SUPPRESSION OF IgE ANTIBODY PRODUCTION IN SJL MICE

I. Nonspecific Suppressor T Cells*

By NAOHIRO WATANABE,‡§ SOMEI KOJIMA,§‖ AND ZOLTAN OVARY

(From the Department of Pathology, New York University Medical Center, New York 10016)

It has been shown that preferential production of IgE antibody may be obtained in certain strains of mice if the adjuvant is $Al(OH)_3$ (1). A high and persistent titer of IgE antibody can thus be obtained using minute doses of antigen and even booster responses can be produced (2). If mice are primed with microgram amounts of dinitrophenylated keyhole limpet hemocyanin (DNP-KLH)[1] with $Al(OH)_3$ as adjuvant and then infected with third stage larvae of *Nippostrongylus brasiliensis* and finally injected with dinitrophenylated protein of *N. brasiliensis* (DNP-Nb) as the challenging agent, high titer antihapten IgE antibody can be obtained (3, 4). In this model, the infection by *N. brasiliensis* stimulates helper T cells which collaborate successfully with hapten-specific B cells (3, 4). 7 days after the injection of DNP-Nb, high antihapten IgE titers are observed in all strains used. High titers of antihapten IgE persist in the circulation for several weeks except in the SJL strain (3).

It has also been shown that antibody production is controlled not only by helper T cells but also by suppressor T cells (5–8). In the present paper the suppressor cells in the SJL strain of mice were investigated. It was found that T cells and also thymocytes from normal SJL mice have a suppressor effect on IgE production. Using F_1 hybrids and backcrosses to SJL mice we could demonstrate that the suppressive function is inherited by a single Mendelian gene, not linked to the *H-2*-gene complex.

Materials and Methods

Antigen. $DNP_{13-4}KLH$ and $DNP_{17}Nb$ were used for immunization. *N. brasiliensis* extract (Nb), dinitrophenylated bovine serum albumin ($DNP_{37}BSA$), and dinitrophenylated ovalbumin ($DNP_{14}Ov$) were used for challenge for passive cutaneous anaphylaxis (PCA) reactions. The subscripts refer to average number of groups per molecule of protein except for Nb and KLH where they refer to 10^5 daltons. Preparation of these antigens has been previously described (3).

Animals. The following strains of 8- to 12-wk-old female mice were used: C57BL/6, AKR/J,

* This investigation was supported by National Institute of Health grant A1-03075-17.

‡ Supported by National Cancer Institute grant 5-PO1 CA 16247-02.

§ Recipient of Fellowship from The Cancer Institute Inc., New York.

‖ Present address: Department of Parasitology, School of Medicine, Chiba University, Chiba, Japan.

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DNP, dinitrophenyl; HBSS, Hanks' balanced salt solution; KLH, keyhole limpet hemocyanin; Nb, *Nippostrongylus brasiliensis* extract; Ov, ovalbumin; PCA, passive cutaneous anaphylaxis.

C3H/J, CBA/J, ASW/J, DBA/1, BALB/c, A/J, and SJL/J. All mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. (BALB/c♂ × SJL♀)F_1 and (BALB/c × SJL)F_1♂ × SJL♀ backcross animals were bred in our laboratory. Male Sprague-Dawley rats, weighing 250–300 g were obtained from Blue Spruce Farms, Altamont, N. Y.

Immunization. Immunization schedules, infections, and boosters were done as published (3). Briefly, five animals in each group were immunized intraperitoneally with 1 μg DNP-KLH mixed with 1 mg $Al(OH)_3$ on day 0. On day 21 the mice were infected subcutaneously by 750 third stage *N. brasiliensis* larvae. Mice were reinjected (challenged) intraperitoneally with 1 μg DNP-Nb mixed with 1 mg $Al(OH)_3$ on day 35 (schedule 1). In some cases, mice were immunized with only 1 μg DNP-KLH in 1 mg $Al(OH)_3$ (schedule 2). Mice were bled weekly beginning 7 days after injection of DNP-Nb from the retro-orbital sinus. 0.2 ml of blood was added to 0.9 ml heparinized saline (10 U/ml), then centrifuged for 10 min at 1,000 *g*. The supernate was considered to be a $^1/_{10}$ dilution.

Irradiation. Immunized, infected, and challenged mice received 540 R of X-ray irradiation from a Gammator M (Radiation Machinery Corp., Parsippany, N. J.) on day 36 or 39. In some cases, unprimed or DNP-KLH-primed mice were irradiated with the same X-ray dose 1 day before cell transfer (see Results).

Cell Transfer. Spleen cells from either noninfected or infected SJL mice were prepared by gentle teasing of spleens in cold sterile Hanks' balanced salt solution (HBSS) (Microbiological Associates, Bethesda, Md.). The cells were washed three times in HBSS and their viability estimated by the trypan blue exclusion test. Mice were intravenously injected with 1–7.5 × 10^7 viable spleen cells. Thymocytes were prepared in a similar fashion.

