normal capacity for immunoglobulin production. Studies on the mechanisms of these short- and long-term suppressions may therefore be expected to provide useful information about some of the sensitive regulation points which keep the entire immune system in balance.

Allotype Suppression in Rabbits

Allotype suppression was originally described in the rabbit. Dray *et al.* immunized female rabbits to allotypic antigens carried on immunoglobulin molecules (i.e., allotypes). Mating these females with homozygous males of the immunizing allotype resulted in heterozygous progeny which were suppressed for production of the paternal type immunoglobulins. It was subsequently

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shown that the suppression is due to exposure to maternal antiallotype antibody since progeny from normal mothers injected with the antibody also become suppressed (Mage and Dray, 1965).

Most of the rabbits suppressed by perinatal exposure to antiallotype antibody eventually do produce some immunoglobulins of the suppressed allotype; however, decreased production of the allotype is generally evident well into adulthood if not throughout the life of the animal. To compensate for the decrease in total immune globulins, which is considerable in the rabbit since the suppressed allotypes occur on the Fd portion of the immunoglobulin molecule and hence in several different immunoglobulin classes, suppressed rabbits either produce more immunoglobulins carrying the nonsuppressed allelic allotype (Mage, 1967) or, in the case of suppressed homozygotes, more of other classes of immunoglobulins (Dubiski, 1967; David and Todd, 1969) coded for by genes of other loci (Appella *et al.*, 1968; Kim and Dray, 1973).

To date, it has been difficult to make progress elucidating the mechanism(s) of allotype suppression in the rabbit, perhaps due to the logistics of rabbit work, or perhaps due to the problems of studying cellular interactions with noninbred animals. Nonetheless, it has been established (among other things) that decrease in circulating allotype reflects a decrease in the number of plasma cells producing that allotype (Lummus *et al.*, 1967) and that suppression decreases the amount of allotype in the antibody produced in response to a particular antigen as well as the amount of allotype in the general immunoglobulins in circulation (Mage, 1967). Rose Mage and co-workers have recently shown that suppressed rabbits also lack antibody-forming-precursor-type cells bearing the suppressed allotype on the cell membrane (Harrison *et al.*, 1973*a*) and these begin to appear as suppression is ending (Harrison *et al.*, 1973*b*).

Allotype Suppression in Mice

Our studies on allotype suppression in the mouse, with which we will largely concern ourselves for the remainder of this review, were stimulated originally by the demonstration of suppression in the rabbit. Following a similar protocol, we immunized female mice of one strain with an allotypic antigen from a second strain, mated males of the second strain to the immune female, and followed the development of the paternal type immunoglobulin in the heterozygous progeny. With the strains used in our first studies we found that the onset of production of paternal type immunoglobulin in the mouse was considerably delayed in progeny of immune mothers, although all suppressed progeny recovered from suppression and showed essentially normal levels of immunoglobulin by about 15 weeks of age (Herzenberg *et al.*, 1967). In later studies we showed that by using a paternal strain which has severe immunoglobulin abnormalities (i.e., SJL/J), we could produce progeny with a long-term or "chronic" suppression (Jacobson and Herzenberg, 1972; Jacobson *et al.*, 1972). In these hybrids (SJL

× BALB/c) the chronic suppression is due to the generation of a transferable population of thymus-derived (T-cells) which actively suppresses production of the allotype (Herzenberg *et al.*, 1971; Herzenberg, 1972; Herzenberg *et al.*, 1973 and see below).

In order to describe and discuss the studies which led to these conclusions, it is perhaps useful at this point to digress slightly for a brief review of the normal production of immunoglobulins in the mouse and some relevant information about mouse allotypy.

Immunoglobulin Allotypes in Mice

Distinct allotypic antigens have been found on heavy chains of four classes of mouse immunoglobulins (Herzenberg *et al.*, 1968). Antigens of two of these classes, Ig-1 on IgG_{2a} heavy chains and Ig-4 on IgG_1 heavy chains, have been used in the allotype suppression studies. BALB/c, the strain generally used as the maternal or antiallotype donor carries Ig-1a and Ig-4a allotypes. C57BL/10, SJL, and several other strains generally used as paternal strains carry Ig-1b and Ig-4b allotypes. Referred to collectively, the immunoglobulins produced by BALB/c (IgG_1, IgG_{2a}, IgG_{2b}, and IgA of allotype "a") are called Iga globulins and the immunoglobulins produced by C57BL/10 or SJL are referred to as Igb globulins. The corresponding shorthand notation for the gene clusters are Ig^a and Ig^b. Low levels of Ig-1b or Ig-4b are readily measurable in the presence of large amounts of Iga globulins by radioimmune assay (Herzenberg and Herzenberg, 1973). For semiquantitative work, we estimate Ig-1b by immunodiffusion in agar gels.

Origins of Immunoglobulins in Young Mice

The young mouse starts to synthesize detectable amounts of its own IgG immunoglobulins sometime around weaning. Prior to this time its circulating IgG comes from maternal IgG which is first passed to the young *in utero* and then passed continually over roughly the first 16 days of life via nursing (Brambell, 1970). In the weanling (about 3 weeks of age), the majority of circulating IgG is still of maternal origin; however, this passively transferred globulin disappears over the next 5 to 8 weeks and is replaced by its natively synthesized counterpart (Herzenberg *et al.*, 1967).

Thus, in heterozygous progeny ($Ig^a.Ig^b$) made by mating BALB/c (Ig^a) females to C57BL/10 (Ig^b) males, most of the IgG in circulation at 3 weeks of age is maternally derived and therefore Iga allotype. This passively transferred immunoglobulin is eliminated at an exponential rate with a half life of approximately 5 to 7 days. Although it is largely gone when the progeny have reached 8 weeks of age, traces may still be found as late as 12 weeks (Herzenberg and Herzenberg, 1966; Herzenberg *et al.*, 1967, Warner and Herzenberg, 1970).

Replacement of the passively acquired maternal IgG by native synthesis begins about 3 weeks of age. Paternal allotypes (for example, Ig-1b in the

heterozygote described above) first appear in circulation sometime between 3 and 5 weeks. Levels then continue to rise until about 12 weeks, when stabilization begins to occur at about the expected adult level (see Fig. 1). Synthesis of the immunoglobulin carrying the maternal allotype is more difficult to measure in young animals because of the large amount of maternally derived IgG present; however, it may be presumed to follow the paternal allotype in that levels of both allotypes in 8-week and older progeny are similar and immunization of progeny starting at 3 weeks yield antibody of both allotypes (unpublished observations).

SHORT-TERM ALLOTYPE SUPPRESSION

Allotype suppression interferes with normal pattern of development described above. Exposure of the neonatal mouse to antibody to the paternal

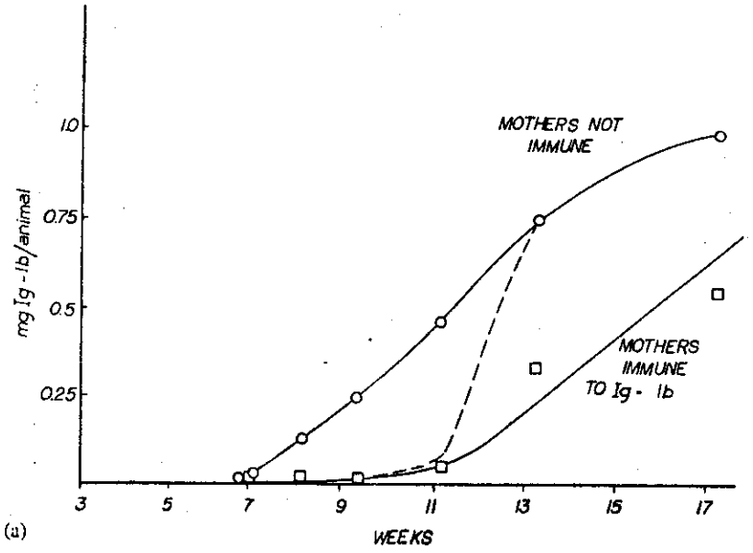


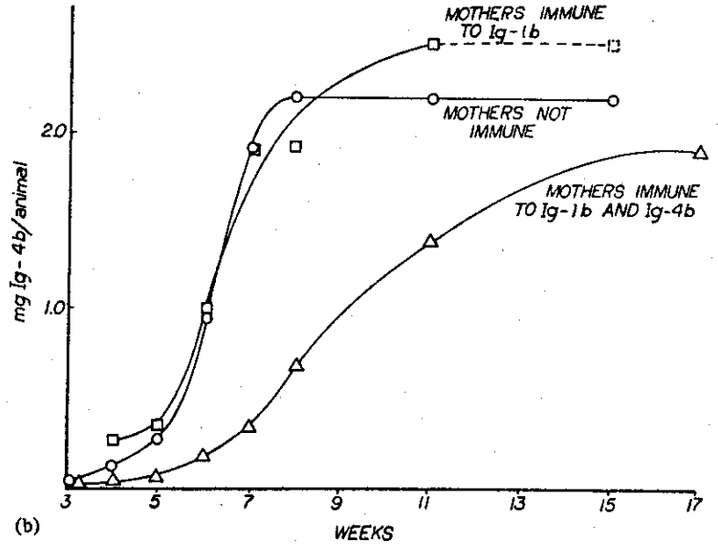
Figure 1. Increase in allotype levels with age in suppressed and normal progeny. Allotype levels in serum were estimated by radioimmune assay. Calculations for total mg allotype/animal were based on an estimated immunoglobulin space equal to 8.8% body weight.

