

## B-CELL INFLUENCES ON THE INDUCTION OF ALLOTYPE SUPPRESSOR T CELLS\*

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In mice, perinatal exposure to a variety of antibodies specific for immunoglobulin heavy (IgH)<sup>1</sup> chain determinants delays onset of (suppresses) Ig production (1, 2), apparently by depleting precursors of Ig-producing cells (3). Antibodies to IgG isotypes (1, 2) and allotypes (4, 5) specifically suppress production of their target Ig. Antibodies to IgM severely deplete the B-cell population in exposed neonates and also suppress IgM, IgG, and IgA production (1, 6), presumably because IgM is present on precursors of IgG- and IgA-producing cells. The duration of suppression, regardless of inducing antibody, appears to be limited in most mouse strains and hybrids by the antibody's persistence (i.e., production of the suppressed Ig is initiated at roughly the same time as the amount of circulating anti-Ig drops below detectability (4, 7) and normal Ig serum levels are reached several weeks later).

A notable exception to this general pattern occurs in the (BALB/c × SJL)<sub>F1</sub> hybrid perinatally exposed to antibodies directed against the paternal Ig-1b allotype (found on IgG<sub>2a</sub>). In these mice, the initial (short term) suppression evolves into a chronic suppression of Ig-1b production (5). Some BALB/c × SJL mice never initiate production of detectable serum levels of Ig-1b. Others (the majority) show an apparent recovery from suppression, in some instances achieving normal Ig-1b serum levels by 16 wk of age. This recovery, however, is short lived. Examined at 5 mo of age or later, more than one-half the exposed mice have ceased detectable Ig-1b production and the remainder have reduced production to subnormal levels.

A similar although substantially less severe chronic suppression of Ig-1a production occurs when the mice are exposed perinatally to anti-Ig-1a antibody (8). Exposure to antibodies against Ig-4 (IgG<sub>1</sub>) allotypes, however, does not induce detectable chronic suppression for these allotypes (8).

The anti-Ig-1b-induced chronic suppression is mediated by a Lyt-1<sup>-</sup>2<sup>+</sup>3<sup>+</sup> suppressor T-cell (Ts) population (9, 10) that recognizes and removes (kills, inactivates) a helper T-cell (Th) population (IgTh) which is distinct from carrier-primed Th and is specifically committed to help Ig-1b memory cells differentiate to antibody-forming cells (afc) (10). Similar Ts-IgTh-B interactions have now been demonstrated in idiotype suppression systems (11, 12).<sup>2</sup>

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<sup>1</sup> Abbreviations used in this paper: afc, antibody-forming cells; anti-BAT, rabbit anti-mouse brain; BSA, bovine serum albumin; CGG, chicken gamma globulin; CTh, carrier-specific Th; DNP, 2,4-dinitrophenyl; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; IgH, immunoglobulin heavy; KLH, keyhole limpet hemocyanin; RIA, radioimmuno assays; Th, helper T cell; Ts, suppressor T cell.

<sup>2</sup> Sercarz, E. E., Manuscript in preparation.

The absence of IgTh for Ig-1b B cells in Ig-1b-suppressed mice (or the presence of Ts) does not appear to impair survival of Ig-1b memory cells in these mice although it does hamper normal memory cell development. Both early (IgD<sup>+</sup>) memory cells and their mature (IgD<sup>-</sup>) progeny develop in appropriately primed suppressed donors. The normally rapid transition from IgD<sup>+</sup> to IgD<sup>-</sup> memory, however, and the accompanying avidity maturation of the memory pool, is severely retarded in these mice so that they retain an inordinately high number of IgD<sup>+</sup> (low avidity) memory cells (13).<sup>3</sup>

This block in Ig-1b B-cell memory maturation, with its concomitant accumulation of early precursors, suggests that Ts indirectly regulate both Ig-1b production and avidity maturation by regulating the supply of Th required for crucial Ig-1b B-cell differentiation events, i.e., IgD<sup>+</sup> to IgD<sup>-</sup> memory and IgD<sup>-</sup> memory to afc.

