SUPPRESSION OF IgE ANTIBODY PRODUCTION
IN SJL MICE

III. Characterization of a Suppressor Substance Extracted from Normal
SJL Spleen Cells*

BY NAOHiro WATANABE$ AND ZOLTAN OvARY
(From the New York University School of Medicine, New York 10016)

Among regulators of immune responses are subpopulations of T cells (1), i.e.
antigen-specific (2–4) and antigen-nonspecific suppressor cells (5, 6). Suppressor
factors from these cells have been extracted and characterized (7–10). Antigen-
specific suppressor cells have also been demonstrated in the case of IgE antibody
responses (11, 12).

High and persistent anti-hapten IgE antibody response was induced in many strains of
mice by appropriate immunization and a nemotode parasite (Nippostrongylus brasiliensis)
infection (13, 14). However, the IgE antibody response was rapidly terminated in SJL
mice. The transient IgE antibody response in SJL mice is inherited as a recessive trait
controlled by a single autosomal gene and is not linked to the H-2 complex (14). When
immunized and infected SJL mice were irradiated (540 R), high titers of anti-hapten IgE
antibody persisted for several weeks. Suppression of IgE response was induced in immu-
nized and irradiated SJL mice by transfer of normal SJL spleen cells. These suppressor T
cells had no specificity for the antigen (14). Moreover, when normal SJL spleen cells were
reated with anti-Ly 1.2 and complement before the transfer, no suppression was ob-
served (15).

The present report shows that the suppression of the IgE response is induced
by an extract from normal SJL spleen cells. The suppressor substance is a heat-
labile high molecular weight protein.

Materials and Methods

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address: Department of Parasitology, Jikei University School of Medicine, Tokyo, Japan.

Abbreviations used in this paper: BSA, bovine serum albumin; HBSS, Hanks' balanced salt
solution; KLH, keyhole limpet hemocyanin; Nb, Nippostrongylus brasiliensis extract; Ov, ovalbu-
min; PCA, passive cutaneous anaphylaxis.
Preparation of these antigens has been previously described (13). The subscripts are omitted in the text.

**Immunological Reagents.** Rabbit globulins were precipitated by (NH₄)₂SO₄ (33% final concentration). Anti-mouse immunoglobulin (Ig) was prepared in rabbits. The rabbits were injected with 2 mg mouse Ig in complete Freund's adjuvant in the foot pads and boosted intravenously (i.v.) with 1 mg mouse Ig in 0.15 M NaCl on three consecutive days monthly. The rabbits were bled 4 mo after the primary immunization. The globulin fraction of the rabbit antisera was separated by (NH₄)₂SO₄ precipitation (as above) and gave strong lines in double diffusion in agar (Ouchterlony) when assayed against mouse Ig preparations.

**Animals.** 8- to 12-wk old female SJL mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Male Sprague-Dawley rats, weighing 250–300 g were obtained from the Blue Spruce Farms, Altamont, N. Y.

**Immunization.** Immunization schedules, infections, and boosters were done as published (14). 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 reinfected (challenged) intraperitoneally with 1 μg DNP-Nb mixed with 1 mg Al(OH)₃ on day 35. 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), and then centrifuged for 10 min at 1,000 g. The supernate was considered to be a 1/10 dilution.

In one experiment to compare a possible carrier specificity, the mice were immunized intraperitoneally with 10 μg Nb mixed with complete Freund's adjuvant or with 1 mg Al(OH)₃ and boosted with 10 μg Nb and the same respective adjuvant 3 wk after immunization. The spleen cells from these mice were harvested 7 days after booster. Spleen cells from mice infected with 750 larvae of N. brasiliensis 3 wk previously were also used as source of suppressor cells.

**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 (1 day after challenge).

**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 was estimated by the trypan blue exclusion test. Mice were i.v. injected with 3 × 10⁷ or 5 × 10⁷ viable spleen cells. Thymocytes and mesenteric lymph node cells were prepared in a similar fashion.

