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MOUSE IMMUNOGLOBULIN ALLOTYPES: CHARACTERIZATION AND USE IN CELLULAR IMMUNOLOGY

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Although mouse immunoglobulin allotypes are intrinsically interesting with respect to the information they provide on the structure and genetic determination of immunoglobulin molecules, it could be argued that their most important use to date has been as markers for studies on the differentiation and function of cells involved in the immune response. Therefore, rather than concentrate entirely on the characterization of the mouse allotypes in this presentation, we would prefer to summarize this material as it has already been published in detail elsewhere [6, 7]. Then we will go on to present some of our recent studies on suppression of allotype synthesis in the mouse. These studies which bear on the cellular events leading to immunoglobulin production, are a good example of the way in which the mouse allotypes have proven useful to the cellular immunologist.

Mouse Immunoglobulins

As in other species, the mouse immunoglobulin molecule is made of a four chain unit, containing two identical heavy (H) chains and two identical light [2] (*) chains. The structure of the H-chains determines the class of the molecule.

Classes were originally identified by physical characteristics such as size and electrophoretic mobility, however, all known classes now may be recognized by class specific antisera raised in another species, usually goat or rabbit. These antisera, which contain antibody specific for a particular

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(*) References are chosen to be useful sources of information on the referenced point and not to indicate priority. Therefore, recent reviews will often be cited. No attempt at exhaustive referencing has been made.

H-chain, generally must be absorbed to remove contaminating anti-light chain antibody (which would react with all classes) and also antibody which reacts with other H-chains.

There are six known H-chain classes in the mouse, each associated with particular biological activities (see table I) and represented by at least one myeloma protein [3, 4, 5]. All inbred mouse strains tested have all of the known immunoglobulin classes, although genetic differences in the classes exist from one strain to another. The genetically different immunoglobulins are called allotypes [8], a term originally defined by Oudin [16].

TABLE I. — **Mouse immunoglobulin classes.**

Class	Locus	Number of alleles known in inbred strains	Number of specificities described	Some biologic activities
$\gamma G_{2a}(\gamma G)$	Ig-1	8	11	Fixes complement, mediates cells lysis and fixes to tissues of other species and mediates local anaphylaxis.
γA	Ig-2	5	4	Does not fix complement, secreted into milk, tears, intestinal lumen, nasal secretions.
$\gamma G_{2b}(\gamma H)$	Ig-3	6	7	Fixes complement, mediates cell lysis, passes placenta.
$\gamma G_1(\gamma F)$	Ig-4	2	2	Does not fix complement, fixes to tissues of same species, mediates local anaphylaxis, passes placenta.
γG_3				Does not fix complement, does not fix to skin, passes placenta.
γM				Fixes complement, mediates cells lysis (more efficient than γG_{2a} and γG_{2b}).

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IDENTIFICATION OF ALLOTYPES

Allotypes within a given class of immunoglobulins are products of alternate forms of the gene, *i. e.* alleles, at the locus determining the structure of the H-chain of that class of immunoglobulins. Typically, allotypes are recognized by antisera raised by immunizing one mouse strain with immunoglobulin from a second strain, however, other means may be used. For example, electrophoretic mobility of the Fc fragment of the H-chain was used to first identify alleles at the Ig-4 (γG_1) locus, although antigenic differences have been identified now for the allotypes in that class as well. Using antisera, allotypes for a given class may be identified either by the presence of a unique antigenic determinant (specificity) or as a unique

combination of antigenic determinants each of which is also found in other combinations in other allotypes.

Anti-allotype antisera often contain several antibody populations. These may react with allotypic determinants on different classes of immunoglobulins or with different determinants on a single class. Heterologous sera also have been shown to detect allotypic differences, however, such sera are not often obtained, probably because allotypes represent such small differences between members of an immunoglobulin class that they are not as effective immunogens as isotypic (species) determinants in heterospecific immunization.

