Role of the Thymus in Induction and Transfer of Vaccination Against Adjuvant Arthritis with a T Lymphocyte Line in Rats

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Abstract

Adjuvant arthritis is an experimental disease of rats induced by immunization to antigens of *Mycobacterium tuberculosis*. Our observation that arthritis could be induced in irradiated rats by the A2 line of T lymphocytes in the absence of mycobacterial antigens suggested that adjuvant arthritis is an autoimmune disease. Moreover, the A2 line could be used to vaccinate unirradiated rats against the subsequent induction of adjuvant arthritis by active immunization to *Mycobacteria*. In the present study we found that thymus cells obtained from A2 vaccinated rats could transfer resistance to adjuvant arthritis to naive rats. This indicates that the mechanism of resistance induced by A2 vaccination is probably immunological and involves thymus-derived lymphocytes.

Introduction

Adjuvant arthritis (AA)[1] can be induced in rats by an intradermal injection of killed *Mycobacterium tuberculosis* (MT) in oil in the form of complete Freund's adjuvant (CFA) (1). AA presents some of the clinical and pathological features of rheumatoid arthritis and has been considered as a model of the human disease (2). From rats immunized with CFA, we have isolated the A2 line of T lymphocytes by virtue of its proliferative response to MT (3). The A2 line cells could induce arthritis following intravenous injection into irradiated recipient rats, which suggests that AA may be caused by an autoimmune attack against joint antigens that are cross-reactive with MT (4). Moreover, A2 line cells could be used to vaccinate unirradiated recipient rats against attempts to induce active AA by subsequent inoculation with CFA (3).

In the studies reported here, we undertook to characterize the mechanisms involved in A2 line-induced vaccination against AA. The major questions examined were: what are the temporal and quantitative parameters of vaccination; is the presence of thymus or spleen required for the vaccination effect; and can line-induced vaccination be adoptively transferred to naive rats?

Methods

Rats. Inbred Lewis rats were obtained from the Animal Breeding Center of this Institute. Unless otherwise stated, 2-mo-old female rats were used throughout the study.

Antigens. Heat-killed *M. tuberculosis* H_{37} Ra was purchased from Difco Laboratories (Detroit, MI). The bacteria were ground with a mortar and pestle under sterile conditions, and suspended either in incomplete Freund's adjuvant to prepare CFA, or in phosphate-buffered saline (PBS) to prepare MT antigen for in vitro experiments. A water soluble preparation of MT was made by suspending 100 mg of ground MT in double-distilled water for 48 h at 4°C with constant stirring. Suspended MT fragments were removed from the water by centrifugation twice at 2,000 rpm for 25 min. The supernatant containing a water soluble fraction of MT was lyophilized and stored at −20°C. Before use, the water soluble MT fraction was dissolved in PBS. This material was used only in the experiment shown in Table III. Concanavalin A (Con A) was purchased from Bio-Yeda (Rehovot, Israel) and ovalbumin (OA) from Sigma Chemical Co., St. Louis, MO. Guinea pig basic protein of myelin (BP) was prepared as described (5).

Culture medium. All cell cultures used Dulbecco's modification of Eagle's medium (Gibco Laboratories, Grand Island, NY). Medium used for restimulation (proliferation medium) was supplemented with 1 mM glutamine (Bio-Lab, Jerusalem, Israel), 2-mercaptoethanol (5×10^{-5} M), gentamycin (40 μg/ml), and 1% fresh autologous rat serum. The medium used to maintain the propagated cell lines and clones in long-term culture (propagation medium) was the proliferation medium supplemented with 15% (vol/vol) of supernatant of Con A-stimulated mouse splenocytes as a source of T cell growth factor (5), 10% horse serum (Gibco Laboratories), 1 mM sodium pyruvate, and nonessential amino acids (Bio-Lab).

Line cells. Line A2 reactive to MT was isolated and maintained as described (3). A single cell suspension was prepared from pooled lymph nodes removed from Lewis rats injected in the hind footpads 9 d earlier with CFA. The cells were resuspended in proliferation medium (5×10^6/ml) and plated in 10-ml petri dishes (Nunc, Kamstrup, Denmark) in the presence of MT (10 μg/ml). After 72 h of incubation at 37°C in a humidified atmosphere plus 7.5% CO_2, the cells were collected, washed three times by mild centrifugation using a table centrifuge, resuspended in propagation medium (2×10^5/ml), and incubated for 48 h in 10-ml petri dishes. The cells were then collected, washed three times, and restimulated by being resuspended in proliferation medium (2×10^5/ml) together with irradiated (1,500 rad) syngeneic thymocytes (15×10^6/ml) and MT (10 μg/ml) in 10-ml petri dishes. After 72 h the cells were collected, washed three times, and transferred for continuous growth in propagation medium as above. Cultures in propagation medium were refreshed every 3–4 d. Once every 2–4 wk, the line cells were restimulated by incubation with MT and irradiated thymocytes for 3 d as above and then transferred back into propagation medium. Line A2 was maintained in this manner for 8 wk before study of its antigen reaction (3) or its ability to mediate arthritis or induce resistance. All A2 line cells which have been used throughout our experiments (3, 4), including the present one, originated