Anti-Thy 1.2 Treatment. Anti-Thy 1.2 and rabbit serum (complement) were kindly supplied by Dr. Shen and Dr. Boyse from the Sloan-Kettering Institute for Cancer Research, New York. Anti-Thy 1.2 treatment was done according to a modified method of Takahashi et al. (9) by Kojima and Ovary (3). The viability of the cells was tested by the trypan blue dye exclusion test. 28% of the cells were stained after anti-Thy 1.2 treatment, and 16% after normal mouse serum treatment. The cell number was adjusted again and 5× 10^7 viable cells per mouse were injected intravenously into the immunized and irradiated recipients.

Titration of Antibody. Titers of IgG_1 and IgE antibody of individual serum or pooled sera from each group were determined by passive cutaneous anaphylactic reactions (10). Female SJL mice were used for IgG_1 antibody titration using a 1.5-h sensitization period (11). Mice were challenged with 500 μg $DNP_{14}Ov$ for antihapten antibody or the same dose of adult Nb for IgG_1 anticarrier antibody titration. Male Sprague-Dawley rats were used for IgE antibody titration (12) using a 2-h sensitization period (11). 1 mg of $DNP_{37}BSA$ or Nb were used as challenging antigens.

Determination of H-2 Antigen. Anti H-2^d serum (C3H anti-BALB/c spleen cell antiserum) was kindly supplied by Dr. Lloyd Old from the Sloan-Kettering Institute for Cancer Research, New York. The hemagglutination test for H-2^d antigen was done according to a modified method (13) of Gorer and Mikulska (14). Briefly, 0.1 ml blood from each mouse was diluted in 0.9 ml heparinized saline (10 U/ml) and centrifuged at 500 rpm for 5 min and washed twice with cold 0.15 M NaCl. After centrifugation, the cell pellet was mixed with 15 drops of inactivated fetal calf serum and 1 ml of cold 0.15 M NaCl. One drop of anti-H-2^d serum dilutions (1/100, 1/400, and 1/800) by 1.8% dextran in 0.15 M NaCl and one drop of cell suspension were mixed in a plastic tray. Hemagglutination was read after incubation at 37°C for 90 min.

Infectivity and Egg Production of N. brasiliensis. Seven mice in each group (8-wk-old female SJL and BALB/c) were infected subcutaneously in the inguinal region with 750 *N. brasiliensis* third stage larvae. 7 days after infection, the small intestines of the mice were removed and the number of adult worms was counted. To test for egg production of *N. brasiliensis* in the mice, feces from each mouse were collected and weighed. The eggs on several slides prepared by the direct thin-smear method were counted and expressed as eggs per gram of feces.

Results

Strain Differences of Antihapten IgE Response. Table I shows the antihapten IgG_1 and IgE antibody responses of different inbred strains of mice immunized according to schedule 1. All strains of mice had persistent and high titers of antihapten IgE antibody, except the SJL strain which had a moderately low

TABLE I
Strain Difference of Antihapten Antibody Response

Strain	H-2 type	Anti-DNP PCA titer*			
		Day 42‡		Day 56‡	
		IgE	IgG_1	IgE	IgG_1
A/J	a	2,560	640	1,280	1,280
C57BL/6	b	2,560	320	2,560	80
BALB/c	d	2,560	640	2,560	320
AKR	k	1,280	640	160	80
C3H	k	1,280	640	10,240	160
CBA	k	1,280	160	10,240	80
DBA/1	q	2,560	1,280	2,560	2,560
ASW	s	2,560	160	640	1,280
SJL	s	320	160	20	160

* Titer determined by pooled serum from five mice of each group.
‡ Days after DNP-KLH immunization.

titer on day 42 and a very low titer on day 56. It should be noted that in ASW mice (same $H\text{-}2^s$ as SJL) the antihapten IgE antibody titer was very high on day 42 and maintained a high level on day 56. The mice with $H\text{-}2^k$, such as AKR, C3H, and CBA, produced similar high titers of antihapten IgE antibody on day 42.

Mice of all strains produced IgG_1 antibody. Some variation in titers was observed in the sera taken on days 42 and 56. However, the important finding was that although in the SJL mice the IgE antihapten antibody titer dropped sharply on day 56, the IgG_1 titer was maintained.

Effect of Irradiation. The effect of irradiation was tested using SJL mice immunized according to schedule 1. The mice were irradiated with a dose of 540 R on day 36 or day 39. On day 42, 7 days after challenge, high anti-DNP IgE titers were obtained in irradiated groups and nonirradiated controls (Fig. 1 A). The group irradiated on day 39 showed higher IgE titers than the group irradiated on day 36 or the controls. On day 49, nonirradiated mice had very low IgE titers, whereas IgE titers were high in irradiated groups, especially in the group irradiated on day 36. These high titers of anti-DNP IgE persisted longer in the group irradiated on day 36 than in the other groups.

Anti-DNP IgG_1 response in the group irradiated on day 36 was consistently lower than the response in either the group irradiated on day 39 or the controls (Fig. 1 B). IgG_1 titers in controls or mice irradiated on day 39 were virtually the same. It should be noted that IgG_1 antibody did not diminish throughout the experiment in any of the three groups.