**Preparation of Spleen Cell Extract.** Spleen cells from five or six normal SJL mice were washed and suspended in 3 ml HBSS (5 × 10⁹ cells/ml). The cells were frozen in dry ice-alcohol and thawed in a 37°C water bath. This procedure was repeated five times. The tubes were then centrifuged at 4,000 g at 4°C for 30 min. After centrifugation the volume of the supernate was 2 ml (equivalent to 7.5 × 10⁹ cells/ml). Extract equivalent to 1.5 or 2 × 10⁸ normal SJL spleen cells was injected i.v. per mouse 5 h after irradiation.

**Absorption with Anti-Mouse Immunoglobulin.** Globulin fraction of rabbit anti-mouse Ig and normal rabbit serum were coupled to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) according to the method of Axen et al. (16). The absorbing activity of these preparations was tested by Ouchterlony test using mouse globulin. The spleen cell extract was added to the same volume of packed, coupled Sepharose 4B and rotated at room temperature for 2 h. The extract was separated from the absorbent after centrifugation at 40 g at 4°C 5 min.

**Protease Treatment.** Protease (Subtilopeptidase-A; Sigma Chemical Co., St. Louis, Mo.) was used. The extract from 12 × 10⁸ normal SJL spleen cells was digested with 70 μg of protease at room temperature for 2 h. The extract equivalent to 1.5 × 10⁸ spleen cells per mouse was injected i.v. immediately after digestion.

**Gel Filtration.** The extract from 10 × 10⁸ normal SJL spleen cells in 1 ml was applied on Sephadex G-100 or G-200 columns (Pharmacia Fine Chemicals, Inc.) of 2.5 × 90 cm and eluted with 0.01 borate-buffered 0.15 M NaCl (pH 8) at 4°C. 2-ml fractions were collected in individual tubes with a flow rate of 10 ml/h. The optical density at 280 μm of each fraction was monitored by an LKB Uvicord Absorptiometer (LKB Instruments, Inc., Rockville, Md.). The pooled fractions were concentrated by positive pressure dialysis and injected i.v. into the mice (see Results).
**Table I**

Suppressive Effect by Spleen Cells from Immunized SJL Donors

| Group* | Source of transferred spleen cells (5 x 10⁷) | Anti-DNP PCA titer† | On day 49§ | On day 63§ |
|--------|---------------------------------------------|---------------------|-------------|-------------|
|        |                                             | IgE    | IgG1    | IgE    | IgG1    |
| I      | No cells transferred (control)              | 400    | 100     | 400    | 10      |
| II     | Mice immunized with 10 μg Nb with 1 mg Al(OH)₃ | 100    | 100     | 0      | 50      |
| III    | Mice immunized with 10 μg Nb in complete Freund's adjuvant | 100    | 100     | 0      | 50      |
| IV     | Mice infected with 750 larvae of *N. brasiliensis* 3 wk previously | 200    | 100     | 200    | 50      |
| V      | Normal SJL mice                             | 200    | 100     | 50     | 50      |

* The recipient mice were immunized as described in the 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.
∥ 0, no PCA reactions with sera diluted 1/10.

**Results**

Suppressive Effect by Spleen Cells from Immunized Mice. Table I shows the anti-DNP IgE and IgG₁ antibody titers on days 49 and 63, 2 and 4 wk after challenge. Irradiated control mice, to which no cells were transferred, showed high titers of anti-DNP IgE antibody (group I). Anti-DNP IgE responses in the recipients receiving normal SJL spleen cells were suppressed, especially on day 63 (group V).

Somewhat stronger suppression was observed in the mice of group II and group III [cells from mice immunized with Nb in Al(OH)₃ or in complete Freund's adjuvant, respectively]. The anti-DNP IgE titer in the mice of group IV (cells from mice infected with *N. brasiliensis*) was only slightly lower than that of group I (controls). Anti-DNP IgG₁ titers in these irradiated mice were low, probably an effect of the irradiation as already discussed (14, 15).

Organ Distribution of Suppressor Cells. The action of cells from normal SJL spleen, mesenteric lymph node, and thymus was compared. 3 x 10⁷ cells were injected into immunized and irradiated recipients on day 37. The anti-DNP IgE titers are shown in Table II.