Figure 1a is reproduced with some modifications from an earlier publication (Herzenberg *et al.*, 1967). Progeny were C57BL/10 X BALB/c hybrids. Dashed curve was calculated on the basis of 25 mg of Ig-1b removed (by maternal antibody), with rate of production set equal to that estimated for controls by equation (1) below. A half-life of 6 days was used. Corrections for the amount of Ig-1b withdrawn in weekly serum sampling were made.

For $t = 0, 1, 2, \ldots .179$, the rate of production of Ig-1b at time t (PRO_t) was found by equation (1) and substituted in equation (2) to determine the amount of Ig-1b expected at time t (Calc Ig_t).

allotype on either IgG_1 or IgG_{2a} globulins specifically delays the onset of synthesis of the IgG_1 or the IgG_{2a} carrying the allotype generally for about 3 weeks. After that, in all strain combinations tested, synthesis of the suppressed allotype or allotypes begins and serum levels climb at a rate similar to that in unsuppressed mice 3 weeks younger.

Most of the data on allotype suppression in mice were collected using BALB/c (Iga) as the mother and the Ig-1b allotype derived from a variety of Igb strains as the suppressed allotype. This particular combination was dictated by the relative ease with which BALB/c mice could be consistently and strongly immunized with Ig-1b. We have now shown, however, that production of Ig-4b, Ig-1a, and Ig-4a is suppressed in similar fashion when neonatal progeny of the appropriate cross are exposed to antibody to the appropriate paternal allotype. The data in Table I summarize these experiments.



$$(1)\quad PRO_{(t+1)} = (Ig_{(t+1)} - Ig_t) + \left(\frac{\ln 2}{T} + k\right) Ig_t \text{ and}$$

$$(2)\quad \text{Calc } Ig_{(t+1)} = \text{Calc } Ig_t + q \cdot PRO_t - \left(\frac{\ln 2}{T} + k\right) \text{Calc } Ig_t - \theta$$

where Ig_t is the observed Ig-1b level in control at time t; T is the half-life in days (taken as 6); k is the fraction of total Ig-1b removed by sampling averaged per day (taken as 0.1/7); q is the fraction by which the rate of production (PRO) is altered; and θ is the amount of Ig-1b withdrawn at $t = 0$, i.e. the amount removed by maternal antibody.

Figure 1b, which shows Ig-4b levels, was drawn from data obtained with a separate group of progeny, in this case (SJL X BALB/c) hybrids.

Table I. Onset of Allotype Synthesis in Suppressed and Normal Mice[1]

Father		Mother			Weeks of age at onset of allotype synthesis			
Strain	Allotype	Strain	Allotype	Immune to	Ig-1a	Ig-4a	Ig-1b	Ig-4b
LP;BAB/14 C57BL/10; CWB/13 101; SJL	Ig^b	BALB/c	Ig^a	Ig-1b			8–10	3–6
Same strains	Ig^b	BALB/c	Ig^a	Nonimmune			3–6	3–6
SJL	Ig^b	BALB/c	Ig^a	Ig-1b + Ig-4b			8–10[2]	8–10
BALB/c	Ig^a	SJL	Ig^b	Ig-1a + Ig-4a	7–9	9–11		
BALB/c	Ig^a	SJL	Ig^b	Nonimmune	3–5	3–5		

[1] A minimum of 20 progeny were used from each mating. Onset of allotype synthesis was scored as the week at which allotype was first seen in immunodiffusion (~0.05 mg/ml). LP, C57BL/10, 101, and SJL were obtained from Jackson Laboratories; BALB/c was obtained from the N.I.H.; BAB/14 is the inbred derivative of 14th backcross generation of a strain congenic with BALB/cN but carrying the Ig^b alleles (originally provided by Dr. Michael Potter); CWB/13 is the inbred derivative of the 13th backcross generation of a strain congenic with C3H.SW (C3H.SW (C3H-H-2^b/SnHz) also carrying the Ig^b alleles.

[2] Some animals (~5%) never show Ig-1 b synthesis.

Since the period of complete suppression is short, it was necessary to show that the absence of circulating allotype was not due mainly to the elimination of newly synthesized allotype resulting from combination with the maternal antibody. The data in Fig. 1a (Herzenberg *et al.*, 1967) show the increase of Ig-1b with age in the sera of a litter of normal animals and a litter of suppressed animals together with a computed curve showing the expected Ig-1b levels if synthesis in the suppressed mice had occurred at the same rate as in normal mice, and maternal antibody merely absorbed the produced immunoglobulin. The "expected" curve shows the level of Ig-1b remaining below the detection threshold for several days longer than normal until the maternal antibody is exhausted. The computed level then rises rapidly to become indistinguishable from levels in normal mice. In contrast, the curve for levels in suppressed mice rises slowly and merges with the levels for normals only when the mice have reached the range for stabilization of adult levels.

Confirmation of this theoretical demonstration of the delay in onset of synthesis of the suppressed allotype comes from the demonstration of small amounts of antiallotype antibody in suppressed mice as late as 8 weeks of age. By this time, if allotype synthesis had been proceeding at a normal rate, enough allotype would have been produced to absorb the original maternal antiallotype antibody several hundred times over (Herzenberg and Herzenberg, 1965; Herzenberg *et al.*, 1967).

Specificity of Suppression

The specificity of antibody to which the young animal is exposed determines the immunoglobulin which is suppressed. This is shown most clearly by the data in Fig. 1b which show levels of Ig-4b with time in three groups of animals: progeny of nonimmune mothers, progeny of mothers immune to Ig-1b, and progeny of mothers immune to Ig-1b and Ig-4b. Suppression for Ig-4b occurs only in the progeny of mothers immune to Ig-4b. Data for suppression of Ig-1b in these animals is not presented; however, progeny of all mothers immunized to Ig-1b showed short-term suppression for that allotype.

Suppression of Antibody Carrying the Suppressed Allotype

Studies on suppression of production of antibody to sheep erythrocytes (SRBC) were conducted with short-term suppressed animals and normal controls. Animals were injected with SRBC at 21 days of age and again at 35 days. At 45 days they were sacrificed and the number of cells in the spleen producing anti SRBC antibody carrying Ig-1a and Ig-1b counted using a localized gel hemolysis assay (Jerne assay) modified to permit estimation of indirect plaques developed with specific antiallotype antibody. Controls produced slightly more

Ig-1a than Ig-1b PFC. The suppressed animals produced few, if any, Ig-1b PFC, although they produced roughly the same number of Ig-1a PFC as the normal controls (see Fig. 2).

It is interesting to note that there was no discernible trend toward increased production of Ig-1a PFC in the suppressed mice, suggesting that at least at this stage, there is no compensating production of the alternate allotype. Direct measurement of Ig-1a levels in suppressed mice past the age when presence of maternal Ig-1a is a significant contribution to the serum Ig-1a also failed to show any evidence of compensation. We also failed to detect a compensatory rise in serum Ig-1a globulins in heterozygotes suppressed for Ig-1b (unpublished observation) although such a rise was detected in suppressed rabbits (Mage and Dray, 1965).

The above essentially summarizes what is currently known about short-term allotype suppression in mice, with the exception of one curious and as yet unexplained observation on the onset of immunoglobulin synthesis both in normal and suppressed mice. Pooling the data for the time of appearance of the first detectable levels of Ig-1b as a function of age in the large group of normal (C57BL/10 X BALB/c)F_1 mice studied in these experiments, we found that the range for time of onset varied over a period of several weeks. The range for individual litters, however, was considerably narrower than for the population as a whole. Frequently we found that all the mice in one litter showed detectable Ig-1b before any of the mice in another litter, to all accounts identical, became Ig-1b positive. The similar results with the suppressed population could be dismissed as due to differences in the amount or quality of suppressing antibody delivered by the various mothers; however, since normal controls also clearly showed a closer correlation of time on onset of immunoglobulin synthesis within litters this finding perhaps suggests the existence of a more general basic mechanism regulating initiation of synthesis (Herzenberg *et al.*, 1967).