Cell Transfer Experiments

ANTI-DNP RESPONSE IN IRRADIATED AND IN NONIRRADIATED RECIPIENTS. Suppression of anti-DNP IgE response occurred after day 42 in nonirradiated mice. This was 3 wk after infection. In order to study the suppressive effect of spleen cells from infected mice, 5×10^7 spleen cells from mice infected 3 wk previously were transferred into irradiated or nonirradiated recipients im-

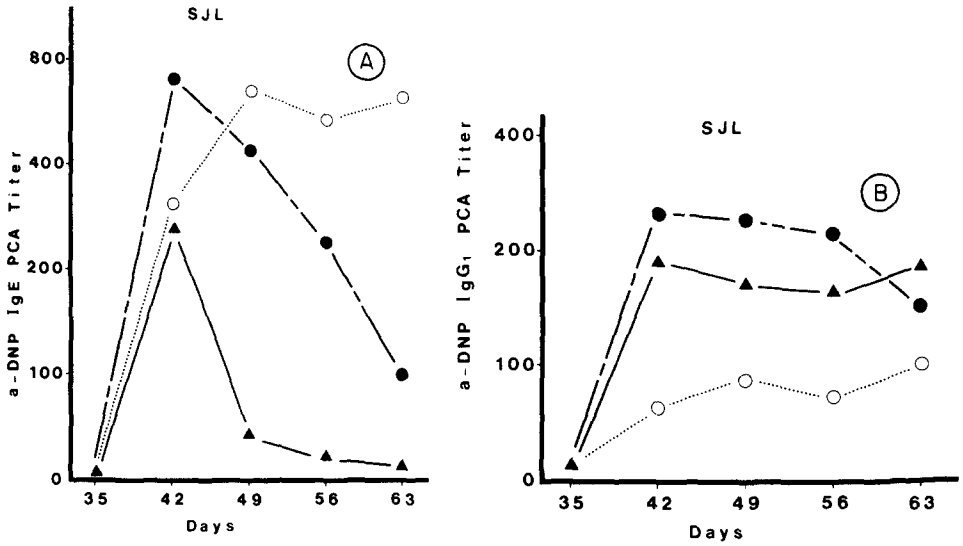

FIG. 1. Antihapten antibody response in irradiated and nonirradiated SJL mice. SJL mice immunized intraperitoneally with 1 μg DNP-KLH mixed with 1 mg $Al(OH)_3$ on day 0 and infected with third stage larvae of *N. brasiliensis* on day 21. Mice were boosted intraperitoneally with 1 μg DNP-Nb plus 1 mg $Al(OH)_3$ on day 35. Groups of five mice were irradiated (540 R) on day 36 (○) or on day 39 (●). No irradiation was given to control mice (▲). (A) Anti-DNP IgE titers. (B) Anti-DNP IgG_1 PCA titers.

munized 35 days previously according to schedule 2. Immediately after cell transfer, the recipient mice were challenged with 10 μg DNP-Nb in 1 mg $Al(OH)_3$ (Table II). Control groups did not produce large amounts of anti-DNP IgE antibody (groups III–V). The mice which were primed with DNP-KLH and received the cells from infected mice, produced anti-DNP IgE 7 days after cell transfer. However, the IgE antibody titer dropped to zero 14 days after challenge (group I, primed but not irradiated). In primed and irradiated recipients (group II) the anti-DNP IgE titer did not change after 14 days.

14 days after transfer, all irradiated animals (groups II, III, and V) had anti-DNP IgE antibody, whereas at this time nonirradiated groups (I and IV) did not have antihapten IgE antibody. This experiment confirmed the previous report (3) that DNP-KLH-primed cells and carrier-specific helper cells were necessary to obtain a strong anti-DNP IgE response. (Compare groups I and IV and groups II and V for DNP-primed cells and groups II and III for helper cells.)

SUPPRESSIVE EFFECT OF NORMAL SJL SPLEEN CELLS. Recipient mice immunized according to schedule 1 were irradiated (540 R) on day 36 and received 5×10^7 spleen cells from normal SJL mice on day 37. Control animals were not injected with normal SJL spleen cells. Controls had high and persistent anti-DNP IgE antibody. The IgE titers in animals receiving spleen cells decreased gradually. Anti-DNP IgG_1 in these two groups was very low (Fig. 2 A).

ASW mice ($H\text{-}2^s$, as SJL) gave high and persistent anti-DNP IgE response (Table I). ASW mice were therefore immunized according to schedule 1 but were not irradiated. On day 37, 7.5×10^7 normal SJL spleen cells were transferred into these mice. Control animals received no spleen cells. On day 42 both groups

TABLE II

Antibody Response in Irradiated and Nonirradiated SJL Recipients

Group	Recipients	Irradiation*	Transferred spleen cells (5×10^7) from:	Challenged with‡	Anti-DNP PCA titer§			
					Day 7‖		Day 14‖	
					IgE	IgG_1	IgE	IgG_1
I	1 μg DNP-KLH with 1 mg $Al(OH)_3$ i.p. primed 5-wk mice	(−)	750 Nb larvae infected 3-wk mice	10 μg DNP-Nb with 1 mg $Al(OH)_3$	200	100	0	200
II	"	540 R	"	"	100	50	100	50
III	"	540 R	Noninfected mice	"	0	0	10	50
IV	Unprimed mice	(−)	750 Nb larvae infected 3-wk mice	"	0	0	0	50
V	Unprimed mice	540 R	"	"	10	0	50	50

* Irradiation was given 1 day before cell transfer.

