Reprinted from:
IMMUNOLOGICAL TOLERANCE
Mechanisms and Potential Therapeutic Applications

ACADEMIC PRESS, INC.
New York San Francisco London

MECHANISM OF ALLOTYPE SUPPRESSION IN MICE

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Within the last year or two immunologists have begun to focus on the role of thymus-derived (T) cells as regulators of antibody production and the immune response in general. Previously, the few studies which demonstrated suppressive effects of T cells on antibody responses were considered either suspect or irrelevant (sometimes even by the investigators doing the studies). Now, however, the number of well-documented instances of T cell suppression has increased to the point where it is becoming necessary to determine not whether, but how, suppressor T cells interact with B cells and cooperator T cells in response to antigen. (See articles in this volume.)

We have been working with a system in which we can generate a T cell population in a hybrid mouse which suppresses production of γG_{2a} immunoglobulins carrying one of the two parental allotypes (reviewed in 1). This system is rather special in that we have thus far been able to demonstrate the suppressor cells in only one hybrid, SJL x BALB/c. Nonetheless, the information on the characteristics of suppressor cells and the methodology for working with them which we have gained with these studies have made this system a useful model for suppression studies in addition to its intrinsic value.

Generally, we use progeny from the cross of SJL male x BALB/c female mice and study suppression of the paternal (Ig-1b) allotype which is carried on γG_{2a} globulins. Suppressed mice are obtained either by exposing very young hybrids (0-3 weeks) to anti-Ig-1b allotype anti-serum (primary suppression) or by transfer of lymphoid tissue from primary suppressed mice to two-week old normal F_1's (secondary suppression).

The suppression is an active (or dominant) phenomenon, i.e. lymphocytes from suppressed animals suppress Ig-1b production by normal spleen cells. It may be measured in mixture-transfer experiments where lymphocytes from suppressed donors are mixed with spleen cells from normal syngeneic donors and transferred to irradiated recipients. Decrease of either Ig-1b antibody-forming cells (Ig-1b anti-DNP-PFC) or serum Ig-1b level have been used as indices for suppression.

The age of onset and extent of suppression is variable in primary suppressed mice (i.e., those exposed to maternal antibody). Basically, there are three categories into which these mice fall. A few percent never initiate production of Ig-1b. Most do produce some allotype between three and five months of age but cease production before six months. The allotype levels in these mice generally remain below detectability after six months, although a small proportion of mice "break" suppression and produce Ig-1b, usually at a low level and for a short period of time. The remainder of exposed mice show allotype levels similar to the second group as young adults, but the allotype production does not become completely suppressed at six months of age. Many of these latter mice are partially suppressed in that their allotype production maintains at a low level throughout life.

(Donor mice for transfer experiments are taken from either of the first two categories and tested for serum Ig-1b levels just before transfer to be certain of complete suppression.)

The mechanism for partial suppression has not been studied directly as yet, although it may represent a good vehicle for determining whether the allotype suppressor T cell functions as a regulator of immunoglobulin synthesis. Data presented below from titration studies with suppressor cells taken from fully suppressed donors suggest this may be the case. However, the picture is more complicated in that the intensity of the suppression appears to be related not only to suppressor dose but to the balance of activities between suppressor and cooperator populations.

The data in Figs. 1 and 2 show that the intensity of suppression is correlated with the dose of suppressor lymphoid tissue. In Fig. 1 the serum Ig-1b levels of normal F1's given varying numbers of suppressor spleen cells at

two weeks is presented as a function of age of animals. Those mice receiving the largest cell dose (1.5×10^7) are completely suppressed. More than 90% never produce any detectable Ig-1b. Those receiving an intermediate dose (5×10^6) produce Ig-1b but remain partially suppressed throughout life. While they never cease allotype production entirely, their serum levels always stay below normal. Those mice receiving the lowest cell dose (10^6) show serum levels which are roughly within normal range. (A non-injected control group was not included with this particular experiment.)

