0022-1767/83/1315-2316$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 131, No. 5, November 1983
Printed in U.S.A.

T LYMPHOCYTE LINE SPECIFIC FOR THYROGLOBULIN PRODUCES OR VACCINATES AGAINST AUTOIMMUNE THYROIDITIS IN MICE

RUTH MARON, RACHEL ZERUBAVEL, AHARON FRIEDMAN, AND IRUN R. COHEN[1]

From the Department of Cell Biology, The Weizmann Institute of Science, P.O. Box 26, Rehovot 76100, Israel

We investigated Ly-1$^+$ T lymphocyte line cells specifically reactive to thyroglobulin (Tg) that were isolated from mice primed with mouse Tg in adjuvant. Intravenous inoculation of as few as 10^5 line cells was sufficient to cause severe and prolonged thyroiditis in recipient mice that were intact, irradiated, or athymic nudes. Disease was independent of circulating Tg antibodies, suggesting that anti-Tg T lymphocytes could cause thyroiditis unaided by antibodies. Thyroiditogenic T lymphocytes could be isolated as cell lines from apparently healthy mice that had been immunized with non-thyroiditogenic bovine Tg in adjuvant, which indicates that autoimmune effector T lymphocytes may develop covertly in the course of immunization with foreign antigens. Finally, a single i.v. inoculation of anti-Tg T lymphocyte line cells attenuated by irradiation vaccinated mice completely against subsequent development of autoimmune thyroiditis produced either by active immunization to Tg or by passive transfer of intact line cells. Vaccinated mice that were protected from inflammatory lesions of thyroiditis still produced high titers of Tg antibodies in response to active immunization. Thus, vaccination specifically inhibited thyroiditogenic T lymphocytes but not helper T lymphocytes required for the production of Tg autoantibodies.

At the root of all autoimmune diseases are lymphocytes that react to normal constituents of the individual. Therefore, it is important to understand the origin of autoreactive lymphocytes, their behavior, and the ways in which they can be controlled.

As tools to approach these goals, we developed long-term lines of specifically autoimmune T lymphocytes that are functional in autoimmune encephalomyelitis (EAE)[2] (1, 2) or autoimmune arthritis (3) in rats. These diseases may be produced by a single i.v. injection of the specific line cells. Alternatively, rats can be vaccinated against subsequent induction of active EAE or arthritis by inoculating them with the autoimmune line cells under suitable conditions (3–5).

We report the development of mouse T lymphocyte line cells that were specifically reactive against mouse thyroglobulin (Tg). These line cells, most of which were Ly-1$^+$2$^-$, caused a most severe thyroiditis within several days of i.v. inoculation in normal recipient mice, in irradiated mice, or in athymic nude mice. Development of thyroiditis was independent of Tg antibodies, suggesting that experimental autoimmune thyroiditis (EAT) may be mediated by T lymphocytes unaided by antibodies (6, 7).

We also isolated EAT effector T lymphocyte lines from mice that did not develop EAT after immunization to bovine Tg. This suggests that covert EAT effector T lymphocytes can arise in the course of non-thyroiditogenic immunization.

Finally, anti-Tg T lymphocytes attenuated by irradiation vaccinated mice against thyroiditis produced by the intact line cells as well as against EAT induced by active immunization to mouse Tg in adjuvant. Mice that were successfully vaccinated against active EAT still produced high titers of Tg antibodies in response to immunization. Hence, vaccination with line cells did not prevent all expressions of autoimmunity to Tg, but only those responsible for inflammatory damage to the thyroid gland.

MATERIALS AND METHODS

Mice. Inbred strains of mice (C3H/eB × C57BL/6)F_1 and CBA/T6T6 and nude mice (nu/nu) of the (C3H/eB × C57BL/6)F_1 strain were supplied by the Animal Breeding Center of the Weizmann Institute.

Antigens. Bovine Tg was purchased from Sigma Chemicals (St. Louis, MO) and murine Tg was prepared from thyroid glands of C3H/eB mice as described (8). Crude murine Tg, unpurified by column chromatography, was used for immunization *in vivo* whereas column-purified murine Tg was used in *in vitro* proliferative tests and radioimmunoassays. Tuberculin (PPD) was purchased from Statens Serum Institute, Copenhagen, Denmark; concanavalin A (Con A) was acquired from Miles-Yeda, Rehovot, Israel.

Immunization to Tg and EAT. EAT was induced as described (7) by two weekly subcutaneous injections of 300 to 400 μg of crude murine Tg or 50 μg of bovine Tg, each emulsified in incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI) to which had been added 7 mg/ml *Mycobacterium tuberculosis*, H37Ra (Difco Laboratories) (CFA). The thyroid glands were removed 4 to 5 wk later and were studied for microscopic evidence of mononuclear cell infiltration. A pathology index of thyroiditis was computed as the average area of thyroid section that was infiltrated: 1 = 25% of the gland, 2 = 50%, 3 = 75%, and 4 = 100%.

To measure proliferative responses of primed lymph node cells (LNC) and to raise lines of T lymphocytes, the draining popliteal lymph nodes were removed 9 days after the second of the two weekly inoculations of murine Tg (150 to 200 μg) into each hind footpad or of bovine Tg (50 μg) in CFA containing 1 mg *M. tuberculosis* H37Ra.

Culture media. Dulbecco's modified Eagle's medium supplemented with glutamine, 2-mercaptoethanol, and gentamicin was used as described (2) except that 1% fresh autologous mouse serum was used in place of rat serum. Propagation medium refers to culture medium supplemented with 15% (v/v) of Con A supernatant as described (2) except that the horse serum was replaced by 10% fetal calf serum (GIBCO, Grand Island, NY: lot 32P 9502).

Proliferative response of primed LNC. Cells from draining popliteal lymph nodes were prepared and tested as described (2), except that the wells contained 5 × 10^5 instead of 1 × 10^6 LNC. Responses were measured as the mean cpm of incorporated [^{3}H] thymidine or as the Δ cpm (mean cpm in test cultures minus mean cpm in control cultures without antigen).

