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)[1] 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 (T_s) (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 (T_s-eff), require the presence of another population of auxiliary T suppressor cells (T_s-aux). The T_s-aux are present in populations of immune lymph node (LN) cells but not in populations of normal LN cells. Depletion of T_s-aux, either by pretreatment with cyclophosphamide (Cy), anti-θ serum + C′ treatment, or by adult thymectomy, rendered the immune LN cells (T_{DH}) nonsuppressible by T_s-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-sulfonic 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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[1] *Abbreviations used in this paper:* anti-MIg, anti-mouse immunoglobulin serum; BA-θ, brain-associated theta antigen; BSS, balanced salt solution; Cy, cyclophosphamide; $DNBSO_3$, 2,4-dinitrobenzene sulfonate; 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; T_{DH}, T cells responsible for passive transfer of immunity; T_{prlf}, T cells responsible for in vitro antigen-driven proliferation; T_s-aux, T cells required for action of efferent blocking suppressor T cells; T_s-aff, afferent blocking suppressor T cells; T_s-eff, efferent blocking suppressor T cells; $TNBSO_3$, 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 50×10^6 cells were injected i.v. into syngeneic recipients. 1 h after transfer, the recipients plus control uninjected 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 100×10^6 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^{-4} inches.

$$\text{Per cent tolerance} = \text{positive control} - \text{experimental/positive control} - \text{negative control} \times 100.$$

Inhibition of Passive Transfer of Contact Sensitivity by Suppressor LN Cells. 50×10^6 tolerant LN cells (LNC) from supraoptimally sensitized mice (T_s) were mixed with 50×10^6 DNFB-immune LNC (T_{DH}) 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^k 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^k serum was prepared by immunizing B.10A(3R) mice with B.10A(5R) spleen and LNC. This serum was tested functionally by treating LNC 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. 10^8 LNC/ml were treated with a 1:10 dilution of normal rabbit serum (NRS), normal mouse serum, anti-Ia^k, anti-I-J^k, 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 secobarbital anesthesia and were controlled by sham operations.

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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 T_{DH} by T_s-eff. Previous work has shown that DNFB-immune T cells (T_{DH}) and their precursors are not sensitive to Cy (12). However, the experiment shown in Fig. 1 indicates that T_{DH} cannot be suppressed if they come from Cy pretreated mice. In this experiment, T_s 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 (T_s-eff) (13). Such suppressors, when cotransferred with T_{DH} from optimally sensitized mice, inhibited the passive transfer of contact sensitivity (B versus A). However, if the T_{DH} were obtained from sensitized mice that had previously been treated with Cy, the T_{DH} were no longer suppressed by T_s (C, D, E versus B). Cy at 200 mg/kg was maximally effective.