The mechanism of Ts induction (as opposed to function) is still an enigma. Preliminary experiments suggest that perinatal anti-Ig-1b exposure of BALB/c × SJL hybrids leads, by 2 wk of age, to the appearance of a Ts population(s) that specifically suppresses Ig-1b production (K. Okumura, and L. A. Herzenberg, unpublished observations). These Ts generally become eclipsed and re-emerge in 6-mo-old animals as a dominant population that chronically suppresses Ig-1b production.

In studies reported here, we focus on the specificity of the inducing antibody and the genetic influences on chronic suppression induction. We show that the Ts induction in SJL-related strains proceeds from exposure to antibodies capable of reacting with Ig present on precursors of Ig-1 afc. Antibodies reactive with Ig-1 allotypic, IgM allotype, and IgM isotypic determinants all induce Ts which specifically suppress Ig-1 production. These data, by severing the isotype specificity of the inducing antiserum (anti-IgM) from the isotype specificity of the suppression (for Ig-1 allotypes), implicate precursor B cells in the regulation of Ts induction in neonates.

Antibody exposures similar to those which we here show induce chronic suppression are known to deplete precursors and suppress Ig production for short periods in most mouse strains (1). Apparently, however, such antibody exposures induce persistent Ts populations in SJL-related allotype heterozygotes. Thus our data suggest that recovery from antibody-mediated disruption of neonatal B-cell differentiation is under genetic control which, in SJL-related mice, favors the establishment of long-lived, mature Ts populations specifically suppressing production of Ig-1 immunoglobulins.

### Materials and Methods

*Mice.* Two pairs of IgH chain congenic mice were used: SJL/JHz (Ig<sup>b</sup> haplotype) and SJA/9Hz (Ig<sup>a</sup> haplotype derived from BALB/c); BALB/cNHz (Ig<sup>a</sup>) and BAB/14Hz (Ig<sup>b</sup> from C57BL/10).

*Production of Maternal Antibody and AntiAllotype Reagents for Radioimmune Assays (RIA).* Methods used here have all been previously described (14, 15). Anti-IgG allotype reagents were tested for specific RIA binding to appropriate myeloma proteins. Contaminating antibodies were removed by solid-phase immunoabsorption. <sup>125</sup>I-labeled reagents for RIA were labeled by the solid-phase method. Anti-IgM and anti-IgD allotype antibodies were tested for specificity by binding to Ig on splenic B cells (measured with the fluorescence-activated cell sorter [FACS]). In addition,

<sup>3</sup> Black, S. J., T. Tokuhisa, L. A. Herzenberg, and L. A. Herzenberg. 1979. T cell regulation and affinity maturation. Manuscript in preparation.

specificity was determined by immunoprecipitation and gel analysis of radiolabeled spleen cell lysates. Goat anti-IgM (a gift from Dr. G. M. Iverson, Yale University, New Haven, Conn.) was rendered specific by solid-phase immunoadsorption and tested by RIA and immunoprecipitation.

Rabbit anti-mouse brain (anti-BAT) used to deplete T cells from splenic populations was prepared as described (16) and absorbed with cells from a B-cell tumor<sup>4</sup> until it had no detectable reactivity against splenic B cells.

*Measurement of Serum Allotype Levels and Allotype Representation in Antibody Responses.* Allotype levels in serum were measured by double diffusion in agar with specific antisera. Allotype representations in anti-2,4-dinitrophenyl hapten (DNP) responses were measured in an indirect (two step) solid-phase RIA assay with DNP<sub>12</sub>bovine serum albumin (BSA) as the coat antigen and specific <sup>125</sup>I-labeled anti-allotype reagents as "second-step" antibodies (14, 15). A titration of a standard SJA × SJL adoptive secondary antiserum was included in each assay. Results are expressed as units of allotype-carrying antibody relative to the standard (1 U equals 1% of standard response). Average values for adoptive secondary responses in experimental groups (three to four recipients) are shown.

*Priming of Donors for Adoptive Transfer Studies.* Keyhole limpet hemocyanin (KLH) and DNP conjugates of KLH were prepared as previously described (10). Donors were primed with 100 μg KLH or DNP-KLH on alum accompanied by 2 × 10<sup>9</sup> killed *Bordetella pertussis* organisms. Mice were used as donors a minimum of 6 wk after priming.