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1. *Abbreviations used in this paper:* AA, adjuvant arthritis; BP, basic protein; CFA, complete Freund's adjuvant; Con A, concanavalin A; EAE, experimental autoimmune encephalomyelitis; EAT, experimental autoimmune thyroiditis; MT, *Mycobacterium tuberculosis;* OA, ovalbumin.

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from the same common pool of lymph node cells. Cloned subline A2b was isolated and maintained as we described (4). Lines Z1a, anti-BP, and C1a anti OA were isolated and maintained as described (5).

Surgical removal of spleen or thymus. Thymectomy or splenectomy of 4–8-wk-old rats was performed under ether anesthesia. Thymectomy was accomplished by suction of the gland through a midline incision made into the upper thorax. Splenectomy was done by exposing the spleen through a lateral incision. The blood vessels were closed by heat or silk ligation. Sham operations involved similar procedures except that the organs were not removed. Incisions were closed by metal clips. Completeness of thymectomy was confirmed at autopsy.

Induction of active AA. To induce active AA rats were inoculated intradermally at the base of the tail with 0.1 ml of CFA containing 10 mg/ml MT (H_{37} Ra, purchased from Difco Laboratories), in incomplete Freund's adjuvant (Difco Laboratories). Severity of arthritis was assessed as previously described (3) and the arthritis score computed as the sum of the degree of inflammation of each of the four limbs graded on a scale of one to four. The maximum arthritis score was thus 16.

Vaccination using line cells. Before being inoculated, line cells were restimulated with their respective antigens in the presence of irradiated (1,500 rad) thymus accessory cells for 3 d as described (3–6). At this time, the irradiated thymus accessory cells have died and disintegrated, so that >90% of the culture is composed of lymphoblasts. Restimulation of line A2 was done routinely before vaccination to obtain the large numbers of line cells needed for the experiments and because activation was essential for vaccination by line cells against experimental autoimmune encephalomyelitis (EAE) (6) or against experimental autoimmune thyroiditis (EAT) (7). The lymphoblasts were collected, separated from residual accessory cells and from cellular debris by density gradients in some experiments (see Table I), washed twice in PBS, and injected in 1 ml of PBS into the tail vein of nonirradiated Lewis rats. In some experiments (see Table I), control rats were inoculated with irradiated thymus accessory cells that had been incubated for 3 d with MT in the absence of line cells. At various times after inoculation the rats were tested for resistance to active induction of AA by challenge with CFA. Vaccination was evident by a decrease in incidence and/or severity of AA compared with control groups. Rats that did not develop arthritis were not included in computation of the mean day of onset and mean arthritis score.

Adoptive transfer of vaccination. Rats were irradiated with 150 rad of gamma irradiation (3) and then inoculated intravenously with various numbers of thymus or spleen cells or with 1.5 ml of serum obtained from rats that had been vaccinated 1 mo earlier by inoculation with 2×10^7 A2 or Z1a line cells. The recipient rats were challenged on the same day with CFA to assay their susceptibility to AA.

Results

Dose response of line-induced vaccination. To define the number of A2 line cells needed to vaccinate recipient rats against AA, we inoculated rats with various numbers of line A2 cells at day 0 and challenged them with CFA 35 d later. As can be seen in Table I, inoculation of 1×10^6 cells had no effect, 5×10^6 cells produced a moderate decrease in clinical severity, while 10×10^6 cells caused a marked decrease in both incidence and severity of disease. Complete protection against AA was achieved by vaccination with 20×10^6 line A2 cells.

Time of onset and duration of vaccination. The time required for line-induced protection to be generated was assessed by challenging recipients of 2×10^7 A2 cells with CFA at various intervals after inoculating the cells. Table II shows that while some degree of protection was found 3 d after inoculating 2×10^7 A2 cells, full protection was found from the 16th d on, and persisted for at least 180 d.

