ACTIVE SUPPRESSION OF IMMUNOGLOBULIN ALLOTYPE SYNTHESIS

III. Identification of T Cells As Responsible for Suppression by Cells from Spleen, Thymus, Lymph Node, and Bone Marrow*

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Jacobson and Herzenberg (1) and Jacobson et al. (2) have shown that (SJL × BALB/c)F_1 mice exposed to antibody to an immunoglobulin allotype early in life often develop a chronic suppression of production of that allotype. The key finding that transfer of a syngeneic mixture of chronically suppressed and normal spleen cells into an irradiated host results in suppression showed that chronically suppressed mice have cells that actively suppress the allotype production.

Thus, when SJL males (Ig^b) are mated to BALB/c females (Ig^a) previously immunized to Ig-1b (the allotype on IgG2a globulins), many of the progeny are actively suppressed for Ig-1b. This suppression is most clearly manifest in mice over the age of 6 mo, when about half the mice are chronically suppressed, i.e., consistently show no detectable Ig-1b in circulation. Before 6 mo, about 95% of the exposed mice synthesize some Ig-1b, although the levels often fluctuate in a range considerably below normal.

In the studies in which Jacobson et al. (2) demonstrated the existence of the actively suppressing cell population, spleen cells from chronically suppressed donors were mixed with spleen cells from normal syngeneic donors (i.e. BALB/c × SJL hybrids not exposed to anti-Ig-1b) and transferred to irradiated (600 R) BALB/c mice in which production of Ig-1b could be followed by measuring serum levels of Ig-1b. Transfer of normal spleen cells alone into such recipients resulted in establishment of Ig-1b synthesis by the 1st wk after transfer and the maintenance of serum levels 50–100-fold greater than the minimum detectable level for the duration of the experiment (greater than 6 mo in some cases). Transfer of the mixture of suppressed and normal cells (or of suppressed cells alone), however, was followed by only a transitory burst of Ig-1b synthesis after which the serum levels fell more or less rapidly depending on the number of suppressed cells in the mixture.

Jacobson et al. also presented data indicating that the suppressor population was present in lymphoid tissue other than spleen since Ig-1b synthesis in re-

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cipients of bone marrow from suppressed animals was suppressed, although the suppression appeared to be less marked. We have extended this work using the mixture-transfer system and present data in this publication showing that suppressor cells are found in all lymphoid tissues and are thymus derived (T cells). We further show that suppressor cells are active in vitro and suppress the development of specific antibody-forming cells of the suppressed allotype in Mishell-Dutton (3) cultures.

Materials and Methods

Mice.—The mice used in suppression studies were derived from matings between SJL/J male and BALB/CN females immunized against the paternal Ig-1b allotype (1). All suppressed mice were tested by Ouchterlony for Ig-1b before use in tissue culture or transfer studies. Only those with <0.01 mg of Ig-1b/ml serum (Ouchterlony negative) were used.

Sheep erythrocyte (SRBC)[1]-primed mice used in tissue culture studies were injected intraperitoneally (i.p.) with 0.1 ml of a 20% suspension of sheep erythrocytes 3–9 mo before sacrifice.

Antisera.—Antiallotype antisera were prepared as previously described (4–6). Antiserum reacting with Ig^a (BALB/c) was prepared in SJL or LP/J mice and reacts with Ig-1a (IgG2a) and Ig-4a (IgG1). Antiserum reacting with Ig^b (SJL allotypes) plaque-forming cells (PFC) was prepared in BALB/c or C3H-SW mice and reacts with Ig-1b (IgG2a) and Ig-4b (IgG1). Antiserum specific for Ig-1b was prepared in BALB/c mice immunized by the "*H-2*" methods.

Anti-Thy-1b antiserum (anti-θ) was produced by immunizing AKR/J mice with C3H thymus according to the method of Reif and Allen (7). It is designated anti-Thy-1b according to the recommendation of the Committee on Standardized Genetics Nomenclature for Mice (8). In most experiments presented, the anti-Thy-1b was an AKR anti-C3H thymus; however, in some experiments (indicated in the text) the anti-Thy-1b was prepared by immunization between a pair of Thy-1 congenic strains (kindly provided by Dr. E. A. Boyse).