As T_{DH} are not sensitive to Cy, we interpret these data to indicate that LN populations from optimally sensitized mice contain not only Cy-resistant T_{DH} but another auxiliary cell that is required for T_s-eff to inhibit the T_{DH}. 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 T_{DH}. To test this, we mixed various populations of cells with T_s from supraoptimally sensitized mice. Fig. 2 shows again that T_s block the passive transfer of sensitivity when mixed with T_{DH} (A) but not when mixed with T_{DH} from Cy pretreated donors (B). To see what populations include auxiliary cells, T_s plus Cy-pretreated T_{DH} were augmented either by normal LNC (C and D) or T_{DH} (E). The results show that the suppressive effects of T_s plus Cy-pretreated T_{DH} are restored by the addition of T_{DH} but not by the addition of even 10×10^7 normal LN cells. This indicates that antigen activation induces not only T_{DH} but also auxiliary cells. (This activity of auxiliary cells must be very high because the addition of T_{DH} to T_s plus Cy-pretreated T_{DH} (E) greatly enhances the suppression (versus B) in spite of the fact that more T_{DH}, 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 T_s (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 T_{DH}. Mice were ATX or sham ATX and 4 wk later were optimally sensitized with DNFB to serve as donors of T_{DH} (and possibly of auxiliary cells). T_s 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 T_{DH} from sham-operated sensitized mice (A) are suppressed by T_s (B). In contrast, T_{DH} from ATX mice (C) are not suppressed when cotransferred with T_s (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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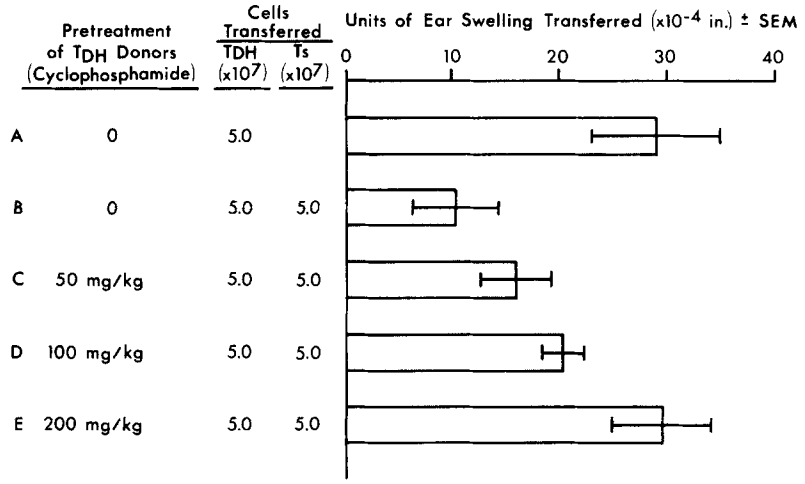

FIG. 1. Failure of T_s-eff to block the passive transfer of immunity when T_{DH} were obtained from Cy pretreated animals.

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 T_{DH}. T_s were obtained from donors that were sensitizied with supraoptimal doses of DNFB 4 d earlier as described in Materials and Methods.

5×10^7 T_s along with 5×10^7 of the various T_{DH} 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 T_{DH} from sensitized mice that had been splenectomized or sham-operated. T_{DH} from sham-operated mice (E) were suppressed by T_s (F), and T_{DH} from splenectomized sensitized mice (G) were also suppressed by T_s (H).

The results shown in Table I directly demonstrate that the auxiliary cell is of the T-cell lineage. T_s (from supraoptimally sensitized mice) and T_{DH} from Cy-pretreated, sensitized mice were transferred to normal recipients along with T_{DH} (from sensitized donors). The latter suspension was treated in vitro with either NRS, anti-brain-associated theta antigen (BA-θ), or anti-MIg serum + C′. T_s fail to inhibit the passive transfer of sensitivity by T_{DH} from Cy-pretreated donors (A), but the addition of T_{DH} treated with NRS + C′ (B) or anti-MIg + C′ (C) restored the suppressive effect. However, the addition of T_{DH} treated with anti-BA-θ + 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 T_{DH}, we will designate it as an auxiliary T-suppressor cell (T_s-aux).

T_s-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 T_s-aux cells also present in sensitized mice. CBA mice were sensitized with either optimal doses of DNFB to serve as donors of T_{DH} or with supraoptimal doses of DNFB to serve as donors of T_s-eff. Before

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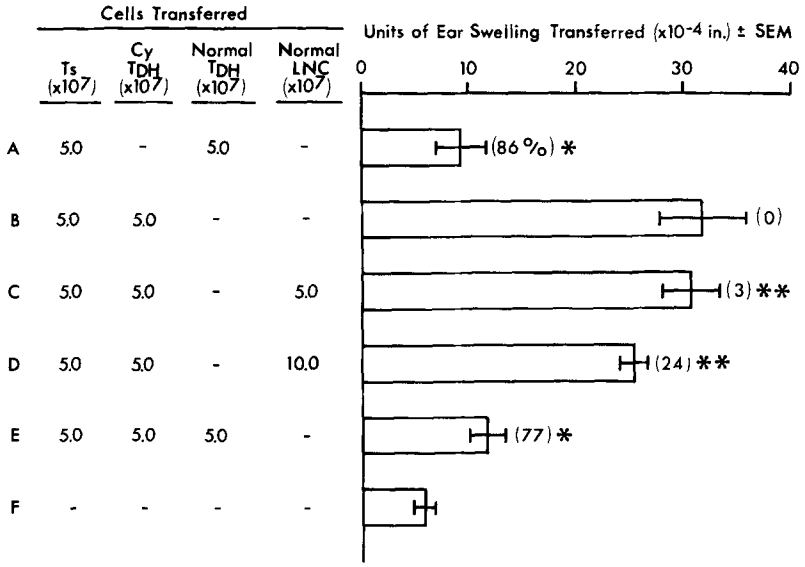

