ACTIVE SUPPRESSION OF IMMUNOGLOBULIN ALLOTYPE SYNTHESIS

II. Transfer of Suppressing Factor with Spleen Cells*

By E. B. JACOBSON,‡ LEONORE A. HERZENBERG, R. RIBLET, and L. A. HERZENBERG

(*From the Department of Genetics, Stanford University School of Medicine, Stanford, California 94305*)

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We have shown, in the previous publication, that when SJL/J (Ig^b) male mice are mated to BALB/c (Ig^a) females immunized against the Ig-*1b* (γG_{2a}) allotype, many of the resultant F_1 heterozygous (Ig^a/Ig^b) progeny are chronically suppressed for Ig-*1b* production. That is, many of the progeny are either unable to produce detectable amounts of circulating Ig-*1b* or, having produced it, are unable to sustain production, so that by about 6 months of age they have become phenotypically negative for the paternal allele. Since repopulation of irradiated (600 R) chronically suppressed mice with spleen cells from nonsuppressed mice did not restore Ig-*1b* production, we conclude that chronic suppression was due to an active process, relatively resistant to irradiation, which prevents expression of the paternal (Ig-*1b*) allele (1).

In this publication we describe the results obtained when cells from chronically suppressed mice are transferred, either alone or together with normal, syngeneic spleen cells, into irradiated hosts of another allotype. These results confirm the concept of an active suppressing mechanism and strongly suggest that it is manifested by cells resident in the lymphoid tissue of the suppressed mice. A discussion of possible mechanisms of active allotype suppression is presented.

Materials and Methods

The materials and methods used in this study are identical with those used in the previous publication (1) with the following exceptions:

Mice.—In addition to the strains described in the previous publication, BAB/14, a strain congenic with BALB/c (Ig^a) but carrying the Ig^b alleles, was used in the work reported here.

Mixture of Suppressed and Normal Cells before Transfer.—Cell suspensions, prepared as described (1), were mixed and stored on ice for no more than 30 min before injection into recipients.

Anti-Sheep Erythrocyte Plaque Formation.—Normal (nonsuppressed) (SJL × BALB/c)F_1

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‡ Present address: Basel Institute for Immunology, Basel, Switzerland.

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recipients were injected intravenously (i.v.),[1] 4–6 hr after irradiation, with primed spleen cells from chronically suppressed mice immunized 8–105 days previously. Control mice were injected with primed cells from nonsuppressed donors. A challenge dose of 4×10^8 sheep erythrocytes (SRBC) was injected simultaneously. The recipients were sacrificed 7 days after transfer and their spleen cells assayed for γG_{2a} plaque-forming cells (PFC) as previously described (2).

TABLE I

Ig-1b Production in Irradiated BALB/c (Ig^a/Ig^a) Recipients of Pooled Cells from Normal or Suppressed Donors

Donor cells* (SJL × BALB/c) F_1 (No. of cells transferred per recipient)	Experiment No.	No. positive for Ig-*1b*/total‡ (weeks after transfer)				Mean level§ in positive mice at week 7	
		2	3	5	7	Ig-*1b*	Ig-*4b*
						mg/ml	*mg/ml*
Suppressed spleen (1.4×10^7)	3	5/10	5/9	1/9	0/9	—	0.91
	7	4/6	4/5	0/5	0/5	—	1.9
	4	10/10	8/9	7/9	4/9	0.5	1.2
	Total	19/26	17/23	8/23	4/23		
Normal spleen (1.4×10^7)	3	2/2	2/2	2/2	2/2	1.47	1.5
	7	1/1	1/1	1/1	1/1	2.1	0.4
	Total	3/3	3/3	3/3	3/3		
Suppressed bone marrow (5×10^6)	3	5/6	4/5	1/5	0/5	—	1.3
	7	4/8	3/7	4/7	4/7	0.8	0.4
	4	1/6	5/6	5/6	4/5	1.4	1.1
	Total	10/20	12/18	10/18	8/17		
Normal bone marrow (5×10^6)	3	2/2	2/2	2/2	2/2	1.7	0.72
	7	2/2	2/2	2/2	2/2	0.9	1.8
	Total	4/4	4/4	4/4	4/4		

* Suppressed spleen and bone marrow were pooled cell suspensions from three mice. Normal and spleen cells were obtained from individual animals. Recipients were irradiated with 600 R total body irradiation approximately 18 hr before transfer.

‡ Tested by double diffusion in agar. By the same method, all of the mice were positive for Ig-*1a*.