Complement.—For in vitro treatment of cells with anti-Thy-1b a pool of serum from young guinea pigs (GPS) was used as a source of complement. Immediately before use, the GPS was absorbed with agarose (80 mg/ml) for 1 h at 4°C to reduce its cytotoxicity for mouse cells (9).

For development of PFC, pools of fresh GPS, absorbed twice (each time for 10 min at 4°C) with sheep erythrocytes, were used at 1:10.

Cell Viability.—Cell viability was estimated by trypan blue exclusion.

Estimation of Immunoglobulin Allotype Levels in Serum.—Levels were estimated by radioimmune assay as previously described (4, 6) or by quantitative immunodiffusion with specific antiallotype antisera. Estimation by the latter technique is indicated in legends for tables and figures.

Assay for PFC.—A modification (3) of the technique described by Jerne et al. (10) was employed for the assay of hemolytic PFC. Duplicate slides were done for each point and the values averaged.

To facilitate counting of indirect PFC, the formation of direct PFC was inhibited by incubation of slides with concanavalin A (0.3 mg/ml) for 1 h at 37°C before incubation with developing antisera, i.e., antiallotype or rabbit antimouse IgG (11). Control slides incubated with concanavalin A but not with developing antisera were done for each culture. In general, PFC formation on these slides was completely inhibited; however, if any "break through" PFC were found, their number was subtracted from the count for indirect PFC on the slides incubated with developing antisera.

[1] *Abbreviations used in this paper:* BSS, balanced salt solution; FCS, fetal calf serum; GPS, guinea pig normal serum; PFC, plaque-forming cells; SRBC, sheep erythrocytes; Thy-1b, previously referred to as AKR anti-θ C3H.

Spleen Cell Culture.—Spleen cells were cultured according to cell culture methods described by Mishell and Dutton (3). The immunizing dose of antigen per culture dish was 30 μl of a 0.05% SRBC suspension. Cells were harvested and assayed for PFC on day 5 of culture. Each culture dish was inoculated with a total of 2×10^7 viable nucleated cells.

Removal of T Cells from Cell Suspensions by Treatment with Anti-Thy-1b Antiserum.—For cell culture, single-cell suspensions at a final cell concentration of 1×10^7 nucleated cells per ml were incubated with anti-Thy-1b (1:40) and GPS (1:10) in 5% fetal calf serum (FCS) in balanced salt solution (BSS) for 45 min at 37°C. The cells were then washed in a large volume of 5% FCS-BSS, resuspended in tissue culture medium, and counted, and the cell concentration was adjusted to $1.5–2 \times 10^7$ viable cells/ml. Control cell suspensions were incubated with GPS alone without anti-Thy-1b and treated similarly. For in vivo transfer experiments, anti-Thy-1b treatment was carried out as previously described (12).

RESULTS

As the data in Fig. 1 show, the ability of spleen cells from normal (BALB × SJL)F_1 mice to sustain production of Ig-1b over a period of weeks after transfer into irradiated (600 R) BALB/c mice is suppressed when spleen cells from genetically identical "chronically" suppressed mice are mixed with the normal cells before transfer. Serum Ig-1b levels in recipients of 10^7 normal cells reach maximum by the 2nd wk after transfer and then are maintained at slightly under that level for the duration of the experiment. Ig-1b levels in recipients of the same number of spleen cells (10^7) from suppressed F_1 donors climb for the 1st 2 wk but then fall rapidly to below the margin of detectability by the 5th or 6th wk after transfer. Ig-1b levels in serum of recipients of 10^7 cells from normal spleens plus 10^7 cells from suppressed spleens (mixed before transfer) also climb and fall and are, in fact, indistinguishable from levels in recipients of suppressed spleen alone.

