ACTIVE SUPPRESSION OF
1-FLUORO-2,4-DINITROBENZENE-IMMUNE
T CELLS

Requirement of an Auxiliary T Cell Induced by Antigen*

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Shortly after the discovery of T- and B-cell interactions in humoral-immune
responses, interest developed in the possible occurrence of interactions between T cells
during cell-mediated-immune responses. T-T interactions occur in virtually all cell-
mediated-immune responses including graft-versus-host (GvH) reactions (1), the
development of cytotoxic T cells (2, 3), mixed lymphocyte reactions (4), responses to
mitogens (5), generation of helper T cells (6, 7), and the development of delayed
hypersensitivity (8). More recently, they have been discovered in the activation of
suppressor T cells (Ts) (9-11).

The purpose of this study was to investigate whether T-T cell interactions were
involved in the expression of suppressor T cells in contact sensitivity system. The
results indicate that suppressor T cells, which block the efferent route of contact
sensitivity (Ts-eff), require the presence of another population of auxiliary T suppressor
cells (Ts-aux). The Ts-aux are present in populations of immune lymph node (LN)
cells but not in populations of normal LN cells. Depletion of Ts-aux, either by
pretreatment with cyclophosphamide (Cy), anti-θ serum + C' treatment, or by adult
thymectomy, rendered the immune LN cells (TDn) nonsuppressible by Ts-eff.

Materials and Methods

Mice. 2- to 4-mo-old female BALB/c mice were obtained from Simonsen Laboratories,
Gilroy, Calif. CBA/J male mice were obtained from The Jackson Laboratory, Bar Harbor,
Maine.

Antigens and Tolerogens. 2,4-dinitrobenzene-1-sulfonyl acid sodium salt (DNBS) was obtained
from Eastman Kodak Co., Rochester, N. Y. 2,4-dinitro-1-fluorobenzene (DNFB) was obtained

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References used in this paper: anti-MIg, anti-mouse immunoglobulin serum; BA-θ, brain-associated
theta antigen; BSS, balanced salt solution; Cy, cyclophosphamide; DNBSO₃, 2,4-dinitrobenzene sulfuric acid;
DNFB, 1-fluoro-2,4-dinitrobenzene; DNP, 2,4-dinitrophenyl; DNP-SC, DNP-haptenated spleen cells;
DTH, delayed-type hypersensitivity; LNC, lymph node cells; NRS, normal rabbit serum; MIg, mouse
immunoglobulin; TDSn, T cells responsible for passive transfer of immunity; TSn, T cells responsible for in
vitro antigen-driven proliferation; TS-aux, T cells required for action of efferent blocking suppressor T
cells; Ts-eff, afferent blocking suppressor T cells; Ts-eff, efferent blocking suppressor T cells; TNBSO₃,
2,4,6-trinitrobenzene sulfonate; TBCB, 1-chloro-2,4,6-trinitrobenzene.
from Sigma Chemical Company, St. Louis, Mo. Picryl chloride (2,4,6-trinitro-1-chlorobenzene) (TNCB) and picryl sulfonic acid (TNBS) were obtained from Matheson, Coleman & Bell, East Rutherford, N. J.

Induction of Immune Suppression with Supraoptimal Doses of Antigen. Animals were sensitized with supraoptimal doses of DNFB that consist of two daily paintings of 150 μl each of 0.5% DNFB as described earlier (12).

Induction of Immune Suppression with DNBS and TNBS. 7 d before transfer, animals were tolerized via i.v. injection of 750 mg/kg of DNBS in saline or 250 mg/kg of TNBS in saline.

Induction and Elicitation of Contact Sensitivity. Optimal contact sensitivity was induced by two daily paintings on the clipped abdomen with 25 μl of 0.5% DNFB. 4 d after the last painting, 20 μl of 0.2% DNFB was applied to the dorsal surface of each ear, and increased ear swelling was measured 24 h later with an engineer's micrometer (12). Sensitization with TNCB was done by once applying 100 μl of 7% TNCB in the same vehicle. The response was elicited by applying 20 μl of 1% TNCB in olive oil to the ears 5 d later.