Terminology

For historical reasons, two bases for notation of mouse immunoglobulins are in use, a practice which often creates confusion for those beginning to work or read in the field. When speaking of antibodies or immunoglobulins as proteins, the class name, for example, γM or γG_{2a} (or IgM or IgG_{2a}), is most often used. When discussing genetic (allelically determined) structural differences between immunoglobulins or antibodies within a given class, the genetic or allotypic notation is used (*e. g.*, Ig-1 is the locus for γG_{2a}. γG_{2a} in the BALB/c strain is called Ig-1a and γG_{2a} in C57BL/10 strain is called Ig-1b). Thus, although logically it would suffice to call those immunoglobulins determined at the Ig-1 locus Ig-1 globulins, they are in any nongenetic context, and often in a genetic context as well, refered to as γG_{2a} (or IgG_{2a}) globulins, making it necessary for the reader to learn both class and allotypic terminology and to shift easily from one to the other if he is to make his way facilely through the mouse immunoglobulin literature (*).

To make matters even worse, the individual immunoglobulin H-chain, whose structure determines both the class and the allotype of the immunoglobulin molecule of which it is a part, and is, in fact, the direct product of a particular Ig allele at a particular Ig locus, is named in Greek symbols in correspondence with the class terminology, *e. g.*, the products of alleles at the Ig-2 locus are γA H-chains and are all called α chains, with no specification of the allotype of the chain.

Attempts to simplify this notational jungle have met with little or no success as yet. Alternate systems have been suggested, but no agreement on nomenclature for mouse immunoglobulins, especially for allotypes, has been reached. Later in this publication we will present a table giving the notation system we generally use together with other commonly used synonyms, however, at this point such detail is unnecessary.

(*) Another notation is used by Potter, Lieberman and some other workers [18]. Although it represents a logical attempt to combine the protein and gene notation, it is not generally used because it does not accord with the World Health Organization internationally accepted nomenclature for human immunoglobulins [15].

Demonstration of allelism

There are certain problems in the proof of allelism at the Ig loci which center around the difficulty in studying a large enough number of mammalian progeny to detect crossing over between closely linked genetic loci. Although upwards of 2,000 progeny of appropriate crosses have been examined in detail, no direct evidence of a crossover between the loci has been found. Thus, by the original definition of a locus as a region of the chromosome defining a particular characteristic and separable by crossing over from regions defining other characteristics, all of the Ig loci would be lumped as one and no meaningful discussion of allelism at each of the loci would be possible.

The current definition of a locus in mammalian systems, however, is not based on a demonstration of crossing over. In analogy with the bacterial cistron a locus is defined as a place on the chromosome at which there exists a DNA sequence (gene) coding for a polypeptide chain. Although deletions and duplications can occur, in general a haploid chromosome set in a species will carry one locus for each of the proteins produced by the species. Therefore, in a diploid organism, each parent donates one structural gene at each locus.

Variations in the DNA sequence of the structural gene which give rise to variations in the protein end product, are called alleles. Conventionally, a locus is not *named* until at least two alleles are known for the locus, so that it can be related, by genetic testing, to other known loci (*).

Four loci have been named for the four mouse immunoglobulin classes in which polymorphic variations, *i. e.*, allotypes, have been described. For each locus, the minimal criteria for allelism were met by genetic testing as follows: Two inbred mouse strains, each with a different H-chain allotype for a given class were crossed to obtain heterozygotes. Both allotypes were found in the heterozygotes. Heterozygotes were then crossed either back to one of the parental strains, to a strain carrying a third allotype, or to themselves. In all cases, the expected ratios for segregating co-dominant alleles were found, indicating that a single haploid set always carried either the allele donated by the mother of the heterozygotes or the allele donated by the father, never both, never neither [8].

Demonstration of genetic linkage

In crosses where the parental strains had allotypic differences at two or more H-chain loci, the genetic testing showed that the loci in a given haploid set were always inherited together indicating that the structural

(*) Even more cautiously, but perhaps reasonably for immunoglobulins whose complete genetic control is to say the least still quite arcane, the reader should store the reservation that some allotype loci may be regulatory ones [19].

genes determining at least four of the mouse H-chain classes are clustered quite closely on the chromosome. This close genetic linkage could have considerable biological importance in the differentiation of immunoglobulin producing cells.