This data, which is similar to that previously presented (2) except that the ratio of suppressed to normal cells transferred is 1:1 instead of 1:4, indicate that suppressed spleen carries a transferable population of cells capable of suppressing production of Ig-1b by normal cells, i.e., by cells not previously exposed to anti Ig-1b antiserum.

The data in Fig. 2 show that the suppressor activity of spleen cells from suppressed animals is destroyed by incubation of the cell suspension with antibody to the Thy-1b antigen, (AKR anti-C3H thymus) plus complement, a procedure that selectively kills thymus-derived (T) cells. Ig-1b levels in suppression controls, that is in recipients of 10^7 normal spleen cells transferred with 10^7 suppressed spleen cells previously incubated with nonimmune AKR serum and complement, rise to 0.12 mg/ml at wk 2 and then fall rapidly to below detectability, as do levels in recipients of nontreated cells in the experiment reported in Fig. 1. In contrast, Ig-1b levels in recipients of 10^7 normal spleen cells transferred with 10^7 spleen cells preincubated with anti-Thy-1b plus complement appear if anything to be higher than Ig-1b levels in recipients of 10^7 normal spleen cells transferred alone. Thus, the ability of spleen cell suspensions from chronically suppressed animals to suppress Ig-1b production is dependent on

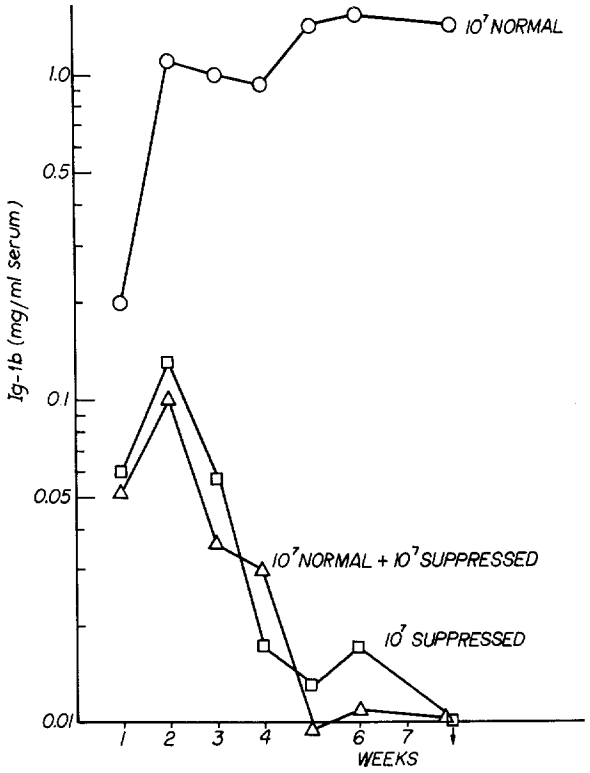

FIG. 1. Mixture-transfer assay for suppression of allotype production. Lethally irradiated (600 R) BALB/c mice (Ig^a) were restored with (SJL × BALB/c)F_1 hybrid spleen cells approximately 18 h after irradiation. (○) received 10^7 spleen cells from normal hybrids; (□) received 10^7 spleen cells from chronically suppressed hybrids; and (△) received a mixture containing 10^7 spleen cells from suppressed hybrids plus 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 five mice.

the presence of a population of cells carrying Thy-1b antigen, i.e., a population of T cells.

Some question has been raised with regard to the specificity of anti-Thy-1b sera prepared by immunizing AKR mice with Thy-1b on thymocytes from C3H mice. The pool of antiserum used here has been used for several studies in this laboratory (12). In all cases, those cells necessary for the immune response that were removed by treatment of a cell population with the antiserum plus complement were completely replaceable by thymocytes. Comparative fluorescence staining studies with congenic anti-Thy-1b and a rabbit antiserum specific for T cells showed no differences between the three sera with respect to the number of spleen cells stained, again demonstrating the specificity of the AKR anti-C3H Thy-1b serum for T cells (unpublished observations, T.