Cy Treatment. Mice were injected i.v. with 200 mg/kg of Cy (Mead, Johnson & Co., Evansville, Ind.) diluted in sterile distilled water.

Passive Transfer of Contact Sensitivity. 3 d after the last painting with the optimal dose (25 μl) of DNFB, single cell suspensions of draining lymph node cells were prepared and 50 × 10⁶ cells were injected i.v. into syngeneic recipients. 1 h after transfer, the recipients plus control un.injected mice were ear challenged with 20 μl of 0.2% DNFB and increased ear swelling measured 24 h later (13).

Transfer of Tolerance. Superficial and mesenteric lymph nodes were obtained from animals sensitized with supraoptimal(150 μl) doses of DNFB or from animals tolerized with DNBS or TNBS. Single-cell suspensions were prepared, washed twice, and 100 × 10⁶ cells were injected i.v. into syngeneic recipients. Positive controls received no cells or an equal number of normal LN cells. All recipients were immediately sensitized with two daily paintings of 25 μl of 0.5% DNFB and were challenged on the ears with 20 μl of 0.2% DNFB 4 d later in the same manner as were the conventionally sensitized mice. The degree of tolerance was calculated as percentage of tolerance according to the following formula by using ear swelling in units of 10⁻² inches.

\[
\text{Per cent tolerance} = \frac{\text{positive control} - \text{experimental}}{\text{positive control}} \times 100
\]

Inhibition of Passive Transfer of Contact Sensitivity by Suppressor LN Cells. 50 × 10⁶ tolerant LN cells (LNC) from supraoptimally sensitized mice (Ts) were mixed with 50 × 10⁶ DNFB-immune LNC (Tm) immediately before i.v. transfer into normal syngeneic recipients. The recipient mice were ear challenged within 1 h after cell transfer and ear swelling was measured 24 h later. The percentage of suppression of passive transfer of sensitivity was calculated by comparing the ear swelling response of mice receiving both immune and tolerant LNC (experimental) to those receiving only immune LNC (positive controls) by using the same formula as was used for determining the percentage of tolerance transferred to naive recipients (see above).

Preparation of Antiserum. Anti-Ia* serum was prepared by giving A.TH mice multiple i.p. injections of A.TL spleen cells. The specificity and cytotoxicity of this serum has been previously described (14). Anti-I-J* serum was prepared by immunizing B.10A(3R) mice with B.10A(5R) spleen and LN. This serum was tested functionally by treating LN from CBA mice with various dilutions of the serum plus fresh rabbit complement. This treatment inhibited the Con A response by more than 90% but had no effect on either the PHA or LPS response (15). Anti-brain-associated θ-serum was prepared in rabbits according to Golb (16). Polyvalent rabbit anti-mouse immunoglobulin serum (anti-MIg) was prepared as previously described (17).

Antiserum Treatment. 10⁶ LNC/ml were treated with a 1:10 dilution of normal rabbit serum (NRS), normal mouse serum, anti-Ia*, anti-I-J*, or rabbit anti-BA-θ serum or a 1:20 dilution of rabbit anti-MIg serum for 45 min at 4°C. The cells were washed once in balanced salt solution (BSS), resuspended in fresh rabbit C′ (1:10) containing 10 μg/ml DNase, and incubated for 30 min at 37°C. The cells were then washed twice in BSS and resuspended in BSS at the appropriate concentration for cell transfer.

Thymectomy and Splenectomy. Thymectomy and splenectomy were performed under secoobarbital anesthesia and were controlled by sham operations.
Identification of Immunoglobulin-Bearing Cells. B cells were determined by immunofluorescent staining with fluorescein-labeled polyvalent rabbit anti-MIg.

