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Regulation of Immunoglobulin and Antibody Production by Allotype Suppressor T Cells in Mice

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Previous studies have shown that perinatal exposure of (SJL × BALB/c)F_1 mice to antibody to the paternal Ig-1b (IgG_{2a}) immunoglobulins generates a population of suppressor T lymphocytes which completely suppress production of Ig-1b globulins in the majority of exposed mice over the age of six months (Jacobson & Herzenberg 1972, Jacobson et al. 1972, Herzenberg & Herzenberg 1974, Herzenberg et al. 1973). The suppressor T cells, which are demonstrable in all lymphoid tissues of suppressed mice, also suppress production of Ig-1b immunoglobulins by co-transferred normal cells in adoptive transfer assays, although co-transfer of sufficient numbers of cooperator T cells from the normal donors can prevent the suppression (Herzenberg & Metzler 1974).

The suppressor population, once induced, is maintained for the life of the animal and often completely suppresses production of the allotype as the animal ages (Jacobson & Herzenberg 1972, Herzenberg & Herzenberg 1974). It is active when transferred to young normal syngeneic mice (i.e., mice never exposed to anti allotype antibody) and may be repeatedly passaged in such mice. When mixed with spleen cells from normal mice and transferred to irradiated recipients, it specifically suppresses production of the target allotype whether measured as reduction of the total serum level of the allotype or reduction of an allotype marked antibody produced in response to specific antigenic stimulation.

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The evidence that the suppressor is a T cell may be summarized as follows: 1) anti Thy 1.2 (Θ) treatment removes suppressor cell activity from suspensions of lymphoid tissues; 2) suppressor activity is recovered in effluents of lymphoid cells passed through nylon wool columns (these columns retain B cells, but not T cells (Julius et al. 1973)); and 3) neonatal thymectomy prevents development of suppression (Herzenberg & Herzenberg 1974).

Thus far we have demonstrated suppressor T cells in only one hybrid (SJL × BALB/c) and long-term ('chronic') allotype suppression in these hybrids for only one H-chain class of immunoglobulins, γG_{2a} which carries the Ig-1a and Ig-1b allotypes. We have looked for 'chronic' Ig-1b suppression in other BALB/c hybrids, and for suppression of γG_1 allotypes in the same hybrid, but have found little or no evidence for 'chronic' suppression except for this case (Herzenberg & Herzenberg 1974). Perhaps this is because suppressor cells exist only in this restricted situation, but we should point out that we have by no means searched exhaustively for conditions to establish 'chronic' suppression in other cases. The ease of obtaining large numbers of completely suppressed animals has tended to channel the major part of our efforts into studying this system as a potential model for regulation of immunoglobulin synthesis by T cells in the hope that information gained here might be useful in systems where suppressor cells are less easily demonstrated.

The reasons for this qualification become apparent when the quantitative aspects of the suppressor activity are considered. The SJL × BALB/c hybrid is similar to all other hybrids between Ig^b carrying males and BALB/c females in that after exposure to maternal anti Ig-1b it shows a short delay ($\sim$ 3weeks) in onset of Ig-1b production. But unlike the other hybrids, the SJL × BALB/c recover poorly from this short-term suppression. Instead, they continue to show varying degrees of impairment of Ig-1b production throughout life. By six months of age, less than half the exposed animals in this cross are able to produce detectable Ig-1b levels in circulation. The rest are completely suppressed (Jacobson & Herzenberg 1972).

The serum Ig-1b levels for a litter of SJL × BALB/c hybrids exposed to maternal anti Ig-1b are shown as a function of age in Table I. There is considerable variability in the degree of suppression seen, from animals who are completely suppressed throughout life to those who show little or no decrease in serum Ig-1b whenever tested. The litter presented in Table I has a larger proportion of completely suppressed mice than is observed when data from many litters are pooled. The usual partition is about 10 per cent completely suppressed, 50 per cent producing some Ig-1b before six months of age but little or none thereafter, and the remainder producing Ig-1b throughout life though often at reduced levels (Jacobson & Herzenberg 1972).

In general, those animals whose Ig-1b levels have dropped below detect-

TABLE I

Ig-1b Production in a Litter of (SJL × BALB/c)F_1 Offspring Born to a BALB/c Female Immune to Ig-1b

Mouse	Age (wk)											
	4	5	6	7	8	9	10	13	19	22	40	48
1–3*	–**	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	.01	–	.01	–	–	–
5	–	.05	–	–	–	–	–	–	..	..	–	..
6	–	–	–	–	–	–	–	–	> .5	Dead		
7	–	–	–	–	–	> .5	> .5	.05	–	–	–	–
8	–	–	–	–	> .5	.05	.05	.05	> .5	–	–	–
9	.05	.05	.01	–	> .5	> .5	> .5	.05	–	.05	–	–
10	.01	.01	.01	.01	> .5	> .5	.14	.14	..	> .5	> .5	> .5
11	–	–	–	–	> .5	> .5	.14	> .5	> .5	> .5	.14	> .5
12	.14	> .5	.14	.05	> .5	> .5	.14	.01	> .5	> .5	> .5	> .5
13	–	–	–	–	> .5	> .5	> .5	–	.05	> .5	> .5	> .5

* These mice, phenotypically negative for the paternal allotype (Ig-1b), were backcrossed to the maternal (Ig^a) strain, and the presence of the paternal (Ig^b) allele was confirmed.

** Levels determined by immunodiffusion. – = < .01. .. = not tested.

ability (< 0.01 mg/ml) by six months of age will remain completely suppressed for life. Some will go through periods when Ig-1b is detectable in their serum, but these episodes are usually brief.

We have adopted the term' chronically' suppressed to describe those animals without detectable Ig-1b in serum even though nearly all exposed animals show some degree of long-term (or chronic) suppression. This hairsplitting definition was required for practical reasons, since we tend to use only 'chronically' suppressed animals for cell transfer studies. These are always tested for Ig-1b just prior to transfer to minimize the chance that a donor is not completely suppressed.

The non 'chronically suppressed' animals have received much less analytical attention thus far although they may prove to be quite useful for regulatory studies. Experience has shown that if their allotype levels have not dipped below detectability by six months of age, the serum Ig-1b will generally be maintained at roughly the six month level for the rest of the animal's life, even if that level is only 1/10 the level in normal hybrids.

This curious ability to maintain relatively constant low allotype levels was the first indication that allotype suppression might represent a regulatory mechanism which is stimulated to a greater or lesser degree in various animals. Further evidence along the same line comes from cell transfer experiments which show that recipients of low doses of suppressor cells often show a similar partial suppression of allotype production over a long period of time.

Transfer of lymphoid tissue from 'chronically suppressed' donors into young, normal syngeneic recipients (2 week) results in long-term suppression of the recipients. The pattern of suppression is shown as a function of cell dose in Table II. At the highest cell dose, no Ig-1b becomes detectable in the serum. At intermediate cell doses, Ig-1b synthesis begins at the normal time (there is no short-term suppression in these animals) but soon falls off. By six months of age, no allotype is detectable in circulation. At the lowest cell doses, none of the animals becomes completely suppressed but many show significant suppression of production, especially after six months of age.

A similar dose dependence occurs in irradiated recipients of mixtures of normal spleen cells with spleen or other lymphoid cells from suppressed donors. Recipients of intermediate doses frequently maintain low-level allotype production over long periods of time (see Table III).