FIG. 2. Antigen stimulation is required for the activation of auxiliary cells.

BALB/c mice sensitized with supraoptimal doses of DNFB 4 d earlier served as donors of T_s. 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 T_{DH} or normal T_{DH}. Normal LN cells were obtained from normal, nonsensitized BALB/c mice. 5 × 10^7 T_s along with 5 × 10^7 of the T_{DH} 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 T_{DH} were treated with anti-Ia^k serum + C′ to remove the Ia^+ cells as described in Materials and Methods.

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

T_s-aux Are Needed for the Activity of T_s-eff but Not T_s-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 = T_s-aff) (19). By contrast, TNBS induces T suppressors that block the expression of contact sensitivity to TNCB (efferent limb blockers = T_s-eff) (20). Supraoptimal doses of DNFB induce both T_s-aff and T_s-eff,[2] but only T_s-eff was assayed in the above experiments.

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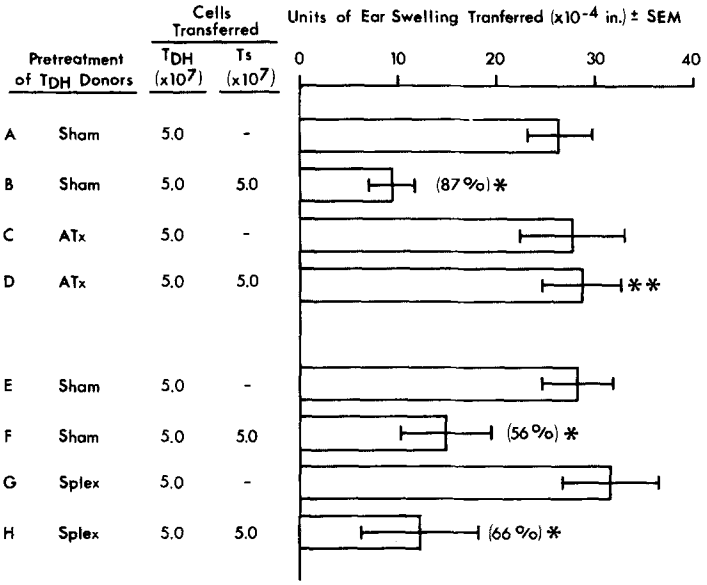

FIG. 3. Effect of adult thymectomy and splenectomy on the induction of the auxiliary cell.

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 T_{DH}. T_s were obtained from animals sensitized with supraoptimal doses of DNFB 4 d earlier. 5×10^7 T_s along with 5×10^7 of the various T_{DH} 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_s-aux was required for the suppressive activity of T_s-aff as well as for T_s-eff. To answer this, we induced the two kinds of suppressors and measured their activity in the standard or nondiscriminating assay for T_s. 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_s-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_s-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_s-aff 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_s-aux in the recipient are needed for the suppressive activity of T_s-eff but not T_s-aff.