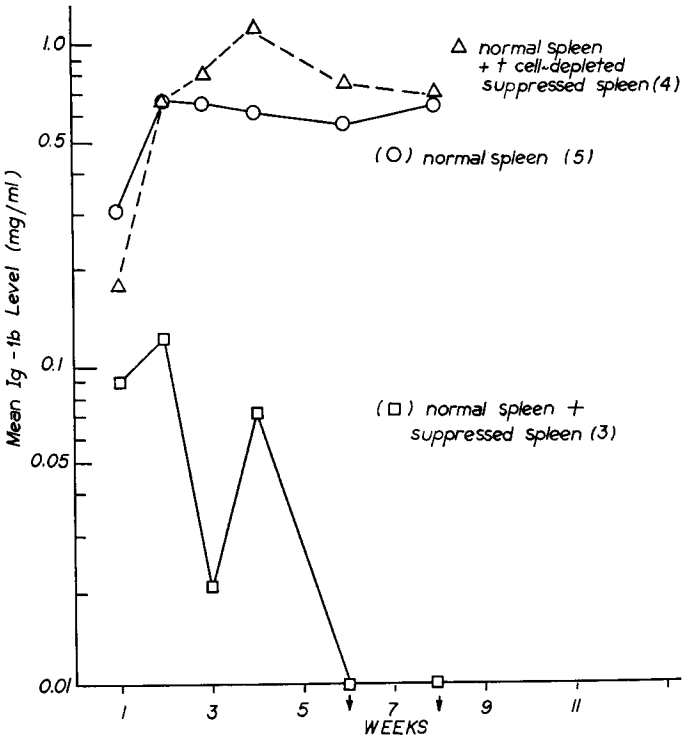

FIG. 2. Depletion of suppressor T cells from spleen by treatment with anti-Thy-1b (anti-θ). Experimental details were the same as described in the legend for Fig. 1. (○) received 10^7 spleen cells from normal (SJL × BALB/c)F_1 hybrids; (□) and (△) received a mixture of 10^7 spleen cells from normal hybrids 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 V.

Masuda). As a final check on specificity however, some key experiments (described below) were repeated with anti-Thy-1b made with a congenic pair. (This serum was generously provided by Dr. E. A. Boyse.)

In Vitro Assay for Suppressor T Cells.—Similar results identifying a T cell in spleens from suppressed animals as responsible for suppressor activity were obtained using a Mishell-Dutton-type in vitro culture system (3), where the ability of cultured spleen cells from normal sheep erythrocyte (SRBC)-primed donors to make indirect PFC developable by anti-Ig-1b was specifically inhibited by the addition to the culture of unprimed spleen cells from suppressed animals.

In the experiments presented in Tables I and II, spleen cells from normal (BALB/c × SJL)F_1 animals primed 3–9 mo previously with 2×10^8 SRBC i.p. were cultured with spleen cells from unprimed normal or unprimed suppressed mice, in the presence of 2×10^5 SRBC. At day 5 of culture, cells were

TABLE I

Active Suppression of the Ig-1b Secondary Response to SRBC in Culture

nos. of spleen cells cultured (× 10^6)			Allotype-developed plaques per 10^6 recovered cells			a/b normalized
SRBC primed Normal	Unprimed Normal	Unprimed Suppressed	a	b	a/b	
20	—	—	908	658	1.4	1
15	5	—	716	343	2.1	1.5
15	—	5	905	83	11	7.7
10	10	—	286	139	2.0	1.5
10	—	10	279	9	31	22

Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 mo 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.

TABLE II

Requirement for T Cells for Active Suppression in Culture

nos. of spleen cells cultured (× 10^6)				Normalized a/b allotype ratio of PFC			
SRBC primed Normal	Unprimed Normal	Unprimed Suppressed	Treatment of suppressed cells	Exp. no. 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	—	10	C′ + anti-Thy-1b	0.67	1.2	1.8	0.77
10	10	—	None	0.67	1.5	1	1.4

Donors of all cells were (SJL × BALB/c)F_1 mice 6-12 mo 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 I). Total b PFC per 10^6 recovered cells for each experiment: no. 7 = 408, no. 8 = 658, no. 9 = 278, no. 10 = 77.

plated on SRBC and the number of indirect PFC, developed with several anti-immunoglobulin antisera, as well as the number of direct PFC were counted.