§ As determined by the method of inhibition of ^{125}I precipitation described in reference 1. For Ig-*1b*, negative mice had less than 0.025 mg/ml; for Ig-*4b*, all mice were positive.

RESULTS

Suppression of Ig-1b Synthesis in Transferred Populations of Suppressed Cells.—Spleen cells or bone marrow (B) cells from chronically suppressed or normal mice were injected i.v. into irradiated BALB/c (non-Ig^b) recipients. The serum levels of Ig-*1b* in these recipients were determined at weekly intervals by means of immunodiffusion in agar.

[1] *Abbreviations used in this paper:* B, bone marrow; i.p., intraperitoneally; i.v., intravenously; PFC, plaque-forming cells; SRBC, sheep erythrocytes.

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Tables I and II show that, while a majority of the recipients injected with spleen cells from suppressed donors had detectable levels of circulating Ig-*1b* early after transfer, these levels decreased with time until no longer detectable. Although a few of the mice were still positive for Ig-*1b* at week 7, the individual test records (not presented) show a pattern of fluctuation of Ig-*1b* levels similar to that seen in untreated suppressed mice.

TABLE II

Ig-1b Production in BALB/c Mice Injected with Suppressed or Normal Spleen Cells from Individual Donors

Donor cells (SJL × BALB/c)F1*	Donor No.	No. positive for Ig-*1b*/total‡ (weeks after transfer)				Mean level in positive mice on week 7	
		1	3	5	7	Ig-*1b*§	Ig-*4b*
						mg/ml	*mg/ml*
Suppressed spleen	1	3/4	3/3	0/3	1/3	0.17	1.2
	2	1/4	2/4	2/4	1/4	0.11	0.85
	3	1/4	1/4	1/4	0/4	—	0.5
	4	4/4	3/4	0/4	0/4	—	0.8
	5	0/4	0/4	0/4	1/4	0.056	0.6
	6	4/4	3/4	0/4	0/4	—	0.5
	7	3/4	2/4	3/4	1/2	0.11	1.0
	8	3/3	3/3	0/3	0/3	—	0.6
	9	3/3	3/3	1/3	1/3	0.42	0.6
	10	3/3	3/3	2/3	2/3	0.42	1.0
	11	3/3	2/2	1/2	1/2	0.8	1.1
	Total	28/40	25/38	10/38	8/36		
Normal spleen	12	4/4	4/4	4/4	4/4	0.85	0.44
	13	4/4	4/4	4/4	4/4	0.75	0.6
	14	3/3	3/3	3/3	3/3	0.75	0.4
	15	3/3	3/3	2/2	2/2	0.4	1.0
	Total	14/14	14/14	13/13	13/13		

* 1.2–1.5 × 10^7 spleen cells from individual mice were injected i.v. into groups of BALB/c mice irradiated with 600 R 18 hr previously.

‡ Ig-*1b* tested by double diffusion in agar.

§ Arithmetic mean of positives only. All mice were positive for Ig-*4b*. Determined by inhibition of ^{125}I precipitation.

Transferred B cells from suppressed mice showed a stronger tendency to sustain Ig-*1b* production after transfer, although many of the recipients of these cells did become suppressed by week 7 (Table I).

Since the recipients of suppressed cells continued to produce Ig-*4b*, another immunoglobulin class (γG_1) carrying the paternal allotype, the disappearance of Ig-*1b* from the sera of these recipients was clearly not due to rejection of the transferred cells. In addition, it can be seen in the tables that cells transferred from normal donors were able to permanently establish Ig-*1b* production. Thus,

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removal of cells from the suppressed host environment and their expansion in a neutral recipient does not allow more than a minimal restoration of Ig-*1b* production.

Since suppression has been shown to be physiologically dominant, i.e. is an active process whereby a known competent cell population is rendered incom-

TABLE III

Ig-1b Production in BAB/14 (Ig^b) Recipients of Normal and Suppressed Cells

Donor cells* (SJL × BALB/c)F_1 (No. of cells transferred per recipient)	Experiment No.	No. positive for Ig-*1b*/total‡ (weeks after transfer)			
		2	3	5	7
Suppressed spleen (1.4 × 10^7)	3	10/10	10/10	1/10§	1/10
	7	2/2	2/2	2/2	1/1
	4	5/5	5/5	4/5	3/5‖
	Total	17/17	17/17	7/17	5/16
Normal spleen (1.4 × 10^7)	3	2/2	2/2	2/2	2/2
	7	1/1	1/1	1/1	1/1
	Total	3/3	3/3	3/3	3/3
Suppressed bone marrow (5 × 10^6)	3	6/6	6/6	6/6	6/6
	7	2/2	2/2	2/2	2/2
	4	6/6	5/5	5/5	5/5
	Total	14/14	13/13	13/13	13/13
Normal bone marrow (5 × 10^6)	3	2/2	1/1	1/1	1/1
	7	1/1	1/1	1/1	1/1
	Total	3/3	2/2	2/2	2/2

* The suppressed spleen and B cell suspensions used in each experiment were pooled from three mice. Normal B and spleen cells were obtained from individual animals. Recipients were irradiated (600 R) approximately 18 hr before transfer.