These experiments provide reasonably good evidence to support the hypothesis that allotype levels in those animals not completely suppressed by perinatal exposure to maternal anti-allotype antibody (i.e., non-chronically suppressed mice) are regulated by smaller numbers of suppressor cells otherwise similar to those found in 'chronically' suppressed mice. In both kinds of

TABLE II

Transfer of Spleen Cells from Suppressed Donors to 2 Week Old Syngeneic Normal Recipients

Suppressed[1] Spleen Cells Transferred ($\times 10^6$)	No. of[2] Recip.	Mean Ig-1b in Serum (mg/ml)[3] Age (Weeks)					
		8	10	12	16	20	24
15	30	0	..	..	0	..	..
5	10	<.06	.2	.4	<.09	<.09	<.08
1	10	.14	.4	<.5	<.5	<.5	<.5

[1] Donors were SJL × BALB/c exposed to maternal anti Ig-1b over 6 months of age with <.01 mg/ml Ig-1b in circulation.
[2] Estimated by immunodiffusion. (0) = <0.1 mg/ml. .. = Not tested.
[3] Recipients were 2 week old normal (SJL × BALB/c) hybrids.

TABLE III

Titration of Suppressor Activity in Spleen of Chronically Suppressed SJL × BALB/c Mice

Spleen Cells Transferred[1]			Mean Ig-1b in serum (mg/ml)[2] Weeks After Transfer				
Normal ($\times 10^6$)	Suppressed ($\times 10^6$)	No. of[3] Recip.	1	2	3	4	6
12	10	4	>.23	.03	0	0	0
12	3	4	>.41	.1	.12	.07	.01
12	1	4	>.5	>.5	>.5	>.41	>.17
12	–	4	>.5	>.5	>.5	>.5	>.5

[1] Suppressed Donors: see legend, Table I
Normal Donors: SJL × BALB/c, 4 months of age.
[3] Recipients: Irradiated (600R) BALB/c, transferred i.v. ~ 18 hours post irradiation.
[2] Estimated by immunodiffusion. (0) = < 0.1 [(0) = < 0.1 mg/ml.]

mice, the size (or activity) of the suppressor population might well be determined early in life (as in the case of the young recipients of suppressor lymphoid cells) although it reaches its full expression only as the animal ages.

To look for suppressor cells in partially suppressed mice we tried using them as donors in a co-transfer experiment (i.e., suppressed spleen plus normal spleen transferred to irradiated recipients). The marginal suppression which resulted was consistent with there being fewer or less active suppressor cells in these donors. But because we were somewhat limited as to the maximum number of cells we could transfer, (and because we didn't work on these

TABLE IV

Specificity of Suppression in the Adoptive Secondary Response to DNP-KLH

SJL × BALB/c Spleen Cells Transferred (× 10^6)			Indirect DNP-PFC*			
DNP-KLH 1° Normal	Unprimed		Plaquing** day	Ig-1a + Ig-4a	Ig-1a	Ig-1b
	Normal	Suppressed				
12	10	—	5	4,500	400	500
12	—	10	5	2,900	520	60
12	10	—	7	6,600	800	600
12	—	10	7	8,700	770	<5

* Indirect DNP-PFC/10^6 pooled recipient spleen cells.

** Recipients were tested for DNP-PFC response on indicated day after transfer.

experiments exhaustively) we were unable to state how many cells might have been present.

Determining the amount of suppressor activity in these animals, however, may tell only part of the story. We have now obtained evidence from experiments on suppression of an adoptive hapten-carrier secondary response which suggests that the level of T cell cooperator activity in suppressed animals also plays an important role in regulating the production of Ig-1b.

Suppression of Ig-1b Adoptive Secondary Response to DNP

In these studies, we used the Ig-1b adoptive secondary response to dinitrophenyl-Keyhole Limpet Hemocyanin (DNP-KLH) since in this assay the doses of suppressor cells, hapten primed B cells and carrier (KLH) primed T cells could be varied independently (Mitchison 1971). The data in Table IV show that the Ig-1b response to DNP (measured as the number of cells making Ig-1b antibody to DNP (Ig-1b DNP-PFC) per 10^6 recipient spleen cells) is suppressed in irradiated BALB/c recipients when spleen cells from chronically suppressed (SJL × BALB/c)F_1 mice are transferred together with primed (DNP-KLH) spleen cells containing both carrier primed cooperator T cells and hapten primed B cells from non-suppressed syngeneic F_1 donors. Recipients of spleen cells from DNP-KLH primed non-suppressed donors made roughly 500 Ig-1b DNP-PFC per million spleen cells, whereas recipients of the same number of these primed non-suppressed cells given, in addition, 10^7 spleen cells from suppressed animals made fewer than 20 Ig-1b DNP-PFC per million on day 7 after transfer.

The activity of these suppressor cells is directed only against the formation of Ig-1b PFC and not against the formation of Ig-1a PFC (also see Table IV). Recipients of 12×10^6 normal (non-suppressed) primed spleen cells plus 10×10^6 normal unprimed (SJL × BALB/c) spleen cells had similar numbers of both Ig-1a and Ig-1b PFC on both day 5 (400 vs. 500) and day 7 (800 vs. 600). Addition of 10×10^6 suppressed unprimed spleen cells, however, resulted in a 100-fold reduction in Ig-1b DNP-PFC by day 7 while leaving the Ig-1a response unaffected. Thus, the secondary Ig-1b DNP response is specifically suppressed and the suppressed mice do not compensate for the decreased production of Ig-1b PFC by increasing the number of Ig-1a PFC in order to maintain their level of γG_{2a} PFC.

Suppressor-Cooperator Antagonism

To study the quantitative interactions between suppressors, cooperators and primed B cells, the adoptive co-transfer assay was modified from the protocol used in the preceding experiments so that numbers of cells of each of these three cell populations could be varied independently and titrated at limiting numbers against the others. Rather than using DNP-KLH primed spleen cells as a source of both carrier primed cooperator T cells and DNP memory B cells, we used spleen cells from short-term KLH primed mice as a source of cooperator T cells and used T-depleted (anti Thy-1 treated) spleen cells from long-term DNP-KLH primed mice as a source of the DNP memory cells. Spleen cell suspensions from unprimed suppressed mice were used as in the previous experiments, as a source of suppressor T cells. None of these cell suspensions showed any measurable IgG DNP-PFC response when transferred alone.

Dose ranges for suspensions of each cell type were established in preliminary experiments. Then in one large experiment, and a repeat experiment, primed B cells, suppressors and cooperators were transferred, each at several doses, to irradiated recipients. The recipients were challenged on day 0 with DNP-KLH and, as in previous experiments, the number of Ig-1a, Ig-1b and total IgG DNP-PFC in recipient spleens were determined 7 days after transfer. Data from these experiments are presented in the following sections.

1) *Titration of T cooperators and B memory cells in the absence of suppressors*

The data in Figures 1a and 1b show that the number of Ig-1b DNP-PFC obtained at doses of cooperator T and B cells below saturation is proportional to both cell doses. At each of the cooperator cell doses, the number of PFC

TABLE V

Titration of 1° B Cells, Cooperator T Cells and Suppressor T Cells

SJL × BALB/c Spleen Cells Transferred (× 10^6)			Indirect DNP-PFC*					
			Experiment 1			Experiment 2		
Normal DNP-KLH 1° T-depleted B	Normal KLH 1° – T_C	Supressed Unprimed – T_S	Total IgG	Ig-1a	Ig-1b Obs.	Ig-1b Exp.	Ig-1b Obs.	Ig-1b Exp.
5	—	—	<10	<10	<10	—	—	—
5	12	—	4320	416	485	780	560	900
5	12	5	3780	402	15	130	170	187
5	12	2.5	4120	398	406	455	489	543
5	12	1.25	4002	345	460	617	510	721
5	6	—	4150	390	420	390	490	450
5	6	5	3700	380	<10	<0	<10	<0
5	6	2.5	3780	390	61	65	78	94
5	6	1.25	3980	380	387	227	290	272
5	3	—	2080	210	190	195	215	225
5	3	5	1780	180	<10	<0	<10	<0
5	3	2.5	1800	189	25	<0	<10	<0
5	3	1.25	1880	201	28	33	40	47
2.5	6	—	1980	193	201	195	201	225
2.5	6	5	1690	187	<10	<0	<10	<0
2.5	6	2.5	1520	160	25	33	38	47
2.5	6	1.25	1680	144	132	114	145	136
2.5	3	—	1410	98	102	98		
2.5	3	5	1320	76	<10	<0		
2.5	3	2.5	1290	81	<10	<0		
2.5	3	1.25	1310	82	18	16		
1.25	6	—	998	121	102	98		
1.25	6	5	1010	131	<10	<0		
1.25	6	2.5	1150	98	<10	16		
1.25	6	1.25	1100	87	56	57		
1.25	3	—	430	39	28	29		
1.25	3	5	421	51	<10	<0		
1.25	3	2.5	421	56	<10	<0		
1.25	3	1.25	491	38	<10	<0		

* Indirect DNP-PFC/10^6 recipient spleen cells.