To simplify the tables, we have presented data here only for indirect PFC developed with antiallotype reagents. Data for direct and total indirect PFC formation, and controls relevant to these figures, were consistent with the allo-

type data. A manuscript describing these and related studies of in vitro suppression in greater detail is in preparation.[2]

As the data in Table I show, the number of PFC developed with anti-b allotype is sharply decreased when the primed normal cells are incubated with unprimed suppressed cells. Whereas incubation of 10^7 primed normal cells with 10^7 unprimed normal cells gives 139 "b" PFC/10^6 recovered cells, incubation of the same number of primed cells with 10^7 unprimed suppressed cells gives only 9 "b" PFC/10^6 recovered cells.

Keeping the total number of cells per culture dish the same (2×10^7) but increasing the number of primed cells added to 1.5×10^7 gave proportionately more PFC, but again the cultures with unprimed suppressed cells (5×10^6) showed substantially fewer Ig-1b PFC, 83, than cultures with a similar number of unprimed normal cells, 343.[3]

To allow comparison of cultures with different ratios of primed and unprimed cells within a given experiment, we calculated the ratio of "a" allotype PFC to "b" allotype PFC from the data presented above. With the addition of unprimed normal cells, this ratio was the same whether there were equal numbers of primed and unprimed or three times as many primed as unprimed, i.e., 2.0 for the former and 2.1 for the latter. With the addition of unprimed suppressor cells, however, the ratio went up to 33 for equal numbers of unprimed suppressed and primed normal and to 11 for 1:3 unprimed suppressed to primed normal. The increase in ratio of "a" allotype PFC to "b" allotype PFC is a direct reflection of the decrease in "b" allotype PFC since in all cases the number of "a" allotype PFC remained essentially constant (see Table I).

To determine whether the cells that suppress in culture are Thy-1b positive, hence T cells, spleen cell suspensions were incubated with anti-Thy-1b antiserum (AKR anti-C3H Thy-1b) and guinea pig complement, washed, resuspended, and added to primed normal cells as above. Data from four such experiments are summarized in Table II. The a/b ratios in each experiment were normalized to the ratio for 2×10^7 primed cells cultured alone in order to facilitate comparison between experiments. The ratio to which each is normalized is given in parentheses on the first line. The b PFC from 2×10^7 primed cells cultured alone for each experiment is given in the legend.

Culture of equal numbers of primed normal plus either unprimed suppressed or unprimed suppressed treated with AKR normal serum and complement show roughly the same high a/b ratio, indicative of suppression of Ig-1b PFC. In contrast, culture of primed normal alone, primed normal plus unprimed normal, or primed normal plus unprimed suppressed treated with AKR anti-

[2] Chan, E., L. A. Herzenberg, and R. I. Mishell. Personal communication.

[3] Since these experiments were done, we have shown that the serum used to develop "b" allotypes PFC can develop Ig-4b (IgG1) as well as Ig-1b (IgG2a) PFC, suggesting that contrary to in vivo data, active suppression in vitro may be effective against allotypes on both of these classes. This fascinating possibility is currently under study.