‡ Ig-*1b* tested by double diffusion in agar. By the same method, all of the mice were positive for Ig-*1a*, with the exception of the two positive mice in experiments 3 and 7. These mice were negative for Ig-*1a* by week 7.

§ Low levels of Ig-*1b* were still detectable in the sera of five of the mice at week 4 (not shown in table).

‖ The level of Ig-*1b* detected in these three mice was very low.

petent (1), the demonstration of initial Ig-*1b* production and subsequent suppression in transferred suppressed cell populations suggests that the active suppressing factor is transferred to the new host along with cells capable of giving rise to Ig-*1b* production. Further support for this contention comes from data on transfers of suppressed and nonsuppressed cell populations into Ig^b homozygous hosts (BAB/14) congenic with BALB/c. These experiments were performed originally as transplantation controls to show persistence of the F_1 (Ig^a/Ig^b) cells in irradiated parents. As executed, using production of Ig-*1a*

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as a marker, F_1 spleen or B cells of either suppressed or normal origin persisted equally well. Surprisingly, it became evident around 5 wk after transfer that many of the irradiated Ig^b homozygous recipients of suppressed spleen cells had lost their ability to produce Ig-*1b* (see Table III).

There appear to be only two possible explanations for these totally unexpected results: (*a*) that the suppressing factor is powerful enough to completely repress the recipients' endogenous production of Ig-*1b*, or (*b*) that overgrowth of the injected cell population has occurred and the recipients have been re-

TABLE IV

Failure of High Levels of Ig-1b to Prevent Suppression in BALB/c Recipients of Suppressed Cells

Injected serum*	Donor cells (No. of cells transferred per recipient)	No. positive for Ig-*1b*‡/total						Mean level in positive mice at week 8	
		Before serum injection	Weeks after transfer						
			0	2	4	6	8	Ig-*1b*§	Ig-*4b*
								mg/ml	*mg/ml*
Ig^b	Suppressed spleen (1.5×10^7)	0/4	4/4	4/4	2/4	1/4	0/3	—	1.6
	Suppressed bone marrow (5×10^6)	0/4	4/4	4/4	3/4	4/4	4/4	0.4	0.98
Ig^a	Suppressed spleen (1.5×10^7)	0/4	4/4	4/4	1/4	1/4	1/4	0.25	1.2
	Suppressed bone marrow (5×10^6)	0/4	4/4	3/3	1/3	3/3	2/3	0.85	1.1
Ig^b	BALB/c bone marrow (5×10^6)	0/4	4/4	4/4	0/4	0/4	0/4		

* Recipients were injected with a total of 1.5 ml of BALB/c (Ig^a) or BAB/14 (Ig^b) serum before irradiation (600 R) and transfer.

‡ Determined by immunodiffusion.

§ Arithmetic mean of positives only. All mice were positive for Ig-*4b*. Determined by inhibition of ^{125}I precipitation.

populated by the donor cells. The latter possibility is most likely, particularly in view of the fact that these mice, more than 3 months after transfer, were still producing Ig-*1a* (and no Ig-*1b*). Rejection of the transferred cells was seen in only 2 of the 16 spleen cell recipients, as evidenced by the disappearance of Ig-*1a* from their sera by week 6 or 7 after transfer. Thus, of the five mice positive for Ig-*1b* at 7 wk, two had rejected the transferred cells and three were producing only minimal amounts (0.1–0.15 mg/ml). Since we have clearly shown the presence of cells capable of producing Ig-*1b* in these cell inocula (Table I), these results verify the concept of a simultaneously transferred suppressing factor, capable of manifesting itself even in an Ig^b homozygous host.

BAB/14 recipients of B cells did not become suppressed. This, taken with

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the lower efficiency of bone marrow's self-suppression shown in Table I, suggests that bone marrow has a smaller or less effective suppressing population than does spleen, although it must be taken into consideration that the number of B cells transferred was always considerably lower than that used in spleen cell transfers.