‡ Antigen was injected immediately after cell transfer.

§ Sera from five mice of each group were pooled and the reciprocal of the highest dilution giving PCA reaction was taken as titer.

‖ Days after cell transfer.

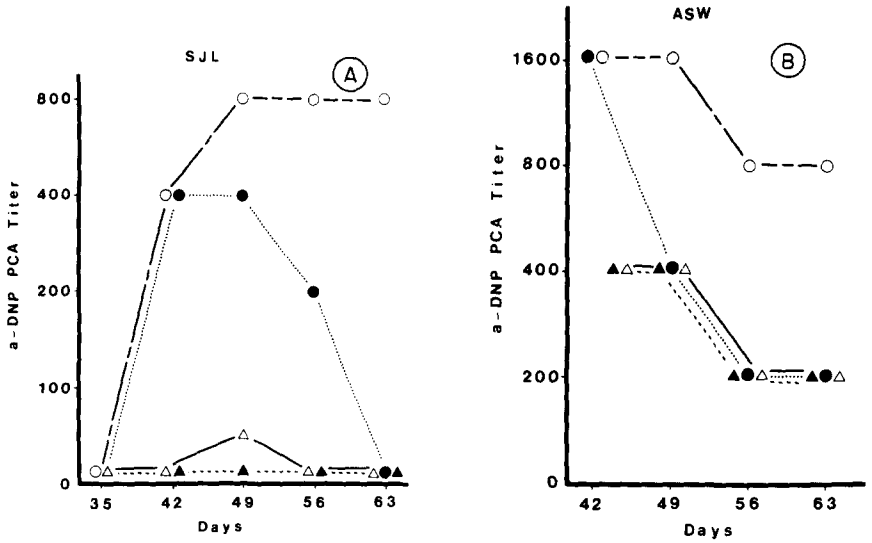

FIG. 2. (A) Antihapten antibody response after cell transfer in SJL mice. SJL mice immunized according to schedule 1 and irradiated on day 36. Anti-DNP IgE (○) and IgG_1 (△) PCA titers of irradiated control animals. Anti-DNP IgE (●) and IgG_1 (▲) PCA titers of mice receiving normal SJL spleen cells. (B) Antihapten antibody response after cell transfer in ASW mice. ASW mice immunized according to schedule 1. Anti-DNP IgE (○) and IgG_1 (△) PCA titer of control group. Anti-DNP IgE (●) and IgG_1 (▲) PCA titers of those mice receiving normal SJL spleen cells.

showed high anti-DNP IgE and IgG_1 responses. After day 42, IgE production in the group receiving SJL spleen cells was diminished as compared to controls. No difference was observed in IgG_1 titers at any time between the two groups (Fig. 2 B).

LACK OF ANTIGEN ACTION ON SUPPRESSOR CELLS. Spleen cells from mice infected 3 wk previously did not show a suppressive effect in irradiated recipients (Table II). On the contrary, these cells did exert a helper effect (3). A helper

TABLE III

*Suppressive Effect by Spleen Cells from Noninfected and Infected SJL mice**

Group	Source of transferred cells	No. of cells transferred	Anti-DNP PCA titer‡			
			Day 49§		Day 63§	
			IgE	IgG_1	IgE	IgG_1
I	(−)	(−)	800	400	600	200
II	Mice infected with 750 Nb larvae 5 days previously	5×10^7	800	200	200	200
III	Mice infected with 750 Nb larvae 5 days previously	1×10^7	800	200	400	200
IV	Noninfected mice	5×10^7	800	200	50	200
V	Noninfected mice	1×10^7	800	200	400	200

* The recipient mice were immunized according to schedule 1 (see Materials and Methods) and irradiated (540 R) on day 36. Cell transfer was done on day 37.

‡ Sera from five mice of each group were pooled and the reciprocal of the highest dilution giving PCA reaction was taken as titer.

§ Days after immunization with DNP-KLH.

effect was not observed with spleen cells from mice infected 5 days previously (3). Therefore, to study the possible action of antigen on spleen cell populations, 1×10^7 or 5×10^7 spleen cells from SJL mice infected 5 days previously or from normal SJL mice were transferred to mice immunized according to schedule 1 and irradiated on day 36 (Table III). Irradiated controls produced persistent anti-DNP IgE antibody. Recipients of 1×10^7 spleen cells from normal mice or from mice infected 5 days previously produced the same amount of anti-DNP IgE antibody even on day 63. However, if the number of transferred cells was increased to 5×10^7, a suppressive effect was observed by day 63 (group IV). Therefore, antigenic stimulation of a suppressive effect by spleen cells after 5 days of infection could be excluded.

EFFECT OF NORMAL SJL THYMOCYTES. In one experiment normal SJL thymocytes were used instead of spleen cells. Anti-DNP IgE antibody in these mice was much lower than in irradiated controls and was comparable to the titers of nonirradiated controls (Table IV).