Results

Evidence That an Auxiliary Cell is Required for Suppression of TDH by Ts-eff. Previous work has shown that DNFB-immune T cells (TDH) and their precursors are not sensitive to Cy (12). However, the experiment shown in Fig. 1 indicates that TDH cannot be suppressed if they come from Cy pretreated mice. In this experiment, Ts were raised in mice sensitized with supraoptimal doses of DNFB and were assayed in a model which measures the activity of suppressors of the efferent limb of sensitivity (Ts-eff) (13). Such suppressors, when cotransferred with TDH from optimally sensitized mice, inhibited the passive transfer of contact sensitivity (B versus A). However, if the TDH were obtained from sensitized mice that had previously been treated with Cy, the TDH were no longer suppressed by Ts (C, D, E versus B). Cy at 200 mg/kg was maximally effective.

As TDH are not sensitive to Cy, we interpret these data to indicate that LN populations from optimally sensitized mice contain not only Cy-resistant TDH but another auxiliary cell that is required for Ts-eff to inhibit the TDH. This intermediate cell is sensitive to Cy.

The Auxiliary Cell Is Not Present in Normal LN Cells but Requires Antigen Activation. We asked whether the auxiliary cell was present in normal LN cells or only in antigen-activated LN cells that also contain TDH. To test this, we mixed various populations of cells with Ts from supraoptimally sensitized mice. Fig. 2 shows again that Ts block the passive transfer of sensitivity when mixed with TDH (A) but not when mixed with TDH from Cy pretreated donors (B). To see what populations include auxiliary cells, Ts plus Cy-pretreated TDH were augmented either by normal LNC (C and D) or TDH (E). The results show that the suppressive effects of Ts plus Cy-pretreated TDH are restored by the addition of Ts (C and D) or by TDH (E). This indicates that antigen activation induces not only TDH but also auxiliary cells. (This activity of auxiliary cells must be very high because the addition of Ts plus Cy-pretreated TDH (E) greatly enhances the suppression (versus B) in spite of the fact that more TDH, as well as auxiliary cells, are being added.)

Evidence That the Auxiliary Cell is a T Cell. Neither adult thymectomy nor splenectomy interferes with the development of contact sensitivity (18, J. W. Moorhead, unpublished observations). In contrast, both procedures interfere with the development of at least some kinds of Ts (18).2 The experiments shown in Fig. 3 were designed to test whether ATX or splenectomy would impair the development of antigen-induced auxiliary cells in populations also containing TDH. Mice were ATX or sham ATX and 4 wk later were optimally sensitized with DNFB to serve as donors of TDH (and possibly of auxiliary cells). Ts were derived from supraoptimally sensitized mice. These two populations were cotransferred to normal recipients that were then ear challenged. The results shown in Fig. 3 indicate that TDH from sham-operated sensitized mice (A) are suppressed by Ts (B). In contrast, TDH from ATX mice (C) are not suppressed when cotransferred with Ts (D).

2 Sy, M.-S., S. D. Miller, J. W. Moorhead, and H. N. Claman. Immune suppression with supraoptimal doses of antigen in contact sensitivity. III. Identification of two distinct populations of suppressor T cells acting on different arms of the immune response. Manuscript submitted for publication.
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### Table 1

| Pretreatment of TDH Donors (Cyclophosphamide) | Cells Transferred | Units of Ear Swelling Transferred ($10^{-4}$ in.) ± SEM |
|---------------------------------------------|-------------------|-----------------------------------------------------|
| A 0                                          | 5.0               |                                                     |
| B 0                                          | 5.0 5.0           |                                                     |
| C 50 mg/kg                                   | 5.0 5.0           |                                                     |
| D 100 mg/kg                                  | 5.0 5.0           |                                                     |
| E 200 mg/kg                                  | 5.0 5.0           |                                                     |

**Fig. 1.** Failure of Ts-eff to block the passive transfer of immunity when TDH were obtained from Cy pretreated animals.