Isolation and propagation of T lymphocyte lines. Suspensions of single cells were prepared from pooled popliteal lymph nodes 9 days after immunization of mice in the hind footpads. The cells were washed in phosphate-buffered saline (PBS) and suspended in culture medium, 5 × 10^6/ml in 25-ml tissue culture flasks (Nunc Products, Denmark; No. 163371; 50 × 10^6 cells/flask) with Tg (50 μg/ml). After 72 hr of incubation at 37°C in air plus 7.5% CO_2, the cells were collected, washed twice in PBS, and resuspended (about 10^6 cells/ml) in 260-ml culture flasks (Nunc, No. 153732) in propagation medium. The propagation medium was replaced by fresh propagation medium every 3 to 4 days and the cultures were split as they became crowded. Every

Received for publication April 27, 1983.
Accepted for publication July 6, 1983.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] I. R. Cohen is the encumbent of the Mauerberger Professional Chair in Immunology.

[2] Abbreviations used in this paper: DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; EAT, experimental autoimmune thyroiditis; Tg, thyroglobulin; LNC, lymph node cells.

10 to 14 days and 3 days before inoculation into mice, cell lines were activated by incubation with Tg (50 μg/ml) and irradiated (1500 R), syngeneic or semisyngeneic normal spleen cells (5×10^6/ml) as accessory cells in culture medium in 25-ml flasks (2×10^5 cells/ml). After 72 hr of incubation, the cells were returned to 260-ml flasks for growth in propagation medium or were inoculated in PBS into recipient mice.

Thyroiditis produced by cell lines. Line cells, intact or irradiated (1500 R) as described (2), were inoculated i.v. or i.p. into syngeneic mice, and the mice were scored for incidence of thyroiditis and pathology index at various times. Uptake of ^{125}I by thyroid glands was measured 24 hr after recipient mice had been inoculated i.p. with ^{125}I (5×10^5 cpm) in 0.45 ml PBS.

Proliferative response of line cells. The test was performed as described (2), except that each well contained 2×10^4 line cells together with 5×10^5 irradiated (1500 R) syngeneic spleen cells and antigen (Tg or PPD, 50 μg/ml; Con A, 5 μg/ml). [^{3}H]Thymidine was added for the last 18 hr of the 72-hr culture.

Tg antibodies. A solid phase radioimmunoassay (9) with the use of purified Tg was employed to measure Tg antibodies. Titers were measured as the last log dilution of serum giving a cpm that was 50% of the maximal cpm.

RESULTS

Anti-Tg line cells induce thyroiditis. To develop lines of T lymphocytes reactive to murine Tg, (C3H/eB × C57BL/6)F_1 mice were primed by inoculation with Tg/CFA. C3H/eB (H-2^k) mice have a high responder and C57BL/6 (H-2^b) have a lower responder EAT phenotype (7, 10). The TF1 line, which we obtained, responded to Tg in the presence of accessory cells of either syngeneic F_1 or parental strain C3H/eB origin; it did not respond well in the presence of C57BL/6 accessory cells (not shown). This line was used for the majority of the experiments presented in this article. Other lines were derived from mice of the H-2^k high responder strains C3H/eB, CBA, CKB, and B6.H-2^k, and studies in which these lines in the various experimental protocols were produced essentially similar results. Table I shows the T lymphocyte proliferative responses of primed LNC and their derivative line TF1 to PPD and to murine Tg. The primed LNC responded to both PPD and Tg, whereas line TF1 responded only to Tg and appeared to lose the PPD-responsive cells present in the initial LNC population. A response to the T lymphocyte mitogen Con A was present in both the primed LNC and the line.

To learn whether line TF1 could produce thyroiditis, we inoculated (C3H/eB × C57BL/6)F_1 mice i.v. with 2×10^6 line cells. Some groups received line cells that had been activated by incubation with Tg or Con A *in vitro* before inoculation and others received activated line cells that had been irradiated (1500 R). Some mice received line cells that were maintained in propagation medium but were not activated. Recipient mice were either intact, irradiated (500 R), or genetically athymic nudes. Thyroiditis was assayed 8 days later by the incidence and pathologic grade of inflammation and in some cases by the uptake of ^{125}I by the thyroid gland. Table II shows that severe thyroiditis was produced by activated TF1 cells, but not by nonactivated or activated and irradiated TF1 cells. A control line selected for its reactivity to bovine insulin produced no thyroid lesions. Recipient mice that had been irradiated (500 R) or that were without functional thymus glands (nude mice) were also susceptible to disease. Tg antibodies were not detectable in any of the mice. These findings indicate that development of thyroiditis did not require the participation of recipient cells that were either radiosensitive or T lymphocytes and that disease was not accompanied by circulating Tg antibodies.

Thyroiditis mediated by TF1 line cells was severe, as indicated by a marked inhibition in thyroid uptake of ^{125}I (Table II) and by the histology of the gland (Fig. 1). In addition to thyroiditis, some mice showed mononuclear cell infiltrations in the tracheal mucosa (Fig. 2) and in the salivary glands, most prominently in perivascular areas and in the surrounding adipose tissue (Fig. 3).

Kinetics of thyroiditis and Tg antibodies. EAT induced by active immunization to Tg/CFA usually requires about 5 or 6 wk for its fullest development (7, 11) and is accompanied by titers of Tg antibody of 1:50,000 or greater (see below). Figure 4 illustrates the onset and duration of thyroiditis and the development of Tg antibodies produced by i.v. inoculation of 10^6 cells of line TF1 into intact or nude recipient mice. An occasional mouse showed thyroiditis 1 day after inoculation, whereas all mice had marked to severe disease after 3 or 4 days. Between 8 and 14 days, the degree of thyroiditis in intact mice partially subsided, but disease persisted for at least 56 days. Severe thyroiditis was evident in the athymic nude mice for at least 42 days.

Tg antibodies were undetectable in the sera of the nude mice throughout the period of observation, but were found at relatively low titers in the sera of the intact recipients at 28, 42, and 56 days. No antibodies were detected at the height of thyroiditis in intact mice.