Excepted (Exp.) Ig-1b DNP-PFC = $k \cdot (B) \cdot (T_C - \alpha \cdot T_S)$. For Experiment 1, $k = 13 \times 10^{-12}$ and $\alpha = 2.0$; for Experiment 2, $k = 15 \times 10^{-12}$ and $\alpha = 1.9$.

increases linearly with primed B cell dose. The slope of each curve is proportional to the cooperator dose up to the highest B cell dose used (5×10^6). Similarly, the number of PFC obtained at each of the B cell doses increases linearly with cooperator T cell dose in the range below cooperator saturation (6×10^6 KLH primed spleen cells or less) and the slope is proportional to the B cell dose.

The cooperator titration at the highest B cell dose, 5×10^6 cells, was extended above saturation. In this range, it shows a sharp departure from the initial linearity. Doubling the T cell dose (from 6 to 12 $\times 10^6$) leads to less than a 15 per cent increase in the number of PFC obtained.

The titration data for Ig-1a and total IgG DNP-PFC in the absence of added suppressors parallels the Ig-1b titration data. The Ig-1a response is the same as the Ig-1b at all cooperator T and B cell doses, and each comprises roughly 10 per cent of the total IgG response (see Table V).

The linearity of the cooperator (T_O) and memory (B) cell titrations in dose ranges below saturation means that the PFC response in these dose ranges is predictable by the equation

$$(1) \qquad PFC = k \cdot (B) \cdot (T_O)$$

where k is an empirical constant which includes the constants for the actual numbers of memory B cells injected as opposed to the total number of T depleted primed spleen cells injected; the efficiency of conversion of memory cells to PFC; the actual number and efficiency of cooperators, etc.

All donors were SJL × BALB/c hybrid mice. Suppressed donors, 6 to 9 months of age, had no detectable Ig-1b in circulation at time of transfer. DNP-KLH primed donors received 100 μg of alum precipitated DNP-KLH 6 to 8 weeks prior to transfer. Primed B cells were obtained by treating spleen suspensions from DNP-KLH primed donors with anti Thy-1 plus complement. KLH primed donors received 100 μg alum precipitated KLH plus *B. pertussis* seven days prior to transfer.

All recipients were BALB/cNHz mice irradiated with 600R approximately 18 hours prior to transfer. Recipients were given 10 μg aqueous DNP-KLH on day of transfer and sacrificed for testing seven days later. Three to five recipients were used per group. Spleens were pooled prior to testing. Spleen size in recipients was similar in all groups.

DNP-PFC were estimated with TNP conjugated sheep erythrocytes in Cunningham chambers. Lysis of indirect DNP-PFC (IgG, Ia-1a and Ig-1b) was facilitated by including the appropriate specific facilitating antiserum with cells. TNP-erythrocytes and complement in the chamber. The number of direct DNP-PFC (no facilitating antiserum) was determined for each cell sample and subtracted from the response in the facilitated chambers to determine the number of indirect DNP-PFC. Direct DNP-PFC for Table V < 50 and Table IV < 150.

Response are expressed as DNP-PFC/10^6 recipient spleen cells.

Ig-1b PFC responses calculated with equation (1) for all doses of (T_C) and (B) in the absence of suppressors are presented in Table V next to the ob-

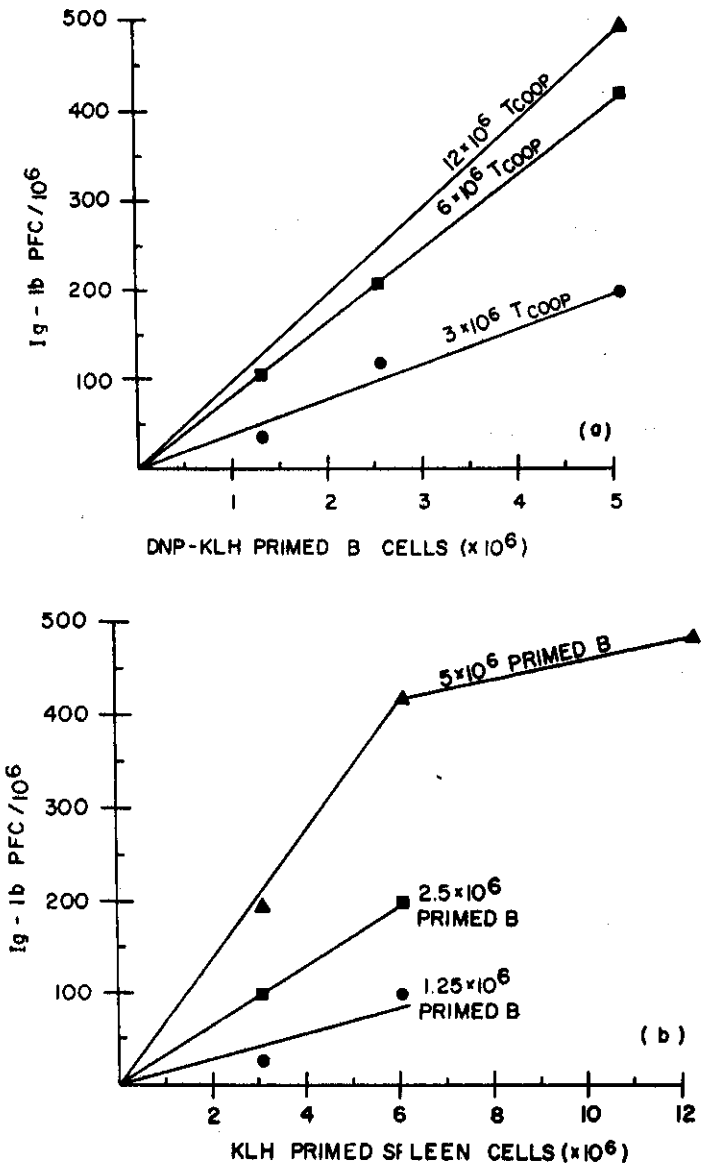

Figure 1. ***Ig-1b DNP-PFC response as a function of increasing dose of primed B cells or carrier primed T cells.*** For source of cells and conditions of transfer, see legend for Table V.

served responses for two titration experiments. As the data show, there is good argreement between the expected and observed responses except for the cooperator (T_O) dose above saturation (12×10^6), where the predicted values considerably exceed observed. (Predicted values for Ig-1b responses in the presence of suppressors are also presented in Table V. These were calculated using a modification of equation (1) which will be discussed in the next section.)

2) *Effect of suppressor T cells on the titrations of cooperator T cells and primed B cells*

There is a striking difference in the effect of suppressor T cells on the titrations of cooperator T cells and primed B cells which has significant implications for the mechanism of suppression. The B cell titrations remain essentially linear in the presence of graded doses of suppressors and show a decrease in slope roughly proportional to the number of suppressed spleen cells added. The cooperator titrations, however, go from linear to S-shaped curves in which the length of the region of increasing slope at low cooperator cell doses is roughly proportional to the suppressor cell dose and the slope of the linear portion is fairly similar to the slope of the linear T cell titration curve obtained in the absence of suppressors. These differences provide the rationale for a modification of equation (1) which accurately predicts the Ig-1b DNP-PFC response in the presence of suppressor cells (see Table V) and thus for a precise description of the quantitative interaction between suppressor and cooperator T cells.