Discussion

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

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TABLE I

The Auxiliary Suppressor Cell is Sensitive to Anti-BA-θ Serum + C′ Treatment

	Number of T_s	Number of Cy T_{DH}	Number of normal T_{DH}	Serum treatment of normal T_{DH} (% Ig+)	Units of ear swelling transferred ($\times 10^{-4}$ inches) ± SEM	Inhibition of passive transfer of immunity
						%
(A)	50×10^6	50×10^6	—	—	35.5 ± 2.8	—
(B)	50×10^6	50×10^6	50×10^6	NRS + C′ (29%)	14.3 ± 1.2	74*
(C)	50×10^6	50×10^6	50×10^6	Anti-MIg + C′ (0%)	14.6 ± 2.4	73*
(D)	50×10^6	50×10^6	50×10^6	Anti-BA-θ + C′ (76%)	37.1 ± 2.1	−6
(E)	—	—	—	—	7.0 ± 1.8	—

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

T_s were obtained from donors that had been sensitized with supraoptimal doses of DNFB 4 d earlier. T_{DH} were obtained from donors (either untreated or Cy pretreated on day −2) that had been sensitized with optimal doses of DNFB 3 d earlier. Before cotransfer to naive recipients, the normal T_{DH} population was first treated with NRS or anti-BA-θ or anti-MIg serum + C′ 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.

sensitivity (T_s-eff), but T_{DH} from cyclophosphamide-pretreated contact-sensitized mice cannot be thus suppressed; (*b*) the inability of T_s-eff to suppress T_{DH} from Cy pretreated mice can be restored by adding T_{DH} but not by adding normal LN cells. We have interpreted these results to indicate that sensitization of mice with DNFB induces not only T_{DH} but also another auxiliary cell. This auxiliary cell is required for T_s-eff to suppress T_{DH} 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_{DH} 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_s-aux); (*d*) this T_s-aux is required for the activity of T_s-eff but not of T_s-aff; 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_s 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_s, high doses of antigen are used to induce the T_s 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 1^+ 2^+ 3^+ Qal^+) to exert suppressive activities. A very similar situation is seen in the experiments of Turkin and Sercarz (9). Feldmann and Kontiainen have also shown that two cells are

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TABLE II

T_s-aux is Sensitive to Anti-Ia^k and Anti-I-J^k Serum + C′ Treatment

	Number of T_s	Number of T_{DH}	Serum treatment of T_{DH}	Units of ear swelling transferred ($\times 10^{-4}$ inches) ± SEM	Inhibition of passive transfer of immunity
					%
(A)	50×10^6	50×10^6	NMS + C′	31.9 ± 4.4	45
(B)	50×10^6	50×10^6	Anti-Ia^k + C′	45.1 ± 5.5	13*
(C)	—	50×10^6	—	50.5 ± 3.2	—
(D)	50×10^6	50×10^6	NMS + C′	34.9 ± 2.2	40
(E)	50×10^6	50×10^6	Anti-I-J^k + C′	49.7 ± 4.6	7*
(F)	—	50×10^6	—	53.2 ± 1.7	—

* Not significantly different from controls.

T_s were obtained from donors that had been sensitized with supraoptimal doses of DNFB 4 d earlier. T_{DH} were obtained from donors that had been sensitized with optimal doses of DNFB 3 d earlier. Before cotransfer to naive recipients, the T_{DH} population was first treated with NMS or anti-Ia or anti-I-J serum + C′ 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 T_s (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 T_s. The question we asked was: what is the target of T_s-eff in the inhibition of expression of contact sensitivity? To answer this, we raised T_s-eff 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_s-eff involved two T cells in the sensitized mouse: the T_{DH} and another T_s-aux cell.

These results most closely agree with those of Tada (22). He found that for a suppressor factor derived from T cells (T_sF) to act on T helper cells (T_H) there was required another T cell from antigen-primed (not suppressed) mice. He believes that this cell accepts the T_sF and only then can it suppress the T_H. The acceptor cell is Ly $1^+ 2^+ 3^+$ and, like our T_s-aux, it is I-J positive. If, indeed, the Tada acceptor cell is the same as our T_s-aux, 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 T_s 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_{prlf}) in response to antigen are a different subset of T cells than those that mediate in vivo delayed hypersensitivity (T_{DH}) and can be distinguished from the latter by their adherence to nylon wool and their sensitivity to killing by anti-Ia serum + C′. The identification of T_s-aux adds another T-cell subpopulation in the list of T cells present in sensitized animals (summarized in Table III). The similarity between T_s-aux and T cells responsible for in vitro antigen-driven proliferation (T_{prlf}) is that both of them are sensitive to anti-Ia