EFFECT OF ANTI-Thy 1.2 TREATMENT. SJL mice immunized according to schedule 1 were irradiated on day 36. On day 37, 5×10^7 SJL spleen cells treated with anti-Thy 1.2 and complement (see Materials and Methods) were transferred intravenously. The control group received normal mouse serum and complement-treated cells (Table V). Anti-Thy 1.2 serum destroyed the suppressive activity of SJL spleen cells (group II compared to group III) on anti-DNP IgE antibody production.

Antibody Response in (BALB/c × SJL)F_1 Mice. BALB/c mice were selected as controls for persistent and high IgE antibody-responding animals. SJL, BALB/c, and male and female (BALB/c × SJL)F_1 mice were immunized according to schedule 1. BALB/c and F_1 mice showed persistent and high antihapten IgE antibody responses. All sera from the F_1 mice were individually titrated on day 63 and the antihapten IgE antibodies were 1/1,000 or higher (not shown). In contrast, antihapten IgE antibody production in SJL mice reached its peak on

TABLE IV
Suppressive Effect of Normal SJL Thymocytes

Group	Irradiation of recipients*	Cells transferred	Anti-DNP PCA titer‡			
			Day 49§		Day 56§	
			IgE	IgG_1	IgE	IgG_1
I	0	(−)	50	200	50	100
II	540 R	(−)	800	200	400	100
III	540 R	2.5×10^7 thymocytes from normal SJL mice	100	200	100	50

* The recipient mice were immunized according to schedule 1 (see Materials and Methods) and irradiated on day 36. Cell transfer was done on day 37.
‡ Sera from five mice of each group were pooled and the reciprocal of the highest dilution giving PCA reaction was taken as titer.
§ Days after immunization with DNP-KLH.

TABLE V
The Effect of Anti-Thy 1.2 Treatment on Suppressive Effect

Group	Spleen cells* treated with:	No. of cells transferred‡	Anti-DNP PCA titer§			
			Day 49‖		Day 63‖	
			IgE	IgG_1	IgE	IgG_1
I	(−)	0	800	400	600	200
II	Anti-Thy 1.2 + C′	5×10^7	800	200	400	100
III	Normal mouse serum + C′	5×10^7	600	100	100	100

* Normal SJL spleen cells.
‡ The recipient mice were immunized according to schedule 1 (see Materials and Methods) and irradiated (540 R) on day 36. The cells were transfered on day 37.
§ Sera from five mice of each group were pooled and the reciprocal of the highest dilution giving PCA was taken as titer.
‖ Days after immunization with DNP-KLH.

day 42 and terminated quickly. There was no detectable antihapten IgE antibody on day 63 in SJL mice (Table VI). Anti-Nb IgE antibody was produced in BALB/c and F_1 mice on day 42, but could not be detected in nonirradiated SJL mice (Table VI). However, in SJL mice which were irradiated on day 36, 1 day after challenge, anti-Nb IgE was demonstrable on day 49 (the titer was 1/100) (not shown).

Antihapten IgG_1 antibody was present in BALB/c, F_1, and in SJL mice on days 42 and 63. Again, this IgG_1 response did not terminate in SJL mice on day 63. Anti-Nb IgG_1 was not detected in any of these mice. There was no difference in antibody production in either sex (Table VI).

Antibody Response in (BALB/c × SJL)F_1♂ × SJL♀ Backcrosses. (BALB/c × SJL)F_1♂ × SJL♀ backcross mice were immunized according to schedule 1. With regard to the antihapten IgE antibody response, the animals were separated into high responders (8 mice, more than 1/800 PCA titer) and low responders (10 mice, less than 1/200 PCA titer) according to their PCA titer on day 42 (Table VII). On day 56, all the high responder mice showed high antihapten IgE

TABLE VI
Antibody Response in (BALB/c × SJL)F_1 Mice

Strain	No. of mice immunized	PCA titer*					
		Day 42‡				Day 63‡	
		Anti-DNP		Anti-Nb		Anti-DNP	
		IgE	IgG_1	IgE	IgG_1	IgE	IgG_1
(BALB/c × SJL)F_1♂	13	4,000	800	100	0§	2,000	800
(BALB/c × SJL)F_1♀	3	4,000	400	ND‖	ND	2,000	800
BALB/c	10	2,000	200	100	0	2,000	400
SJL	8	500	400	0	0	0	100

* Titer determined by pooled serum from each group.
‡ Days after DNP-KLH immunization.
§ 0, no PCA reaction with sera diluted 1/10.
‖ ND, not done.

antibody titers. However, the titers in almost all of the low responders were less than 1/10. The difference between the two groups is significant $P < 0.001$ for both days. All high responders had anti-Nb antibody (on day 42 or on day 56). Only one low responder had anti-Nb IgE antibody. The PCA titers of anti-Nb IgE antibody were lower than those of antihapten IgE antibody.

The division of backcross mice into two groups, i.e. high and low responders, is apparent even after primary DNP-KLH immunization. 21 days after injection of 1 μg DNP-KLH in 1 mg $Al(OH)_3$, nine mice had antihapten IgE antibody titers of 1/100 or higher and seven mice had titers of only 1/10 or less (not shown). The mice were infected on day 21 and boosted on day 35 as described above. The antihapten IgE antibody titers on day 42 were more than 1/1,600 in the high responder mice (those which had high antihapten IgE antibody titers on day 21). The other mice (low responders after the primary immunization) had antihapten IgE antibody titers which were less than 1/200.