Intact mice inoculated i.p. with 10^6 line TF1 cells had about the same clinical course of thyroiditis as did mice that had been inoculated i.v. (not shown). Thus, inoculation of line cells led to the relatively quick onset of persisting thyroiditis. The late appearance of low titers of Tg antibodies only in intact mice suggests that the Tg antibodies may have developed secondarily to the thyroiditis.

TABLE I

T lymphocyte proliferative responses of Tg-primed LNC and TF1 line cells[a]

Cells	Proliferative Response (cpm × 10^{-3})			
	No antigen	PPD	Tg	Con A
LNC	4	87	49	82
Line TF1	6	7	65	73

[a] (C3H/eB × C57BL/6)F_1 mice were immunized with Tg/CFA and proliferative responses were assayed by using cells obtained from the draining lymph nodes or cells of line TF1 isolated from such primed lymph nodes.

TABLE II

Thyroiditis produced by line TF1[a]

Line Cells	Activation	Recipient Mice	EAT			
			Incidence (%)	Pathologic index	Thyroid ^{125}I uptake (cpm × 10^{-3})	Tg antibody titer
None	None	Intact	0	0	24	0
TF1	None	Intact	0	0	N.D.[b]	0
	Tg	Intact	100	4	2.8	0
	Con A	Intact	100	4	N.D.	0
	Tg	Irradiated	100	4	N.D.	0
	Tg + irradiation	Intact	0	0	N.D.	0
	Tg	Nude	100	4	N.D.	0
Anti-insulin	Insulin	Intact	0	0	N.D.	0

[a] (C3H/eB × C57BL/6)F_1 mice were inoculated with 2×10^6 line cells and were studied 8 days later for EAT. Each group contained five to 10 mice.
[b] Not determined.

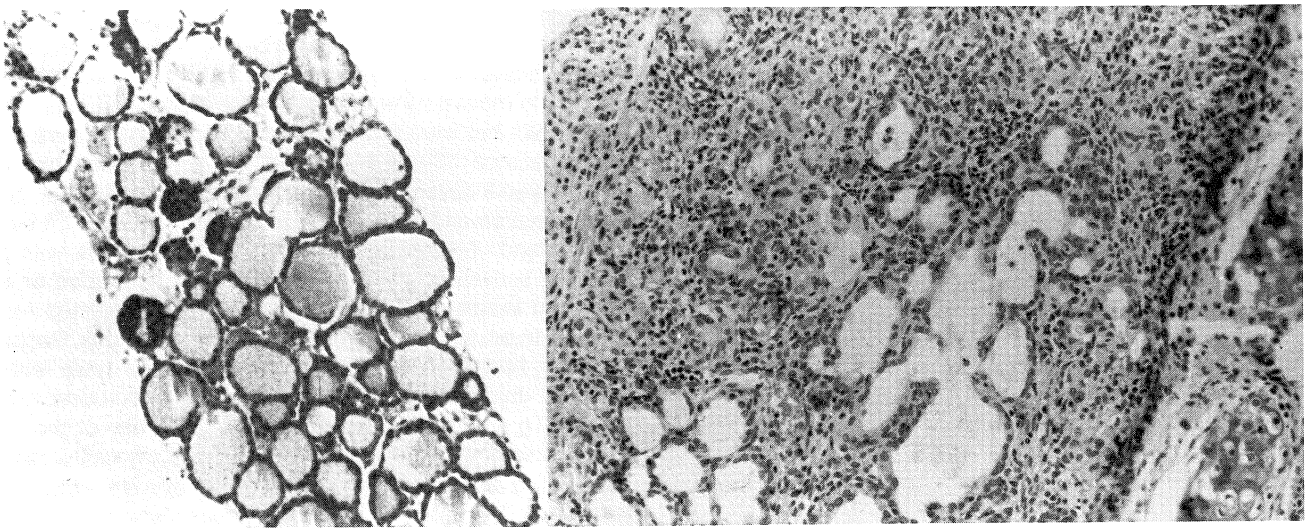

Figure 1. Thyroiditis mediated by line TF1. Histologic sections of normal thyroid (left) and thyroid obtained from a mouse inoculated with 2×10^6 TF1 line cells 8 days before study (right)