In Fig. 2 Ig-1b levels are given as a function of time after transfer of mixtures of a constant number of normal F_1 cells with varying numbers of cells from spleens of suppressed F_1's. With no suppressor cells added, the normal cells establish Ig-1b production and serum levels remain high throughout the life of the recipient. When the mice receive suppressor cells in addition to normal cells, there is an initial burst of allotype synthesis followed by a fall in serum allotype level. The final level obtained is progressively lower as the suppressor dose increases. Thus, in both cases, the intensity of suppression depends on the size of the suppressor population in a given animal.

Indications that "cooperating" T cells also play a role in determining the intensity of suppression come from studies with suppression of production of antibody marked with Ig-1b allotype (Ig-1b anti-DNP) in a hapten-carrier system. These show that there is a marked antagonism between suppressors and cooperators such that either increasing the number of cooperators or decreasing the number of suppressors will result in a greater Ig-1b anti-DNP-PFC response. (2)

The data in Table I show that the Ig-1b adoptive secondary response to the DNP-KLH is completely suppressed by 10^7 cells from suppressed spleen. In this experiment 1.2×10^7 DNP-KLH-primed normal spleen cells were mixed with varying doses of unprimed suppressor cells and transferred to irradiated (600 R) BALB/c animals. Recipients were challenged with DNP-KLH on day 1 after transfer and sacrificed for estimation of PFC on day 7. As the data in the table show, the lowest dose of suppressed spleen had no affect on the Ig-1b Pfc response, intermediate doses partially suppressed, and the highest dose suppressed completely.

TABLE I

SUPPRESSION OF Ig-1b ANTI-DNP RESPONSE

DNP-KLH 1^0 Normal Spleen x 10^6	Unprimed Spleen Normal x 10^6	Unprimed Spleen Suppressed x 10^6	PFC/10^6 Direct	PFC/10^6 Ig-1a +Ig-4a	PFC/10^6 Ig-1b
12	10	-	34	3800	400
12		1.1	29	4300	410
12		3.3	40	3300	170
12		6.7	40	2300	120
12		10	42	3300	17

100 µg alum-pptd DNP-KLH + 2 x 10^9 B. pertussis injected i.p. 1-3 months prior to transfer.

10 µg DNP-KLH boost i.v. 1 day after transfer.

Donors: (SJL x BALB/c)F_1; recipients BALB/c irradiated (600R) ~ 18 hours prior to transfer.

To examine the interaction between suppressors and cooperators, we moved to a heterologous hapten-carrier system so that cooperator dose could be varied. DNP-KLH-primed normal F_1 spleen was used as the source of DNP-primed B cells. Ovalbumin-primed normal F_1 spleen was used as the source of cooperators. Spleens from suppressed F_1 mice (as above) provided the source of suppressor cells. Irradiated (600 R) BALB/c recipients were challenged one day after transfer with DNP-ovalbumin. PFC were measured on day 7 as before.

Two separate experiments with the heterologous hapten-carrier system are presented in Table II (in each case three doses of carrier-primed spleen was used). As the data shows, when no suppressor cells were added, the number of Ig-1b PFC increases with increasing numbers of cooperators. Formation of these PFC is completely suppressed, regardless of cooperator dose, when transferred recipients also received a large number of suppressor cells. Suppression is partially reversed, however, in recipients of smaller numbers of suppressor cells and larger numbers of cooperators.

Thus, the extent of suppression of Ig-1b PFC anti-DNP appears to depend on the balance between the activity of carrier-primed T-cooperator population and the activity of the suppressor population. In the presence of suppressors, more cooperators are needed to achieve a PFC response of a given level. Similarly, in the presence of larger numbers of cooperators, more suppressors are needed to achieve a given level of suppression.