BALB/c mice were pretreated with various doses of Cy or nothing on day -2. On days 0 and 1, they were sensitized with the optimal dose of DNFB and served as donors of TDH. Ts were obtained from donors that were sensitized with supraoptimal doses of DNFB 4 d earlier as described in Materials and Methods.

5 × 10^7 Ts along with 5 × 10^7 of the various TDH populations were transferred into normal BALB/c recipients. The recipients were ear challenged within 1 h after cell transfer and increased ear swelling measured 24 h later.

The lower half of Fig. 3 shows the results obtained by using TDH from sensitized mice that had been splenectomized or sham-operated. TDH from sham-operated mice (E) were suppressed by Ts (F), and TDH from splenectomized sensitized mice (G) were also suppressed by Ts (H).

The results shown in Table I directly demonstrate that the auxiliary cell is of the T-cell lineage. T~ (from supraoptimally sensitized mice) and TDH from Cy-pretreated, sensitized mice were transferred to normal recipients along with TDH (from sensitized donors). The latter suspension was treated in vitro with either NRS, anti-brain-associated theta antigen (BA-0), or anti-MIg serum + C'. T~ fail to inhibit the passive transfer of sensitivity by TDH from Cy-pretreated donors (A), but the addition of TDH treated with NRS + C' (B) or anti-MIg + C' (C) restored the suppressive effect. However, the addition of TDH treated with anti-BA-0 + C' (D) fails to restore suppression, indicating that the auxiliary cell is a T cell.

The results, taken together, show that the auxiliary cell is present in the LN cells of sensitized splenectomized mice but is not demonstrable in the lymph nodes 4 wk after adult thymectomy. Because the auxiliary cell is sensitive to both ATX and anti-θ serum treatment, it is apparently a T cell. As it is required for suppression of TDH, we will designate it as an auxiliary T-suppressor cell (Ts-aux).

Ts-aux Express Ia Antigens. In a previous report, Moorhead has shown that the T cells which mediate the passive transfer of contact sensitivity are Ia^- (14), so it was of interest to find out the Ia phenotype of these Ts-aux cells also present in sensitized mice. CBA mice were sensitized with either optimal doses of DNFB to serve as donors of TDH or with supraoptimal doses of DNFB to serve as donors of Ts-eff. Before
Fig. 2. Antigen stimulation is required for the activation of auxiliary cells.

BALB/c mice sensitized with supraoptimal doses of DNFB 4 d earlier served as donors of Tα.
Other groups of BALB/c mice were either pretreated with 200 mg/kg Cy or nothing on day −2.
They were then sensitized with optimal doses of DNFB on days 0 and 1 and served as donors of Cy
TDM or normal TDM. Normal LN cells were obtained from normal, nonsensitized BALB/c mice. 5
× 10^7 Ts along with 5 × 10^7 of the TDH or normal LN cells were transferred to normal syngeneic
recipients. The recipients were ear challenged within 1 h after cell transfer and increased ear
swelling measured 24 h later. Numbers in parentheses represent the percentages of suppression of
control passive transfer calculated as described in Materials and Methods. *, significantly different
from controls P < 0.001; **, not significantly different from controls.

cotransfer, the TDH were treated with anti-Iαk serum + C’ to remove the Iα+ cells as
described in Materials and Methods.

The results of the experiment are shown in Table II. When the TDH was first treated
with NMS + C’ and then cotransferred with Tα-eff, the ability of TDH to transfer
sensitivity was suppressed by 45% (A versus C). However, if the cells were first treated
with anti-Iαk serum + C’, there was only 13% suppression (B) which is not significantly
different from control group animals receiving 50 × 10^6 TDH alone (C). These
experiments indicated that removal of Iα+ cells rendered TDH insensitive to suppression
by Tα-eff. In other words, the Tα-aux are Iα+ T cells. To further characterize the I
region markers on these Tα-aux cells, anti-I-Jk serum was used, and the results of the
experiment are shown in the second half of Table I. TDH cells treated with anti-I-Jk
serum + C’ were also insensitive to suppression by Tα-eff. This indicates that Tα-aux
express determinants encoded by the I-J subregion.