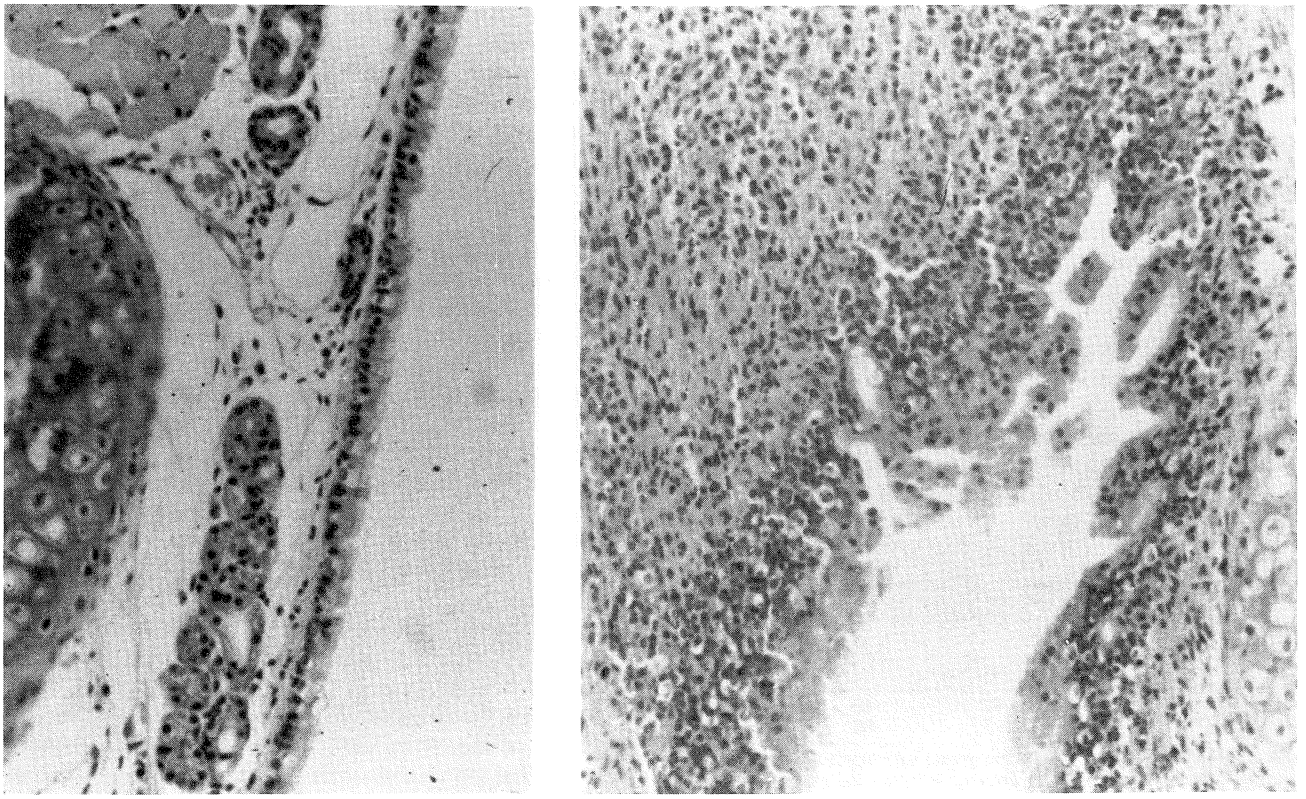

Figure 2. Tracheitis mediated by line TF1. Histologic sections of normal tracheal mucosa (left) and tracheal mucosa obtained from a mouse inoculated with 2×10^6 TF1 line cells 8 days before study (right). Note that the lumen of the affected trachea is severely compromised by a profuse inflammatory infiltrate in the mucosa and submucosa.

Dose response. Dose-response characteristics of line TF1 are shown in Table III. As few as 10^4 cells of this or other anti-Tg lines were noted to cause disease in some mice, but 10^5 or more cells were usually required to produce marked thyroiditis.

Line TF1 cells are T lymphocytes. The surface markers of line cells were characterized by using specific antibodies and a fluorescence-activated cell sorter (2). The proportion of marker-positive cells is shown in Table IV. Ninety percent or more of the cells were found to bear the Thy-1 and Ly-1 markers and a small percentage demonstrated the Ly-2 marker. About 15% of line TF1 cells were positive for Ia^k. Other functionally active lines showed the same distribution of Thy-1, Ly-1, and Ly-2 markers, but some had a greater proportion (up to 85%) of cells positive for Ia^k (not shown). We found no difference between these surface markers on activated compared to non-activated line cells.

Autoimmune effector T lymphocytes isolated after non-thyroiditogenic immunization with bovine Tg. In studies of EAE we found that autoimmune effector T lymphocyte lines could be derived from rats that were clinically well after spontaneous recovery from EAE, or that had been immunized to myelin basic protein in a non-encephalitogenic form (12). This observation suggested that potential autoimmune effector T lymphocytes could develop and persist without necessarily causing disease.