Figure 2 shows the Ig-1b DNP-PFC response as a function of KLH primed spleen dose (T_O) at three dose levels of suppressed (T_S) spleen cells. To avoid presentation of essentially duplicate T_O titration curves at each of the B cell doses used, we have normalized the response to Ig-1b PFC/(5×10^6) B cells transferred and put all of the data from the experiment into this one figure. This is possible because, as the coincidence of the normalized point shows, the number of PFC obtained with any given pair of suppressor and cooperator doses is always proportional to the B cell dose. Both in this and other experiments, where we have extended the B cell dose range another two-fold, the T_O titration curves in the presence or absence of suppressor cells superimpose when the response is expressed as PFC/B cell transferred.

The strict proportionality between Ig-1b PFC response and B cell dose at various cooperator and suppressor cell doses suggests that the PFC response is an index of the cooperator activity available to facilitate B cell differentiation. Even at limiting doses of suppressors, increasing the B cell dose does not alter the fraction of the response which is suppressed (although it does pro-

portionally increase the total response). Since, as the data in Figure 2 show, increasing the cooperator dose compensates for the presence of suppressors and reduces the fraction of the response which is suppressed, it seems likely that B cells do not interact directly with suppressors but instead reflect the outcome of some type of interaction between suppressors and cooperators.

The quantitative nature of this interaction is descernible from the shape of the cooperator T cell titration curves shown in Figure 2. In the absence of suppressors, the response is proportional to cooperator dose between 0 and 6×10^6 T_C, (i.e., is linear with T_C and passes through the origin). Above 6×10^6 T_C, there is only a small increase in response with additional T_C, indicating that T_C saturation is reached at approximately at this dose. When suppressors are present, the response is no longer proportional to T_C dose. Instead, there is no detectable response with increasing T_C dose until a critical T_C dose is reached after which further addition of cooperators increases the response at roughly the same rate as in the nonsuppressed control until saturation for cooperators is reached. Thus, at low T_C doses the suppressor cells appear to neutralize (or remove) most of the cooperator activity so that the cooperator dose remains effectively at zero until the neutralization capacity of the suppressors is satisfied. Since the neutralization point (i.e., the displacement of the linear portion of the curve) is essentially proportional to the sup-

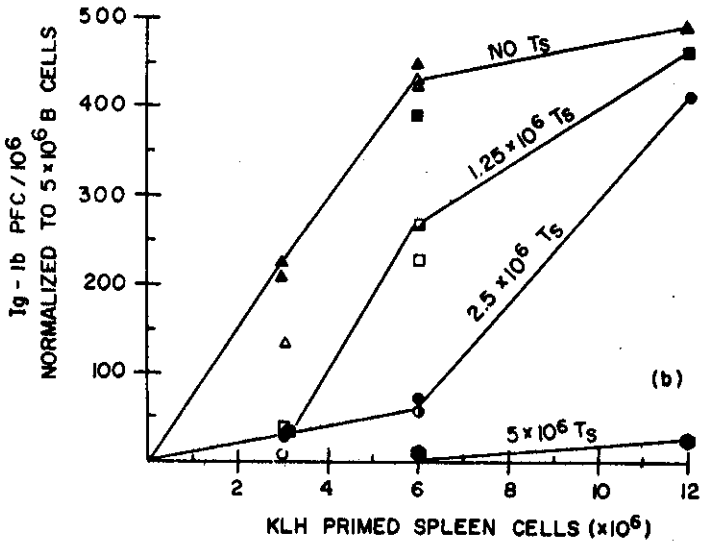

Figure 2. *Effect of addition of suppressor T cells on the Ig-1b DNP-PFC response, plotted as a function of increasing dose of carrier primed T cells.* Open symbols are for response with 5×10^6 B cells; closed and half-closed symbols are for response normalized to 5×10^6 B cells. For source of cells and conditions of transfer, see legend for Table V.

pressor dose, these data suggest that the removal of cooperator activity is proportional to suppressor concentration.

If cooperator activity (T_C) is indeed removed in proportion to the dose of spleen cells from suppressed mice (T_S) and if the Ig-1b response is determined by the residual cooperator activity, then equation (1), which predicts the PFC response at various T_C doses, should predict the Ig-1b PFC response in the presence of suppressor cells provided a term is included to correct for the loss of cooperator activity due to suppressors. Thus, if

(2) $$T_C \text{ lost} = \alpha \cdot T_S$$

where α is a proportionality constant which relates the relative effectiveness of the pools of cooperator and suppressor spleens cells used in the experiment then the residual cooperator activity is equal to the difference between the injected cooperator dose (T_C) and (T_C lost), i.e.,

(3) $$T_C \text{ left} = T_C - \alpha \cdot T_S$$

and the Ig-1b PFC response in the presence of suppressors will be predicted by the equation

(4) $$PFC = k \cdot (B) \cdot (T_C - \alpha \cdot T_S)$$

This equation may also be stated

(5) $$\frac{PFC}{B} = k \cdot (T_C - \alpha \cdot T_S)$$

3) *Estimation of loss of cooperator activity due to suppressor T cells*

The proportionality constant, α, from equations (4) and (5) can be evaluated either by substituting data points in the equation or by determining the amount of cooperator activity lost directly from the data (see below) and plotting this figure as a function of suppressor cell dose. According to equation (2), plotting (T_C lost) as a function of T_S should yield a straight line passing through the origin with a slope equal to α. Since plotting the data in this fashion also tests the basic assumption in this model, (i.e., that loss of cooperator activity is proportional to suppressor cell dose, and therefore that α is a constant), this graph is presented in Figure 3.

The amount of cooperator activity lost (T_C lost) was determined by subtracting the number of cooperator T cells required to give the observed response at each combination of cell dose from the cooperator dose injected using the non-suppressed cooperator dose response curve as a standard. Thus, at 5×10^6 B, 6×10^6 T_C and 2.5×10^6 T_S the number of PFC observed gave a response equal to that given by 0.8×10^6 T_C in the absence of added suppressor cells, i.e., (T_C left) $= 0.8 \times 10^6$. Subtracting this number from the number of T_C injected (6×10^6), (T_C lost) was found to be 5.2×10^6.

Similar calculations were made for all dose combinations with which the response was above background (> 10 Ig-lb PFC/10^6) but less than the saturation response.

As predicted, plotting (T_C lost) as function of (T_S) for both experiments shown in Table V yielded straight lines passing through the origin. The slopes of these lines, α_1 and α_2, differed only slightly, which is consistent with the similarity in cooperator activity and suppressor activity shown by the pools of KLH primed spleen cells and suppressed spleen cells used in the experiments. Thus, the loss in cooperator activity is proportional to the number of suppressed spleen cells transferred (T_S) over the entire range of suppressor concentrations.