Backcross mice were also separated into high and low responders according to the antihapten IgG_1 antibody response. The high responder mice (those which had high antihapten and anti-Nb IgE titers) produced large amounts of antihapten IgG_1 antibody. The differences in antihapten IgG_1 titers between the two groups were significant $0.01 < P < 0.02$ for sera on day 42 and $0.001 < P < 0.01$ for sera on day 56. However, antihapten IgG_1 antibody production did not terminate even in the low responder mice.

Anti-Nb IgG_1 antibody response was also tested with several sera on day 42. However, none of the tested sera provoked PCA reaction at 1/10 dilution.

The presence of H-2^d antigen was tested in backcross mice using erythrocytes. The results are shown in Table VII. 12 mice were positive for the H-2^d antigen and 6 mice were negative. However, there was no correlation between IgE or IgG_1 antibody response and H-2^d antigen.

Infectivity of N. brasiliensis in BALB/c and SJL Mice. BALB/c and SJL mice were injected subcutaneously with 750 third stage larvae of *N. brasiliensis*. The eggs appeared in the feces within 6 days after the injection of larvae (Fig. 3). The peak of egg production was reached on day 7 in both strains of mice. The

TABLE VII
Antibody Response in Backcross Mice

Mice	PCA titer							
	Day 42*				Day 56*			
	Anti-DNP		Anti-Nb		Anti-DNP		Anti-Nb IgE	Anti-H-2^d HA‡
	IgE	IgG_1	IgE	IgG_1	IgE	IgG_1		
♀1	50	800	0§	ND‖	0	1,600	ND	+
2	200	1,600	50	ND	50	400	ND	+
3	50	100	0	ND	50	50	ND	+
4	50	800	0	0	0	800	0	−
5	50	100	0	ND	0	800	ND	−
6	0	100	0	0	0	200	0	−
♂1	50	400	0	0	0	400	0	+
2	0	100	0	ND	0	100	ND	+
3	0	200	0	ND	0	400	ND	−
4	200	800	0	0	0	400	0	+
♀7	3,200	1,600	100	0	1,600	1,600	100	+
8	3,200	1,600	100	ND	400	1,600	ND	−
♂5	3,200	3,200	0	0	3,200	3,200	50	+
6	800	1,600	0	0	3,200	1,600	50	+
7	3,200	1,600	100	ND	3,200	1,600	ND	+
8	1,600	400	100	ND	3,200	400	ND	+
9	1,600	800	100	0	3,200	800	100	+
10	1,600	400	100	ND	3,200	800	ND	−

* Days after DNP-KLH immunization.
‡ +, positive hemagglutination reaction with anti-H-2^d sera diluted 1/800.
§ 0, no PCA reactions with sera diluted 1/50 except for anti-Nb IgG1 where the dilution was 1/10.
‖ ND, not done.

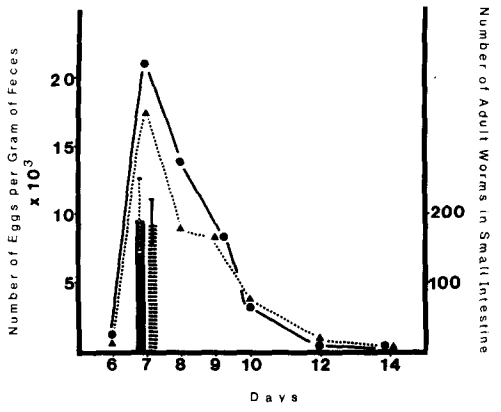

FIG. 3. Egg production and infectivity of *N. brasiliensis* in SJL and BALB/c mice. Egg production of *N. brasiliensis* in SJL (▲) and BALB/c (●). Number of adult *N. brasiliensis* in small intestine of SJL (■) and BALB/c (▤). Vertical bars represent the range.

number of eggs at the peak was slightly more in BALB/c mice than in SJL mice. The kinetics of egg production show similar patterns in each strain. It was difficult to detect the eggs in the animal feces after day 18 by this method.

The number of adult worms in the small intestine of infected mice was counted 7 days after injection of *N. brasiliensis* larvae. Virtually the same number of adult worms was obtained from each strain of mouse.

Discussion

In the SJL strain, antihapten IgE antibody production was rapidly terminated (Fig. 1 A). Normal spleen cells from SJL mice injected into antihapten IgE-producing animals were capable of terminating this IgE response in irradiated SJL (Fig. 2 A) or nonirradiated ASW mice (Fig. 2 B). ASW mice were used as recipients because they are also H-2^s as the SJL strain.

It had to be ruled out that the difference between SJL mice and other strains in IgE antibody production is not due to differences in handling the infestation by *N. brasiliensis*. There was no difference in the number of adult worms in the small intestine and the number of eggs per gram of feces of infested SJL and BALB/c mice. It is of interest in this regard that SJL mice did not produce anti-Nb IgE antibody.