The simplest explanation for short-term suppression is the elimination or diversion of precursor cells due to reaction with the maternal antiallotype antibody. Once the antibody disappears, or drops below a critical level, synthesis of the suppressed allotype is initiated and then develops from that time according to the normal timetable. How the precursors are removed or diverted, however, is as yet unclear.

CHRONIC ALLOTYPE SUPPRESSION

Immunologic Abnormalities of SJL/J Mice

Our studies on allotype suppression in mice took a different tack with the discovery that progeny produced by mating SJL/J (also Igb) males to BALB/c females immunized to Ig-1b showed not only short-term suppression for Ig-1b,

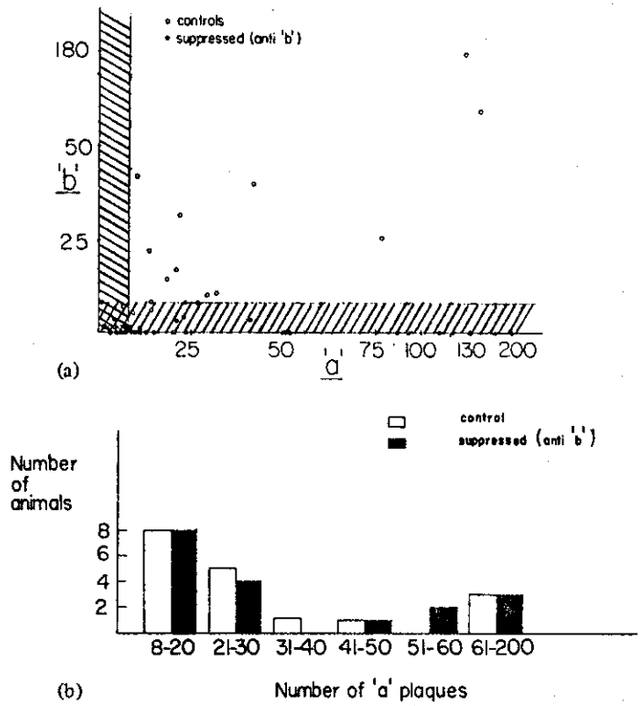


Figure 2. Lack of compensation for suppressed allotype. Figs. 2a and 2b are drawn from the same data. C57B1/10 ♂ X BALB/c ♀ litters of hybrids from normal mothers or mothers immune to Ig-1b were immunized with 4 X 10^8 SRBC at 21 and 35 days of age. Animals were sacrificed at 45 days of age and their spleens assayed for direct PFC and indirect PFC developed with anti-Ig-1a and anti-Ig-1b.

In Fig. 2a, the number of "b" allotype plaques in each animal is plotted as a function of the number of "a" allotype plaques in the same animal.

In Fig. 2b, the similar distribution of "a" allotype plaques in suppressed and normal progeny is shown.

but also chronic long-term suppression in which more than half the progeny at 6 months of age had no detectable Ig-1b in circulation (Jacobson and Herzenberg, 1972). This strain combination was selected for study because SJL/J inbred animals show severe abnormalities of the immune system. Not only do SJL/J animals show significant enlarged lymph nodes from birth (Old and Carswell, personal communication), but virtually all mice over the age of one year develop a pleiomorphic reticulum cell sarcoma [classified as Type B by Dr. Thelma Dunn (Dunn and Derringer, 1968)]. Animals greater than 6 months of age also show marked gammopathies, ranging from virtual absence of all immunoglobulin

classes to extraordinary elevations of immunoglobulins of one or more immunoglobulin classes (Wanebo *et al.*, 1966).

While the elevated immunoglobulins in SJL often appear similar to the restricted electrophoretic mobility "spike" seen in animals with plasmacytomas, the progressive increase in the protein level with growth of the tumor seen with plasmacytomas is often not observed in SJL disease. Instead, "spikes" may appear and rise to quite high levels only to disappear again. New spikes may arise or the animal may show a general elevation of immunoglobulins or a depletion of same. While some animals never show "spikes" others may show several, in sequence or at the same time (unpublished observation).

The mechanisms which regulate this discoordinated immunoglobulin synthesis in SJL mice are at present completely unknown and relatively unstudied. It has been observed, however, that the IgG_{2a} and IgG_1 immunoglobulin classes appear to be primarily affected. We have recently shown that SJL mice are excellent producers of antiallotype antibody, and we have found that some mice produce restricted heterogeneity antibodies to these antigens (unpublished observation).

Chronic Allotype Suppression in (SJL × BALB/c) Hybrids

Aware, then, that SJL mice show immunologic abnormalities suggestive of regulatory defects, we mated males of this strain to both normal BALB/c females and BALB/c females immune to Ig-1b allotypes and followed the onset of Ig-1b synthesis in the progeny as we had previously with C57BL/10 × BALB/c progeny. No significant differences between the two hybrids were observed during the short-term suppression period, i.e., for the first 8 to 11 weeks. The curves for onset of Ig-1b synthesis in the normal SJL × BALB/c progeny superimposed on the curve for normal C57BL/10 × BALB/c as did the early portion of the curves for onset of synthesis in the two types of suppressed progeny. (Jacobson and Herzenberg, 1972) As time progressed, however, the SJL × BALB/c progeny from immune mothers began to show marked differences in the pattern of recovery from suppression compared to their suppressed C57BL/10 × BALB/c counterparts.

To begin with, several suppressed progeny from the SJL × BALB/c cross never showed detectable Ig-1b levels in circulation, although they were tested weekly until over 30 weeks of age. Other progeny which appeared to have recovered from suppression and had initiated Ig-1b synthesis reversed field and stopped production, so that their levels once again dropped below detectability. From 10 to 24 weeks of age many of the progeny alternated between synthesis and nonsynthesis, thus displaying irregular variations in Ig-1b, with shifts from undetectable levels to normal adult levels and back again being not uncommon.

At about 20 to 24 weeks of age, the Ig-1b levels appeared generally to stabilize with about half the progeny showing no detectable Ig-1b and the other half ranging from trace amounts to full adult levels. While some shifting still

occurred after this time, by and large, animals which showed no detectable Ig-1b at 6 months of age tended to remain suppressed (Jacobson and Herzenberg, 1972). These animals, which were dubbed "chronically suppressed," were used in the subsequent experiments which led to the discovery of a thymus-derived cell (T-cell) responsible for this form of allotype suppression.

Examination of the SJL × BALB/c hybrid for abnormalities of the immune system similar to those seen in SJL shows the hybrid to be somewhat abnormal but to a much lesser extent than the parental SJL. The hybrid does not develop tumors, nor does it develop the sharp restricted mobility immunoglobulin "spikes" characteristic of the parental serum. There is, however, a tendency toward generalized elevation of immunoglobulin levels. No differences in serum electrophoretic patterns were observed between suppressed and normal progeny except for the absence of the suppressed allotype (unpublished observation).

SJL Genome and Chronic Suppression

The importance of the SJL genome to the development of chronic allotype suppression is demonstrated by the data in Table II. Of 7 Igb strains tested, only

Table II. Chronic Suppression of Ig-1b Immunoglobulin Synthesis[1]

F_1 hybrid		No. suppressed/total (at 7 months)	
Father (Ig^b)	Mother (Ig^a)	Mother immune to Ig-1b	Nonimmune mother
SJL	BALB/c	100/199	0/152
LP	BALB/c	0/23	0/10
C57BL/10	BALB/c	0/29	0/6
101	BALB/c	1/17	0/5
BAB/14	BALB/c	0/20	0/10
CWB/13	BALB/c	0/19	0/10
B10.S	BALB/c	1/81	0/21
SJL	129	0/23	

[1] Animals were scored for Ig-1b production by immunodiffusion. B10.S is a strain congenic with C57BL/10 carrying $H\text{-}2^s$ (i.e., C57BL/10-H-2^s) obtained from Jackson Laboratories. For other strain descriptions, see legend for Table I.

SJL progeny developed chronic suppression. All others recovered from short-term suppression at approximately the same time and subsequently showed no difference in Ig-1b levels between control and suppressed groups. At 6 months of age, only SJL hybrids were suppressed.