C3H Thy-1b all show low a/b ratios, showing that the anti-Thy-1b treatment has removed suppressor activity from the suppressed spleen population. These experiments have been repeated with congenic anti-Thy-1b. The data will be presented elsewhere.[2]

Distribution of Suppressor T Cell in Lymphoid Tissues.—To assay for cell-associated suppressor activity in various lymphoid organs, 10^6 and 10^7 cells from each of the tissues were mixed with 10^7 spleen cells from normal animals and transferred into irradiated (600 R) BALB/c mice (in vivo mixture-transfer assay). As the data in Table III show, both thymus and lymph nodes from sup-

TABLE III

Suppressor Cells in Lymphoid Tissues

Normal donor	Suppressed donor			Ig-1b levels					
no. of spleen cells	Tissue	no. of cells transferred	no. of recipients	wk after transfer					
				1	2	3	4	6	8
				mg/ml					
10^7	ND*	ND*	3	0.24	>0.38	>0.5	>0.5	>0.5	>0.5
10^7	Spleen	10^7	4	<0.04	–	–	–	–	–
10^7	"	10^6	4	0.4	0.13	<0.03	–	<0.02	0.04
10^7	Thymus	10^7	4	0.02	–	–	–	–	–
10^7	"	10^6	4	>0.3	>0.3	>0.32	0.12	0.15	0.08
10^7	Lymph node	10^7	4	0.03	–	–	–	–	–
10^7	" "	10^6	4	0.4	0.11	0.08	<0.03	<0.02	–

Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 mo old. Suppressed donors were exposed to maternal anti-Ig-1b perinatally. Ig-1b levels were determined by immunodiffusion. Minus signs (–) = <0.01 mg of Ig-1b/ml. Recipients were BALB/c mice irradiated with 600 R~ 18 h before transfer.

* ND, not done.

pressed animals contain roughly the same suppressor activity as spleen at either cell concentration, thymus being just somewhat less active at 10^6 cells transferred. Normal tissue showed no suppressor activity.

Similar results were obtained with bone marrow as the source of suppressor cells. In the experiment presented in Table IV, 5×10^6 or 10^7 bone marrow cells from suppressed animals were able to suppress as well, respectively, as 3×10^6 or 10^7 spleen cells. Normal bone marrow (10^7) cells did not suppress at all.

Since bone marrow is expected to contain very few Thy-1b-positive cells, the sensitivity to anti-Thy-1b serum of the suppressor cell population in bone marrow was tested. As the data in Table V show, suppressor activity was lost after exposure of the cells to AKR anti-C3H Thy-1b plus complement but not exposure to AKR normal serum plus complement, indicating that just as in the spleen, the Thy-1b-positive population in bone marrow contains the cells responsible for suppression. The results for treatment of suppressed bone marrow with congenic anti-Thy-1b were similar (see Table V).

If a generous estimate of 10% Thy-1b-positive cells is assigned to bone

TABLE IV

Suppressor Cells in Bone Marrow

nos. of cells transferred ($\times 10^6$)				no. of mice	Mean Ig-1b levels				
Spleen		Bone marrow			wk after transfer				
Normal	Suppressed	Normal	Suppressed		1	2	3	4	6
					mg/ml				
12				8	0.2	>0.4	>0.4	>0.5	>0.3
12	3			4	0.1	0.07	<0.02	<0.04	<0.04
12	10			5	0.1	0.03	<0.02	—	—
12		10		4	0.3	0.4	>0.5	>0.5	>0.5
12			1	4	0.07	0.4	0.4	0.3	0.1
12			5	4	0.12	0.07	0.02	—	—
12			10	4	0.06	0.04	0.02	—	—

Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 mo 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 h before transfer.

marrow, and a minimum estimate of 30% Thy-1b-positive cells in spleen, comparison of the dosage data for spleen and bone marrow (see Table IV) indicates a minimum of twofold enrichment of suppressor cells or suppressor cell activity in the Thy-1b-positive cells in bone marrow.

DISCUSSION

In this and the preceding two publications (1, 2) on chronic allotype suppression, we have established several points that may be briefly summarized as follows:[4]

(*a*) Production of Ig-1b immunoglobulin is suppressed in (SJL × BALB/c)F_1 hybrids exposed early in life to antibody for the Ig-1b allotype. Most animals produce some Ig-1b and show complete suppression only after 6 mo of age.

(*b*) Suppression is an active process. Lymphoid tissues from chronically suppressed animals suppress Ig-1b production when mixed with spleen cells from normal (never exposed to anti-Ig-1b) syngeneic animals, both in vivo and in vitro.