Suppression of Transferred Populations in Presence of High Levels of Circulating Ig-1b.—Although latent production of anti–Ig-*1b* or the presence of cell-associated cryptic anti–Ig-*1b* was ruled out earlier as a mechanism for maintenance of chronic suppression in intact animals, the effect of high circulating levels of Ig-*1b* was again investigated, using a transferred population of suppressed cells, to verify this point under more stringent conditions. Before irradiation and transfer, prospective BALB/c recipients were injected with normal Ig^b (BAB/14) serum to create high levels of circulating Ig-*1b* at the time of transfer. A control group was injected with Ig^b serum and BALB/c normal bone marrow, merely to determine the length of time of detectability of the injected serum. The results of this experiment, shown in Table IV, indicate that the presence of circulating Ig-*1b* does not prevent suppression of the transferred cells, as was subsequently confirmed by the results obtained with BAB/14 mice.

Suppression of Ig-1b Synthesis in Transferred Mixtures of Syngeneic Suppressed and Normal Cells.—In both this and the preceding publication (1), we have presented experiments in which normal, Ig-*1b*–producing cell populations were incidentally combined in vivo with suppressed cells and appeared to become suppressed as a result. Taken as a whole, these findings strongly suggest the existence of an active cell-associated factor which is able to suppress Ig-*1b* synthesis in populations of normal cells never exposed to maternal anti–Ig-*1b*. To test this hypothesis more directly, spleen cells from chronically suppressed mice were mixed with spleen cells from normal syngeneic F_1 mice in vitro and then transferred into irradiated BALB/c (Ig-*1a*) hosts.

The results of this experiment are presented in Fig. 1, where the average Ig-*1b* levels in the sera of three groups of recipients are plotted as a function of time after transfer. These results demonstrate conclusively that cells from a suppressed spleen are able to suppress Ig-*1b* production by normal spleen cells. In the "normal control" group, which received 1.6×10^7 spleen cells from the normal F_1 donor, the serum level of Ig-*1b* starts at 0.38 mg/ml, rises to approximately 1 mg/ml by the third week, and then falls to a stable 0.5 mg/ml by week 7. In the "suppressed control" group, which received 1.2×10^7 spleen cells from the suppressed donor, the Ig-*1b* serum level is at 0.1 mg/ml at 1 wk after transfer and falls steadily thereafter, dropping below the threshold for detection at about 7 wk.

In the "mixture" group, which received 1.2×10^7 normal cells plus 0.3×10^7 suppressed cells, the Ig-*1b* level at 1 wk reaches 0.18 mg/ml, a value intermediate between suppressed (0.1) and control (0.38), and then falls in a manner similar to the "suppressed control" group, although not quite as sharply.

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Initially, each group consisted of five recipients. However, one animal in the suppressed control group and one in the mixture group were excluded because the serum level of Ig-*4b* fell below detectability, indicating rejection of the spleen cell graft by the irradiated BALB/c recipient.