Figure 3 also shows that loss of cooperator activity is independent of the cooperator dose. Regardless of whether the suppressor cells were transferred together with 3×10^6 or 1.2×10^7 cooperator spleen cells, for every 10^6 suppressed spleen cells transferred the cooperator activity lost was equivalent to that present in approximately 2×10^6 cooperator spleen cells. Thus although the extent of suppression is influenced by cooperator dose because the response is determined by residual cooperator activity, the amount of cooperator activity lost depends only on the cooperator neutralization capac-

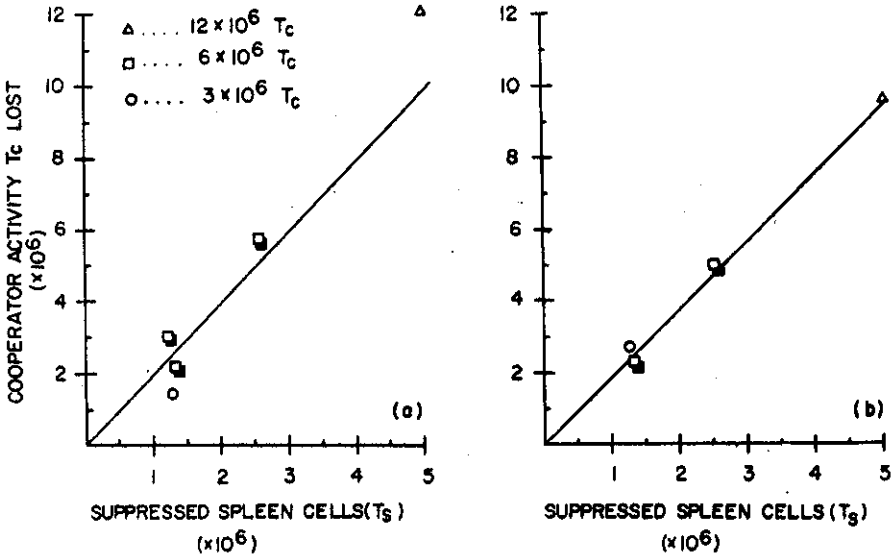

Figure 3. *Loss of cooperator T cell activity as a function of increasing suppressor T cell dose.* Loss of activity estimated by taking DNP-PFC response as an index of residual cooperator activity and subtracting this from the injected cooperator dose (see text for details). Open symbols are for response with 5×10^6 B cells; closed symbols are for responses normalized to 5×10^6 B cells.

ity of the suppressor cells transferred. Furthermore, since there is a fixed amount of cooperator activity lost per suppressor cell transferred, the suppressor cells must be stoichiometrically removing cooperator activity, and, even at low doses, must be functioning at maximum capacity.

4) *Comparison of observed and predicted responses*

In Table V, the predicted Ig-1b PFC responses based on equation (5) are presented next to the observed responses in two experiments. In Figure 4, theoretical response curves calculated with equation (5) are shown with the observed responses in Experiment 2. The data show quite good agreement between observed and expected responses at all combinations of cooperator, primed B cell and suppressor cell doses except where the expected response is above the number of PFC obtained at the saturating T cell dose. Even when the injected T_C dose is above saturation, as long as the number of suppressor cells reduces the level of cooperator activity to within the linear cooperator titration range, the Ig-1b PFC response is reasonably accurately predicted. Although, because of space limitation, we have presented the data from only two titration experiments, these findings are buttressed by similar

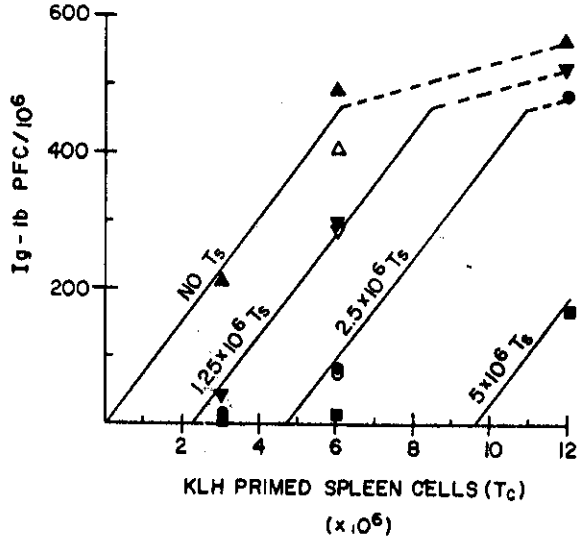

Figure 4. *Theoretically drawn curves for Ig-1b DNP-PFC response in presence of suppressor T cells with experimental data from suppressor-cooperator titration experiment.* Data was taken from Table V, Experiment 2. Open symbols are for responses with 5×10^6 B cells; closed symbols are for responses normalized to 5×10^6 B cells. Solid portion of curves were drawn from the equation $(PFC)/B = (15 \times 10^{-12}) \cdot (T_C - 1.9 \cdot T_S)$. Dotted portion of curves represents responses above T_C saturation which are out of range for the equation.

evidence from a number of additional experiments, in some where the dose range for B cells was extended another two-fold and in others where α was increased (up to 50-fold) by removal of non-suppressing cells but was constant for each preparation titrated. Thus, it appears that, if the Ig-1b PFC response may be taken as an index of residual cooperator activity, the mechanism of suppression of allotype synthesis by allotype suppressor T cells involves the stoichiometric removal of cooperator activity.

Reservations in Accepting Cooperator Neutralization Hypothesis

The question of how (or whether) allotype suppressor T cells reduce cooperator activity is a puzzle at this point. It is tempting to conclude that suppressor cells or their products (Jacobson 1973) directly inactivate cooperator cells or their products (Taussig 1974, Feldmann & Nossal 1972) since the data fit so well with this type of model as opposed, for example, to models in which suppressors compete with cooperators for a trigger site on a B cell or inactivate cooperator triggered B cells (see below). But aside from the fact that complex cell interaction such as that presented here is sufficiently fraught with interpretative difficulties to render any conclusion suspect, there is a basic problem with the concept of cooperator neutralization which must be resolved before such a hypothesis could be seriously accepted: for this model to work in allotype suppression, cooperator T cells or T cell factors capable of facilitating the differentiation of Ig-1b memory cells to Ig-1b producing cells would have to be committed to cooperate only with those cells.

The need to endow the target cooperator cells with specificity for Ig-1b derives from the well established fact that suppressor T cells generated by perinatal exposure to anti Ig-1b antibody suppress only Ig-1b production. Data presented in this publication, for example, show clearly that only Ig-1b DNP-PFC are suppressed. Ig-1a and γG_1 DNP-PFC production is unhampered at suppressor cell doses which completely eradicate the Ig-1b response. Thus, if suppressors directly neutralize cooperators or their products the cooperator activity removed must be responsible only for facilitation of Ig-1b memory B cell differentiation to Ig-1b producers.

This requirement for allotype specific cooperator T cells makes the cooperator neutralization hypothesis difficult, although not impossible, to accept. Data on immune deviation (Liu et al. 1974) and on cooperation in the IgE response (Kishimoto & Ishizaka 1973) suggest the existence of class specific cooperators. But no evidence whatever supports the postulation of allotype specific helpers. In fact, to the contrary, many laboratories (including our own) have shown that cooperation occurs easily between B cells and T cells each taken from one of a pair of allotype congenic strains. However, from

existing data we cannot exclude the idea that B cell products are involved in conferring specificity on the T cell, nor can we exclude even rather large quantitative differences in the effectiveness of cooperation across an allotype barrier. Therefore, it is possible that allotype specific T cells do exist, and that suppressors act by attacking these cells or their products.*

Sufficiently little is known about the way suppressor T cells recognize their targets that a hypothesis in which suppressors recognize cooperators, or cooperative factors, can be easily accommodated. At present we know only that although the suppressors are generated in response to exposure to anti Ig-1b antisera, suppression is not blocked by large amounts of circulating Ig-1b. In earlier reports (Jacobson & Herzenberg 1972), we have shown that the onset of suppression in mice perinatally exposed to anti allotype serum often occurs relatively late in life when the mouse has been actively synthesizing Ig-1b for some time and has a high serum Ig-1b level. We have also shown that Ig-1b immunoglobulin passively transferred to completely suppressed mice does not 'break' suppression, and that adoptive transfer of the Ig-1b DNP-PFC response is suppressed by suppressor T cells even when the transfer is made into irradiated recipients from a strain, BAB/14 (BALB/c-Ig^b), which have large amounts of circulating *b* allotype immunoglobulins including Ig-1b. While these data don't exclude surface bound Ig-1b as the way in which the suppressor T cell recognized its (T cell?) target, other kinds of recognition mechanisms certainly are possible within the current confines.