Tα-aux Are Needed for the Activity of Tα-eff but Not Tα-aff. Previous work in other
laboratories and our own has shown that two kinds of suppressor cells can be
distinguished on the basis of their activities in suppressing different limbs of the
contact sensitivity response. In particular, DNBS induces T suppressors that block the
induction of sensitivity to DNFB (afferent limb blockers = Tα-aff) (19). By contrast,
TNBS induces T suppressors that block the expression of contact sensitivity to TNCB
(efferent limb blockers = Tα-eff) (20). Supraoptimal doses of DNFB induce both Tα-
aff and Tα-eff, but only Tα-eff was assayed in the above experiments.
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Cells Transferred

Units of Ear Swelling Transferred (x10^-4 in.) ± SEM

| Pretreatment of TDH Donors | TDH (x10^7) | Ts (x10^7) |
|---------------------------|-------------|------------|
| A  | Sham 5.0 - |  |
| B  | Sham 5.0 5.0 | (87%) * |
| C  | ATx 5.0 -  |  |
| D  | ATx 5.0 5.0 | ** |
| E  | Sham 5.0 - |  |
| F  | Sham 5.0 5.0 | (56%) * |
| G  | Splex 5.0 - |  |
| H  | Splex 5.0 5.0 | (66%) * |

Fig. 3. Effect of adult thymectomy and splenectomy on the induction of the auxiliary cell.

BALB/c mice were thymectomized or sham thymectomized (groups A–D) as described in Materials and Methods. Other groups of mice were splenectomized or sham splenectomized (groups E–H) as described in Materials and Methods. 4 wk after thymectomy or 1 wk after splenectomy, these mice were sensitized with optimal doses of DNFB and served as donors of TDH. Ts were obtained from animals sensitized with supraoptimal doses of DNFB 4 d earlier. 5 x 10^7 Ts along with 5 x 10^7 of the various TDH populations were transferred to normal syngeneic recipients. The recipients were ear challenged within 1 h after cell transfer and increased ear swelling measured 24 h later. Numbers in parentheses represent the percentages of suppression of control passive transfer calculated as described in Materials and Methods. *, Significantly different from controls P < 0.001; **, not significantly different from controls.

We asked whether the T^-aux was required for the suppressive activity of T^-eff as well as for T^-eff. To answer this, we induced the two kinds of suppressors and measured their activity in the standard or nondiscriminating assay for T^-eff. In this assay, suppressors are transferred to normal mice which are then sensitized and later ear challenged, thus activating both afferent and efferent limbs of the response in the recipient. The requirement for T^-aux in the recipient was tested for by pretreatment of the recipient with Cy. The results are shown in Fig. 4. In the top experiment, T^-eff were induced in donors by injection of TNBS. These suppressors were able to inhibit the sensitization and challenge of normal recipients (B versus A). If, however, the recipients were pretreated with Cy before cell transfer, sensitization, and challenge, the transferred suppressors were ineffective (group C). The lower experiment shows contrasting results when T^-eff were induced in donors by injection of DNBs. These suppressors were able to inhibit the sensitization and challenge of both normal and Cy pretreated recipients. Taken together, these results show that T^-aux in the recipient are needed for the suppressive activity of T^-eff but not T^-eff.