*Cell Preparations.* B cells were prepared by incubating whole spleen cells with rabbit anti-BAT for 30 min at 4°C. The cells were then pelleted through fetal calf serum (FCS), resuspended in guinea pig serum (complement), incubated at 37°C for 45 min, and washed before use. T-cell-enriched populations of splenic lymphocytes were prepared by passing spleen cells through nylon wool columns (10). Erythrocyte-depleted spleens were obtained by incubating spleen cells for 2 min at 4°C in hemolytic Gey's (NH<sub>4</sub>Cl, 0.7%) balanced salt solution. All cell preparations were in minimal essential medium, usually with 5% FCS.

*Adoptive Transfer.* Primed or suppressed spleen cells from SJL mice, SJA mice, or (SJL × SJA)F<sub>1</sub> mice were mixed before injection into SJL recipients which had been x-irradiated (800 rad) 18 h previously (17). Recipients were challenged with 10 μg DNP-KLH (aqueous) at the time of transfer; 7 and 14 d later serum was collected from these animals and the anti-DNP activity was assessed in an RIA.

*Antibody Exposures of Neonates for Suppression Induction.* Generally, F<sub>1</sub> hybrids between SJL and BALB/c were exposed to maternal antibodies against paternal allotypes. Mothers were immunized, mated, and boosted approximately monthly with some adjustments in schedule during gestation and nursing. In some experiments, goat anti-IgM or mouse anti-Ig-1b allotype was passively administered to neonates at days 5 and 9 after birth.

## Results

To facilitate suppression-induction studies, most of the studies reported here were conducted with highly suppressible SJA × SJL hybrids made by crossing the SJL strain, which carries the Ig<sup>b</sup> haplotype, to its allotype congenic strain, SJA, which carries the Ig<sup>a</sup> haplotype derived from BALB/c. Perinatal exposure of these hybrids to anti-Ig-1b (IgG<sub>2a</sub> allotype) induces a chronic suppression of Ig-1b production similar to the suppression induced in BALB/c × SJL but substantially more severe (see Table I). Both the earlier onset and the universal development of chronic suppression demonstrate the heightened sensitivity of these hybrids to suppression induction.

Precursor (B cell)-reactive anti-Ig antisera were tested for ability to induce chronic

<sup>4</sup> Knapp, M. R., P. P. Jones, S. J. Black, E. S. Vitetta, S. Slavin, and S. Strober. 1979. Characterization of a spontaneous murine B cell leukemia (BCL<sub>1</sub>). I. Cell surface expression of IgM, Ia, and FcR. *J. Exp. Med.* In press.

TABLE I  
*Ig-1 Suppression is Stronger in SJA × SJL Than in BALB/c × SJL*

Perinatal anti-Ig-1b exposure			Ig-1b suppression (No. of progeny)					
Hybrid	Source	μl equiv	At 12 wk			At 24 wk		
			None	Part	Full	None	Part	Full
SJA × SJL	Maternal*	>200‡	0	4	31	0	0	35
SJA × SJL	Injected	200	0	0	4	0	0	4
SJA × SJL	Injected	20	4	0	0	4	0	0
SJA × SJL	Injected	2	4	0	0	4	0	0
BALB/c × SJL	Maternal*	>200†	15	80	5	10	20	70
	Injected	300	6	5	3	6	2	6
	NMS		12	0	0	12	0	0

\* The mother (SJA or BALB/c) was immunized with SJL Ig-1b antibodies and mated when serum anti-Ig-1b levels were high.

‡ Compared to μl of injected anti-Ig-1b. Anti-Ig-1b was injected 5 and 9 d after birth into SJA × SJL mice and 7, 14, and 21 d after birth into BALB/c × SJL mice.

§ These data were derived from observations on several hundred mice and are normalized to 100.