Originally, because of the specificity of suppression, we thought it likely that suppressors or suppressive factors reduce Ig-1b production by competing with cooperators or cooperator factors for some site, perhaps on B cells, which must be occupied by the cooperator for the successful triggering of differentiation of Ig-1b memory cells to Ig-1b DNP-PFC. This hypothesis, however, is not supported by the data from the titration experiments. If the loss of cooperator activity were due to competition between cooperators and suppressors then loss should be dependent in part on cooperator concentration. It should not be strictly proportional to suppressor cell dose as Figure 4 shows it is. Similarly, the Ig-1b response in suppressed recipients should not be predictable from equations (4) or (5).

Quite different responses are predicted by the equation for a competitive interaction between suppressors and cooperators. In this case, if β is a constant which (like α above) corrects the relative efficiencies of the suppressor and cooperator populations, then the fraction of sites occupied by cooperators (as opposed to suppressors) is given by the term

$$\frac{T_C}{T_C + \beta \cdot T_S}$$

* See page 82.

and the total number of sites captured by cooperators is equal to the product of the fraction of sites occupied by cooperator T cells and the dose of cooperators. Thus, if the PFC response is proportional to the sites occupied by cooperators, the equation

$$\frac{PFC}{B} = k \cdot (T_C) \cdot \frac{T_C}{(T_C + \beta \cdot T_S)}$$

should fit the data from the titration experiments. Attempts to use this equation to predict the Ig-1b responses shown in Table V however, were completely unsuccessful. β could not be evaluated as a constant, nor could it be given any simple regular function. This is consistent with the observation that suppression is independent of T_C dose. Thus the data from the experiments appear to be inconsistent with a hypothesis in which suppressor T cells compete with cooperator T cells.

Suppressor Cooperator Interaction at B Cell Surface

The specificity problem could also be resolved by envisioning a non-competitive type interaction of suppressor and cooperators (or their factors) on the B cell, e.g. at the surface. The suppressor could attack a cooperator product attached to an Ig-1b bearing cell, recognizing its target by virtue of closely juxtaposed surface bound Ig-1b. This seems unlikely since the extent of suppression is apparently totally independent of B cell dose. If the suppressor cell interacts with the B cell, it is difficult to frame a hypothesis which accounts both for the inability of increasing B cell doses to reverse suppression at limiting suppressor cell doses and the ability of cooperator T cells to reverse suppression at doses above saturation when B cell stimulation is maximal and the number of B cells being triggered is presumably constant. Despite these reservations, this type of hypothesis at present seems the most viable alternative to postulating an allotype specific cooperator T cell.

Mechanism of Suppression in Intact Suppressed Mice

In the previous experiments, the cells responsible for mounting the immune response in the adoptive recipient (i.e., primed B cells and cooperator T cells) were taken from non-suppressed donors and the suppressor T cells from unprimed donors so that the effects of the suppressor T cells on differentiation of memory B cells to Ig-1b antibody producing cells could be studied independent of any effect the suppressor cells might exert on priming of these populations. We have now completed a study with DNP-KLH primed suppressed mice which shows that the mechanism of suppression in intact DNP-KLH primed suppressed mice is indistinguishable from that found in the

adoptive co-transfer system. Our data indicate that B cell priming occurs normally in these mice although suppressor T cells are present throughout the priming period. Therefore, the observed inability of intact primed suppressed mice to produce Ig-1b antibody must result from the prevention of differentiation of B memory cells to Ig-1b antibody producing cells, just as it does in the co-transfer assay.

In earlier work, we demonstrated that when SJL × BALB/c mice are completely suppressed for Ig-1b allotype production, they have no detectable Ig-1b in serum, no Ig-1b producing plasma cells in spleen or lymph nodes, and only a very low probability of regaining the ability to produce circulating Ig-1b except in small amounts for relatively short periods of time (Jacobson & Herzenberg 1972, Herzenberg & Herzenberg 1974). Nevertheless these mice have normal numbers of Ig-1b bearing lymphocytes (Herzenberg et al. 1974). Consistent with this observation, current studies show that DNP-KLH primed suppressed mice develop normal Ig-1b memory which is carried by the Ig-1b bearing cells and is demonstrable in an adoptive secondary response if the suppressor T cells are removed prior to transfer (Okumura et al. 1975). To demonstrate that the Ig-1b bearing cells carry Ig-1b memory, we used the Fluorescence Activated Cell Sorter (FACS)[1] (Bonner et al. 1972) to isolate these cells from spleens of DNP-KLH primed suppressed mice and showed in adoptive transfer that they contain essentially all of the memory B cells which give rise to Ig-1b plaque forming cells (PFC) but no memory B cells for other IgG-PFC (Okumura et al. 1975). Thus in suppressed mice as well, we have narrowed the locus of suppressor cell attack to the latter part of the B cell differentiation pathway, somewhere after the appearance of the Ig-1b bearing memory cells but before differentiation of these cells proceeds to antibody producing cells. In other words, whether in both adoptive co-transfer and in intact suppressed mice, suppressor cells interrupt B cell differentiation at a stage very close to the point where cooperator cells interact to facilitate memory cell differentiation.

Finding normal Ig-1b memory cell development in suppressed mice confirms a suspicion we have had for some time that these mice have reasonably mature precursors of Ig-1b producing cells (Okumura et al. 1975). Data from early experiments (Okumura et al. 1975) showed that both intact (antibody exposed) suppressed animals and suppressed recipients of spleen cells from these animals retain the capacity for Ig-1b production. Occasionally spontaneous, short-term 'breaks' in suppression are found in a fair number of intact suppressed animals if these are tested frequently enough over a long period of time. Furthermore, when spleen cells from suppressed mice are transferred to irradiated BALB/c recipients, although these recipients soon become suppressed, sufficient Ig-1b is produced during the first week after

transfer to frequently render the serum level in these recipients indistinguishable from the Ig-1b levels in recipients of normal cells.

In contrast with Ig-1b production in recipients of normal spleen, the initial burst of Ig-1b production in recipients of suppressed spleen is short-lived. By 4 to 6 weeks after transfer, recipients of normal spleen have established and maintain a stable serum Ig-1b level about equal to the level in normal hybrids, while suppressed spleen recipients generally have no detectable Ig-1b in serum by this time. Transfer of spleen from these latter recipients, once they have become suppressed, again reveals a cryptic ability to produce Ig-1b. The second passage recipients show the same short burst of Ig-1b production prior to the onset of suppression as primary passage recipients, even when the second transfer is done as late as 12 weeks after the first.

Since suppressed animals have normal numbers of mature precursor B cells which can rapidly differentiate in the absence of suppressor cells to Ig-1b producing cells, the burst in Ig-1b production observed after adoptive transfer and the spontaneous breaks in suppression most likely represent the differentiation of the Ig-1b precursors allowed by a temporary alleviation of the suppression. The evidence from studies on suppressor: cooperator neutralization suggest that the conditions which allow Ig-1b precursor differentiation involve either increased cooperator or decreased suppressor activity. In the case of the adoptive transfer assay, the Ig-1b production may also be stimulated by allogenic factors produced because hybrid (SJL × BALB/c) cells are being transferred into parental (BALB/c) mice, which, although heavily irradiated, still contain some cells capable of reacting to the hybrid. The absence of the Ig-1b burst in transfers to syngeneic irradiated recipients (unpublished observation) is consistent with this hypothesis, as is the demonstration by Cinader et al. (1974) that parental cells injected into suppressed hybrids stimulate Ig-1b production, (i.e., breaks suppression). However, if allogeneic factors are involved in establishing conditions whereby Ig-1b precursors can differentiate to producers, or in stimulatory differentiation or expansion, then suppressor cells apparently are able to regulate the effects of these factors since a high enough dose of suppressors will suppress the initial Ig-1b burst.