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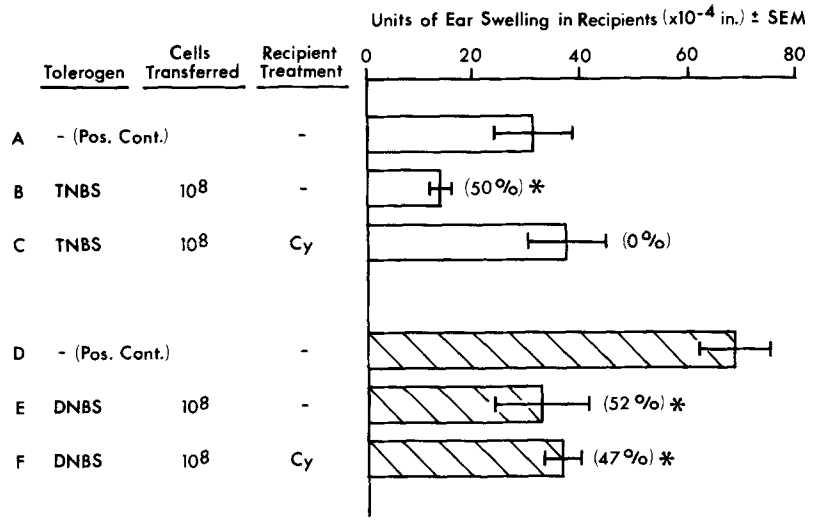

FIG. 4. Efferent blocking T_s (T_s-eff), but not afferent blocking T_s (T_s-aff), require T_s-aux to exhibit suppression.

TNBS T_s (T_s-eff) and DNBS T_s (T_s-aff) were induced by i.v. injection of TNBS or DNBS 7 d previously as described in Materials and Methods. 10^8 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 (T_{prif}) resistance to ATX and Cy pretreatment although the other (T_s-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, T_{DH} are generated without the apparent production of active T_s. Nevertheless, this antigen stimulation not only generates T_{DH} but also a population of T_s-aux. These auxiliary suppressor cells are required for the down-regulation of T_{DH} by T_s-eff. In these experiments, the T_s-aux are immunologically silent unless exogenous T_s-eff are supplied, whereupon their presence is demonstrated because the T_s-eff cannot inhibit the T_{DH} without them. In this context, it is extremely pertinent that antigen stimulation not only induces a T_{DH} but also a T_s-aux which is the instrument for the immunoregulation of those T_{DH}.

Summary

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

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TABLE III

T-Cell Subpopulations in Animals Sensitized with Optimal Doses of DNFB

	T_{DH}*	T_{prif}‡	T_s-aux§
Sensitive to adult thymectomy‖	No	No	Yes
I-a determinants	No	Yes	Yes
I-J determinants	No	ND	Yes
Sensitive to pretreatment with cyclophosphamide	No	No¶	Yes
Adhere to nylon wool column	No	Yes	Yes¶
Sensitive to splenectomy	No	ND	No

ND, Not determined.
* T cell responsible for in vivo transfer of ear swelling.
‡ T cell responsible for in vitro antigen-driven proliferation.
§ T cell required for action of efferent blocking suppressor T cells.
‖ 4 wk after adult thymectomy.
¶ Unpublished observation.

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 (T_s-aux). It is not sensitive to splenectomy and it carries I-J determinants. T_s-aux are required for the activity of suppressors of the efferent limb (T_s-eff) but not of suppressors of the afferent limb (T_s-aff). Thus, in the feedback loops in contact sensitivity, the generation of T_{DH} is coordinated with the development of auxiliary T_s which are essential for the suppression of those T_{DH}.

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