TABLE II  
*Antibodies Reactive with Precursor Ig Induce Chronic Suppression for Ig-1 Allotypes*

Perinatal antibody exposure of (SJA × SJL) <sub>F1</sub> hybrids			Ig-1 suppression at 24 wk					
Source	Antibodies	μl equiv	Ig-1a			Ig-1b		
			None	Part	Full	None	Part	Full
Maternal	Anti-Ig-5b (δ) + anti-Ig-6b (μ)	>200	41	0	0	0	0	41
Injected	Goat-anti-μ	40	7	2	3	0	0	12
Maternal	Anti-Ig-5a (δ) + anti-Ig-6a (μ)	>200	6	5	8	19	0	0

allotype suppression by perinatally exposing SJA × SJL to injected or maternally transmitted anti-IgM and anti-IgD. None of the antisera used had detectable levels of anti-Ig-1 allotype antibody as measured by solid-phase radioimmune binding. Allotype levels in exposed progeny (and controls) were measured frequently throughout life. As before, the levels found after the mice reached 20 wk of age proved to be the best measure of suppression induction; therefore results are presented as the number of progeny displaying the indicated serum allotype levels at 24 wk of age.

Data in Table II show that specific chronic suppression for Ig-1a and Ig-1b is inducible by perinatal exposure to antisera reactive with IgM and IgD. Goat anti-mouse IgM, which reacts with all IgM immunoglobulins in the allotype heterozygote, induces chronic suppression for both Ig-1a and Ig-1b in the exposed progeny. A combination of anti-Ig-5a and anti-Ig-6a (IgD and IgM allotypes) induces suppression for Ig-1a but not Ig-1b. Conversely, anti-Ig-5b and anti-Ig-6b induces suppression for Ig-1b but not for Ig-1a. None of these sera induce chronic suppression for IgG<sub>1</sub> immunoglobulins (Ig-4a and Ig-4b). Thus, perinatal exposure to antibodies reactive with IgM immunoglobulins leads to a specific chronic Ig-1 suppression. (The role of anti-IgD allotype exposure is indeterminate in these experiments.) The haplotype

TABLE III  
*Titration of Ig-1b Suppression Activity from Anti-Precursor-Ig Exposed SJA × SJL\**

DNP-KLH primed normal	SJA × SJL spleen cells (× 10 <sup>6</sup> )		Anti-DNP response (RIA)‡	
	Unprimed		Ig-1a	Ig-1b
	Sup- pressed	Treatment		
7.5	—	—	70	69
7.5	5	—	54	3
7.5	1	—	48	2
7.5	0.5	—	50	2
7.5	0.1	—	61	46
7.5	2	Nylon-passed	82	2
7.5	2	anti-BAT + C'	86	83
7.5	20	Nonsuppressed spleen	62	62

\* Suppressed by exposure to maternal anti-Ig-5b and anti-Ig-6b.

‡ Units of a standard SJA × SJL anti-DNP serum. One unit = 1% of the binding activity of the standard. Response measured 7 d after transfer.

specificity of the suppression induction with anti-Ig-6 allotype antisera argue for precursor reactivity of inducing antibody being involved in suppressor induction.

Chronic suppression for Ig-1a is considerably more difficult to induce than for Ig-1b (see Table II). The difference in severity of suppression between Ig-1a and Ig-1b cannot be accounted for by differences in the inducing antiserum because they also occur in mice exposed to the same inducing serum, i.e., goat anti-IgM. These findings are consistent with observations in BALB/c × SJL hybrids. In these mice, where Ig-1b suppression itself is less severe, mice fully suppressed for Ig-1a can be obtained only extremely rarely (data not shown). In both hybrids, however, the ratio of Ig-1a: Ig-1b suppressed mice appears to be the same and thus independent of the general severity of suppression.

The chronic suppression in antiprecursor-Ig-exposed SJA × SJL is caused by the induction of Ts like those in anti-Ig-1b-exposed BALB/c × SJL (10). Data in Table III show that anti-BAT-sensitive, nylon-passed Ts in SJA × SJL chronically suppressed for Ig-1b specifically suppress Ig-1b anti-DNP production by co-transferred syngeneic DNP-KLH-primed spleen cells in an adoptive secondary assay. These Ts suppress by attacking Th committed to help Ig-1b B cells (see Table IV) (i.e., suppression is completely reversed by addition of T cells from DNP-KLH-primed nonsuppressed SJL [Ig<sup>b</sup>] donors but not by B cells from the same donors or by T cells from KLH-primed SJA [Ig<sup>a</sup>] spleens [which lack Th for Ig-1b B cells]).