Allotype suppression in Other Mouse Strains

As mentioned earlier, we have made some attempts to demonstrate the induction of allotype suppressor T cells in mouse strains and hybrid combinations other than SJL × BALB/c, but have been roundly unsuccessful. This may be because the suppressor T cells are unique to this hybrid; however, it is also possible that our failure was for quantitative reasons. It may be that in most mouse strains it is difficult to find conditions where the level of suppres-

sor activity could be raised high enough to overcome the antagonistic effects of the cooperator T cells present or, conversely, where the level of cooperator activity is low enough to allow the suppressor population to be fully expressed. There is some evidence from studies published some years ago (Herzenberg & Metzler 1974, Herzenberg et al. 1967) on short-term allotype suppression in C57BL/10 × BALB/c mice which suggest that this quantitative interplay may mask a population of suppressor cells induced by exposure to anti allotype antibody.

(C57BL/10 × BALB/c)F_1 mice exposed to maternal anti allotype antibody show a delay of about 3 weeks in onset of allotype production. After that the serum allotype levels increase rapidly. But although the serum levels in these animals climb at roughly the same rate as the controls and eventually reach normal range, calculation of the actual rate of allotype production in these animals suggests an impairment of production as late as 15 weeks of age.

In Figure 5, the two solid curves show the Ig-1b levels in serum averaged for three litters of controls and three litters of mice exposed to maternal antibody (Herzenberg & Metzler 1974). The dashed curve (which joins the controls at 10 weeks) shows the estimated allotype levels for the suppressed mice if production of allotype proceeded at the same rate as controls once the maternal antibody had removed as much allotype as it could absorb. The point of the figure was to demonstrate that 'mopping up' by maternal antibody could not explain allotype suppression. But the method used to estimate theoretical allotype levels also allowed estimation of the parameters which would approximate the observed data, and thereby allowed us to recognize a persistent difference in the rate of allotype synthesis between control and suppressed mice.

To make this comparison, we set up an equation ((see legend for Figure 1) which expressed the rate of allotype production as a function of the observed total allotype level (corrected for the fluid space of the animal) and the amount of allotype lost because of catabolism and test bleeding. Assuming a 6 day half-life (which was consistent with observed values) and an extracellular fluid space of 8.8 per cent body weight, we determined the rate of synthesis in the controls. Then by including another variable, i.e., loss due to maternal antibody, we tried various combinations of loss and rates of synthesis to approximate the curve for the suppressed animals.

We found that in order to account for the continued lag between the suppressed animals and the controls, it was necessary to postulate that the suppressed animals produced the allotype at half the rate determined for the controls. In no way could we approximate the suppressed curve by assuming the normal rate of production in suppressed mice after the release of suppres-

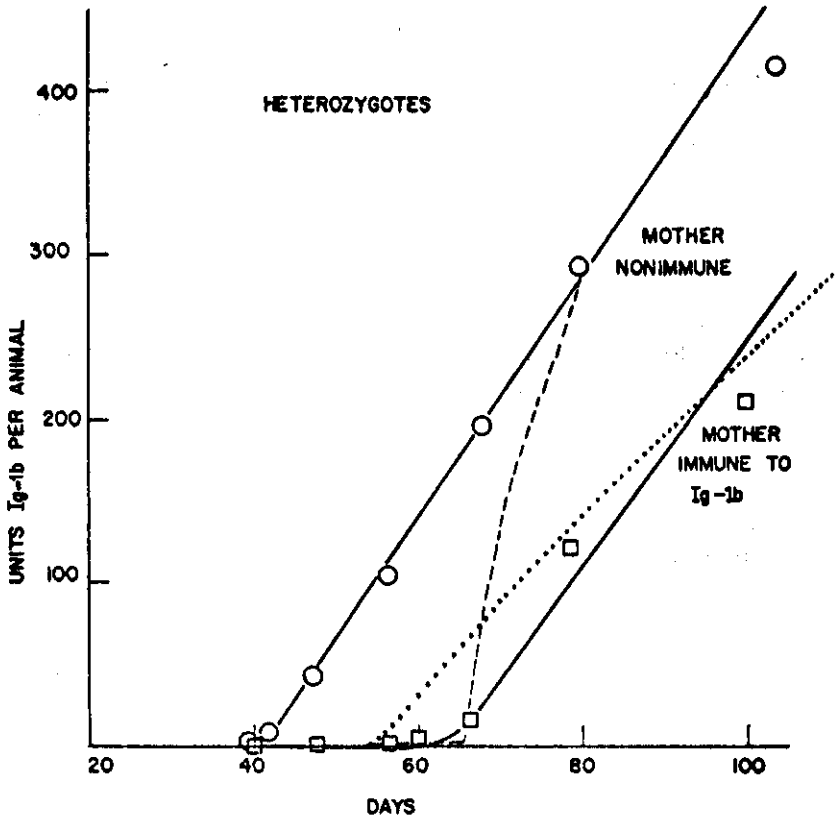

Figure 5. Calculated increase in Ig-1b level in heterozygotes. Solid curves are observed data points. Dashed curve (– – –) was calculated on the basis of 500 units of Ig-1b removed but no change from the rate of production of Ig-1b found for the controls. Dotted curve (· · ·) was calculated on the basis of 150 units of Ig-1b removed and the rate of production of Ig-1b equal to one-half the control rate. A half-life of 6 days was used for both curves. Corrections for the amount of Ig-1b withdrawn in serum sampling were made. For $t = 0, 1, 2, \ldots, 179$, the rate of production of Ig-1b at time $t(\mathrm{PRO}_t)$ was found by equation (1) and substituted in equation (2) to determine the amount of Ig-1b expected at time $t(\mathrm{Calc\ Ig}_t)$.

$$(1) \qquad \mathrm{PRO}_{(t+1)} = (\mathrm{Ig}_{(t+1)} - \mathrm{Ig}_t) + \left(\frac{\ln 2}{\mathrm{T}} + \mathrm{k}\right)\mathrm{Ig}_t \quad \text{and}$$

$$(2) \qquad \mathrm{Calc\ Ig}_{(t+1)} = \mathrm{Calc\ Ig}_t + \mathrm{q}\cdot\mathrm{PRO}_t - \left(\frac{\ln 2}{\mathrm{T}} + k\right)\mathrm{Calc\ Ig}_t - \partial$$

where Ig_t is the observed Ig-1b level in control at time t; T, the half-life in days (6, in curves in figure); k, the fraction of total Ig-1b present in the animal removed by sampling averaged per day (0.1/7 in curves in figure); q, the fraction by which the rate of production (PRO) is altered; and ∂, the amount of Ig-1b withdrawn at $t = 0$.
Reprinted from (5) and (17)

sion. Thus these mice may have a population of allotype suppressor T cells which impairs allotype production but does not suppress it entirely.

General Consideration on the Difficulties of Demonstrating Suppressor T Cells

The quantitative interactions between primed B cells and suppressor and cooperator T cells in allotype suppression discussed earlier could explain why suppressor T cells in general are difficult to demonstrate. We have shown using the adoptive secondary response to DNP, that when various numbers of unprimed suppressed spleen cells and KLH-primed (cooperator) spleen cells are co-transferred to irradiated mice together with a constant number of DNP-primed B cells, the extent of suppression of the Ig-1b DNP-PFC response is determined by the doses of suppressor and cooperator spleen cells. Adding more DNP-primed B cells increases the number of Ig-1b PFC produced but does not affect the fraction of the total response suppressed so long as the suppressor and cooperator doses remain unchanged. This independence of suppression from primed B cell concentration suggests that the mechanism of suppression involves a contest between suppressor T cells and cooperator T cells, the results of which determine the fraction of available Ig-1b bearing (memory) B cells which differentiate to Ig-1b producing cells.