(*c*) Suppression is dependent on the presence of T cells from the suppressed animal. Elimination of T cells (Thy-1b-bearing cells) from the suppressor lymphoid tissue before mixture with the normal (target) spleen cells abrogates suppression.

(*d*) The effector T cells that suppress arise at an as yet undefined time in (SJL × BALB/c)F_1 hybrid animals treated with anti-Ig-1b before weaning.

[4] A more complete summary is presented in a recent review by L. A. Herzenberg and L. A. Herzenberg (13).

TABLE V

Anti-Thy-1b (Theta) Sensitivity of Suppressor Cells in Bone Marrow

Exp. no.	no. of cells transferred ($\times 10^6$)			Treatment	no. of mice	Mean Ig-1b level wk after transfer						
	Spleen	Bone marrow										
	Normal	Normal	Suppressed			1	2	3	4	5	6	8
						mg/ml						
I	12			—	4	0.2	>0.5	>0.5	>0.5	>0.5	>0.4	>0.5
	12	10		—	4	0.4	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
	12		10	Anti-Thy-1b + C′	4	0.1	>0.5	>0.5	>0.4	>0.4	>0.4	>0.4
	12		10	NMS + C′	4	0.07	0.1	<0.04	<0.02	—*	<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
	12		9	Anti-Thy-1b + C′	6	0.1	>0.5	>0.5	>0.5		>0.5	>0.5
	12		9	Congenic anti-Thy-1b + C′	6	0.3	>0.5	>0.5	>0.5		>0.5	>0.5
	12		9	NMS + C′	4	0.1	0.1	0.2	0.2		0.05	0.05

Donors of all cells were (SJL × BALB/c)F_1 mice 6–12 mo 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 h before transfer. In exp. I, AKR anti-Thy-1b or AKR normal serum was used. In exp. II, congenic anti-Thy-1b (from Dr. E. A. Boyse) was used in addition.

* — = <0.01 mg of Ig-1b/ml serum.

(*e*) These cells are readily demonstrated in spleen, lymph node, thymus, and bone marrow by transfer of these tissues from chronically suppressed animals, i.e., from animals, generally over the age of 6 mo, in which Ig-1b synthesis has either begun and terminated or never begun at all.

(*f*) The suppressor cells survive indefinitely as a population, either by division or recruitment. They have been demonstrated in chronically suppressed animals over 18 mo of age and have been taken through at least two passages in irradiated hosts.

From this summary it is clear that while studies to date clearly implicate a T cell in suppression of Ig-1b globulin synthesis, its role in the process is far from understood. We have shown that the thymus, which contains few B cells, is as good a source of suppressor activity as spleen; that T cell-depleted suppressor spleen does not suppress although it is in the presence of an abundance of T cells from the concomitantly transferred normal spleen (target) population; and that suppression is transferred by cells but not by serum. Thus, it is unlikely that the T cells from suppressed animals are serving as cooperators for B cells able to produce an antibody that is the suppressing agent. Exclusion of this hypothesis, however, leaves a wide range of possibilities in which the T cells from suppressed animals are directly responsible for suppression either through cell-to-cell interaction or the production of a mediating product.

In an earlier publication (2), where we described the active suppression of immunoglobulin allotype synthesis, we discussed the pathway of differentiation of bone marrow-derived cells (B cells) from uncommitted precursor cells to immunoglobulin-producing cells committed to the production of a unique immunoglobulin molecule of a particular H chain class and allotype. We concluded that for the specific suppression of Ig-1b synthesis to occur in a heterozygote, the suppressing agent must recognize a B cell at some stage during differentiation when it is identifiable either as committed or able to be committed to Ig-1b synthesis. This stage, we felt, probably occurs after the decision to make Ig^b (as opposed to Ig^a) proteins has taken place, i.e., after allelic exclusion has taken place. The generation of this recognition and its maintenance in the T cell line must in some way be related to the presence of antiallotype serum in the young animal. Once established, however, it is apparently independent of the continued presence of the antiallotype.