It is possible that the BALB/c genome also contributes an element critical to the establishment of chronic suppression. Progeny of 129 (Iga) mothers immunized to Ig-1b mated to SJL males showed short-term suppression but failed to develop any evidence of chronic suppression, suggesting that not all Iga strains can replace BALB/c. Some caution, however, must be used in the interpretation of this result. It could be due to subtle differences between 129 and BALB/c in quality or quantity of anti-Ig-1b passed from mother to offspring. It would perhaps be better to inject young SJL X 129 hybrids born from normal mothers with a known anti-Ig-1b serum, since we have shown that injection of normal SJL X BALB/c hybrids with anti-Ig-1b is just as effective as maternal transfer in generating chronically suppressed progeny (see Table III); however, to date, neither this experiment nor any experiments with other SJL hybrids have been conducted.

Whether BALB/c is unique in its contribution to the hybrid or not, it would appear that it does make a significant contribution toward the development of chronic suppression. SJL inbred animals exposed to conditions which would suppress hybrids show only short-term suppression. Neither injection of antiallotype antibody into SJL inbreds foster nursed on normal BALB/c mothers nor direct transfer of maternal antibody by foster nursing inbreds on BALB/c mothers immunized to Ig-1b induces chronic suppression in the inbred SJL animals. There was no evidence of chronic suppression in three SJL animals which survived transplantation at the late blastocyst stage into an immunized

Table III. Failure to Develop Chronic Suppression in SJL Inbred Mice[1]

Strain	Treatment	Chronic suppressed total
SJL	Egg transplant into BALB/c immune to Ig-1b	0/3
	Foster nurse on BALB/c immune to Ig-1b	0/37
SJL	Injected with anti-Ig-1b	0/4
(SJL X BALB/c)F_1	Injected with anti-Ig-1b	11/20

[1] Animals scored for Ig-1b production by immunodiffusion.

BALB/c mother and grew to adulthood, although the animals were tested regularly until over 30 months of age (see Table III).

There also appears to be a restriction on the class of immunoglobulin which may be chronically suppressed, even in the SJL X BALB/c hybrid. In reciprocal crosses (i.e., SJL females immune to Iga allotypes mated to BALB/c males) chronic suppression for Ig-1a is observed, although many fewer progeny exhibit total suppression at 6 months of age (see Table IV). On the other hand, when mothers of either strain were immunized to Ig-4 allotypes (on IgG_1) as well as Ig-1 allotypes (on IgG_{2a}) the progeny showed chronic suppression only for allotypes determined at the Ig-1 locus. The Ig-4a and Ig-4b did show short-term suppression, but all animals recovered and were producing normal levels of Ig-4 by 6 months of age (see Tables I and IV).

As yet we have very few clues as to where these various genetic observations fit in the overall picture of chronic allotype suppression. Nonetheless, they should be borne in mind during consideration of the following studies in which a T-cell responsible for chronic suppression of Ig-1b production is described, so that a comprehensive hypothesis for the origin of this T-cell may be constructed.

Active Factor Responsible for Chronic Suppression

The first evidence that there was an active factor responsible for allotype suppression came from studies in which lethally irradiated (600R) chronically

Table IV. Failure to Develop Chronic Suppressions for Ig-4a or Ig-4b

Strain	Mother immune to	Number of mice showing suppression at 24 weeks or older/total			
		Ig-1b	Ig-4b	Ig-1a	Ig-4a
(SJL X BALB/c)F_1	Ig-1b	105/216[1]	0/17[2]	—	—
	Ig-1b and Ig-4b	8/18	0/18	—	—
	Nonimmune	0/165	0/11[2]	—	—
(BALB/c X SJL)F_1	Ig-1a and Ig-4a			24/143	0/79
	Nonimmune			0/19	0/19

[1] Animals scored for Ig-1b, Ig-1a, and Ig-4a production by immunodiffusion. Ig-4b was measured by radioimmune assay. Number of suppressed/total tested.
[2] All animals, tested individually reached adult level by 12 weeks. Pools tested to 36 weeks showed average level equal to adult level.

suppressed SJL X BALB/c hybrids 6 months of age or older were reconstituted with spleen from normal syngeneic donors. Although the grafts were accepted and the animals apparently healthy, no Ig-1b appeared in circulation (Jacobson and Herzenberg, 1972). Thus, normal adult cells capable of producing Ig-1b *in situ* were unable to produce the allotype when transferred into an irradiated suppressed host, suggesting the presence of a radiation resistant factor which could prevent Ig-1b production by the transferred tissue.

Spleen or bone marrow transferred from suppressed donors into nonsuppressed irradiated recipients (in this case BALB/c) also failed to produce Ig-1b (Herzenberg *et al.*, 1971).

BALB/c mice were used as recipients in these experiments (as well as in most of those which follow) because of the difficulties in following de novo Ig-1b production by transferred tissue in an animal with Ig-1b in circulation. Although transferring F_1 tissue into irradiated parents is not always successful, we were fortunate in this strain combination that irradiation of recipients with 600R and transfer of a minimum of 10^7 F_1 cells was fully successful in better than 95% of animals. This is demonstrated in suppressed recipients by the continued production of Ig-4b, a nonsuppressed Igb allotype.

The course of suppression in recipients of suppressed spleen or bone marrow showed a curious pattern. In recipients of suppressed cells, a burst of Ig-1b synthesis almost invariably appeared in the first 2 weeks after transfer. By 6 to 8 weeks after transfer, no detectable Ig-1b is produced in recipients of suppressed tissue, although recipients of normal tissue produce consistently high levels of Ig-1b for the duration of the experiment (at least 20 weeks) and recipients of either suppressed or normal tissues produce high levels of Ig-4b throughout the experiment as well.

Mixture-Transfer Assay for Suppression

The key experiment proving that suppressed mice contain an active, dominant cell-associated factor which suppresses Ig-1b production by normal cells otherwise capable of Ig-1b production was that in which spleen from suppressed hybrids was mixed *in vitro* with spleen from syngeneic normal hybrids prior to transfer into the irradiated BALB/c "indicator" hosts. When 10^7 spleen cells of each type mixed prior to transfer were injected, the levels of Ig-1b in serum of the transferred recipients were indistinguishable from the levels in recipients of 10^7 suppressed cells transferred alone, and considerably below levels in recipients of 10^7 normal hybrid cells. At 6 to 7 weeks post transfer, the recipients of suppressed or of suppressed plus normal cells no longer produced any Ig-1b (see Fig. 3).

In a similar experiment, recipients of 4×10^6 suppressed cells mixed with 1.2×10^7 normal cells were suppressed, although not as well as recipients of 1.2×10^7 suppressed cells transferred alone (Jacobson *et al.*, 1972). We have since found that the degree of suppression is more closely related to the number of suppressed cells in the mixture, since results with graded numbers of suppressed cells are roughly similar whether 1.0, 1.2, or even 1.5×10^7 normal cells are used in the mixture.

There is considerable variability in the amount of Ig-1b produced in individual recipients of the same suspension of suppressed cells or mixtures of sup-

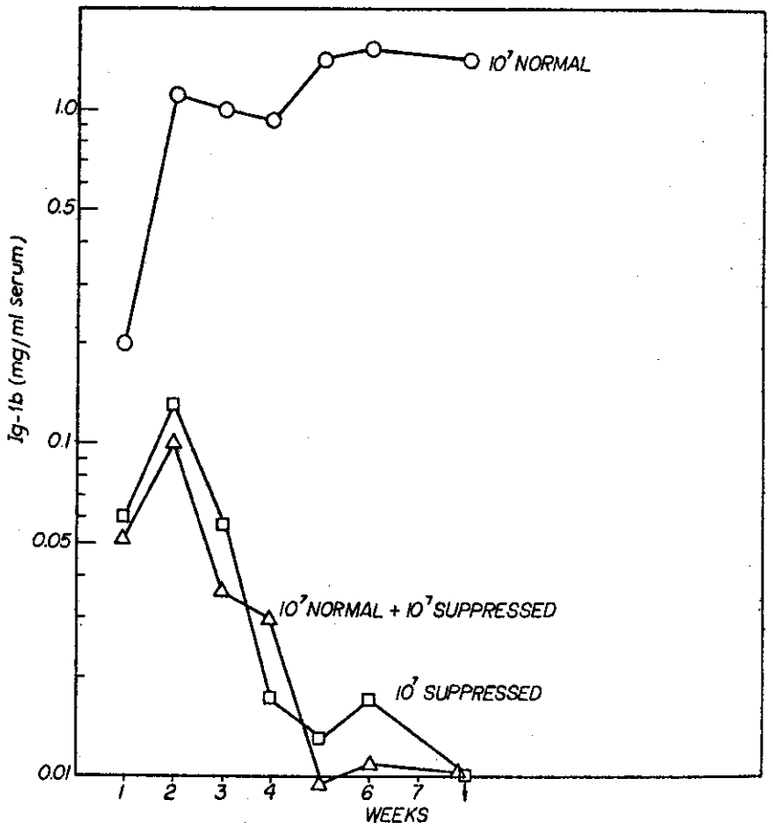


Figure 3. Mixture-transfer assay for suppression of allotype production. Lethally irradiated (600R) BALB/c (Ig^a) were restored with (SJL X BALB/c) hybrid spleen cells approximately 18 hours after irradiation. (○) received 10^7 spleen cells from normal hybrids; (□) received 10^7 spleen cells from chronically suppressed hybrids; and (△) received a mixture containing 10^7 spleen cells from suppressed hybrids and 10^7 spleen cells from normal hybrids. Day 0 = day of transfer. Ig-1b levels were estimated on weekly bleeds by radioimmune assay. Each point is the average of determinations from 5 mice. (Herzenberg *et al.*, 1973).