The nature of the cooperator: suppressor interaction is still unclear; however, the quantitative relationship between suppressor and cooperator doses in the allotype suppression system is reasonably well established by the data presented in this publication. Regardless of mechanism, the data indicate that effectively each suppressor cell neutralized (removes) a fixed amount of cooperator activity, no matter what the dose of cooperators injected. In other words, the apparent quantitative relationships behave as though suppressors stoichiometrically remove cooperator activity.

The effects on immune response caused by this type of quantitative interaction are considerably different than those generally expected in cell interaction studies and can cause confusion in attempts to demonstrate suppressor cells. For example, suppressor capacity is fixed so that a given number of suppressors cannot be expected to suppress the same proportion of a response if the magnitude of the response varies. This, and several other implications of the quantitation described here may be important for the identification of suppressor cells in systems where a similar mechanism is operant.

The usual assay for suppressor cells requires that the cells from a putatively suppressed donor be able to suppress an immune response mounted by co-transferred or co-cultured cells from normal donors, either primed or unprimed as the case may be. While the inability to suppress in this kind of assay is reasonably interpreted as evidence for the absence of suppressor cells,

the quantitative aspects of the antagonism between suppressors and cooperators in the allotype suppression system suggest that unless certain critical conditions are met in the co-transfer assay, suppressor cells which exist in actively suppressed donors could easily go undetected. Such donors in fact, may appear under some circumstances simply to have a reduced number of cooperator T cells.

One of the problems with the co-transfer assay involves the amount of cooperator activity contributed by the normal donor. We have shown that when the cooperator dose from this donor is greater than required to obtain the maximum response (i.e., is above saturation), the addition of increasing numbers of suppressor T cells will have no apparent effect on the response until a sufficient number of suppressors are added to reduce the effective cooperator activity to below saturating levels. Since the number of B cells necessary for an adequate adoptive secondary response in many systems often forces the dose of primed spleen cells (from the normal donor) to well above the saturating cooperator T cell dose for that spleen cell preparation, the co-transfer of considerable numbers of suppressor cells may fail to reduce the response detectably.

For example, in the adoptive secondary response to DNP described in this publication, transfer of 10^7 spleen cells from our usual long-term DNP-KLH primed donors will transfer nearly twice the number of cooperator T cell required to achieve the maximum response. With this dose of primed spleen cells, even as potent a population of suppressor T cells as that present in allotype suppressed mice shows no suppression when less than 2×10^6 spleen cells from suppressed donors are co-transferred.

A second problem with co-transfer assays involves the presence of cooperator T cells in the suppressed donor. In the allotype suppression co-transfer studies, only the normal (non-suppressed) donors of B or cooperator T cells are antigen primed. The suppressor cell donors for the co-transfer are not exposed to the test antigen and do not have any demonstrable cooperator T cell activity. In systems where antigen specific suppressors are under study, the situation is more complicated. Exposure to antigen in suppressive doses may also generate cooperator activity. Similarly, where total allotype production is used as an index of suppression, the animal may have a substantial pre-existing cooperator population.

If the suppressor activity generated by the antigenic exposure is adequate to neutralize most or all of the cooperator activity generated the net effect in the intact animal will be suppression of response to the test antigen. But since cooperators also neutralize suppressors, the lymphoid tissue of the suppressed animal may not contain enough excess suppressors to neutralize the added cooperator load in a co-transfer assay with cells from a non-suppressed

primed donor, especially if the assay is not very sensitive. In fact, in antigen induced suppression, if the suppression is complete and some cooperator activity is left in the suppressed donor, this activity will be demonstrable if a sensitive assay for cooperator detection is used, (i.e., co-transfer with primed B cells). Thus, failure to demonstrate suppressor cells may not mean that the suppressor cells are not present in the intact animal.

The basis for this conclusion may be more precisely understood by considering the equation which predicts the response to DNP at various doses of B cells (B), cooperator T cells (T_C) and allotype suppressor T cells (T_S). We have shown that cooperator activity is lost in proportion to the suppressor cell dose (i.e., (T_C lost) $= \alpha \cdot T_S$) and that the response per B cell injected is given by the equation.

$$\frac{PFC}{B} = k \cdot (T_C - \alpha \cdot T_S)$$

as long as the amount of T_C activity left, i.e., $(T_C - \alpha \cdot T_S)$ falls below the saturating dose of T_C. Regardless of the T_C dose, if $\alpha \cdot T_S$ is large enough to reduce T_C below saturation, the response will be suppressed. Conversely, if $\alpha \cdot T_S$ is not large enough to reduce T_C below saturation, then no suppression will be observed. Thus, at doses of T_C above saturation, suppressors will be consumed with no obvious effect on the response.

In cases where antigen has induced both T_C and T_S or where the native T_C level is high, unless $(T_C - \alpha \cdot T_S)$ is a negative value, (i.e., $\alpha \cdot T_S > T_C$), cells from the suppressed animal will not suppress the response by co-transferred normal cells since all of the cooperator neutralizing capacity will be consumed by the suppressed donor's cooperators. If $(T_C - \alpha \cdot T_S)$ is positive, (e.g., in a partially suppressed donor) the remaining cooperator activity will simply add to that contributed by the normal donor.

Further exploration of the ramifications of the quantitative interaction seen in the allotype suppression system shows that the number of suppressor cells being produced in response to an antigenic stimulus can appear to drop if the number of cooperator cells increases, i.e., if T_C increases, $(T_C - \alpha \cdot T_S)$ can go from a negative to a positive value although the number of suppressor cells being generated remains constant; also, addition of suppressors from two cell sources can mimic a synergy if neither population alone is able to reduce the co-transferred cooperator dose to below saturation but the sum of the suppressive activity in the two populations is adequate to do so.

Isolation and Characterization of Suppressor T Cells

Ideally, the solution to the problems of recognizing suppressor T cells in the presence of cooperator T cells (and *vice versa*, for that matter) should be

solvable by separating these cells from one another and testing their activities independently. Similarly, determining whether suppressor T cells (or factors) interact with cooperator T cells (or factors) would be greatly facilitated if these cells could be mixed and isolated at will. For these reasons, and also because identification of the allotype suppressor T cells as belonging to a known functional subset of T cells may give important clues as to the mechanism of suppression, we have recently begun work on characterizing these cells with respect to T cell surface antigenic markers and physical properties.

Data from these studies is still too fragmentary to give a clear picture of the suppressor T cells responsible for allotype suppression. At this point, based on FACS separations, we can state that the suppressor cells are larger than roughly 90 percent of splenic lymphocytes and stain dully (although definitely) with a serum which specifically stains T lymphocytes (Herzenberg et al. 1975). Whether these or other criteria will allow us to separate suppressor from cooperator T cells, however, remains to be determined.

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Note in proof to page 73

Since submission of this manuscript we have obtained experimental evidence supporting the 'cooperator removal' hypothesis advanced here. We have now shown that:

1) Ig-1b cooperator activity in spleens of non-suppressed carrier-primed mice is selectively removed by culturing spleen cell suspensions from these mice for 24 hours with a 48-hour tissue culture supernatant from suppressed non-primed spleens. Ig-1a and other IgG cooperator activity is unimpaired by the treatment.

2) No Ig-1b cooperator activity is demonstrable in spleen cell suspensions from carrier primed suppressed mice. Suppressor T cells were removed prior to testing the suspensions for cooperator activity in a hapten-carrier adoptive transfer assay. Suppressor cells were removed by size separation or by cytotoxic treatment with an antiserum (anti Ly 2.2 and Ly 3.2) which kills suppressor but not cooperator T cells. Ig-1a and other IgG cooperator activity (again) is unimpaired by these treatments.

If Ig-1b cooperator activity were present it should have been unmasked by removal of the suppressor prior to transfer; however the suppressor-depleted spleen cells failed to show an Ig-1b response. Thus suppressor T cells appear to specifically remove Ig-1b cooperator activity in intact primed suppressed mice.

Reference to above note:

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