Thus far, the testing of a large number of progeny (over 2,000) has yielded no direct evidence of crossing over between H-chain loci. Nonetheless, the existence of a number of cross-reacting allotypes at each locus are probably signs of past crossovers. It is likely that continued progeny testing will eventually directly demonstrate crossing over in the mouse Ig region since in humans, where the immunoglobulin genes are also closely linked, at least one family has been found where crossing over has occurred [14].

Efforts to link the Ig region to other mapped genetic markers in the mouse have thus far been unsuccessful (*), although crosses with markers in all of the known linkage groups in the mouse have been studied. Since male heterozygotes demonstrate both alleles, Ig cannot be sex-linked.

Alleles and specificities of the Ig locus

A summary description of the six known immunoglobulin H-chain classes, including associated biological activities, is presented in table I. Both the notation we use and Potter and Lieberman's alternate notation are given. As the table shows, allelic differences are known for only 4 of the classes: γG_{2a}, γA, γG_{2b} and γG_1, defining the loci Ig-1 through Ig-4 respectively.

The known alleles and distribution of allotype specificities at each of the Ig loci are presented in tables II, III, IV and V. Each Ig-1 allele in

Table II. — **The Ig-1 Locus (*).**

Type strain	Allele	Specificities										
BALB/c	$Ig\text{-}1^a$	1(G7)	2G(8)	—	—	—	6	7	8	10(G1)	—	12(G6)
C57BL/10J	$Ig\text{-}1^b$	—	—	—	4	—	—	7	—	—	—	—
DBA/2J	$Ig\text{-}1^c$	—	2(G8)	3	—	—	—	7	—	—	—	—
AKR/J	$Ig\text{-}1^d$	1(G7)	2(G8)	—	—	5	—	7	—	—	—	12(G6)
A/J	$Ig\text{-}1^e$	1(G7)	2(G8)	—	—	5	6	7	8	—	—	12(G6)
CE/J	$Ig\text{-}1^f$	1(G7)	2(G8)	—	—	—	—	—	8	—	11	—
RIIIJ	$Ig\text{-}1^g$	—	2(G8)	3	—	—	—	—	—	—	—	—
SEA/Gn	$Ig\text{-}1^h$	1(G7)	2(G8)	—	—	—	6	7	—	10(G1)	—	12(G6)

(*) Ig-1 determines γG_{2a} immunoglobulin H chains.
Potter-Lieberman determinant names given in parentheses [18]. Specificity 12 described only by Potter et Lieberman.
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(*) Linkage to a locus for immunological responsiveness to dextran has recently been found [21].

TABLE III. — **The Ig-2 Locus (*).**

Type strains	Alleles	Specificities			
BALB/cJ, SEA/Gn	Ig-$2^{a,h}$	—	2(A12)	3(A13)	4(A14)
C57BL/10J	Ig-2^{b}	—	—	—	—
DBA/2J, RIII/J	Ig-$2^{c,g}$		—	—	—
AKR/J, A/J	Ig-$2^{d,e}$	—	—	3(A13)	—
CE/J	Ig-2^{f}	—	—	—	4(A14)

(*) Ig-2 determines γA immunoglobulin H chains.
Potter-Lieberman determinant names given in parentheses [18[. Specificities 3 and 4 have been described only by Potter et Lieberman.
Reprinted from [6].

TABLE IV. — **The Ig-3 Locus (*).**

Type strains	Alleles	Specificities						
BALB/cJ, DBA/2J, SEA/Gn	Ig-$3^{a,c,h}$	1	2(H11)	—	4	7	8	—
C57BL/10J	Ig-3^{b}	—	—	—	4	7	8	9(H9)
AKR/J	Ig-3^{d}	1	—	3	—	7	8	—
A/J	Ig-3^{e}	1	—	3	—	7	—	—
CE/J	Ig-3^{f}	1	2(H11)	3	4	—	—	—
RIII/J	Ig-3^{g}	1	2(H11)	—	4?	—	—	—

(*) Ig-3 determines γG_{2b} immunoglobulin H chains.
Potter-Lieberman determinant names are given in parentheses [18]. Specificity 9 has been described only by Potter et Lieberman.
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table II is assigned to a type strain whose immunoglobulin, by definition, is the standard for comparison for that allele.