There was considerably more variation in the Ig-*1b* serum levels in the mix-

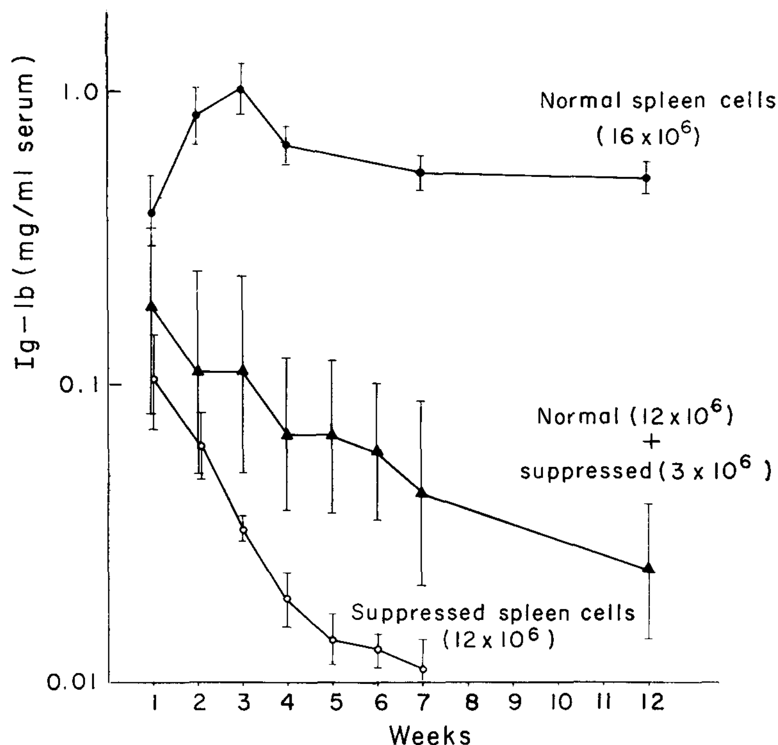

FIG. 1. Suppression of Ig-*1b* production in lethally irradiated Ig^a (BALB/c) recipients of a syngeneic mixture of normal and chronically suppressed (SJL × BALB/c)F_1 spleen cells. Recipients irradiated with 600 R total body irradiation approximately 18 hr before transfer. Ig-*1b* levels determined by inhibition of ^{125}I precipitation. Each point is the geometric average of Ig-*1b* level in four or five recipients. Bars represent ±SEM.

ture group than in either of the control groups, hence the larger standard errors of the means for this group. None of the mixture animals, however, aside from the first 2 wk after transfer, ever showed levels of Ig-*1b* which exceeded one-half the level of the lowest normal control, despite the fact that the mixture animals received 1.2×10^7 normal cells while the normal controls received 1.6×10^7 cells from the same suspension.

Persistence of Transferred Cells from Chronically Suppressed F_1 (SJL × BALB/c) in Parental (BALB/c) Recipients.—Using a recipient from the preceding experiment, the persistence of F_1 cells after disappearance of the sup-

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pressed Ig-*1b* (SJL allotype) marker was demonstrated by a second passage into irradiated BALB/c mice. At 12 wk after transfer, a recipient of 1.2×10^7 spleen cells from a chronically suppressed F_1 (suppressed control group) was sacrificed; 10^7 spleen cells from this mouse were injected alone into each of five irradiated BALB/c mice, and, in combination with 10^7 normal F_1 spleen cells, into another five recipients. Three control mice each received 10^7 normal cells. As the data in Table V show, those animals receiving only passaged suppressed cells made low, but clearly measurable, Ig-*1b*. At 2 wk, the levels were approximately equal to those for the similar time point in the first passage. Thus the disappearance of circulating Ig-*1b* in an original recipient is due to suppression of Ig-*1b* production rather than to rejection of the spleen cell graft.

TABLE V

Survival of Active Suppression through Passage

Donor cells	No. of cells	No. of recipients*	Ig-*1b* (weeks)		
			1	2	10
			mg/ml	*mg/ml*	*mg/ml*
Normal (SJL × BALB/c)F_1 spleen	10^7	3	0.06 (0.05–0.08)‡	0.68 (0.64–0.73)	0.51 (0.49–0.54)
Suppressed§ spleen (2nd passage)	10^7	5	0.02 (0.02–0.02)	0.09 (0.07–0.11)	0.07 (0.04–0.15)
Normal + suppressed (2nd passage)	10^7 + 10^7	5	0.05 (0.04–0.06)	0.20 (0.18–0.23)	0.11 (0.08–0.15)

* All transfers into (600 R) irradiated BALB/c (Ig-*1a*) recipients.

‡ Geometric mean (±SE).

§ Chronically suppressed (SJL × BALB/c)F_1 spleen transferred into irradiated (600 R) BALB/c (Ig-*1a*) recipient and then, 12 wk later, retransferred into similar recipients.

Not only does the ability to produce Ig-*1b* reappear in the second transfer of suppressed spleen cells, but along with it is carried the ability to suppress Ig-*1b* production. The inability of the group receiving a mixture of 10^7 normal and 10^7 suppressed cells to produce levels equivalent to the group receiving 10^7 normal cells indicates the survival of cells capable of bringing about suppression in the second passage.

As in preceding experiments, graft survival was also verified by measuring levels of Ig-*4b*. As the data in Table VI show, 13 of the 15 irradiated (600 R) BALB/c recipients of F_1 (SJL × BALB/c) cells had levels of circulating Ig-*4b* at 7 and 12 wk after transfer comparable to or higher than normal intact F_1 animals. The remaining two were negative for Ig-*4b* by 7 wk after transfer, indicating rejection of the graft. As stated above, these two animals were excluded from the experiment.