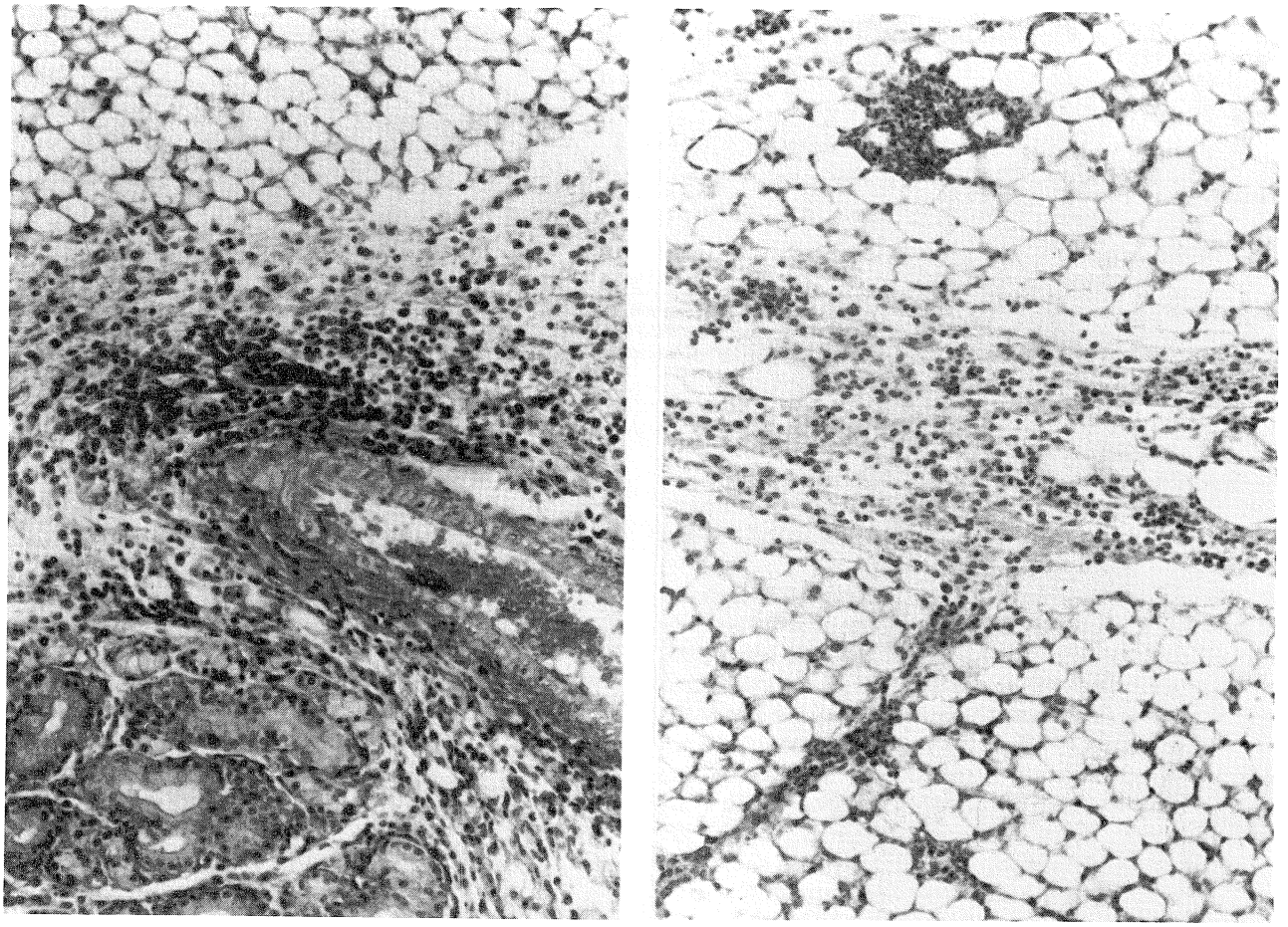

Figure 3. Perivasculitis (left) and peniculitis (right) of the salivary gland mediated by line TF1.

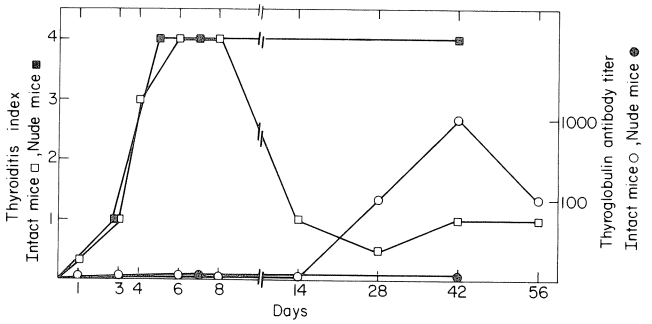

Figure 4. Kinetics of thyroiditis and Tg antibodies after inoculation with line TF1. (C3H/eB × C57BL/6)F_1 mice, intact or nude, were inoculated with 10^6 line cells and studied for development of thyroiditis by histologic examination and Tg antibodies by radioimmunoassay. Each point represents the average of groups of three to five mice.

TABLE III

Dose response to line TF1[a]

Number of TF1 Cells	Thyroiditis	
	Incidence	Pathology index
10^4	1/4	0.5
5×10^4	0/4	0
10^5	3/4	2.5
5×10^5	4/4	3.7
10^6	2/2	4
2×10^6	2/2	4

[a] (C3H/eB × C57BL/6)F_1 mice were inoculated i.v. with varying numbers of line TF1 cells and were observed for thyroiditis 8 days later.

TABLE IV

Surface markers of TF1 line cells[a]

Marker	Percent Positive Cells
Thy-1	96
Ly-1	90
Ly-2	3
Ia^k	15

[a] Surface markers were studied by using monoclonal antibodies and a fluorescence-activated cell sorter.

Therefore we sought covert autoimmune effector T lymphocytes in the mouse thyroiditis model. CBA mice were immunized with either murine Tg/CFA that is thyroiditogenic or bovine Tg/CFA that does not usually lead to EAT. Table V shows that the primed LNC of the mice responded to murine and bovine Tg in a relatively immunospecific manner; those immunized to murine Tg had higher proliferative responses to murine Tg than they did to bovine Tg, and vice versa. The primed LNC of both groups of mice responded to PPD. High titers of antibodies that did not distinguish between murine and bovine Tg were detected in the sera of the mice. As expected, only the mice that had been immunized with murine Tg developed EAT.

T lymphocyte lines were then isolated from these primed LNC; line TCM was selected for its response to murine Tg from mice that had been primed with murine Tg/CFA, and line TCB was selected for its response to bovine Tg from those that had been primed with bovine Tg/CFA. Table V shows the proliferative responses of the line cells and their ability to induce thyroiditis *in vivo*. The lines showed no responsiveness to the PPD, but each had a significant degree of specificity to a particular Tg. Upon inoculation into syngeneic CBA mice, neither line induced Tg antibodies, but both caused marked thyroiditis in all recipients.

TABLE V

Tg-primed LNC and Tg lines: proliferative responses in vitro and antibody titers and thyroiditis in vivo[a]

Cells	Tg Specificity	PPD	In Vitro: Proliferation Response (Δ cpm $\times 10^{-3}$)		In Vivo			
			Tg murine	Tg bovine	Tg antibody titer		Thyroiditis	
					Murine	Bovine	Incidence	Pathology index
Primed LNC	Murine	56	12	0	10^{-5}	10^{-4}	10/10	2
	Bovine	60	9	61	10^{-4}	10^{-4}	0/10	0
Lines:								
TCM	Murine	0	147	34	0	0	7/7	3
TCB	Bovine	0	2	113	0	0	14/14	2

[a] CBA mice were immunized to murine or bovine Tg and lines TCM and TCB were isolated from the primed LNC. Proliferative response of the primed LNC and their daugher line cells were studied. Tg antibodies and thyroiditis were assayed in mice that had been actively immunized with Tg/CFA 5 wk before and in mice that had received 3×10^6 line cells 8 days before assay. Each line was activated by incubation with its specific antigen in the presence of antigen-presenting cells.

Thus, line TCB was able to produce thyroiditis, although it seemed to have minimal proliferative reactivity in response to murine Tg *in vitro* and had been obtained from well mice. Hence, potential autoimmune effector T lymphocytes developed covertly in mice immunized with non-thyroiditogenic bovine Tg/CFA and could be rescued as a line of T lymphocytes.

Attenuated line TF1 vaccinates against EAT. In the EAE model in rats, we found that line cells attenuated by irradiation or mitomycin C could be used to induce resistance to active EAE (4), but not to EAE mediated by the line cells (5). To investigate the possibility of vaccination against EAT in mice, we inoculated mice i.v. with irradiated TF1 line cells and challenged them 3 wk later with Tg/CFA or an i.v. inoculation of 3×10^6 intact TF1 line cells. The results in Table VI show that unvaccinated mice developed a high incidence of thyroiditis after inoculation with either Tg/CFA or intact line cells. Irradiated line cells caused no thyroiditis, but mice receiving such cells were protected against the development of thyroiditis produced either by intact line TF1 or by immunization with Tg/CFA. Thus, attentuated line cells were able to vaccinate mice against autoimmune thyroiditis.