Since the Ig loci are closely linked genetically, and since the largest number of alleles have been described at the Ig-1 locus, the type strain assigned to each Ig-1 allele is also assigned as the type strain for the entire Ig chromosome region of which that allele is a part, and alleles at subsequently discovered loci in the Ig region are named consistently with the Ig-1 allele. For example, the BALB/c strain which is Ig-1a, is assigned the Ig^{a} chromosome region, and therefore, by definition, as new Ig loci are recognized, the allele carried by BALB/c is designated by a superscript « a », *i. e.*, Ig-2^{a} is the allele at the Ig-2 locus in BALB/c. It is important to note that the assignment of Ig-1^{a} and Ig-2^{a} to BALB/c is not in any way meant to imply that BALB/c γG_{2a} is *structurally* more closely related to BALB/c γA than to γA molecules determined by other alleles at the Ig-2 locus.

At Ig-2 through Ig-4, although some alleles at each of the loci have been defined, allotypic differences have not (as yet ?) been recognized

TABLE V. — **The Ig-4 Locus (*).**

Type	Strains	Alleles	Specificities
BALB/c	DBA/2J	Ig-$4^{a,c,d,e,g,h}$	1 —
AKR/J	A/J, CE/J		
R111/J	SEA/Gn		
C57BL/10J		Ig-4^{b}	— 2

(*) The Ig-4 locus determines γG_1 immunoglobulin H chains.
Reprinted from [6].

between immunoglobulins in all of the type strains. In these cases the recognized alleles are formally named as a composite of the alleles of the undifferentiated type strains, *e. g.*, Ig-$2^{a,h}$, although in general usage Ig-2^a is used to describe the allele for γA of BALB/c origin. This type of notation system was adopted, among other reasons, in the expectation that as the search for new antisera continues, some sera may be found which diffe-

TABLE VI. — **Distribution of Ig-1 alleles in inbred mouse strains.**

Ig-1^a			Ig-1^b		Ig-1^c
BALB/cJ (*)	C58/J	PL/J	C57BL/10J (*)		DBA/2J (*)
BALB/CGa	F/Ao	POLY1/Ao	BAB/PoHz (***)		DBA/1J
BUB/Bn	H-2G/Go	POLY2/AO	B10.D2(new)Hz		I/Ao
CBA/J	JK/Bi	PRUNT/Ao	B10.D2(old)Hz		JB/Di
CHI/Ao	MA/H	ST/J	C57BL/H	SM/J	RF/J
C3H/Hz	MA/MyJ	STR/N	CWB/Hz (**)	STA/Je	SWR/J
C3H.SW/Hz	NZY/B1	T6/H	C57BL/Ka	WB/Be	
C57BR/cdJ	PBR/Ao	129/RrGa	C57BL/6J	WC/Re	
C57L/J			H-2H/Go	WH/Re	
			H-21/Go	WK/Re	
			LP/J	58N/Sn	
			SJL/J	101/R1	

Ig-1^d	Ig-1^e	Ig-1^f	Ig-1^g	Ig-1^h
AKR/J (*)	A/J (*)	CE/J (*)	RIII/J (*)	SEA/Gn (*)
AL/N	NZB/B1	DE/J	DA/Hu	BOP/J
	NZO/B1	N/Ao	FZ/Ci	BSL/Di
	NZW	NH/N	STB/Je	P/J
				SEC/Gn

(*) Type strain for each allele is italicized.
(**) CWB, C3H and C3H.SW form a congenic trio : C3H = $H2^b$, Ig^a ; C3H.SW = $H2^b$, Ig^a ; and CWB = $H2^b$, Ig^b.
(***) BAB is congenic with BALB/c ; BALB/c = Ig^a ; BAB = Ig^b. BAB are derived by further backcrossing from BALB/c-2, received from Dr. M. Potter at the 13th backcross generation. Congenic strains are almost identical genetically except at the indicated loci.
Reprinted from [6].

rentiate between the proteins produced by the different type strains. However, it is quite possible that two type strains which carry different Ig-1 alleles could have identical Ig-4 alleles. As yet, no example of the reverse case has been found, *i. e.*, two strains with different alleles at Ig-2, Ig-3 or Ig-4 having the same Ig-1.