Suppression of Ig-1b Anti-Sheep Erythrocyte Plaque-Forming Cells.—The

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suppression of Ig-*1b* production may also be demonstrated in terms of the inability of suppressed cell populations to produce Ig-*1b* antibody to SRBC. Fig. 2 shows the results obtained after transfer of primed spleen cells from suppressed and normal mice into irradiated normal syngeneic recipients. A chal-

TABLE VI
Persistence of Spleen Cell Grafts as Measured by Production of a Nonsuppressed Paternal Immunoglobulin

Cell donor	Recipient No.	Ig-*4b* levels in recipients* (weeks after transfer)			Persistence of graft
		1	7	12	
Normal	1	0.58‡	1.3	1.3	+
	2	0.39	1.9	1.2	+
	3	0.43	3.2	2.0	+
	4	0.36	1.4	1.1	+
	5	0.54	1.3	N.D.	+
	$\bar{X}$	0.45§	1.7	1.4	
Suppressed + normal	6	0.54	<0.1	<0.1	−‖
	7	0.77	1.5	2.1	+
	8	0.58	2.9	1.9	+
	9	0.51	1.2	0.9	+
	10	0.61	3.2	1.6	+
	$\bar{X}$	0.61	2.0	1.5	
Suppressed	11	0.83	1.2	N.D.	+
	12	0.61	<0.1	<0.1	−
	13	0.96	1.3	2.4	+
	14	0.54	0.8	1.6	+
	15	0.70	1.5	1.7	+
	$\bar{X}$	0.71	1.2	1.9	
Normal intact F_1	$\bar{X}$¶	0.9			

* Ig-*4b* levels in individual recipients from mixture experiment described earlier. Spleen cells from normal (SJL × BALB/c)F_1, chronically suppressed (SJL × BALB/c)F_1, and 3:1 normal:suppressed mixture were transferred into (600 R) irradiated BALB/c recipients. N.D., not determined.

‡ Ig-*4b* mg/ml serum, determined by inhibition of ^{125}I precipitation.

§ Geometric mean.

‖ Data from animals in which graft did not persist were excluded from all calculations.

¶ Mean of 18 normal F_1 animals of greater than 3 months of age.

lenge dose of SRBC was given at time of transfer. The values given are for 7S-producing PFC only. The anti-*b* allotype antiserum used develops only plaques made by Ig-*1b*–producing antibody-forming cells (i.e. γG_{2a} PFC of *b* allotype). The anti-*a* allotype serum used reacts with both Ig-*1a* and Ig-*4a* and therefore develops both γG_{2a} and γG_1 plaques, which probably accounts for the high *a*/*b* ratio of PFC in cell suspensions from normal heterozygotes.

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Comparison of suppressed and nonsuppressed donors shows that while the recipients of spleen cells from preimmunized normal donors made both *a* and *b* plaques, recipients of spleen cells from primed suppressed donors made normal numbers of *a* plaques but few or no detectable *b* plaques (see Fig. 2).

The appearance of a low number of Ig-1*b* antibody-forming cells in some of the recipients of primed suppressed spleen cells is consistent with the appearance of normal Ig-*1b* in the sera of recipients of nonimmunized suppressed cells early after transfer. The *b* response seen in these mice probably constitutes a low 7S

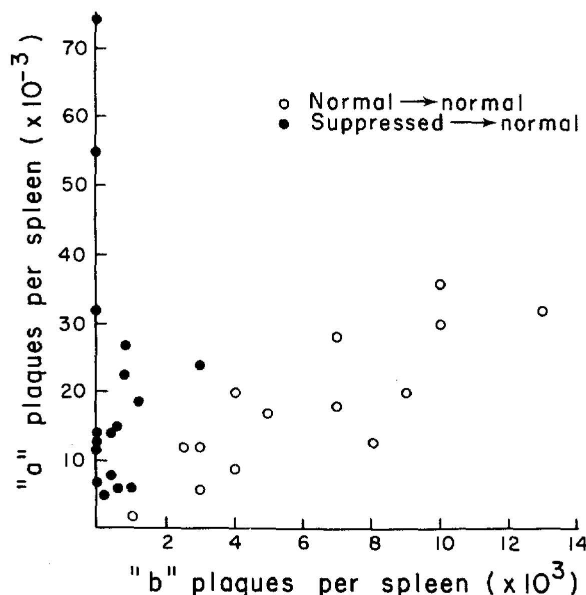

FIG. 2. Development of 7S PFC in irradiated BALB/c recipients of primed cells from (SJL × BALB/c)F_1 (Ig^a/Ig^b) mice. Donor cells were obtained from normal (○) or chronically suppressed (●) heterozygotes immunized 4–105 days previously with 4 × 10^8 SRBC. A challenge dose of 4 × 10^8 SRBC was injected together with the primed cells, and the number of antibody-forming cells was determined 7 days later.

primary response and does not reflect the presence of primed Ig-*1b*-producing cells in suppressed mice.