Data presented on the last several lines of Table IV, testing the ability of noncarrier primed cells to reverse suppression, add another dimension to the mechanisms of suppression and help regulating Ig-1b antibody responses. They show that the Th responsible for reversing suppression, i.e., the Ts targets are distinct from carrier-specific Th. Chicken gamma globulin (CGG)-primed SJL spleen cells, which lack carrier-primed Th for the DNP-KLH antigen used, reverse suppression essentially as well as KLH-primed SJL spleen. Similarly, primed SJA spleen cells, however, do not. Thus, two helper populations, one carrier specific and the other Ig (allotype) specific,

TABLE IV  
*Suppression of Ig-1b Responses is Reversed by T Cells From Ig<sup>b</sup> but not Ig<sup>a</sup> Donors*

SJA × SJL spleen cells (× 10 <sup>6</sup> )		Additional cells*‡			Anti-DNP response§	
DNP-KLH 1° normal	Unprimed sup- pressed	Strain	Priming	Type	Ig-1b	Ig-1a
7.6	—	—	—	—	62	61
7.6	1	—	—	—	2	51
7.6	1	SJL	DNP-KLH	B	1	82
7.6	1	SJL	DNP-KLH	T	54	49
7.6	1	SJL	CGG	T	33	25
7.6	1	SJA	CGG	T	2	75
7.6	1	SJA	KLH	T	2	56

\* From 2 × 10<sup>7</sup> spleen.

‡ CGG: chicken gamma globulin; T: nylon-passed spleen; B: anti-BAT plus C' treated spleen (T-depleted).

§ See legend, Table III.

|| Suppressed by exposure to maternal anti-Ig-5b and Ig-6b.

TABLE V  
*T-Depleted Adult SJL Spleen Cells Prevent Neonatal Suppression Induction*

Antiprecursor-Ig exposed neonates*		Ig-1b suppression at 12 and 24 wk		
Injected material	Dose	None	Part	Full
SJL T-depleted adult spleen‡	10 <sup>7</sup> cells	8	0	0
SJA T-depleted adult spleen‡	10 <sup>7</sup> cells	0	0	7
SJL normal serum	μ	0	0	6
SJA normal serum		0	0	3

\* Nursing progeny of mothers producing anti-Ig-5b and anti-Ig-6b injected as indicated at 5 and 9 d of age.

‡ Anti-BAT + C'.

appear to be required for Ig-1b memory cell expression. The carrier-specific Th (CTh) can be supplied by either Ig<sup>a</sup> or Ig<sup>b</sup> haplotype mice; however, the Ig-specific helper must be supplied by Ig<sup>b</sup> mice (for the Ig-1b response). Analogous CTh and IgTh populations have been demonstrated in the Ars and A5A idiosyncrasy suppression-help systems (11, 12).<sup>1</sup>

Unlike Ts expression, the induction of suppression by exposure of SJL × SJA to maternal anti-Ig-5b and Ig-6b can be prevented by neonatal introduction of T-depleted adult SJL spleen cells (see Table V). This prevention of suppression induction cannot be a result of incidental introduction of Ig-1b produced by cells in the injected population because direct injection of relatively large amounts of serum Ig-1b (or Ig-1a) immunoglobulins does not alter the course of suppression (see Table V). Furthermore, the reversal does not appear to be a result of removal of the maternally derived antibody because similar amounts of this antibody are present in sera from 3-wk-old spleen-cell-injected mice and suppressed controls. FACS titration curves for these sera (17) were essentially superimposable (data not shown). Thus although the number of mice tested is relatively small, the data strongly suggest that B cells in adult spleen prevent induction or establishment of allotype-specific Ts. However, the use of T-depleted populations here leaves open the possibility that residual cells (surviving T

or non-T, non-B) rather than B cells are actually responsible for preventing suppression.