pressed and normal cells. This is particularly true when marginal numbers of suppressing cells are given (i.e., 3-5 X 10^6 spleen). It is not infrequent to see one or two recipients out of five in such a group become completely suppressed as though these mice had received a full suppressing dose, whereas others in the same group might be only partially suppressed throughout the experiment. Such variability is related perhaps to the escapes from suppression seen in the original suppressed hybrids, both before and after 6 months of age (Jacobson and Herzenberg, 1972, and unpublished observation).

Suppressor T-cells in Lymphoid Tissues

Cells with suppressing activity are found not only in spleen of suppressed hybrids but in thymus, lymph node, and bone marrow. A survey of these tissues in the mixture-transfer assay described above showed that lymph nodes and thymus (parathymic nodes removed) suppress about equally with spleen, on a per cell basis, whereas bone marrow is roughly twofold better (see Tables V and VI).

Experiments with spleen and bone marrow showed that in each of these tissues suppressor activity is associated with T-cells. Cell suspensions from each of the tissues were treated with antiserum to Thy-1b[1] (an antigen found only on thymocytes and T-cells often referred to as theta (Reif and Allen, 1964)) in the presence of complement prior to mixture with normal spleen and transfer into irradiated BALB/c hosts (as above). In each case all suppressing activity was destroyed (see Fig. 4 and Table VII).

The results with spleen were confirmed *in vitro* using Mishell-Dutton cultures (Mishell and Dutton, 1967). Mixture of suppressed spleen with normal syngeneic F_1 spleen primed to SRBC suppressed the formation of Ig-1b PFC while leaving Ig-1a PFC unaffected. Treatment of the suppressed spleen with anti-Thy-1b in the presence of complement prior to mixture and culture destroyed the suppressing activity as it did in the *in vivo* experiments (see Tables VIII and IX).

The demonstration that suppression requires the presence of a T-cell from suppressed animals leaves open the possibility that a bone-marrow-derived cell (B cell) is also required. Such a cell might, for example, produce a suppressing antibody in the presence of T-cells from the suppressed animal. Two lines of evidence, however, make this possibility highly unlikely. First, B-cells generally cooperate with any T-cells if a sufficient number are present. Therefore, transfer of T-cell depleted suppressed spleen should have suppressed, even if somewhat

[1] Nomenclature following recommendation of the Committee on Standardized Nomenclature for the Mouse (Staats, J., 1972).

Table V. Suppressor Cells in Lymphoid Tissues[1]

Normal donor	Suppressed donor		No. of	Ig-1b levels (mg/ml)						
Spleen	Tissue	No. of cells transferred	recipients	Weeks after transfer						
No. of cells				1	2	3	4	6	8	
10^7	Not done	Not done	3	0.24	>0.38	>0.5	>0.5	>0.5	>0.5	
"	Spleen	10^7	4	<0.04	-	-	-	-	-	
"	Spleen	10^6	4	0.4	0.13	<0.03	-	<0.02	0.04	
"	Thymus	10^7	4	0.02	-	-	-	-	-	
"	Thymus	10^6	4	>0.3	>0.3	>0.32	0.12	0.15	0.08	
"	Lymph node	10^7	4	0.03	-	-	-	-	-	
"	Lymph node	10^6	4	0.4	0.11	0.08	<0.03	<0.02	-	

[1] Donors of all cells were (SJL X BALB/c)F_1 mice 6–12 months old. Suppressed donors were exposed to maternal anti-Ig-1b perinatally. Ig-1b levels were determined by immunodiffusion. - = <0.01 mg of Ig-1b/ml. Recipients were BALB/c mice irradiated with 600R ~ 18 hours prior to transfer (Herzenberg *et al.*, 1973*a*).

Table VI. Suppressor Cells in Bone Marrow[1]

Number of cells transferred (× 10^6)					Mean Ig-1b levels (mg/ml)				
Spleen		Bone marrow			Weeks after transfer				
Normal	Suppressed	Normal	Suppressed	No. of mice	1	2	3	4	6
12				8	0.2	>0.4	>0.4	>0.5	>0.3
"	3			4	0.1	0.07	<0.02	<0.04	<0.04
"	10			5	0.1	0.03	<0.02	–	–
"		10		4	0.3	0.4	>0.5	>0.5	>0.5
"			1	4	0.07	0.4	0.4	0.3	0.1
"			5	4	0.12	0.07	0.02	–	–
"			10	4	0.06	0.04	0.02	–	–

[1] Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 months old. Suppressed donors were exposed to maternal anti-Ig-1b perinatally. Ig-1b levels were determined by immunodiffusion. – = <0.01 mg of Ig-1b/ml. Recipients are BALB/c mice irradiated with 600 R 18 hours prior to transfer (Herzenberg *et al.*, 1973).

Table VII. Anti-Thy-1b (theta) Sensitivity of Suppressor Cells in Bone Marrow[1]

Experiment No.	Number of cells transferred (× 10^6)			Treatment	No. of mice	Mean Ig-1b level (mg/ml)						
	Spleen	Bone marrow				Weeks after transfer						
	Normal	Normal	Suppressed			1	2	3	4	5	6	8
I	12			–	4	0.2	>0.5	>0.5	>0.5	>0.5	>0.4	>0.5
	"	10		–	4	0.4	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
	"		10	Anti-Thy-1b + C'	4	0.1	>0.5	>0.5	>0.4	>0.4	>0.4	>0.4
	"		10	NMS + C'	4	0.07	0.1	<0.04	<0.02	–[2]	<0.02	<0.04
			10	–	4	0.07	0.04	–	–	–	–	–
II	12			–	4	0.1	>0.5	>0.4	>0.5		>0.5	>0.5
	"		9	Anti-Thy-1b + C'	6	0.1	>0.5	>0.5	>0.5		>0.5	>0.5
	"		9	Congenic Anti-Thy-1b + C'	6	0.3	>0.5	>0.5	>0.5		>0.5	>0.5
	"		9	NMS + C'	4	0.1	0.1	0.2	0.2		0.05	0.05

[1] Donors of all cells were (SJL × BALB/c)F_1 mice 6 – 2 months old. Suppressed donors were exposed to maternal anti-Ig-1b perinatally. Where indicated under "Treatment," cell suspensions from suppressed donors were incubated with guinea pig serum and anti-Thy-1b or AKR normal serum, at 37°C for 45 min, sedimented, washed, counted, mixed with syngeneic normal cells and injected i.v. Ig-1b levels were estimated by immunodiffusion. Recipients were BALB/c mice irradiated with 600 R ~ 18 hours prior to transfer. In experiment 1, AKR anti-Thy-1b or AKR normal serum was used. In experiment II, congenic anti-Thy-1b (from Dr. E. A. Boyse) was used in addition (Herzenberg *et al.*, 1973).
[2] – = <0.01 mg of Ig-1b/ml.