In addition to histologic examination of the mice for thyroiditis, we studied their sera to learn the effect of vaccination against thyroiditis on the development of Tg antibodies. Table VI shows that mice that were fully vaccinated against active thyroiditis produced Tg antibodies to the same high titer as did the unvaccinated controls. The titration curves illustrated in Figure 5 suggest that the avidity as well as the concentration of Tg antibodies were the same in disease-free vaccinated mice and in unvaccinated mice that developed EAT. Hence, irradiated anti-Tg line cells vaccinated mice against inflammation of their thyroid glands but did not prevent the production of Tg autoantibodies.

TABLE VI

Attenuated line TF1 vaccinates against EAT[a]

Vaccination	Thyroiditogenic Challenge	Tg Antibody Titer	Thyroiditis	
			Incidence (%)	Pathology index
None	Tg/CFA	5×10^4	90	1.5
None	TF1	0	100	4
TF1	None	0	0	0
TF1	TF1	0	0	0
TF1	Tg/CFA	5×10^4	0	0

[a] Groups of four to 10 $(C3HeB \times C57BL/6)F_1$ mice were vaccinated by a single i.v. inoculation of 5×10^6 irradiated (1500 R) TF1 line cells. Three weeks later, some groups were inoculated with 3×10^6 intact TF1 line cells and studied 7 days later for thyroiditis and Tg antibody titers. Other groups of mice were immunized to Tg/CFA twice at an interval of 1 wk and were studied for EAT and Tg antibodies 3 wk after the second immunization. Each group contained 10 mice.

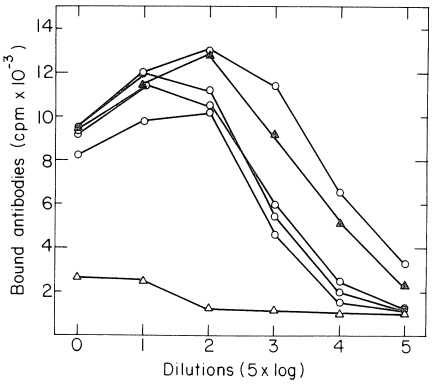

Figure 5. Tg antibodies in vaccinated mice. Tg antibody titration curves were made on sera obtained from mice described in the legend to Table VI. The open triangles are a pool of control sera obtained from unimmunized mice; the closed triangles sera from unvaccinated mice that had been immunized with Tg/CFA and developed EAT. The open circles are individual sera obtained from mice that had been vaccinated with irradiated TF1 cells and were resistant to induction of EAT by immunization with Tg/CFA.

DISCUSSION

Induction of thyroiditis. The effector mechanism responsible for the lesions of EAT has not been identified unequivocally. Some investigators proposed that EAT was mediated by antibodies (13, 14), whereas other researchers concluded that effector T lymphocytes were responsible. Evidence for the latter was provided by the finding that the EAT effector mechanism showed H-2 gene restriction at the level of the target thyroid gland (6, 7). Further support for the role of effector T lymphocytes derives from the present observation that lines of anti-Tg T lymphocytes could mediate thyroiditis in as short a time as 1 day in the apparent absence of circulating antibody. The induction of thyroiditis in irradiated recipient mice also argues against the participation of host B lymphocytes. Such Tg antibodies that did develop in intact recipient mice appeared relatively late and in low titer and may have been produced in response to endogenous Tg leaking from a damaged gland.

It is conceivable however, that EAT may be produced by more than one mechanism and that cyotoxic antibodies may play a role in active EAT induced by immunization with Tg/CFA. Nevertheless, the line cells were derived from mice primed with Tg/CFA and at the very least we must conclude that in the course of active EAT there appear T lymphocytes that are potentially thyroiditogenic without the aid of antibodies.

Autoimmune thyroiditis has been transferred by using about 10^9 LNC from Tg-primed rats (15) or guinea pigs (16). Recently, it has been possible to reduce the numbers of transferred cells in guinea pigs to 3 to 5×10^7 by activating them *in vitro* before transfer (17). Induction of thyroiditis by around 10^5 anti-Tg T lymphocyte line cells suggests that such selected populations are perhaps 10,000-fold more efficient in producing disease than are primed LNC. It is interesting that mediation of disease

required that the line cells be activated by antigen or mitogen before inoculation. A similar requirement was observed in the EAE model in which we found that activation led to changes in the lectin receptors of line cells and in their migration to the target organ (18).

More study is needed to elucidate how anti-Tg line cells cause thyroiditis, but it seems that they do not need the assistance of additional T lymphocytes (19) because severe disease developed in irradiated or in athymic nude mice. The Ly-1$^+$ phenotype of the vast majority of the line cells suggests that they might mediate delayed-type hypersensitivity (DTH) through the secretion of lymphokines and activation of macrophages (20). Alternatively, it is possible that the small minority of Ly-2$^+$ presumably cytotoxic cells did the job. The latter would fit our earlier observations based on the EAT phenotypes of H-2 mutant mice of a critical role for the H-2K (7, 21, 22) or H-2D loci (22) in genetic control of EAT. Mutations at H-2K or H-2D, however, can create target antigens for the rejection of skin grafts (23), a reaction that also appears to be mediated by Ly-1$^+$ DTH T lymphocytes (24). Thus, it is conceivable that DTH T lymphocytes might be able to recognize Tg in association with H-2 K/D gene products. These questions should be resolved by studying clones of thyroiditogenic T lymphocytes derived from the lines.

Cloning might also help explain why the TF1 line cells caused lesions in a few mice in the tracheal mucosa and salivary glands in addition to thyroiditis. It is possible that the Tg was contaminated with specific antigens from these adjacent tissues and that the TF1 line, in addition to anti-Tg cells, contained T lymphocytes specific for these other antigens. It is also possible that Tg-like antigens were present in the other affected organs or that the very violent inflammatory response nonspecifically spilled over into adjacent structures.