Discussion

The major findings in this paper are: (a) immune LN cells (TDH) from contact sensitized mice can be inhibited by T suppressors which block the efferent limb of


**Table I**  
The Auxiliary Suppressor Cell is Sensitive to Anti-BA-θ Serum + C' Treatment

| Number of T<sub>s</sub> | Number of Cy T<sub>DH</sub> | Number of normal T<sub>DH</sub> | Serum treatment of normal T<sub>DH</sub> (% Ig+) | Units of ear swelling inhibition (× 10<sup>-4</sup> inches) ± SEM | Inhibition of passive transfer of immunity % |
|-------------------------|-----------------------------|-------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------|
| (A) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | NRS + C' (29%) | 35.5 ± 2.8 | 74* |
| (B) 50 × 10<sup>6</sup> | 50 × 10<sup>5</sup> | 50 × 10<sup>6</sup> | Anti-Mlg + C' (0%) | 14.6 ± 2.4 | 73* |
| (C) 50 × 10<sup>6</sup> | 50 × 10<sup>5</sup> | 50 × 10<sup>6</sup> | Anti-BA-θ + C' (76%) | 37.1 ± 2.1 | -6 |
| (E) 7.0 ± 1.8 | -- | -- | -- | 7.0 ± 1.8 | -- |

* Significant suppression as compared to control values (P < 0.01).

**Sensitivity (T<sub>s-eff</sub>)**, but T<sub>DH</sub> from cyclophosphamide-pretreated contact-sensitized mice cannot be thus suppressed; (b) the inability of T<sub>s-eff</sub> to suppress T<sub>DH</sub> from Cy pretreated mice can be restored by adding T<sub>DH</sub> but not by adding normal LN cells. We have interpreted these results to indicate that sensitization of mice with DNFB induces not only T<sub>DH</sub> but also another auxiliary cell. This auxiliary cell is required for T<sub>s-eff</sub> to suppress T<sub>DH</sub> and has precursors that are sensitive to Cy; (c) the auxiliary cell cannot be shown 4 wk after adult thymectomy or after treatment of T<sub>DH</sub> cells with anti-BA-θ + C', but is unaffected by splenectomy. Thus, it appears to be a short-lived thymus-derived cell which we have called a suppressor auxiliary cell (T<sub>s-aux</sub>); (d) this T<sub>s-aux</sub> is required for the activity of T<sub>s-eff</sub> but not of T<sub>s-eff</sub>; and (e) this cell carries I-J determinants.

As outlined in the introduction, the continued investigation of the field of immunoregulation has uncovered a growing number of instances involving T-T-cell interactions. To try to clarify a complex subject, it is important to make certain distinctions. These will help to relate our current findings to some published work and will also show dissimilarities with other experiments.

First, it is important to distinguish (when possible) between T-T interactions in the induction of suppressor cells versus T-T interactions in the expression of suppressor cells. Second, it may be important to analyze the roles of T<sub>s</sub> by testing them in the context of optimal sensitization programs rather than in models where supraoptimal doses of antigen are involved. Actually, however, both of these problems are often inherent in a given experimental protocol. In many explorations of T<sub>s</sub> high doses of antigen are used to induce the T<sub>s</sub> that are analyzed in the context of supraoptimal antigen, and the net result of all the T-T interactions is suppression.

Thus, the experiments of Eardley et al. (11) indicate that T-helper cells (stimulated by supraoptimal doses of antigen) induce another subset of T cells (Ly<sup>1+</sup> <sup>2+</sup> <sup>3+</sup> Qal<sup>+</sup>) to exert suppressive activities. A very similar situation is seen in the experiments of Turkin and Sercarz (9). Feldmann and Konttinen have also shown that two cells are
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**Table II**

| Number of T<sub>s</sub> | Number of T<sub>DH</sub> | Serum treatment of T<sub>DH</sub> | Units of ear swelling transferred (× 10<sup>-5</sup> inches) ± SEM | Inhibition of passive transfer of immunity |
|------------------------|--------------------------|-------------------------------|-------------------------------------------------|----------------------------------------|
| (A) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | NMS + C<sup>+</sup> | 31.9 ± 4.4 | 45% |
| (B) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | Anti-Ia<sup>k</sup> + C<sup>+</sup> | 45.1 ± 5.5 | 13% |
| (C) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | — | 50.5 ± 3.2 | — |
| (D) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | NMS + C<sup>+</sup> | 34.9 ± 2.2 | 40% |
| (E) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | Anti-I-J<sup>k</sup> + C<sup>+</sup> | 49.7 ± 4.6 | 7% |
| (F) 50 × 10<sup>6</sup> | 50 × 10<sup>6</sup> | — | 53.2 ± 1.7 | — |

*Not significantly different from controls.