In table VI, a catalogue of 70 inbred mouse strains listed according to Ig-1 allele is presented. Since in the many cases where testing of inbred strains has been possible, the other Ig alleles have been shown to conform to the Ig-1 allotype, it is reasonable to assume that if an inbred strain is $Ig\text{-}1^a$, it is Ig^a, etc.

Allotypic specificities

Each allele at a given locus is actually defined by the unique combination of antigenic specificities found on the immunoglobulin it determines (see tables II through V). These specificities represent the reactions of the immunoglobulins of a given class with a panel of anti-allotype antisera, and therefore represent structural differences between the immunoglobulin H-chains determined by the alleles at a given locus.

The specificities *for each locus* are numbered according to the order of their discovery. Therefore, specificity 3 for the Ig-1 locus has no structural relation to specificity 3 for the Ig-2 locus. When specificities at more than one locus are being discussed, the more complete designation, Ig-1.3 or Ig-3.3 should be used to prevent confusion.

Allotypes in cellular immunology: suppression of synthesis of immunoglobulins

Two types of suppression of synthesis of immunoglobulins bearing allotypic markers have been described in the mouse. Herzenberg *et al.* [9] showed that exposure of young C57BL/10 ♂ × BALB/c ♀ hybrids or young C57BL/10 inbred mice to antibody to the C57BL/10 γG_{2a} immunoglobulin allotype (*i. e.* to BALB/c anti Ig-1b) results in a short-term suppression of Ig-1b production which last until the animals are about eight weeks of age. The suppressed mice all then begin production of Ig-1b and within a month or so show serum Ig-1b levels indistinguishable from levels in normal syngeneic controls.

This short-term or transient allotype suppression contrasts markedly with the second type of allotype suppression in which allotype production is often permanently impaired. Jacobson and Herzenberg [12] and Jacobson *et al.* [13] have reported that young (SJL × BALB/c)F_1 hybrid mice exposed to antibody to either parental γG_{2a} allotype (anti Ig-1a or Ig-1b) not only show short-term suppression but often later develop a chronic suppression of production of the target allotype. The key finding that transfer of a syngeneic mixture of chronically suppressed and normal spleen

cells into an irradiated host results in suppression of allotype production by the normal spleen showed that spleens of chronically suppressed mice have cells which actively suppress the allotype production.

Herzenberg *et al.* [10] subsequently showed that the active suppressing cell is a thymus-derived (T) lymphocyte present in spleen, thymus, lymph nodes and bone marrow of chronically suppressed animals. While less is known about the factors responsible for short-term suppression, mainly because of the difficulties involved in studying a phenomenon which lasts only a few weeks, a comparison of the two types of suppression suggests that short-term suppression is mediated by a different mechanism than the active T-cell mechanism found in chronic suppression (see table VII).

TABLE VII. — **Comparison of short-term and chronic suppression.**

	Short-term suppression	Chronic suppression
Strain restrictions	None found	Works with (SJL × BALB/c)F_1. Requirement for SJL parent definite does not work with SJL inbred
Immunoglobulins affected	γG_{2a} : Ig-1a and Ig-1b γG_1 : Ig-4a and Ig-4b	γG_{2a} : Ig-1a and Ig-1b γG_1 : not affected
Age when manifested	1 to 2 months	Over 3 months (generally)
Induced by	Exposure of young mice to anti-allotype antibody	*1)* Short-term suppression or *2)* transfer of lymphoid tissue from chronically suppressed donors to young syngeneic mice
Effect of thymectomy Neonatal Adult	 None found None found	 Prevents chronic suppression None found
Effector	Anti-allotype antibody ?	Active suppressor T-cells
Mechanism	Temporary elimination of B-cell precursors ?	Prevention of differentiation of precursors (B-cell) to allotype producers ?