There was no great difference between controls (group I) and the experimental groups on day 42. However, on day 63, the suppressive effect was evident and equally strong in groups II and IV (spleen and mesenteric lymph node cells, respectively) and evident but less marked in group III (thymocytes).
**Suppressor Cells in SJL Mice**

### Table II

**Organ Distribution of Suppressor Cells**

| Group* | Transferred cells from (3 × 10⁷): | Anti-DNP IgE PCA titer† | Day 49§ | Day 63§ |
|--------|----------------------------------|------------------------|--------|--------|
| I      | No cells transferred (control)   | 800                    | 800    |        |
| II     | Spleen                          | 800                    | 50     |        |
| III    | Thymus                          | 800                    | 200    |        |
| IV     | Mesentric lymph node            | 400                    | 50     |        |

* The recipient mice were immunized as described in the 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.

**Suppression of IgE Response by the Spleen Cell Extract.** Extract corresponding to 1.5 or 2 × 10⁸ spleen cells was injected i.v. into the recipients (5 h after irradiation). As shown in Fig. 1A, nonirradiated mice showed only a transient anti-DNP IgE antibody response. When the immunized mice were irradiated persistent high titers of IgE antibody were obtained.

The spleen cell extract was as effective in IgE antibody suppression as the viable cells. Higher doses produced greater suppression. IgG₁ antibody response persisted in all the groups, even in the group which was irradiated (Fig. 1B). There was no great difference between the mice receiving and not receiving the extract. However, as the titers were low, it is difficult to precisely evaluate the effect of the extract on IgG₁ suppression.

**Characterization of the Spleen Cell Extract**

**Absorption with anti-mouse Ig.** The results are shown in Table III. After absorption each recipient received the extract equivalent to 1.5 × 10⁸ spleen cells. The extract absorbed with anti-mouse Ig (group III) and that absorbed with normal globulin (group IV) induced almost the same suppressive effect.

**Heat treatment.** The suppressive effect of the extract was destroyed by heating at 56°C for 2 h (Table IV, compare group III to group II).

**Protease treatment.** Protease treatment destroyed the suppressive activity of the extract (Table V).

**Gel filtration.** The extract obtained from 10 × 10⁸ spleen cells in a 3 ml volume was applied to the Sephadex G-200 column (see Materials and Methods). The elution profile showed two peaks. One peak at 160 ml corresponded to the void volume as determined by blue dextran as a marker. The second peak was at 284 ml. The contents of the tubes from 140 to 180 ml were pooled and designated as fraction I (void volume). Contents from tubes 181 to 240 ml were pooled and designated fraction II. Fraction III (the second peak) was the pool from 241 to 300 ml. Each fraction was concentrated to 1.5 ml. If all activity would have been in only one fraction then 0.25 ml of this fraction would correspond to the activity of 1.66 × 10⁸ cells. Each fraction was injected i.v. into the mice (0.25 ml per mouse). As shown in Table VI only fraction I, corresponding to the void volume, could induce suppression (group I). Similarly, only the fraction corresponding to the
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Fig. 1. Anti-DNP antibody response in SJL mice. (●), nonirradiated mice. (○), mice irradiated with 540 R. (■), irradiated mice (640 R) injected with extract of normal SJL spleen cells corresponding to \(1.5 \times 10^8\) cells. (□), irradiated mice (540 R) injected with the same extract but corresponding to \(2 \times 10^8\) cells. (A), anti-DNP IgE titers and (B), anti-DNP IgG1 titers.

TABLE III
Suppressive Effect of Spleen Cell Extract Absorbed with Anti-Mouse Ig

| Group* | Injected with:                          | Anti-DNP IgE titer† on day 63§ |
|--------|----------------------------------------|-------------------------------|
| I      | HBSS                                   | 600                           |
| II     | Extract equivalent to \(1.5 \times 10^8\) cells | 50                           |
| III    | Extract equivalent to \(1.5 \times 10^8\) cells absorbed with rabbit anti-mouse Ig | 200                           |
| IV     | Extract equivalent to \(1.5 \times 10^8\) cells absorbed with normal rabbit globulin | 200                           |

* The recipient mice were immunized as described in the Materials and Methods and irradiated (540 R) on day 36.
† 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.

void volume after passage on Sephadex G-100 column had a suppressive effect (results not shown).