Ts were obtained from donors that had been sensitized with supraoptimal doses of DNFB 4 d earlier. T<sub>DH</sub> were obtained from donors that had been sensitized with optimal doses of DNFB 3 d earlier. Before cotransfer to naive recipients, the T<sub>DH</sub> population was first treated with NMS or anti-Ia or anti-I-J serum + C<sup>+</sup> as described in Materials and Methods. Recipients were ear challenged within 1 h after cell transfer and increases in ear swelling measured 24 h later.

required for development of T suppressors by high doses of antigen (10). In each of these models, the intact system involves supraoptimal doses of antigen and a final result (when individual cell populations are not isolated and tested) of suppression. In these cases, it is not easy to determine whether the T-T interactions are occurring during the generation or expression of Ts (or both).

Our experiments allow a somewhat more precise localization of the T-T interactions. The protocol was not concerned with cell interactions in the generation of Ts. The question we asked was: what is the target of T<sub>s-eff</sub> in the inhibition of expression of contact sensitivity? To answer this, we raised T<sub>s-eff</sub> in one animal and tested their ability to suppress the expression of optional contact sensitization in another animal. We found that the target of the T<sub>s-eff</sub> involved two T cells in the sensitized mouse: the T<sub>DH</sub> and another T<sub>s-aux</sub> cell.

These results most closely agree with those of Tada (22). He found that for a suppressor factor derived from T cells (T<sub>s,F</sub>) to act on T helper cells (T<sub>H</sub>) there was required another T cell from antigen-primed (not suppressed) mice. He believes that this cell accepts the T<sub>s,F</sub> and only then can it suppress the T<sub>H</sub>. The acceptor cell is Ly<sup>1</sup> 2<sup>+</sup> 3<sup>+</sup> and, like our T<sub>s-aux</sub>, it is I-J positive. If, indeed, the Tada acceptor cell is the same as our T<sub>s-aux</sub>, we would expect its precursors to be sensitive to cyclophosphamide and to adult thymectomy.

It is now clear that mice optimally sensitized with contact allergens (where no Ts are found) still have several different kinds of antigen-specific activated T cells. Earlier, Moorhead described two distinct populations of T cells in DNFB sensitized animals (14). The T cells that proliferate in vitro (T<sub>pvl</sub>) in response to antigen are a different subset of T cells than those that mediate in vivo delayed hypersensitivity (T<sub>DH</sub>) and can be distinguished from the latter by their adherence to nylon wool and their sensitivity to killing by anti-Ia serum + C<sup>+</sup>. The identification of T<sub>s-aux</sub> adds another T-cell subpopulation in the list of T cells present in sensitized animals (summarized in Table III). The similarity between T<sub>s-aux</sub> and T cells responsible for in vitro antigen-driven proliferation (T<sub>pvl</sub>) is that both of them are sensitive to anti-Ia
Summary

We investigated T-T-cell interactions in the suppression of contact sensitivity. Suppressor cells that block the efferent limb of sensitivity (Ts-eff) can inhibit the passive transfer of contact sensitivity mediated by 1-fluoro-2,4-dinitrobenzene immune cells (TDH). But, Ts-eff cannot block the passive transfer of TDH which comes from cyclophosphamide (Cy) pretreated sensitized mice. We interpret these results to indicate that lymph node cells from sensitized mice contain not only TDH but also another intermediate cell which is required for the suppression of TDH by Ts-eff. This

| Tolerogen | Cells Transferred | Recipient Treatment | Units of Ear Swelling in Recipients (x10^-4 in.) ± SEM |
|-----------|------------------|---------------------|-----------------------------------------------------|
| A         | (Pos. Cont.)     | -                   |                                                     |
| B         | TNBS 10^8        | -                   |                                                     |
| C         | TNBS 10^8        | Cy                  |                                                     |
| D         | (Pos. Cont.)     | -                   |                                                     |
| E         | DNBS 10^8        | -                   |                                                     |
| F         | DNBS 10^8        | Cy                  |                                                     |

FIG. 4. Efferent blocking Ts (Ts-eff), but not afferent blocking Ts (Ts-aff), require Ts-aux to exhibit suppression.