There is not sufficient evidence available, as yet, to determine the mechanism by which the suppressor T cells are generated or how they interact with the B precursor cell to suppress Ig-1b synthesis. It is possible that the 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 and are then continually eliminated by T cells acting in their normal surveillance role. While this hypothesis has much to recommend it in that it assigns a role to suppressor T cells consistent with a known T cell function, a number of accommodations must be made in order to explain the data.

Why, for example, if we are dealing with a sensitized T cell population able to reject Ig-1b precursors as a foreign graft, does Ig-1b synthesis become established in most animals before 6 mo of age and complete rejection only occur in animals over 6 mo 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 6 mo of age who do not become completely suppressed (1)?

An alternate approach to the mechanism of suppression is to focus on the role of T cells in the antigen-driven differentiation of B cells from precursors to IgG antibody-producing cells (14). In general, this differentiation requires interaction with a cooperating T cell capable of recognizing either the antigen or a carrier to which the antigen has been attached (15). Therefore, response to an antigen may be limited not only by the number of B cell precursors able to differentiate to produce antibody to that 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 exist that actively regulate the flow of B cells from precursors to antibody-forming cells. The data we have presented on suppression of Ig-1b synthesis is 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 T cells, collectively responsible perhaps for the active control of the levels of antibody 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 cell by suppressors results in prevention of further differentiation of the B cell. While under ordinary circumstances the presence of suppressors and cooperators adheres to a finely tuned balance, the presence of antiallotype serum in the young animals could shift this balance in favor of overregulation of Ig-1b synthesis. In (SJL $\times$ BALB/c)F_1 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 that specifically suppress Ig-1b precursors from differentiating to producers.

Although this model in many ways more easily explains the partial suppression of allotype synthesis frequently observed and certain other features of the "kinetics" of suppression, e.g., temporary release from suppression (1), it perhaps strains the imagination to postulate suppressor T cells for all classes and possibly all antibody specificities that an animal can produce. It will remain for further study to decide which model, if either, more closely approximates the truth.

Interestingly, there have been reports recently of a number of systems in which T cells actively suppress antibody production (16–19) although in these cases, T cells have not been shown to selectively inhibit an immune response. The system described here offers the first example of suppressor T cells with specificity. Just one of the two allotypes on just one of the classes of IgG immunoglobulins in heterozygotes is suppressed. However, the generality of this suppressive mechanism and its role in regulation of antibody production is still an open question. The limitations of induction of active (T cell) allotype suppression to the particular genetic background of (BALB/c $\times$ SJL)F_1 animals may be a clue that should be kept in mind in further genetic and cellular immunologic explorations.

SUMMARY

Thymus-derived cells (T cells) that actively suppress production of IgG2a immunoglobulins carrying the Ig-1b allotype have been found in adult (SJL $\times$ BALB/c)F_1 mice exposed to anti-Ig-1b early in life. The suppression is specific for Ig-1b. The allelic product, Ig-1a, is unaffected.

Spleen, lymph node, bone marrow, or thymus cells from suppressed mice

suppress production of Ig-1b by syngeneic spleen cells from normal F_1 mice. When a mixture of suppressed and normal cells is transferred into lethally irradiated BALB/c mice, there is a short burst of Ig-1b production after which Ig-1b levels in the recipient fall rapidly below detectability. Pretreatment of the cells from the suppressed mice with antiserum specific for T cells (anti-Thy-1b) plus complement before mixture destroys the suppressing activity.

Similar results with suppressor cells were obtained in vitro using Mishell-Dutton cultures. Mixture of spleen cells from suppressed animals with sheep erythrocyte (SRBC)-primed syngeneic normal spleen before culture suppresses Ig-1b plaque-forming cell (PFC) formation while leaving Ig-1a PFC unaffected. Treatment of the suppressed spleen with anti-Thy-1b before transfer removes the suppressing activity.

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