The mechanism of the suppressor-cooperator antagonism is difficult to infer from these types of experiments. These data could equally well be interpreted as fitting either a model which postulates a competition between suppressor and cooperator for a site on a DNP antibody-forming cell precursor or a model which sees the suppressor as acting on the B cell after or before the cooperator has started it on the road to antibody production. In the latter case, the antagonism would depend on increasing numbers of cooperators "turning on" increasing numbers of B cells, therefore, requiring more suppressors for equivalent suppression. Another model which fits the data would postulate a direct T-T interaction prior to B cell stimulation. Whatever the case, it is clear that any mechanism for suppression must take into account the cooperator-suppressor antagonism.

TABLE II

ANTAGONISM BETWEEN SUPPRESSOR AND COOPERATOR T CELLS

		Unprimed Suppressed Spleen ($\times 10^6$)	Ovalbumin (Carrier) 1^0 Normal Spleen * ($\times 10^6$) 1	3	10
Expt.	1	0 **	23	72	246
		1	0	0	64
		3	0	0	0
			2.7	8	24
Expt.	2	0	80	290	330
		1.6	0	45	50
		4	0	0	0

* All animals received 12 x 10^6 hapten-primed (DNP-KLH) spleen cells together with carrier-primed spleen and unprimed suppressed spleen as indicated. Carrier-primed animals received 100 μg alum precipitated ovalbumin i.p. 4 to 8 weeks before transfer. Hapten primed animals: See legend for Table I. Recipients (600 R) irradiated BALB/c were boosted with 10 μg DNP-ovalbumin 1 day after transfer.

** 0 means <5 ppm.

There is another aspect of suppression, not necessarily directly related to the suppressor cooperator antagonism, which I would like to bring up at this point. Suppressor T cells are found in all lymphoid tissues of the mouse, even thymus and bone marrow. Localization of suppressors in these latter tissues raise interesting questions with respect to what is known about the overall distribution of T cells in the tissues.

With respect to thymus it is not unreasonable to ask how the suppressor cells get there. As data in Table III shows, 10^7 thymocytes suppress about as well as 10^7 spleen cells. (Parathymic nodes were removed.) Since the thymus is thought of as primarily an exporter of T cells and since cells with immunologic memory are seldom found in the thymus, the existence of specific suppressor activity is unexpected.

The finding of suppressor activity in the bone marrow is even more surprising considering the notoriously low T cell content of bone marrow. It has been shown to be sensitive to anti-Thy-1 treatment (1) and therefore, meets the standard criterion for a T cell. Quantitatively, the activity of bone marrow is about equal to suppressor activity of spleen suggesting that the few T cells in bone marrow are highly enriched for suppressors.

To test for this enrichment directly, we isolated T cells from bone marrow and spleen by nylon wool passage and compared their activity. As the data in Table IV show, the suppressor activity per T cell is roughly the same (or slightly greater) in the original tissue and the isolated T population. However, the bone marrow T cell population has considerably more suppressive activity per T cell than the splenic T cell population (e.g. compare 4×10^3 T cells isolated from bone marrow with 6.4×10^5 T cells isolated from spleen).

It is not clear why bone marrow T should be enriched for suppressor activity. It may be that suppressor cells home to the bone marrow because of the presence of large numbers of Ig-1b precursors. It may also be that the high suppressor activity in bone marrow represents not so much a homing phenomenon as the absence of other T cell types (e.g. GVH precursors) which may be selectively excluded from bone marrow. Neither of these explanations is particularly satisfactory, and I raise the question here in the hope of stimulating discussion which could lead to some experiments which might help to explain this finding.

TABLE III

SUPPRESSOR CELLS IN LYMPHOID TISSUES

Normal Donor		Suppressed Donor	No. of	Ig-1b levels (mg/ml) weeks after transfer					
Spleen	Tissue	No. of Cells Transferred	Recip.	1	2	3	4	6	8
10^7	-	-	3	.24	>.38	>.5	>.5	>.5	>.5
"	Spleen	10^7	4	<.04	.01	<.01	<.01	<.01	<.01
"	"	10^6	4	.4	.13	<.03	<.01	<.02	.04
"	Thymus	10^7	4	.02	<.01	<.01	<.01	<.01	<.01
"	"	10^6	4	>.3	>.3	>.32	.12	.15	.08

See legend for Figure 2 for details of transfer.