TNBS Ts (Ts-eff) and DNBS Ts (Ts-aff) were induced by i.v. injection of TNBS or DNBS 7 d previously as described in Materials and Methods. 10^6 LN cells from each tolerant group were transferred into either normal or Cy pretreated (200 mg/kg on day -2) syngeneic recipients. Recipient mice along with normal controls were then sensitized and ear challenged with either TNCB (groups A-C) or DNFB (groups D-F) as described in Materials and Methods, and increased ear swelling measured 24 h after challenge. Numbers in parentheses represent the percentages of suppression of control passive transfer calculated as described in Materials and Methods. * Significantly different from controls P < 0.001.

serum + C' treatment and both adhere to nylon wool; however, they can be distinguished by ones (Tpr~r) resistance to ATX and Cy pretreatment although the other (T~aux) is sensitive to both ATX and Cy.

The final point to be made with regard to these experiments concerns the mechanism of negative feedback regulation in contact sensitivity. In contact sensitization to DNFB with optimal doses of antigen, TDH are generated without the apparent production of active Ts. Nevertheless, this antigen stimulation not only generates TDH but also a population of T~aux. These auxiliary suppressor cells are required for the down-regulation of TDH by Ts-eff. In these experiments, the T~aux are immunologically silent unless exogenous T~eff are supplied, whereupon their presence is demonstrated because the T~eff cannot inhibit the TDH without them. In this context, it is extremely pertinent that antigen stimulation not only induces a TDH but also a T~aux which is the instrument for the immunoregulation of those TDH.

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The final point to be made with regard to these experiments concerns the mechanism of negative feedback regulation in contact sensitivity. In contact sensitization to DNFB with optimal doses of antigen, TDH are generated without the apparent production of active Ts. Nevertheless, this antigen stimulation not only generates TDH but also a population of T~aux. These auxiliary suppressor cells are required for the down-regulation of TDH by Ts-eff. In these experiments, the T~aux are immunologically silent unless exogenous T~eff are supplied, whereupon their presence is demonstrated because the T~eff cannot inhibit the TDH without them. In this context, it is extremely pertinent that antigen stimulation not only induces a TDH but also a T~aux which is the instrument for the immunoregulation of those TDH.

Summary

We investigated T-T-cell interactions in the suppression of contact sensitivity. Suppressor cells that block the efferent limb of sensitivity (Ts-eff) can inhibit the passive transfer of contact sensitivity mediated by 1-fluoro-2,4-dinitrobenzene immune cells (TDH). But, Ts-eff cannot block the passive transfer of TDH which comes from cyclophosphamide (Cy) pretreated sensitized mice. We interpret these results to indicate that lymph node cells from sensitized mice contain not only TDH but also another intermediate cell which is required for the suppression of TDH by Ts-eff. This
intermediate cell is sensitive to cyclophosphamide and requires antigen activation for its development. It is sensitive to adult thymectomy and anti-brain associated theta serum and is therefore designated as an auxiliary T-suppressor cell (Tₐ-aux). It is not sensitive to splenectomy and it carries I-J determinants. Tₐ-aux are required for the activity of suppressors of the efferent limb (Tₐ-eff) but not of suppressors of the afferent limb (Tₐ-eff). Thus, in the feedback loops in contact sensitivity, the generation of TDH is coordinated with the development of auxiliary Tₐ which are essential for the suppression of those TDH.

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