Covert autoimmunity. As a corollary to clonal selection, Burnet (25) proposed that self-tolerance and the absence of autoimmunity required the prompt elimination of self-recognizing lymphocytes. Despite the logic of this notion, the use of line technology to rescue quiescent autoimmune T lymphocytes has expanded our perception of the extent of covert, subclinical autoimmunity. In the EAE model, we have found that suppressed effector cells persisted in the thymus, spleen, and lymph nodes of rats that had recovered from active EAE and were resistant to induction of second attacks (12), that rats genetically resistant to active EAE could generate covert EAE effector T lymphocytes (26), that such cells also developed after non-encephalitogenic immunization with basic protein in incomplete adjuvant (12), and that passively transferred effector T lymphocyte line cells persisted (27) in the thymus for months after recovery from EAE mediated by the line cells. In the present investigation, the thyroiditogenic TCB line was isolated from clinically well CBA mice that had been immunized with non-thyroiditogenic bovine Tg/CFA.

It is paradoxical that line TCB showed minimal evidence of a proliferative response to murine Tg *in vitro*, although the cells must have been able to recognize murine Tg *in vivo*, in the ill recipient mice. It is conceivable that thyroiditogenic lymphokines or cytotoxicity may have been produced *in vivo* independently of proliferation *in vitro*. Other explanations are possible, such as masking or destruction of murine-bovine Tg cross-reactive determinants *in vitro* or the production of disease by a small minority of cross-reactive T lymphocytes. In any case, it is evident that T lymphocytes with the potential for autoreactivity may arise covertly. The quiescence of these cells implies the existence of regulatory mechanisms (28).

Vaccination against thyroiditis. Vaccination is the use of a pathogenic agent, usually attenuated, to endow the individual with a measure of resistance to disease caused by the particular agent. In this sense, inoculation with irradiated line cells can be seen as a type of vaccination against autoimmunity. We have successfully used antigen-specific T lymphocyte line cells to vaccinate rats against EAE (4, 5) or against adjuvant arthritis (3). It was observed that vaccination produced resistance to the development of active EAE induced by immunization with basic protein in CFA, but there was no resistance to EAE caused by inoculation of anti-basic protein line cells (5). It is remarkable that vaccination of mice with irradiated TF1 line cells prevented thyroiditis mediated by the intact TF1 line cells as well as that induced by immunization with Tg/CFA. This suggests that the actions of preformed effector T lymphocytes as well as their differentiation may be inhibited by vaccination. That vaccination against thyroiditis was more comprehensive than vaccination against encephalomyelitis might be attributable to differences intrinsic to rats and mice, to the various lines, or to diverse types of autoimmune disease. In any case, it is evident that vaccination with line cells is not limited to modulation of EAE or arthritis in rats but can be achieved in other species. Preliminary results in the adjuvant arthritis model suggest that it may be possible to use antigen-specific line cells to treat autoimmunity after it has been induced and not only to prevent disease. It remains to be seen whether thyroiditis can also be treated in this manner.

The mechanisms of resistance that are activated by vaccination have not been elucidated fully but could involve anti-receptor (anti-idiotypic) immunity (29) or suppressor cells (30). Anti-receptor immunity appears to function in acquired resistance to EAE (12). Anti-receptor immunity is also compatible with the selective effect of vaccination against inflammation of the thyroid gland, without suppression of the production of Tg antibodies. It is conceivable that the attentuated effector T lymphocytes of the TF1 line vaccinated the recipients against receptor idiotypes specific for effector lymphocytes but not against idiotypes found on the helper T cells required for production of Tg antibodies. This would suggest the possibility that different antigenic determinants on the Tg molecule are recognized by functionally different classes of T lymphocytes. Other explanations are conceivable, however, and studies with cloned T lymphocytes and anti-idiotypic antibodies might resolve the question. In any case, vaccination with attenuated antigen-specific T lymphocytes appears to have a remarkable degree of immunologic specificity.

Heterologous anti-idiotypic antibodies have been successfully used to partially prevent induction of autoimmune tubulointerstitial nephritis in guinea pigs (31) and, combined with sublethal irradiation, to alleviate spontaneous autoimmune thyroiditis in rats (32). These antibodies failed to alter significantly the induction of autoimmune encephalomyelitis in rats (33). Active immunization against acetylcholine receptor antibodies was partially successful in preventing experimental autoimmune myasthenia gravis in rabbits (34). Although these models differ substantially from the one described here, the results indicate that anti-idiotypic immunity may be used to modify autoimmune processes (35). The relative effectiveness of using preformed anti-idiotype antibodies, compared to inducing active anti-idiotypic responses with T lymphocyte or antibody idiotypes, should be investigated.

REFERENCES

1. Ben-Nun, A., H. Wekerle, and I. R. Cohen. 1981. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur. J. Immunol. 11:195.
2. Ben-Nun, A., and I. R. Cohen. 1982. Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. J. Immunol. 129:303.
3. Holoshitz, J., Y. Naparstek, A. Ben-Nun, and I. R. Cohen. 1983. Lines of T