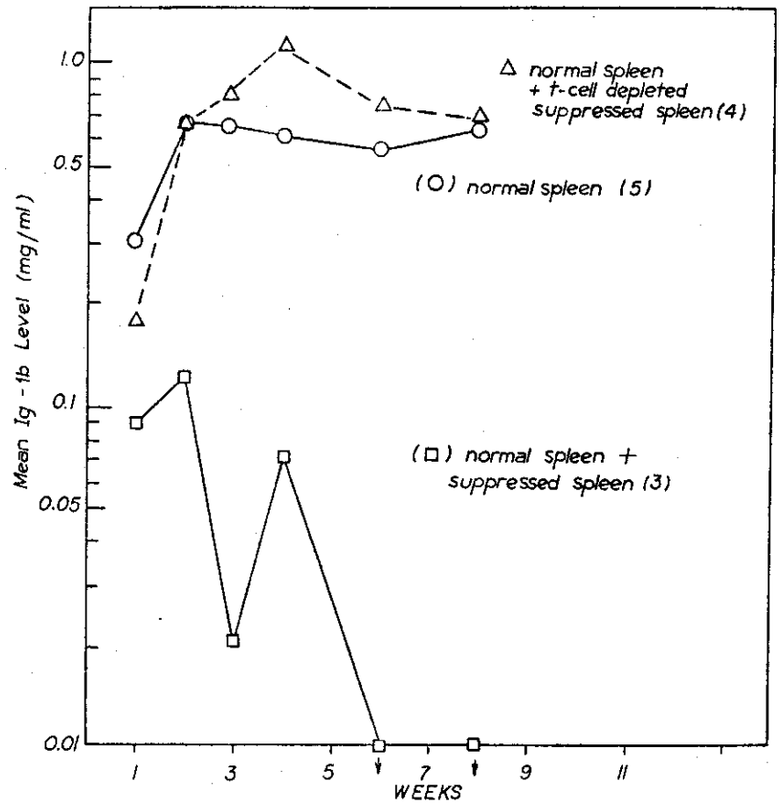


Figure 4. Depletion of suppressor T-cells from spleen by treatment with anti-Thy-1b (anti-θ). Experimental details were the same as described in the legend for Fig. 3. (○) received 10^7 spleen cells from normal (SJL × BALB/c) hybrids; (▫) and (△) received a mixture of 10^7 spleen cells from normal hybrids plus 10^7 spleen cells from suppressed hybrids. Cells from suppressed hybrids were treated with anti-Thy-1b plus complement (△) or with AKR normal serum plus complement (▫) as described in legend for Table VII (Herzenberg *et al.*, 1973*a*).

less effectively, when transferred with whole normal spleen. Second, although thymus suppresses about equally to spleen, very few B-cells are found in thymus.

Since bone marrow contains very few T-cells, the demonstration that bone marrow has a high suppressor activity makes the T-cell depletion experiments with bone marrow particularly crucial. The data in Table VII show the results of treatment of bone marrow with two antisera to Thy-1b, one prepared in AKR mice against Thy-1b carried on C3H cells and the other[2] prepared in one

[2] Kindly provided by Dr. E. A. Boyse.

Table VIII. Active Suppression of the Ig-1b Secondary Response to SRBC in Culture[1]

Number of spleen cells cultured (× 10^6)			Allotype developed plaques 10^6 recovered cells			a/b normalized
SRBC primed	Unprimed					
Normal	Normal	Suppressed	a	b	a/b	
20	-	-	908	658	1.4	1
15	5	-	716	343	2.1	1.6
15	-	5	905	83	11	7.7
10	10	-	286	139	2.0	1.5
10	-	10	279	9	33	25

[1] Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 months old. Suppressed donors were exposed to maternal anti-Ig-1b perinatally. The a/b ratios were normalized by setting the ratio for primed cells alone equal to 1 (Herzenberg *et al.*, 1973*a*).

Table IX. Requirement for T-Cells For Active Suppression in Culture[1]

Numbers of spleen cells cultured (× 10^6)			Treatment of suppressed cells	Normalized a/b allotype ratio of PFC			
SRBC primed	Unprimed			Experiment number			
Normal	Normal	Suppressed		7	8	9	10
20	-	-	None	1 (2.9)	1 (1.4)	1 (2.3)	1 (1.7)
10	-	10	None	6.3	22	5	15
10	-	10	C′	4.7	18	5.9	5.4
10	-	-	C′+ anti-thy-1b	0.67	1.2	1.8	0.77
10	10	-	None	0.67	1.5	1	1.4

[1] Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 months old. Suppressed donors were exposed to maternal anti-Ig-1b perinatally. Where indicated under "Treatment," cell suspensions from suppressed donors were incubated with guinea pig serum and AKR anti-Thy-1b or guinea pig serum alone, 37°C for 45 min, sedimented, washed, counted, mixed with primed normal cells and cultured. Results with AKR anti-Thy-1b were confirmed with congenic anti-Thy-1b in another experiment. The a/b ratios were normalized for each experiment by setting the a/b ratio for primed cells alone equal to 1 (see Table VIII) Total b PFC per 10^6 recovered cells for each experiment: No. 7 = 408, No. 8 = 658, No. 9 = 278, No. 10 = 77 (Herzenberg *et al.*, 1973*a*).

Table X. Retransfer of Suppressor Cells from Irradiated BALB/c Recipients of a Mixture of Suppressed and Normal Spleen (Mixture-Transfer Recipients)[1]

Number of cells transferred (× 10^6)				Mean Ig-1b levels (mg/ml) in serum						
	Suppressed		No. of recipients	Weeks after transfer						
Normal spleen	Spleen	Bone marrow		3	4	5	6	8	12	
12			2	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	
	10		3	<0.07	0.01	–	–	–	–	
	3		3	0.14	0.11	0.05	–	–	–	
		10	3	0.08	0.05	0.04	0.02	–	0.07	
		3	3	0.14	0.27	0.11	>0.35	>0.23	>0.07	
		1	3	>0.5	>0.4	>0.4	0.14	0.08	0.07	

[1] Suppressed donors were BALB/c recipients 11 weeks prior to this transfer of a mixture of suppressed and normal cells as in Fig. 3. All were negative for Ig-1b. Normal donors were (SJL × BALB/c) hybrids. Recipients in this experiment were, as usual, BALB/c irradiated with 600R ~ 18 hours prior to transfer. Ig-1b levels were determined by immunodiffusion. – = <0.01 mg Ig-1b/ml.

member of a pair of Thy-1 congenic strains against the Thy-1b of the other strain. Suppressing activity is destroyed by both sera.

A rough calculation suggests that there is considerably more suppressing activity per T-cell in bone marrow than per T-cell in spleen. Setting a conservatively high estimate of T-cells in bone marrow at 10% and a conservatively low estimate of T-cells in spleen at 30%, the observation that bone marrow suppresses as well as spleen on a total cell basis suggests that the T-cell population in bone marrow is, at a minimum, 3 times more effective in suppressing.

The significance of this concentration of suppressor activity in bone marrow is not clear; however, we have shown recently that bone marrow of BALB/c recipients of mixtures of suppressed and normal spleen (i.e., recipients in mixture-transfer experiments) contains suppressor cells which must have settled there after transfer. To demonstrate this we extended an earlier observation that spleen cells taken from recipients after they were completely suppressed (11 weeks) are able to suppress when repassaged in a mixture-transfer experiment. As the data in Table X show, both bone marrow and spleen from transferred BALB/c recipients suppress about as well as their counterparts taken directly from chronic suppressed hybrids.

Chronic Suppression by Transfer of Suppressor Cells to Young Syngeneic Hybrids

In the transfer experiments discussed thus far, T-cells from various lymphoid tissues of chronic suppressed SJL × BALB/c hybrids have been shown to actively suppress Ig-1b production by normal syngeneic spleen when the two tissues are transferred together into an irradiated nonsyngeneic host. We have also shown, however, that lymphoid tissues from chronic suppressed donors suppress Ig-1b production when transferred into young, intact syngeneic hybrids.

Table XI. Chronic Suppression by Passage of Suppressor T-Cells into Young Syngeneic Hosts[1]

Recipient	Age at transfer	Suppressor tissue	No. of cells	Chronic suppressed/ total
(SJL × BALB/c)F_1	2–3 weeks	Spleen	1.5×10^7	53/58
"	"	Thymus	"	5/6
"	"	Bone marrow	"	6/9

[1] Animals were scored for chronic suppression at 24 weeks of age.

of immune mothers developed chronic suppression, suggesting that the thymus is necessary only for development of chronic suppression and not for the expression of suppressor cell activity (see Table XIII). This conclusion is supported by the demonstration that spleen from suppressed donors is active in a mixture-transfer experiment when the BALB/c recipients have been thymectomized one month prior to irradiation and transfer (see Table XIV).