On the one hand it has been shown that helper T cells are relatively radioresistant (15). On the other hand, it has also been shown that suppressor T cells are very radiosensitive (16, 17). Irradiation data on survival of SJL mice has been published (18); 540 R is not lethal in this strain. Further, this dose of irradiation generally did not inhibit antibody production. Therefore, it did not destroy either carrier-specific helper-committed T cells or hapten-specific committed B cells. Irradiated controls consistently produced high and persistent titers of hapten-specific IgE antibody.

The day of irradiation after challenge is important. Animals irradiated on day 36 (1 day after challenge) produced lower titers of hapten-specific IgE antibody on day 42 than mice irradiated on day 39 (4 days after challenge). The antihapten IgE antibody titer in mice irradiated on day 36 increased later and persisted longer and at higher titers than in mice irradiated on day 39. It is possible that some of the IgE-producing B cells are damaged by irradiation and this would explain the lower titers at day 42 of hapten-specific IgE antibody in mice irradiated on day 36. However, this possible damage is surely quickly overcome, because the IgE antibody titer subsequently increased and persisted (Fig. 1 A). Destruction by radiation (540 R) of suppressor T cells early after challenge is indicated by the results (Fig. 1 A).

Transfer experiments also showed the destruction of suppressor cells by irradiation and demonstrated that 3 wk after infection with the number of cells transferred no suppressive effect was detectable. It is possible that the presence of large numbers of carrier-specific helper T cells masks the activity of suppressor cells (Table II). Previous transfer experiments demonstrated that antigen did not prepare effective helper cells very rapidly (3) and that the antihapten IgE production in animals receiving cells 5 days after infection was still inhibited (Table III).

That suppressor cells are indeed T cells is shown by the suppressive effect of

thymocytes (Table IV) and by the lack of suppressive effect of spleen cells transferred after treatment with anti-Thy 1.2 and complement (Table V). In contrast to the suppressive effect on antihapten IgE production by normal SJL T cells, no appreciable effects have been observed on production of IgG_1 antihapten antibody.

F_1 hybrids (BALB/c × SJL) produce high titer antihapten antibodies for a prolonged time (Table VI). This is unlike the SJL parent, but similar to the BALB/c parent. Therefore, the presence of suppressor T cells must be a recessive trait. In the backcross mice of the (BALB/c × SJL)F_1 to the SJL parent, 55% of the progeny had low titer antihapten IgE antibodies and the production of this antibody was terminated rapidly in these mice, precisely as in the SJL parent strain (Table VII). The proportion of backcross mice having this characteristic is in accord with the inheritance of the expression of suppressor activity of T cells by one autosomal recessive gene. No linkage of inheritance of suppressor T-cell activity was found with the *H-2*-gene complex (Table VII).

When backcross mice [(BALB/c × SJL)F_1 × SJL] were immunized it was found that about half of the animals not only produce lower amounts of antihapten IgE antibody, but also lower amounts of antihapten IgG_1 antibody. This "low responder" characteristic is much more pronounced for antihapten IgE antibody ($P < 0.001$ at day 42; $P < 0.001$ at day 56) than for antihapten IgG_1 antibody ($0.01 < P < 0.02$ at day 42; and $0.001 < P < 0.01$ at day 56) (Table VII). The low responder characteristic was found not only in animals immunized according to schedule 1 (Table VII), but also in the primary immune response.

In nonirradiated SJL mice, anti-Nb IgE was not produced at any time. However, after irradiation anti-Nb IgE antibody was present in the sera on day 49. Anti-Nb IgE antibody was regularly obtained in "high responder" mice though the titers were lower than the titers of antihapten IgE antibody in the same animals. Only one of the low responder mice produced anti-Nb IgE antibody. No anti-Nb IgG_1 was found in any of the backcross animals tested.

The production of antihapten IgG_1 antibody is much lower in the SJL strain, but the production is much more persistent than the production of antihapten IgE antibody. The action of suppressor T cells in SJL mice is mainly on the production of IgE antibody. The low and high responder characteristics of the backcross animals for IgE and IgG_1 production might be due to suppressor T-cell activity or it could be due to another gene governing IgE and IgG_1 antibody production. If so, this gene must be closely linked to that regulating suppressor T-cell activity as early termination of IgE antibody and the low responder trait segregate together.

The SJL strain has very interesting traits. This strain developes reticulum cell sarcomas upon aging (19, 20). It actively suppresses allotype production (21–23), has other T- and B-cell defects (24), and as shown by the present study has suppressor T cells acting on the production of IgE antibody.

Summary

High titer and persistent antihapten IgE production in SJL mice can be obtained using appropriate immunization and radiation. Nonirradiated mice rapidly terminate this antihapten IgE production. Radiation was not necessary

to prolong antihapten IgE production in other strains of mice. Termination can be obtained even in irradiated SJL mice by transferring normal SJL spleen cells. That the suppressor cells are T cells is shown by using thymocytes or cells treated with anti-Thy 1.2 and complement. No appreciable suppressive effect by normal spleen cells could be demonstrated on IgG_1 production in SJL mice. The characteristic of low and transient IgE antibody response in SJL mice is inherited as a recessive trait controlled by a single Mendelian autosomal gene and is not linked to the *H-2*-gene complex. This characteristic does not depend on the infectivity of *Nippostrongylus brasiliensis*, the effect of anticarrier antibody, or the recognition of antigen.