lymphocytes induce or vaccinate against autoimmune arthritis. Science 219:56.
4. Ben-Nun, A., H. Wekerle, and I. R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis using attenuated cells of a T lymphocyte line reactive against myelin basic protein. Nature 292:160.
5. Ben-Nun, A., and I. R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis (EAE): attenuated autoimmune T lymphocytes confer resistance to induction of active EAE but not EAE mediated by the intact T lymphocyte line. Eur. J. Immunol. 11:949.
6. Ben-Nun, A., R. Maron, Y. Ron, and I. R. Cohen. 1980. H-2 gene products influence susceptibility of target thyroid gland to damage in experimental autoimmune thyroiditis. Eur. J. Immunol. 10:156.
7. Maron, R., and I. R. Cohen. 1980. H-2K mutation controls immune response phenotype of autoimmune thyroiditis. Critical expression of mutant gene product in both thymus and thyroid glands. J. Exp. Med. 152:1115.
8. Tomazic, V., and N. R. Rose. 1976. Autoimmune murine thyroiditis. VII. Role of different thyroid antigens in the induction of experimental autoimmune thyroiditis. Immunology 30:63.
9. Eshhar, Z., G. Strassman, T. Waks, and E. Mozes. 1979. *In vitro* and *in vivo* induction of effector T cells mediating DTH response to a protein and a synthetic polypeptide antigen. Cell. Immunol. 47:378.
10. Vladutiu, A. O., and N. R. Rose. 1971. Autoimmune murine thyroiditis. Relation to histocompatibility (H-2) type. Science 174:1137.
11. Rose, N. R., F. J. Twarog, and A. J. Crowle. 1971. Murine thyroiditis: importance of adjuvant and mouse strain for the induction of thyroid lesions. J. Immunol. 106:698.
12. Ben-Nun, A., and I. R. Cohen. 1982. Spontaneous remission and acquired resistance to autoimmune encephalomyelitis (EAE) are associated with suppression of T cell reactivity. Suppressed EAE effector cells recovered as T cell lines. J. Immunol. 128:1450.
13. Clagett, J. A., and W. O. Weigle. 1974. Roles of T and B lymphocytes in the termination of unresponsiveness to autologous thyroglobulin in mice. J. Exp. Med. 139:643.
14. Tomazic, V., and N. R. Rose. 1975. Autoimmune murine thyroiditis. VII. Induction of the thyroid lesions by passive transfer of immune serum. Clin. Immunol. Immunopathol. 4:511.
15. Twarog, F., and N. R. Rose. 1970. Transfer of autoimmune thyroiditis of the rat with lymph node cells. J. Immunol. 104:1467.
16. Sharp, G. C., H. Mullen, and M. Kyriakos. 1974. Production of augmented experimental autoimmune thyroiditis lesions by combined transfer of antiserum and lymph node cells. J. Immunol. 112:478.
17. Braley-Mullen, H., J. G. Tompson, G. C. Sharp, and M. Kyriakos. 1981. Adoptive transfer of experimental autoimmune thyroiditis (EAT) with *in vitro* activated lymph node cells from thyroglobulin-sensitized guinea pigs: characterization of the cell that transfers EAT. J. Immunol. 127:1767.
18. Naparstek, Y., A. Ben-Nun, J. Holoshitz, *et al*. 1983. T lymphocyte lines producing or vaccinating against autoimmune encephalomyelitis (EAE). Functional activation induces PNA receptors and accumulation in the brain and thymus of line cells. Eur. J. Immunol. 13:418.
19. Cohen, I. R., and S. Livnat. 1976. The cell-mediated immune response: interactions between initiator and recruited T lymphocytes. Transplant. Rev. 29:24.
20. Shell, G. 1978. T cells, T cell recognition and the major histocompatibility complex. Transplant. Rev. 38:5.
21. Maron, R., and I. R. Cohen. 1979. Mutation at H-2K locus influences susceptibility to autoimmune thyroiditis. Nature 279:715.
22. Maron, R., J. Klein, and I. R. Cohen. 1982. Mutations at H-2K or H-2D alter immune response phenotype of autoimmune thyroiditis. Immunogenetics 15:625.
23. McKenzie, I. F. C., T. Pang, and R. V. Blanden. 1977. The use of H-2 mutants as models for the study of T cell activation. Immunol. Rev. 35:181.
24. Loveland, B. E., P. M. Hogarth, R. H. Ceredis, and I. F. C. McKenzie. 1981. Cells mediating graft rejection in the mouse I Lyt^{-1} cells mediate skin graft rejection. J. Exp. Med. 153:185.
25. Burnet, F. M. 1959. The Clonal Selection Theory of Acquired Immunity. Vanderbilt University Press, Nashville.
26. Ben-Nun, A., S. Eisenstein, and I. R. Cohen. 1982. Experimental autoimmune encephalomyelitis (EAE) in genetically resistant rats: PVG rats resist active induction of EAE but are susceptible to and can generate EAE effector T cell lines. J. Immunol. 129:918.
27. Naparstek, Y., J. Holoshitz, S. Eisenstein, *et al*. 1982. Effector T lymphocyte line cells migrate to the thymus and persist there. Nature 300:262.
28. Cohen, I. R., and H. Wekerle. 1973. Regulation of autosensitization: the immune activation and specific inhibition of self-recognizing T lymphocytes. J. Exp. Med. 137:224.
29. Binz, H., and H. Wigzell. 1976. Specific transplantation tolerance by autoimmunization against the individuals own naturally occurring idiotypic, antigen-binding receptors. J. Exp. Med. 144:1438.
30. Sercarz, E. E., R. L. Yowell, D. Turkin, A. Miller, B. A. Araneo, and L. Adorini. 1978. Different functional specific repertoires for suppressor and helper T cells. Immunol. Rev. 39:108.
31. Brown, C. A., K. Carey, and R. B. Colvin. 1979. Inhibition of autoimmune tubulointerstitial nephritis in guinea pigs by heterologous antisera containing anti-idiotype antibodies. J. Immunol. 123:2102.
32. Zanetti, M., and P. E. Bigazzi. 1981. Anti-idiotypic immunity and autoimmunity. 1. *In vitro* and *in vivo* effects of anti-idiotypic antibodies to spontaneously occurring autoantibodies to rat thyroglobulin. Eur. J. Immunol. 11:187.
33. Fritz, R. B., and A. E. Desjardins. 1982. Idiotypes of Lewis rat antibodies to encephalitogenic peptides of guinea pig myelin basic protein: *in vitro* and *in vivo* studies. J. Immunol. 128:247.
34. Fuchs, S., C. Feingold, D. Bartfeld, D. Mochly-Rosen, I. Schmidt-Hopfeld, and R. Tarrab-Hazdai. 1982. Molecular approaches to myasthenia gravis. *In* Disorders of the Motor Unit. D. L. Schotland, ed. John Wiley and Sons, New York. P. 245.
35. Cohen, I. R. 1983. Autoimmunity: physiological and pernicious. *In* Advances in Internal Medicine, vol. 29. G. H. Stollerman, ed. Year Book Medical Publishers. In press.