Table XIII. Effect of Thymectomy on Development of Chronic Suppression[1]

	Age at Tx	Chronic suppressed/total
NTx	1–2 days	0/4
Sham N Tx	"	6/6
A Tx	6–8 weeks	21/25
Sham A Tx	"	11/12

[1] Litters of (SJL × BALB/c) hybrids exposed perinatally to maternal anti-Ig-1b were divided equally between sham and thymectomized animals. Most animals classified as chronic suppressed were negative for Ig-1b after 6 months of age. Some showed sporadic low levels of the allotype.

The four neonatally thymectomized animals showed continued high (normal) levels of Ig-1b starting at 10–12 weeks of age, i.e., after short-term suppression was over.

Table XIV. Transfer of Mixture of Suppressed and Normal Spleen into ATx Irradiated BALB/c[1]

Recipient (BALB/c)	Number of cells transferred (× 10^6)		Number of recipients suppressed/total
	Normal spleen	Suppressed spleen	
ATx	13	10	3/3
Sham	"	"	4/4
Normal	"	"	3/3
ATx	13	-	0/3
Sham	"	-	0/3
Normal	"	-	0/3

[1] Recipients were BALB/c mice thymectomized or sham thymectomized at 12 weeks of age, irradiated (600R) 4 weeks later, and injected with cell suspension ~ 18 hours after irradiation. Suppressed mice showed typical pattern in mixture-transfer experiment (see Fig. 3).

MECHANISM OF CHRONIC ALLOTYPE SUPPRESSION

Most of the studies completed so far on chronic allotype suppression have involved the identification of T-cells as responsible for chronic allotype suppression and the investigation of conditions under which suppression occurs. In addition, we have made some progress toward understanding the mechanism by which T-cells suppress. While the data are still incomplete, when taken in conjunction with what is known about the normal pathway of differentiation of immunoglobulin producing cells and the known properties of other types of T-cells, several interesting possible hypotheses arise. Before proceeding to a discussion of these, however, we will briefly review the events in the B-cell line leading to antibody synthesis and the role of T-cells in the immune system in order to bring together a number of relevant considerations.

B-Cell Differentiation

The normal process of differentiation to synthesis of γG or γA immunoglobulin (or antibody) involves the commitment of an originally totipotent (with respect to species of immunoglobulins) B-stem cell to the production of a large amount of a unique immunoglobulin molecule. During this process, one of the two autosomal chromosomes carrying H-chain determinants is selected, the variable region is determined, and one of the four linked genes for H-chain constant regions (in the mouse Ig-1 to Ig-4, specifying γG_{2a}, γA, γG_{2b}, and γG_1, respectively) paired to it in a manner which allows for the transcription of the entire unit as a single messenger. In similar fashion a unique light-chain messenger is transcribed. Light and heavy chains are then produced, assembled, and exported, while the cell differentiates further to a mature plasma cell. While there is evidence (Pernis *et al.*, 1971; Wang *et al.*, 1970) which indicates that during this process some or all cells may go through a stage of γM production before selection of the final γG or γA class without change in the variable region, this does not substantially alter the above description.

Almost nothing is known of the mechanisms involved in this process. If the insertion of a γM step proves correct, then chromosome exclusion and variable region choice occur earlier than selection of H-chain class. In any event, at the end of the process a single cell produces a unique immunoglobulin whose H-chain is determined by a gene (or genes) on only one of the two parental chromosomes (Pernis *et al.*, 1965). No other such system for exclusion of autosomal genes is known in mammalian cells.

In an allotype heterozygote, such chromosomal (or allelic) exclusion leads to the presence of two populations of immunoglobulin-producing cells for any given class, one making immunoglobulins of paternal allotype and the other of maternal allotype. Usually an animal has a characteristic ratio of cells producing

each of the parental allotypes, and circulating immunoglobulins reflect this cellular ratio (Cebra, 1968). While there is little understanding at present of how this ratio is maintained, the regularity with which certain allotype (and classes) predominate over others suggests that there is more than just random selection operating.

It is usually assumed that all immunoglobulins in circulation in normal animals are antibodies produced in response to antigenic stimulation. This assumption is buttressed by the demonstration that animals raised in a germ-free environment show very low IgG levels (Gustafsson and Laurell, 1959). Although the low levels could be explained by factors other than lack of antigenic exposure, the studies with germ-free animals suggest that the presence of an immunoglobulin molecule in circulation is evidence of antigenic stimulation, i.e., that a progenitor B-cell committed to production of that immunoglobulin was stimulated by antigen to give rise to cells producing the immunoglobulin.

T-Cell Functions

Combination of B-cells with antigen is a necessary but generally not sufficient condition for antibody production to ensue.[1] The antigen driven differentiation of B-cells from precursors to IgG antibody producing cells most often requires interaction with a (thymus-derived) "cooperating" T-cell capable of recognizing either the antigen or a carrier to which the antigen has been attached. Some antigens are known which stimulate antibody production in the apparent absence of T-cells. For example, neonatally thymectomized or irradiated adult thymectomized mice reconstituted with either fetal liver or bone marrow, respond to polymerized flagellin, as do genetically athymic (nu/nu) mice. Since a small number of Thy-1 (theta) positive cells, presumably T-cells, are always found in these animals and polymerized flagellin is a very good antigen, the possibility formally remains that thymic independent antigens are those capable of extraordinarily efficient use of cooperators (Rajewsky *et al.*, 1969; Miller and Mitchell, 1969; Davies, 1969; Claman and Chaperon, 1969; Taylor, 1969, Mäkelä *et al.*, 1971; Warner, 1972; Mitchison, 1967; Paul *et al.*, 1967).

Interestingly, T-cell depleted animals such as those described in the preceding paragraph produce substantial levels of IgG even though the IgG antibody response shows a virtually absolute dependence on T-cell cooperation. T-cell depleted animals have been shown to respond to antigens with IgM antibody but not with IgG antibody production. This again raises the question of whether all

[1] A complete review with extensive referencing on T- and B-cell interactions is beyond the scope of this work. The short list of references presented at the end of each paragraph is intended to be minimal rather than comprehensive.

IgG is, in fact, produced in response to antigenic stimulation (Wortis, 1971; Tyan and Herzenberg, 1968; Warner, 1972; Grumet, 1972; Mitchell *et al.*, 1972*a*).

The cooperating T-cell has been extensively studied over the last several years, but as yet the mechanism of cooperation has not been firmly established. For our purposes here it is sufficient to state that (at least for most antigens) B-cells capable of binding the antigen must interact with T-cells capable of recognizing the antigen, although the two cell types probably do not recognize the same antigenic determinants. This interaction could be directly between B- and T-cells or between B-cells and a factor produced by T-cells stimulated by antigen (Feldmann, 1972; Iverson, 1972).

T-cells serve a second major function in the immune system. They are the effectors of cellular immunity. T-cell depleted animals are unable to reject homografts or to show other manifestations of cellular immunity, such as delayed hypersensitivity. These functions may be restored in depleted animals by reconstitution with thymus, spleen, or lymph nodes from normal animals. Reconstitution with bone marrow, which contains many fewer T-cells, is effective but only marginally so (Warner *et al.*, 1962; Raff, 1971).

Immunologic memory appears to be carried in both T- and B-cell lines. Although in many cases a primed B-cell population can produce a secondary response in cooperation with a sufficient number of unprimed T-cells, priming renders the T-cell population far more effective for cooperation. Similarly, although an allograft will be rejected by an animal never previously exposed to the allograft antigens, the rejection of a second graft is considerably more rapid and vigorous. Spleen and lymph node appear to be the principal location of both B- and T-cell populations carrying immunologic memory. The thymus and bone marrow seem to serve more as reservoirs of stem and progenitor cells, since transfer of these tissues from primed animals mainly yields a primary type response (Jacobson *et al.*, 1970, Mitchell *et al.*, 1971, Mitchell *et al.*, 1972*a*; Cunningham and Sercarz, 1971; Davies, 1969; Raff, 1970).

Suppression of Immunoglobulins in Other Systems

Various types of suppression of antibody or immunoglobulin synthesis have been reported by other laboratories in addition to the short- and long-term allotype suppression described earlier in this chapter.

Injection of neonates with heterologous anti-immunoglobulin antibody specific for a particular immunoglobulin class suppresses the production of antibodies and immunoglobulin of that and other classes perhaps by a mechanism similar to short-term suppression (Manning and Jutila, 1972; Lawton *et al.*, 1972). The suppression lasts for shorter or longer periods and covers other classes, depending on the specificity of the antiserum and the injection protocol. Similarly, treatment of spleen cell cultures with heterologous anticlass antibody

suppresses the *in vitro* anti-SRBC response in that class (Pierce *et al.*, 1972). A "feedback" inhibition of the IgM response to a particular antigen as the result of presence of IgG antibody to the same antigen has also been shown, but whether this relates to suppression of immunoglobulin synthesis by anti-immunoglobulin reagents is unclear (Henry and Jerne, 1967).