The authors thank Mr. Csaba de Szalay, Mr. Bruce Kaplan, and Mrs. Zsuzsanna Fekete de Nagyivany for their excellent technical help; Dr. S. Salvatore Caiazza for his help in the preparation of the manuscript; and Mr. Frank Reilly for his secretarial assistance.

Received for publication 6 November 1975.

References

1. Revoltella, R., and Z. Ovary. 1969. Reaginic antibody production in different mouse strains. *Immunology*. 17:45.
2. Vaz, E. M., N. M., Vaz, and B. B. Levine. 1971. Persistent formation of reagins in mice injected with low doses of ovalbumin. *Immunology*. 21:11.
3. Kojima, S., and Z. Ovary. 1975. Effect of *Nippostrongylus brasiliensis* infection on anti-hapten IgE antibody response in the mouse. I. Induction of carrier specific helper cells. *Cell. Immunol.* 15:274.
4. Kojima, S., and Z. Ovary. 1974. Induction of carrier specific helper activity by *Nippostrongylus brasiliensis* infection in anti-hapten IgE antibody response in the mouse. *Fed. Proc.* 33:756.
5. Gershon, R. K. 1974. T cell control of antibody production. *Contemp. Top. Immunobiol.* 3:1.
6. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. VI. Inhibitory effect of thymocytes on the homocytotropic antibody response. *J. Immunol.* 107:1682.
7. Kishimoto, T., and K. Ishizaka. 1974. Regulation of antibody response in vitro. VIII. Multiplicity of soluble factors released from carrier-specific cells. *J. Immunol.* 112:1685.
8. Basten, A., J. F. A. P. Miller, J. Sprent, and C. Cheers. 1974. Cell-to-cell interaction in the immune response. X. T-cell-dependent suppression in tolerant mice. *J. Exp. Med.* 140:199.
9. Takahashi, T., E. A. Carswell, and G. J. Thorbecke. 1970. Surface antigens of immunocompetent cells. I. Effect of θ and PC.1 alloantisera on the ability of spleen cells to transfer immune responses. *J. Exp. Med.* 132:1181.
10. Ovary, Z. 1964. Passive cutaneous anaphylaxis. *In* Immunological Methods, C.I.O.M.S. Symposium. J. F. Ackroyd, editor. Academic Press, Inc., New York. 97.
11. Ovary, Z., S. S. Caiazza, and S. Kojima. 1975. PCA reactions with mouse antibodies in mice and rats. *Int. Arch. Allergy Appl. Immunol.* 48:16.
12. Mota, I., and D. Wong. 1969. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci.* 8:813.
13. Ovary, Z., T. W. Vris, C. de Szalay, N. M. Vaz, and C. A. Iritani. 1973. Independent

segregation of the H-2 locus and the locus for responsiveness to histamine-sensitizing factor. *Proc. Natl. Acad. Sci. U. S. A.* **70**:2500.

14. Gorer, P. A., and Z. B. Mikulska. 1954. Antibody response to tumor inoculation improved methods of antibody detection. *Cancer Res.* **14**:651.
15. Hamaoka, T., D. H. Katz, and B. Benacerraf. 1972. Radioresistance of carrier-specific helper thymus-derived lymphocytes in mice. *Proc. Natl. Acad. Sci. U. S. A.* **69**:3453.
16. Tada, T., M. Taniguchi, and K. Okumura. 1971. Regulation of homocytotropic antibody formation in the rat. II. Effect of x-irradiation. *J. Immunol.* **106**:1012.
17. Kapp, J. A., C. W. Pierce, S. Schlossman, and B. Benacerraf. 1974. Genetic control of immune responses in vitro. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid-L-alanine-L-tyrosine (GAT). *J. Exp. Med.* **140**:648.
18. Lerman, S. P., J. Chapman, E. A. Carswell, and G. J. Thorbecke. 1974. Unique properties of transplantable SJL/J reticulum cell sarcomas (RCS). *Fed. Proc.* **33**:616.
19. Siegler, R., and M. A. Rich. 1968. Pathogenesis of reticulum cell sarcoma in mice. *J. Natl. Cancer Inst.* **41**:125.
20. Murphy, E. D. 1969. Transplantation behavior of Hodgkin's like reticulum cell neoplasmas of strain SJL/J mice and results of tumor reinoculation. *J. Natl. Cancer Inst.* **42**:797.
21. Jacobson, E. B., and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL × BALB/c)F_1 mice. *J. Exp. Med.* **135**:1151.
22. Jacobson, E. B., L. A. Herzenberg, R. Riblet, and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. *J. Exp. Med.* **135**:1163.
23. Herzenberg, L. A., E. L. Chan, M. M. Ravitch, R. Riblet, and L. A. Herzenberg. 1973. Active suppression of immunoglobulin allotype synthesis. III. Identification of T cells as responsible for suppression by cells from spleen, thymus, lymph node, and bone marrow. *J. Exp. Med.* **137**:1311.
24. Mozes, E., R. Isac, and M. J. Taussig. 1975. Antigen-specific T-cell factors in the genetic control of the immune response to poly(Tyr,Glu)-polyDLAla-polyLys. Evidence for T- and B-cell defects in SJL mice. *J. Exp. Med.* **141**:703.