### Discussion

Evidence presented here showing that perinatal exposure to anti-IgM induces suppressor T cells which specifically suppress Ig-1 (IgG<sub>2a</sub>) production in SJA × SJL mice suggests that precursor (B cell) depletion may play a major role in induction of chronic allotype suppression. Such suppression has previously been induced by exposure to antibody reactive with the suppressed Ig-1 allotype. This exposure could be expected, by extrapolation of evidence obtained from other studies (1, 6), to temporarily deplete precursors committed to production of the suppressed allotypes. However, because the specificity of the inducing antiserum used and the chronic suppression obtained were the same (i.e., anti-Ig-1b and Ig-1b), precursor depletion was only one of several induction hypotheses that appeared plausible.

Here we have examined three cases where an exposing antiserum which does not react with Ig-1 determinants induces specific chronic suppression for Ig-1 production. In each case, the B-cell precursor-progeny relationship provides the only known connection between the specificity of the inducing serum and the specificity of suppression; whenever the inducing antibody is capable of reacting with Ig on or produced by precursors, it induces a T<sub>s</sub> population capable of suppressing production of Ig-1 by linearly descended (haplotype-excluded) progeny. This correlation suggests that precursor depletion, a well-known consequence of perinatal exposure to anti-Ig antibodies (1, 3), initiates T<sub>s</sub> induction in the hybrids studied here.

It is difficult to see how temporary perinatal B-cell depletion could induce (or heighten the expression of) a persistent allotype T<sub>s</sub> population that suppresses by depleting allotype-specific Th. Perinatal anti-IgM exposure, however, has been shown to deplete one of the two helper-cell populations jointly required for memory B-cell expression (18) (in addition to depleting B cells). Thus we suggest that temporary B-cell depletion in the neonate appears to lead to a failure in helper induction which, in SJL-related mice, allows establishment of a long-lived T<sub>s</sub> population committed to preventing the appearance of the missing helper population. This conclusion is consistent with evidence presented here showing that introduction of appropriate T-depleted adult spleen cells into antiprecursor-Ig-exposed neonates prevents T<sub>s</sub> establishment. Such regulatory interactions admittedly appear tortuous; nonetheless, they may indeed be the evolutionary solution to the organism's need to regulate processes within as complex and heterogeneous a system as that responsible for antibody responses.<sup>5</sup>

Additional insights into this matter may be obtained through development of an understanding of the genetic factors that restrict chronic suppression (anti-Ig-induced) to SJL-related strains. Data presented here clearly demonstrate the importance of the SJL genome in determining the extent of chronic suppression. The allotypically heterozygous hybrid between SJL allotype congenic strains, i.e., the SJA × SJL, is substantially more suppressible than the outcrossed BALB/c × SJL hybrid; and this

<sup>5</sup> Herzenberg, L. A., S. J. Black, and L. A. Herzenberg. 1979. Regulatory circuits and antibody responses. Submitted for publication.

hybrid, we have shown earlier (8), is substantially (or infinitely) more suppressible than hybrids between BALB/c and a variety of  $Ig^b$  haplotype strains other than SJL. Thus, one or more semidominant SJL genes are involved in the control of T<sub>s</sub> induction or their expression once stably induced.

SJL alleles within or closely linked to the major histocompatibility complex (MHC) also appear to contribute to suppressibility (8, 19).<sup>2</sup> Weak but detectable chronic suppression is induced in the (B10.S × BALB/c)<sub>F1</sub> hybrids but not in hybrids between immune BALB/c mother and the C57BL/10 strain which carries the H-2<sup>b</sup> haplotype (both B10.S and SJL carry the H-2<sup>s</sup> haplotype). Thus the SJL MHC haplotype contributes towards suppressibility but induction of full chronic suppression requires participation by non-MHC-linked SJL gene(s) as well.