Examples of active cellular suppression of antibody response, which is perhaps analogous to T-cell mediated chronic allotype suppression, have recently been reported. Two of these, which have been well documented, are the responses to polyvinylpyrollidone and to pneumococcal polysaccharide, both "thymic independent" antigens (Baker *et al.*, 1970; Kerbel and Eidinger, 1972). The suppression in these cases has been interpreted to be T-cell mediated suppression of IgM response. No IgG response occurs. Evidence for "active tolerance" to T-dependent antigens appears to be more difficult to obtain; however, data suggesting this may occur for IgE antibody in rats (Okamura and Tada, 1971), and for a variety of other systems in mouse (Gershon, 1973), have been reported.

The demonstration of apparent analogies to chronic allotype suppression suggests that perhaps the T-cell mediated suppression demonstrable in SJL X BALB/c hybrids may have a broader biological significance than its restriction to one class of immunoglobulin and one hybrid cross would indicate. It is intriguing to speculate that suppressor cells play a role in the regulation of antibody synthesis and overall immunoglobulin levels, but evaluation of this possibility will have to wait until there is more evidence with which to establish a firmer concept of the mechanism(s) of suppression.

In chronic allotype suppression, we have established that exposure to antiallotype antibody is necessary for the development of chronic suppression. Once the suppressor T-cell population is established, however, its maintenance and activity do not require the continued presence of antiallotype antibody.

The suppressor T-cells could be required as cooperators for B-cells primed to produce an unknown antibody which then is the suppressing agent. This is unlikely, however, since (1) the thymus, which contains few B-cells, is as good a source of suppressor activity as spleen; (2) T-cell depleted suppressor spleen does not suppress, although it is in the presence of an abundance of T-cells from a concomitantly transferred normal spleen population; and (3) no evidence for a circulating suppressing factor has been found.

A more likely interpretation of the data is that the suppressor T-cell exerts its influence by interaction with a precursor cell originally destined to produce the suppressed allotype. The suppressor cell may either kill the precursor, hold it static by preventing its differentiation to immunoglobulin production, or divert it to another pathway of differentiation. This interaction could be a direct T-cell to B-cell interaction (e.g., at a membrane level), or it could be mediated either by a factor elaborated by the suppressor or other cells activated by the suppressor. In either case, the requirement for suppressor T-cells does suggest that if this is the case, then the T-cell must initiate the interaction.

Since the suppression is specific for Ig-1b production in an Ig^a/Ig^b heterozygote, it is likely that the suppressor T-cells act on B-cells which have differentiated to a point at which they are committed to Ig-1b production and are recognizable as such. This is most likely after allelic exclusion has taken place and the cell is committed to production of a unique antibody molecule.

Antibody forming cell precursors are known to synthesize small amounts of the antibody molecule to which the cell is committed and to display this antibody on the cell surface, where it can be detected either by antigen binding or heterologous antiallotype and anti-immunoglobulin reagents (Wigzell *et al.*, 1971; Rabellino *et al.*, 1971; Raff *et al.*, 1970; Julius *et al.*, 1972). Short-term suppression probably results from combination of the exposed antibody with antiallotype or anticlass specific antibody. The marker which the T-cell suppressors recognize must be somewhat more complex than the Ig-1b allotype as it occurs in circulation, since large amounts of circulating Ig-1b do not block the onset of suppression. We have proposed that Ig-1b bound to the cell surface displays or creates a new antigen, which is the recognition clue for the suppressor T-cell, but this marker could be antigenically distinct from the free Ig-1b molecule and simply occur uniquely on Ig-1b committed B-cells.

Suppressor T-Cells as Killers?

Perhaps the most significant question still to be answered about suppressor T-cells is the identification of their normal role in the animal. A conservative hypothesis pictures these cells as part of the population of T-cells responsible for cellular immunity (immune surveillance). It is possible that exposure to antiallotype antibody removes Ig-1b precursors from the young animal for a sufficient period past the "tolerance" limit so that new Ig-1b precursors, when they appear, are no longer recognized as self. T-cells seeing these "nonself" cells become primed to them as if to an allograft. Once this occurs, new Ig-1b precursors will be eliminated as they arise by the primed T-cells acting in their normal surveillance role.

This hypothesis has much to recommend it in that it assigns a role to suppressor T-cells consistent with a known T-cell function; however, a number of accommodations must be made in order to make this hypothesis consistent with all the data. A seesaw balance between rejecting T-cells and Ig-1b precursors which tips towards rejection when the animals reach 6 months of age must be postulated. Otherwise, if there is a sensitized T-cell population able to reject Ig-1b precursors as a foreign graft, why does Ig-1b synthesis become established in most animals prior to 6 months of age, and complete rejection only occur in animals over 6 months of age? Similarly, why is a partially suppressed Ig-1b level

frequently maintained, not only in young animals, but often for the life of those animals over six months of age who do not become completely suppressed. While a balanced rejection state in which antibody to cellular antigens ("blocking antibody") prevents rejection by T-cells has recently been suggested in other systems (Hellström, 1972), evidence for its existence as a general phenomenon applicability here is still lacking.

Another inconsistency is the demonstration of high suppressor activity in bone marrow which is in general a poor source of cells for cellular immune reactions. This finding can be reconciled with the hypothesis by postulating that suppressor T-cells have specifically migrated into the bone marrow in large numbers because target cells occur there; however, such arguments do raise some serious, albeit not fatal objections, to the hypothesis. Additional questions are why chronic suppression only occurs in SJL X BALB/c hybrids and why only for γG_{2a} globulins.

Suppressor T-Cells as Regulators?

A more novel approach to the mechanism of chronic allotype suppression is to focus on the inherent regulatory role of T-cells in the differentiation of B-cells from precursors to IgG antibody producing cells. Since this differentiation generally requires the cooperation of a T-cell, antibody production may be limited not only by the number of B-cell precursors able to differentiate to produce antibody to a given antigen, but as well by the number of T-cells available to cooperate with the B-cell precursors in order to facilitate their differentiation to antibody forming cells. Put another way, the requirement for cooperation with T-cells before antibody production can proceed creates a pressure point at which the extent of the antibody response may be controlled by the availability of cooperating T-cells.

While this type of T-cell control over B-cell function is essentially passive, i.e., a decrease of cooperators results in a decrease of the response, it is possible that another class of T-cells exists which actively regulates the flow of B-cells from precursors to antibody forming cells, perhaps by preventing or aborting cooperation. The data we have presented on suppression of Ig-1b synthesis are as well explained by postulating that the T-cell responsible for the active suppression of Ig-1b is one of a group of many specific suppressor (regulator) T-cells, collectively responsible for the active control of the levels of antibody of the various classes in serum.

A simple model for the mechanism of this kind of regulation could be competition for B-cells between cooperators and suppressors, where blocking of the interaction between cooperators and B-cells by suppressors results in prevention of further differentiation of the B-cell. Under ordinary circumstances, the presence of suppressors and cooperators adheres to a finely tuned balance which

maintains the relatively uniform immunoglobulin levels found in mice of the same age and strain. The presence of antiallotype serum in the young animals, however, could shift this balance in favor of overregulation of Ig-1b synthesis. In SJL X BALB/c animals which, like the SJL parent, show an abnormal regulation of IgG immunoglobulin synthesis, this early shift could result in the establishment of an overdeveloped population of regulator (suppressor) T-cells which specifically suppress Ig-1b precursors from differentiating to producers.

While the animal is young and its immune response is at its peak, cooperators would be expected to have a numerical advantage and, therefore, be able to override suppressors. On the other hand, during periods of relative immunologic inactivity suppressors would once again be dominant.

As the animal ages and cooperator function declines, the degree of overregulation originally established would determine whether the animal will become completely or partially suppressed.

A competition model such as this depends on the validity of assuming that all B-cell differentiation to IgG production is T-cell dependent, a point which is hardly well established. The inability to obtain chronic suppression for γG_1 could be explained, however, if the γG_1 production were not entirely T-cell dependent, whereas the γG_{2a} production was.

This model is admittedly fanciful. Nevertheless, it does deal more easily with some of the objections to viewing chronic allotype suppression as an induced autoimmunity. No telling argument currently exists for determining which of the two hypotheses is correct, and we therefore leave the decision up to the personal preference of the reader until decisive evidence bearing on the question is available.

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