ULTRACENTRIFUGATION. Ultracentrifugation of the extract was performed to examine the possibility of a virus as a suppressor substance. The suppressive effect of the extract remained in the supernate after centrifugation at 100,000 g for 90 min (Table VII, group I).
**SUPPRESSOR CELLS IN SJL MICE**

**Table IV**

*Effect of Heat on Suppressive Activity of the Extract*

| Group* | Injected with:                               | Anti-DNP IgE titer on day 63$ |
|--------|---------------------------------------------|-------------------------------|
| I      | HBSS                                        | 400                           |
| II     | Extract equivalent to $2 \times 10^8$ cells | 50                            |
| III    | Extract equivalent to $2 \times 10^8$ cells heated at 56°C for 2 h | 800                           |

* The recipient mice were immunized as described in the Materials and Methods and irradiated (540 R) on day 36.
† 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**

*Suppressive Effect of the Spleen Cell Extract after Protease Treatment*

| Group* | Injected with:                               | Anti-DNP IgE titer on day 63$ |
|--------|---------------------------------------------|-------------------------------|
| I      | HBSS                                        | 400                           |
| II     | Extract equivalent to $2 \times 10^8$ cells | 50                            |
| III    | Extract equivalent to $2 \times 10^8$ cells treated with protease | 400                           |

* The recipient mice were immunized as described in the Materials and Methods and irradiated (540 R) on day 36.
† 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 VI**

*Suppressive Effect of the Fractions Obtained by Sephadex G-200 Filtration*

| Group* | Injected with: | Anti-DNP IgE titer on day 56$ |
|--------|----------------|-------------------------------|
| I      | HBSS           | 800                           |
| II     | Fraction II    | 0                             |
| III    | Fraction II    | 800                           |
| IV     | Fraction III   | 400                           |

* The recipient mice were immunized as described in the Materials and Methods and irradiated (540 R) on day 36.
† 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.
‖ See text.

**Discussion**

These experiments confirmed previous results in which it has been shown that normal SJL spleen cells had a suppressing effect on anti-DNP IgE antibody production (14) (Table I; compare IgE titers on day 63 between group I and group
TABLE VII
Suppressive Effect of the Extract after Ultracentrifugation

| Group* | Injected with: | Anti-DNP IgE titer$ on day 63§ |
|--------|----------------|----------------------------------|
| I      | HBSS           | 400                              |
| II     | Supernate of extract equivalent to $1.5 \times 10^8$ cells after centrifugation for 90 min at 100,000 g | 50 |
| III    | Extract equivalent to $1.5 \times 10^8$ cells | 50 |

* The recipient mice were immunized as described in the Materials and Methods and irradiated (540 R) on day 36.

† 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.

V). Somewhat greater suppression was obtained with spleen cells from mice immunized with Nb and Al(OH)$_3$ or complete Freund’s adjuvant (Table I; compare group V with groups II and III). Therefore, it is possible that in addition to the antigen-nonspecific suppression by normal SJL spleen cells another suppressive mechanism is also operating, i.e., the type of suppression observed in other strains of mice on antibody production (2-4). However, our studies were directed to characterize the antigen-nonspecific suppressive effect by normal SJL spleen cells.

When spleen cells obtained from mice infected with *N. brasiliensis* larvae were used the suppression was much less evident (Table I; compare group IV with group V). It is probable that the suppressive effect in this case is masked by carrier-specific helper T cells (13).

The anti-DNP IgG$_1$ titers in these irradiated mice were low. As discussed previously (14) this is probably attributable to the irradiation.