Recent studies examining genetic control of a spontaneously induced chronic suppression of  $IgG_{2a}$  allotypes which occurs in BALB/c-related strains (reference 19, and A. Dowsett, manuscript in preparation) also demonstrate requirements for both MHC-linked and MHC-nonlinked gene(s) in suppression induction/establishment. Dowsett, in studies for his PhD dissertation conducted in our laboratory, has shown that the joint presence of BALB/c alleles at at least two genetically distant loci predisposes mice to the spontaneous induction of specific chronic Ig-1 allotype suppression, weaker but apparently similar to suppression induced in the BALB/c × SJL hybrid by perinatal exposure to anti-Ig-1b. One of these alleles, which segregates with the H-2<sup>d</sup> (BALB/c) MHC haplotype, shows recessive expression; the other(s), as yet unmapped, appear to be dominant.

The differentiability sensitivity of Ig-1a and Ig-1b to either spontaneous or antibody-induced chronic suppression indicates yet another genetic control on suppression induction/expression (19). In all cases, Ig-1b suppression is stronger than Ig-1a. The failure to obtain suppression for  $IgG_1$  (Ig-4) in any of the systems studied may indicate that  $IgG_1$  is yet further “down the ladder” and its regulation is undetectable in the strains used here.

Finally, we have shown some time ago that inbred SJL mice exposed to anti-Ig-1b by foster nursing on immune BALB/c mothers or by gestation (after zygote transplantation) in such mothers induced short-term but not chronic suppression in exposed progeny (8). These results must be reconciled with evidence presented here showing universal severe Ig-1b suppression induced by similar antibody exposure in SJA × SJL hybrids that theoretically differ from SJL inbred mice only by being heterozygous at  $IgH$  loci. They suggest either that  $Ig^b$  homozygotes are less susceptible to suppression induction or that dominant BALB/c genes, linked or nonlinked to the  $IgH$  region but important for suppression induction, are still carried by the SJA strain (which derived its  $Ig^a$  haplotype from BALB/c and was inbred after nine backcrosses to SJL).

In sum, then, diverse and apparently interactive genetic differences in  $IgG_{2a}$  (Ig-1 allotype) regulation distinguish SJL and BALB/c strains from other mice. These differences, we suggest, are responsible either singly or in concert for conversion of short-term anti-Ig-induced suppression into chronic Ig-1 allotype suppression. This suppression is characterized by the indefinite persistence of T<sub>s</sub> that prevent Ig-1 production by attacking Th specifically committed to helping Ig-1 B cells. Therefore, because antibody exposures that affect precursors for Ig-1-producing cells induce chronic suppression, we propose that at least certain of the genetic “lesions” in these



strains influence the potential for belated establishment (recovery) of the normal, B-cell-initiated, T-cell-mediated support system for IgG<sub>2a</sub> production.

### Summary

Allotype suppressor T-cell (Ts) populations that persist for the life of the animal arise in (BALB/c × SJL)F<sub>1</sub> hybrids exposed perinatally to antibody to the paternal (Ig-1b) allotype on IgG<sub>2a</sub>-isotype immunoglobulin H chains. These Ts suppress Ig-1b production by depleting the supply of allotype-specific helper T cells (Th) required, in addition to carrier-specific Th, for the latter stages of Ig-1b memory B-cell differentiation.

In this publication, we show that specific Ig-1 allotype Ts are induced by perinatal exposure to antisera which interfere with normal B-cell maturation, i.e., by antibodies reactive with surface IgM on immature precursors of IgG<sub>2a</sub> memory cells. Antibodies to IgM (Ig-6) allotypes carried on precursors induce specific suppression for the IgG<sub>2a</sub> allotype produced by progeny of the target precursor. Anti-Ig-6a and anti-Ig-6b induce Ts that specifically suppress Ig-1a and Ig-1b, respectively. Heterologous (goat) anti-IgM induces suppression for both IgG<sub>2a</sub> immunoglobulins (Ig-1a and Ig-1b). Ts activity in these antiprecursor-Ig-suppressed mice is expressed in adoptive transfer assays and, as with anti-Ig-1b-induced Ts, is rendered ineffective by cotransfer of adequate numbers of T cells but not B cells from nonsuppressed mice. The Ts induction, in contrast with Ts expression, is reversed by the introduction of appropriate adult B-cell populations from nonsuppressed donors.