Injection of Suppressed Serum into Normal Mice.—Since suppressed cells were capable of carrying over their ability to suppress into secondary hosts, an attempt was made to "passage suppression" by serum alone.

Groups of young normal (SJL × BALB/c)F_1 mice, age 3–4 wk, were injected intraperitoneally (i.p.) with a serum pool obtained from chronically suppressed mice. The mice were injected every 2 days with 0.1–0.3 ml of serum for 3 wk and were bled and tested at weekly intervals. Control mice were injected with the same amounts of normal BALB/c serum. No reduction in the level of Ig-*1b*

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in the recipients of suppressed serum resulted from this regimen; hence if suppression is mediated by a circulating serum factor, it is either unstable or a more subtle means of detecting it must be found.

DISCUSSION

The normal process of differentiation to synthesis of γG or γA immunoglobulin (or antibody) involves the commitment of an originally totipotent B stem cell to the production of a large amount of a unique immunoglobulin molecule. During this process, one of the two autosomal chromosomes carrying H-chain determinants is selected, the variable region is determined, and one of the four linked genes for H-chain constant regions (in the mouse Ig-*1* to Ig-*4*, specifying γG_{2a}, γA, γG_{2b}, and γG_1, respectively) paired to it in a manner which allows for the transcription of the entire unit as a single messenger. In similar fashion a unique light-chain messenger is transcribed. Light and heavy chains are then produced, assembled, and exported, while the cell differentiates further to a mature plasma cell. While recent evidence (3, 4) indicates that during this process some or all cells may go through a stage of γM production before selection of the final γG or γA class without change in the variable region, this does not substantially alter the above description.

Almost nothing is known of the mechanisms involved in this process. If the insertion of a γM step proves correct, then chromosome exclusion and variable region choice occur earlier than selection of H-chain class. In any event, at the end of the process, a single cell produces a unique immunoglobulin whose H-chain is determined by a gene (or genes) on only one of the two parental chromosomes (5). No other such system for exclusion of autosomal genes is known in mammalian cells.

In an allotype heterozygote, such chromosomal (or allelic) exclusion leads to the presence of two populations of immunoglobulin-producing cells for any given class, one making immunoglobulins of paternal allotype and the other of maternal allotype. Usually an animal has a characteristic ratio of cells producing each of the parental allotypes, and circulating immunoglobulins reflect this cellular ratio (6). While there is little understanding at present of how this ratio is maintained, the regularity with which certain allotypes (and classes) predominate over others suggests that there is more than just random selection operating.

A better understanding of the way in which this normal balance of immunoglobulin-producing cells is established may be obtained by studying the mechanism(s) of allotype suppression, which creates a disruption of this process, leading to a specific decrease both in the number of cells making the suppressed allotype (7) and the amount of that allotype found in circulation (8, 7, 1). Unfortunately, such studies have been severely hampered since rabbits, which exhibit long-term (chronic) suppression, are not inbred, while inbred mice showed only short-term suppression, lasting until 8–10 wk of age (8). The dis-

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covery of a mouse strain combination, SJL♂ × BALB/c ♀, in which at least half the progeny of mothers immunized against a paternal allotype become phenotypically negative (chronically suppressed) for that allotype at some time during their life (1) has resolved this problem.

In this publication we have presented evidence that spleen cells from chronically suppressed mice transferred into irradiated "indicator" hosts show an immediate period of production of the paternal (Ig—*1b*) allotype, indicating clearly that Ig-*1b* progenitors are present and capable of differentiating to production in an appropriate environment. Synthesis of the suppressed allotype terminates, however, within 1–3 wk, even though transferred cells (as demonstrated by other markers) survive indefinitely and spleen cells from normal animals transferred into identical hosts continue to produce Ig-*1b*. Thus, either the cells are intrinsically unable to sustain Ig-*1b* production or another cell type in the transferred suppressed spleen population prevents Ig-*1b* production after a short period. The latter possibility was the most likely, in view of the inability of normal spleen cells to sustain Ig-*1b* production in suppressed hosts (1). This possibility was tested more directly by transferring a mixture of syngeneic spleen cells from suppressed and normal animals. As in the case of transfer of suppressed spleen cells alone, Ig-*1b* production by the mixture of cells occurred immediately after transfer and decreased rapidly (or ceased) shortly thereafter. In subsequent experiments (not presented here) we have found that the amount of Ig-*1b* produced depends in part on the ratio of suppressed spleen cells to normal spleen cells.[2]