Mesenteric lymph node cells and spleen cells were more effective in IgE antibody suppression than thymus cells. This fact was expected, as the antigen-nonspecific suppressor T cells from normal SJL mice are of the Ly-1 subclass (15) and it is known that the majority of thymus cells is of the Ly 1, 2, 3 subclass (20). Therefore, it was decided that spleen cells would be used for extraction of the substance responsible for suppression.

The spleen cell extract injected into anti-DNP IgE producing SJL mice was very effective in suppressing IgE production (Fig. 1 A). Extract corresponding to $1.5 \times 10^8$ cells reduced the titer considerably (from $1/400$ on day 56 to $1/20$ on day 63) when mice which did not receive the extract had still a high titer ($1/600$). Extract corresponding to $2 \times 10^8$ cells was even more effective. On anti-DNP IgG$_1$ production the extract seems to be much less effective. This point needs further investigation but our aims were to study the role of the extract on IgE production and not on IgG$_1$.

The suppressive substance in the extract was not absorbed by rabbit antismouse immunoglobulin (Table III; compare groups III and IV with group I). The efficacy of the extract is destroyed by exposure to heat (56°C for 2 h) (Table IV) and it was destroyed by protease (Table V).

The heat-labile protein responsible for suppression has a mol wt higher than
300,000 daltons as it is eluted in the void volume by filtration of Sephadex G-200 (Table VI) and G-100. However, it is not precipitated by ultracentrifugation at 100,000 g for 90 min. Therefore, it is improbable that the suppressive effect is due to a virus. It is possible but highly improbable that the suppressive substance extracted from normal SJL spleen cells could be an agglomerate of smaller molecular weight proteins. If it were an agglomerate one would expect some suppressive activity also in fractions II or III (Table VI), which was not the case.

Suppression of IgE antibody production in mice was demonstrated using urea-denatured antigen (21). This suppression is different from the one which was investigated by us previously because the former is antigen specific and the latter, observed in the SJL strain of mice, is antigen nonspecific. In another species, the rat, antigen-specific IgE suppression was also demonstrated (11).

Suppression of antibody formation using the plaque-forming cell techniques was shown in mice (2, 4). In these cases, the suppressive factor is antigen specific and its production is controlled by the $H-2$ complex (4). The suppressor factor is a relatively small molecular weight protein (mol wt between 35,000 and 55,000 daltons). Again the suppressor factor reported in this paper is different from that quoted above because of the molecular weight, and the antigen specificity. Antigen-nonspecific suppressor substance obtained by stimulation with concanavalin A was reported using the plaque-forming cell assay (10). Here again the mol wt is between 48,000 and 67,000 daltons (10).

Another important point to be considered is the time lag between injection of the extract and the suppression of IgE antibody titers. The suppression occurred with an important time lag; generally about 3 wk after injection of the extract. If the extract would have direct enzymatic action on IgE and not an action on the production of IgE one would expect no time lag. We propose therefore that the extract has an action on the IgE production. Because of the time lag it is possible that the extract has an action not directly on the anti-DNP IgE antibody-producing B cell but on some other cell which in turn influences the antibody-producing B cell.

Summary

SJL mice were immunized with 1 $\mu$g dinitrophenylated keyhole limpet hemocyanin in 1 mg Al(OH)$_3$. The mice were infected 21 days later with 750 third stage larvae of *Nippostrongylus brasiliensis*. On day 35, 14 days after infection, they were injected with 1 $\mu$g DNP-N. brasiliensis extract (Nb) in 1 mg Al(OH)$_3$. In order to obtain high titer and persistent anti-DNP IgE antibody the mice were irradiated (540 R) 1 day after injection of DNP-Nb. Suppression of anti-DNP IgE antibody production was induced by spleen cells from normal SJL mice. Suppression of IgE antibody response is also obtained by an extract from normal SJL spleen cells. The suppressor substance from normal SJL spleen cell extract is a heat-labile protein, and is not absorbed by anti-mouse immunoglobulin. The mol wt of this substance is larger than 300,000 daltons as determined by gel filtration on Sephadex G-200, but after ultracentrifugation, the supernate still has suppressive activity on IgE antibody production.

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