For documentation of points covered in this summary, see [11].

Chronic suppression is restricted to hybrids in which one parent strain is SJL. It may, in fact, be restricted only to BALB/c × SJL hybrids. It appears to affect only one class of immunoglobulins, γG_{2a}, although in reciprocal crosses either parental allotype of this class may be chronically suppressed. It occurs generally in older hybrids which have been exposed to maternal anti allotype serum and have therefore passed through a period of short-term suppression, however, it may also be produced by transfer of T-lymphocytes from chronically suppressed donors to young normal hybrids. It does not occur in SJL/J inbred animals, even when they are implanted as embryos in BALB/c mothers immune to the SJL allotype.

Short-term suppression, on the other hand, is less restrictive in that it occurs in all strain hybrid combinations we have tested as well as in inbred mice exposed to the appropriate anti allotype antibody. Further, it affects allotypes on both γG_1 and γG_{2a} (the two immunoglobulin classes for which sufficiently strong anti allotype sera are available).

Attempts to demonstrate active suppression by T-cells in short-term suppression have thus far been unsuccessful. Neonatal thymectomy, which abrogates chronic suppression, does not relieve short-term suppression in the same animals. Nor, for that matter, does it prolong the suppression period. Adult thymectomy has no discernible effect either on short-term or chronic suppression. Transfer experiments to determine directly whether active suppressors are present during short-term suppression are in progress in our laboratory; however, the results from the thymectomy experiments suggest strongly that T-cells will not be implicated.

On this same point, we have found that transfer of suppressor cells from older chronically suppressed mice to 1 to 3 week old syngeneic normal hybrids (*i. e.*, to hybrids never exposed to anti allotype antibody) results in the chronic suppression of allotype synthesis in nearly all of the recipients. Many of these never produce any detectable allotype, although a reasonable number do initiate synthesis before becoming suppressed. It is significant that in those animals in which synthesis is initiated, it begins at 3 to 5 weeks of age which is the normal time for onset of Ig-1b synthesis rather than beginning at 8 to 10 weeks, which is the time for onset of synthesis in short-term suppression. Thus, while chronic suppressing T-cells can suppress a young animal during the period of initiation of allotype synthesis, these cells tend to be more active as the animals age, suggesting again that the chronic suppressing cells are not responsible for short-term suppression.

The tentative exclusion of suppressor T-cells as the mechanism for short-term allotype suppression still leaves a wide open field for speculation as to how exposure of the neonate to anti allotype antibody brings about short-term suppression. The hypothesis we favor is that short-term suppression results from the destruction or diversion of B-cell precursors of allotype producing cells, perhaps by the direct interaction between precursors and anti allotype antibody. This continues until the antibody concentration drops below some critical level, after which newly created precursors survive in increasing numbers and are stimulated to differentiation to allotype producers. Production of a small amount of allotype then clears the remaining anti-allotype antibody from circulation and short-term suppression is over. In all strain combinations except SJL $\times$ BALB/c (where chronic suppression occurs) allotype production then increases normally until adult levels are reached.

It is tempting to speculate that the induction of the chronic suppressing T-cell is a consequence of the short-term suppression of the genetically susceptible hybrid. At the present time, however, there is no evidence that short-term suppression is required for, rather than co-existent with the induction of suppressors. One hypothesis which we have discussed in the

past [11] is that the early absence of Ig-1b precursors for a prolonged period may prevent the recognition of the precursors as self. Their subsequent appearance would then lead to an autoimmune, T-cell mediated sensitization of the animal, which would in turn lead to the killing (rejection) of newly arising Ig-1b precursor by T-cells acting in their recognized surveillance role.