TABLE IV

SUPPRESSOR T CELLS IN SPLEEN AND BONE MARROW

Normal (SJL x BALB/c) Spleen	Suppressed[1] (SJL x BALB/c) Tissue	Treatment[2]	# of cells transferred Total ($\times 10^6$)	T-cell content[3] ($\times 10^6$)	No. of Recip.	Mean Serum Ig-1b (mg/ml)[5] weeks after transfer 1	2	3	4	6	8	10
1.2×10^7	-	-	-	-	4	>.5	>.5	>.5	>.5	>.5	>.5	>.5
"	spleen	-	10	2.3	4	>.2	.1	-	-	-	-	-
"	"	nylon	4	3.2	3	.1	.05	-	-	-	-	-
"	"	-	3	0.7	4	.1	.1	.09	<.05	-	-	-
"	"	nylon	0.8	0.64	4	>.4	>.5	>.5	>.4	.06	<.07	<.04
"	bone marrow	-	9	0.05	4	.2	.1	.08	<.05	-	-	-
"	"	nylon	1	0.05	4	>.2	>.3	.1	-	-	-	-
"	"	-	1.3	0.006	4	>.4	>.4	>.5	.1	<.08	<.04	-
"	"	nylon	0.1	0.004	4	>.2	>.3	>.2	.1	-	<.05	-

1 Donors were SJL x BALB/c hybrids over 6 months of age exposed perinatally to maternal anti-Ig-1b.
2 Treated cell suspension passed through nylon wool to remove B cells.
3 T cell content determined by fluorescent staining.
4 Recipients were 600 R irradiated BALB/c mice.
5 Ig-1b levels determined by immunodiffusion. (-) = <0.01 mg/ml serum.

ACKNOWLEDGMENTS

This work was supported by N.I.H. grants HD-02187-12 and CA-04681-15.

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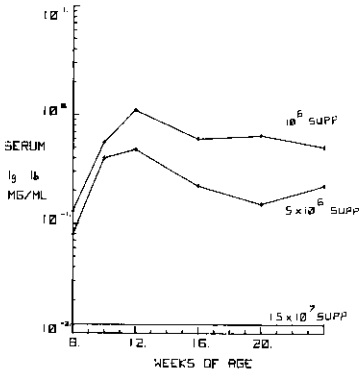

Fig. 1. Transfer of suppressor cells to young syngeneic F_1 mice. Donors of spleen cells were completely suppressed SJL x BALB/c F_1 mice over 6 months of age exposed perinatally to maternal anti-Ig-1b. Recipients were 2 week old normal SJL x BALB/c F_1 mice. Cells were injected i.p. Recipients were not irradiated. Ig-1b levels in serum were determined by radioimmune assay.

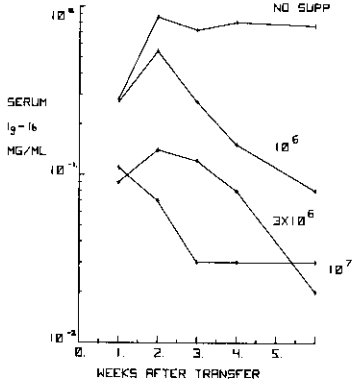

Fig. 2. Dose dependence of suppression in mixture-transfer assay. Recipients are 600 R irradiated BALB/c mice. All recipients receive 1.2×10^7 normal F_1 cells in addition to graded numbers of spleen cells from suppressed donors, i.e. SJL x BALB/c F_1 mice over 6 months of age exposed perinatally to maternal anti-Ig-1b. Cell suspensions were mixed immediately prior to transfer. Serum Ig-1b level determined by radioimmune assay.