Taken together, these data suggest that the development of mature B cells plays a central role in the early establishment of the balance between helper cells and suppressor cells that determines whether Ts or Th will dominate in regulating Ig-1b production in adult animals.

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### References

1. Lawton, A. R., and M. D. Cooper. 1973. Modification of B lymphocyte differentiation by anti-immunoglobulins. *Contemp. Top. Immunobiol.* **3**:193.
2. Manning, D. D. 1975. Heavy chain isotype suppression: A review of the immunosuppressive effects of heterologous anti-Ig heavy chain antisera. *J. Reticuloendothel. Soc.* **18**:63.
3. Raff, M. C., J. J. T. Owen, M. D. Cooper, A. R. Lawton, M. Megson, and W. E. Gathings. 1975. Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin suppression in vitro. Possible implications of B-cell tolerance to self. *J. Exp. Med.* **142**:1052.
4. Herzenberg, L. A., L. A. Herzenberg, R. C. Goodlin, and E. C. Rivera. 1967. Immunoglobulin synthesis in mice: suppression by anti-allotype antibody. *J. Exp. Med.* **126**:701.
5. Jacobson, E. B., and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL × BALB/c)F<sub>1</sub> mice. *J. Exp. Med.* **135**:1151.

6. Lawton, A. R., R. Asofsky, M. B. Hylton, and M. D. Cooper. 1972. Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to  $\mu$ -chain. *J. Exp. Med.* **135**:277.
7. Manning, D. D. 1974. Recovery from anti-Ig induced immunosuppression. *J. Immunol.* **113**:455.
8. Herzenberg, L. A., and L. A. Herzenberg. 1973. Short-term and chronic allotype suppression in mice. *Contemp. Top. Immunobiol.* **3**:41.
9. Herzenberg, L. A., and C. Metzler. 1974. Antibody induced suppressor T cells. In *The Immune System: Genes, Receptors, Signals*. E. E. Sercarz, editor. Academic Press, Inc. New York. 455.
10. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F-W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. Demonstration of allotype specific helper cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144**:330.
11. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotypic selectivity in the cooperation between T and B lymphocytes. *Eur. J. Immunol.* **8**:846.
12. Woodland, R., and H. Cantor. 1978. Idiotypic-specific T helper cells are required to induce idiotype-positive B memory cells to secrete antibody. *Eur. J. Immunol.* **8**:600.
13. Black, S. J., T. Tokuhisa, and L. A. Herzenberg. 1978. Regulation of memory B cell differentiation. In *B Lymphocytes in the Immune Response*. M. Cooper, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier North-Holland, New York. In press.
14. Tsu, T. T., and L. A. Herzenberg. 1979. Solid-phase radioimmune assays (RIA). In *Selected Methods in Cellular Immunology*. Barbara B. Mishell and Stanley M. Shiigi, editors. W. H. Freeman & Company Publishers, San Francisco, Calif. In press.
15. Herzenberg, L. A., and L. A. Herzenberg. 1978. Mouse immunoglobulin allotypes: Description and specific methodology. In *Handbook of Experimental Immunology*, 3rd Edition. D. M. Weir, editor. Blackwell Scientific Publications Ltd., Oxford, England. 12.1-12.23.
16. Golub, E. S. 1971. Brain-associated  $\theta$  antigen: Reactivity of rabbit anti-mouse brain with mouse lymphoid cells. *Cell. Immunol.* **2**:353.
17. Black, S. J., W. van der Loo, M. R. Loken, and L. A. Herzenberg. 1978. Expression of IgD by murine lymphocytes: loss of surface IgD is a maturation marker. *J. Exp. Med.* **147**:984-996.
18. Janeway, C. A., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent, antigen specific helper T cell. *Proc. Natl. Acad. Sci. U.S.A.* **74**:4582.
19. Herzenberg, L. A., A. P. Dowsett, S. J. Black, and L. A. Herzenberg. 1979. Genetic and serologic requirements for induction of spontaneous and anti-Ig induced allotype suppression. In *B Lymphocytes in the Immune Response*. M. Cooper, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier North-Holland, Inc., New York. In press.