An alternate hypothesis discussed in the same review [11] assigned a novel role to suppressor T-cells, postulating that the suppressor did not interfere with the appearance of precursors but instead prevented their differentiation to allotype producing cells. It viewed the induction of the suppressor as dependent upon exposure of the animal to anti allotype antibody but left undefined the way in which the antibody brought about the induction.

It is obvious that to decide between these hypotheses, as well as to shed light on the differences between short-term and chronic suppression, it would be useful to be able to study the precursors of Ig-1b producing cells directly. We therefore tried using immunofluorescent techniques to stain spleen cell suspensions for cells bearing membrane bound Ig-1b, since such cells bearing membrane bound allotype have been shown in other systems to be the precursors of allotype producers. This proved to be a fairly difficult task until several weeks ago when we succeeded in staining mouse spleen cells for membrane bound Ig-1b.

Since development of the staining technique we have completed two experiments with suppressed animals. In both experiments (see table VIII)

TABLE VIII. — **Ig-1b-Bearing spleen cells in suppressed and normal SJL × BALB/c hybrids.**

Experiment		% Stained
I	Normal	.95
	Suppressed	.80
II	Normal	1.0
	Suppressed	.50

To stain for cells bearing membrane bound Ig-1b, spleens were disrupted by passing through 50 gauge wire mesh, treated with hemolytic Gey's solution, then pelleted through fetal calf serum (FCS) to remove erythrocyte ghosts. Dulbecco's phosphate buffer medium was used for all cell suspensions and staining mixtures. Cells were pelleted at 290*g* for 5 minutes in all instances. The spleen cell pellet was resuspended and passed through glass wool to remove most dead cells and macrophages.

Then 2×10^7 cells were pelleted and resuspended in 100 μl of rabbit anti-mouse $Ig\text{-}1^b$ (33 % ammonium sulfate precipitate prepared in this laboeatory from antiserum kindly supplied by Dr. J. Coe, Rocky Mountain Laboratory) at 0.1 mg/ml in the presence of 33 % normal (Ig^a) mouse serum and incubated at 0-4° C for 20 minutes. The reaction mixture was pelleted through 1.5 ml of FCS and subsequently washed once in 2 ml of buffer. This pellet was resuspended in 100 μl of fluorescein-conjugated goat anti-rabbit Ig (kindly supplied by Dr. C. Todd and fluorescein-conjugated in this laboratory) at 0.2 mg/ml and incubated at 0-4° for 20 minutes. The cells were then washed after the first layer incubation and the final pellet resuspended in 50 μl of FCS. Smears were prepared, air dried and fixed in 95 % ethanol for 15 minutes. Slides were mounted in glycerol: phosphate buffered saline (9:1) and sealed.

substantial numbers of cells with membrane bound Ig-1b were found in pooled spleen cells from 6 to 8 months old chronically suppressed animals. Thus, although the suppressed animals produce no detectable serum Ig-1b, their spleens contain cells which are likely precursors of Ig-1b producing cells.

Interpretation of this data must be considered tentative until the assumption that cells with membrane bound Ig-1b are producers is verified. However, the results of these experiments suggest that suppressor T-cells in chronically suppressed animals do not suppress by destruction of precursors. Rather, the mechanism of chronic allotype suppression would appear to involve the regulation of B-cell differentiation from early stage precursor to producer.

Viewing the suppressor cell as a regulator of initiation of differentiation simplifies the interpretation of the characteristic sporadic Ig-1b production seen in chronically suppressed animals [12]. With precursor cells present, a short relaxation of suppression could allow some precursors to pass the regulating barrier and thus give rise to producers. Allotype production would then continue until these producers are exhausted, even if newly arisen (less differentiated) precursors are concurrently being prevented by suppressor cells from starting along this differentiative pathway. This avoids the necessity of resorting to a problematic partial or balanced rejection theory to explain the occasional escapes from suppression when chronic suppressed animals are watched closely over a long period of time. Of course, what the actual mechanism of suppr ssion is remains for future work to determine.

Key-words: Immunoglobulin, Allotypy, Cellular immunology; Mouse, Immunosuppression, Suppressor T-cells.

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