Thus spleens of suppressed animals not only contain a population of progenitors of Ig-*1b*–producing cells but also contain a population of cells capable of preventing these progenitors from expressing their potentiality. Whether the suppressors act by direct cell-to-cell interaction, or produce a factor which conditions the environment, is as yet unclear. While the lag in expression of suppression might indicate the latter, as yet it has not been possible to suppress Ig-*1b* production by transfer of large amounts of serum from suppressed animals. In any event, since injection of large amounts of Ig^b serum before cell transfer does not relieve suppression, we can be fairly certain that if a serum factor is being produced, it is not simply antibody to Ig-*1b* as it appears on circulating globulins. This does not rule out the existence of an antigen unique to the progenitors of Ig-*1b*–producing cells, e.g. cell-bound Ig-*1b* with a different configuration, which identifies the progenitors for the suppressor.

The fact that retransfer of suppressed spleen cells alone and in mixtures with normal spleen cells leads to a repeat of the transfer sequence, i.e. Ig-*1b* production and subsequent termination, suggests strongly that the suppressing cells are able to proliferate and that the effectors of suppression seen in the recipients are similar to those in the original suppressed animals. The specificity of the sup-

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[2] Herzenberg, Leonore A., R. Riblet, and Leonard A. Herzenberg. Manuscript in preparation.

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pressor population for Ig-*1b*-producing cells reflects the specificity of the original maternal antibody, which we have shown to react detectably only with Ig-*1b*.

From the data available thus far it is not possible to determine whether the transferred Ig-*1b* progenitor cells have already passed through the allelic exclusion stage, no less whether they are already committed to Ig-*1b* production and await only the trigger to begin differentiation toward actively synthesizing plasma cells. It is possible that the suppressor cell exerts a static action on cells just before the final differentiational pathway, but equally possible that the suppressor destroys or diverts any cell which becomes committed to production of the suppressed allotype. We can state only that along the normal pathway from uncommitted progenitor to Ig-*1b* producer, the cell passes through a stage where it is identifiable as committed or able to be committed to Ig-*1b* production, and that the suppressor (cell or factor) can recognize the cell at this stage and prevent it from continuing its progression to synthesis of Ig-*1b*.

The nature and mode of action of the suppressor also remains to be elucidated. Preliminary experiments indicate that it is a thymus-derived (θ-bearing) cell present in reasonable numbers in thymus as well as in spleen from 6-month-old suppressed mice.[2] As mentioned earlier, this cell appears to be capable of proliferation. How it arises as a consequence of exposure of the young animal to anti-allotype antibody, and whether it has a normal counterpart which, for example, regulates the level of Ig-*1b* in serum, has yet to be determined.

Preliminary evidence suggests that chronic allotype suppression in mice can be induced only when SJL mice are used as one of the parent strains. Since SJL mice are known to suffer from severe fluctuating disgammaglobulinemias (9) and to ultimately die of reticulum cell sarcoma (10), it is possible that the heterozygote has a faulty immunoglobulin regulation system as well, which reacts to perturbation by the initial maternal Ig-*1b* by producing an overactive Ig-*1b* regulating mechanism.

SUMMARY

The mechanism of chronic allotype suppression in (SJL × BALB/c)F_1 mice has been investigated by means of cell transfer studies.

These mice are phenotypically negative for serum Ig-*1b*, the paternal allotype determinant on γG_{2a} immunoglobulin, as a result of perinatal exposure to maternal anti–Ig-*1b*.

When spleen or bone marrow (B) cells from suppressed mice were injected into irradiated BALB/c "indicator" hosts, detectable levels of Ig-*1b* were demonstrated in the sera of a majority of the recipients early after transfer. These results indicate that Ig-*1b*-producing cells or their precursors are present in the lymphoid tissues of suppressed mice, even though they are not expressed.

Within 5–7 wk, it was no longer possible to detect Ig-*1b* in the sera of these hosts, although cells producing another paternal allotype (Ig-*4b*) were shown to persist. Control BALB/c mice, injected with spleen and B cells from normal mice, continued to produce high levels of immunoglobulin carrying this allotype.

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The disappearance of serum Ig-*1b* occurred most frequently in the recipients of suppressed spleen cells.

Similar results were obtained using a mixture of spleen cells from normal and suppressed mice. Ig-*1b* production in the recipient mice ceased within a few weeks, even though the majority of cells in the mixture were obtained from normal (nonsuppressed) donors.

The data are interpreted as evidence that chronic allotype suppression in mice is actively maintained by cells which are resident in the lymphoid tissues, splenic cells being the most effective. These cells are capable of proliferating in a new host and exerting their suppressive influence on Ig-*1b*-producing cells and/or their precursors.

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