MT carry-over is not responsible for vaccination. It has been shown that administration of small doses of MT antigens can induce resistance to AA (8). Therefore, a series of experiments were designed to test whether the vaccination effect could be attributed to MT antigens carried-over with the A2 line cells into the recipient rats. The results of these experiments are tabulated in Table III. Groups 2 and 3 illustrate that density gradient separation of residual accessory cells that might have carried processed MT did not interfere with the ability of line A2 to vaccinate rats against AA. This was confirmed by experimental group 4, which was successfully vaccinated with A2 line cells that had been activated by the mitogen Con A and were therefore free of any possible contamination with MT. Group 5 shows that water soluble MT was as effective as particulate MT. Hence, incubation of A2 with particulate material was not a prerequisite for vaccination. Group 6 shows that incubating the arthritogenic clone of A2 (4) with MT did not lead to successful vaccination, although this clone responded to MT (4) and could have carried over any MT antigens picked up in culture. Group 7 illustrates that vaccination could not be effected by irradiated accessory thymocytes incubated with MT in the absence of line A2. Therefore, the vaccination effect was seen to be a function of the A2 line cells and was independent of possible MT carry-over.

Adoptive transfer of vaccination. To learn whether the state of vaccination could be transferred, we irradiated recipient rats

Table I. Dose-Response of Line A2-induced Protection Against AA

A2 line cells injected intravenously at day 0 ($\times 10^{-6}$)	AA induced with CFA at day 35		
	Incidence	Mean day of onset	Mean arthritis score
		±*SD*	±*SD*
0	5/5	14.2±0.4	13.6±0.9
1	5/5	17.0±0.7	12.0±0.7
5	5/5	17.4±1.5	7.4±1.1
10	1/5	19.0	2.0
20	0/5	—	—

3-mo-old male Lewis rats were inoculated intravenously with various numbers of A2 line cells and their susceptibility to AA was tested by immunizing them with CFA 35 d later.

Table II. Time Course of Line A2-induced Protection Against AA

Challenge with CFA after inoculation with line A2 (*d*)	AA		
	Incidence	Mean day of onset	Mean arthritis score
		±*SD*	±*SD*
Not inoculated	10/10	13.9±1.0	8.9±0.9
0	9/10	14.1±1.2	8.7±1.8
3	3/5	14.7±0.6	4.7±0.6
16	0/5	—	—
30	0/5	—	—
52	0/5	—	—
180	0/12	—	—

Lewis rats were tested for susceptibility to induction of AA by immunization with CFA at various times after inoculation with 2×10^7 A2 line cells. Day 0 signifies that the line cells and the CFA were administered on the same day.

Table III. Carryover of MT is Not Responsible for Vaccination

Inoculum					
Group	T cells	Irradiated thymocytes	Antigen/ mitogen	Separation of thymocytes	CFA challenge percentage incidence of AA
1	None	None	None	No	90
2	A2	Yes	MT	No	0
3	A2	Yes	MT	Yes	0
4	A2	Yes	Con A	No	0
5	A2	Yes	MT-H_2O	No	0
6	A2b	Yes	MT	No	83
7	None	Yes	MT	No	100

Cells (2×10^5/ml) of line A2 or cloned subline A2b were incubated with MT (10 μg/ml), water soluble extract of MT (MT-H_2O; 50 μg/ml), or Con A (2.5 μg/ml) in the presence of accessory cells in the form of irradiated (1,500 rad) thymocytes for 72 h. Control cultures included irradiated thymocytes, which were incubated with MT in the absence of line cells. After 72 h the cultures were collected and washed to eliminate cell debris and accessory cells. Cultures in group 3 were subjected to density separation (5). The blasts were counted, and 2×10^7 blasts or an equivalent number of irradiated thymocytes were injected intravenously into groups of 6–10 nonirradiated Lewis rats. After 35 d, all the rats were challenged with CFA and observed for the appearance of AA.

with 150 rad and inoculated them intravenously with 1.5 ml of serum or with 10^9 spleen or thymus cells obtained from normal donor rats or from donors that had been vaccinated 1 mo earlier by intravenous inoculation with 2×10^7 A2 line cells. The recipients were challenged with CFA to induce active AA. Fig. 1 shows that the recipients of serum from A2 vaccinated rats showed enhanced expression of active AA. In contrast, resistance to AA was transferred by thymus cells of donors that had been vaccinated with line A2. Spleen cells obtained from vaccinated rats and thymus or spleen cells from control rats had no effect on development of AA. Resistance to AA was not detectable following transfer into nonirradiated recipients (not shown).

Fig. 2 shows a dose-response experiment in which recipients were inoculated with 1×10^8, 2.5×10^8, 5×10^8, or 1×10^9 donor thymus cells of rats that had been vaccinated against AA using A2 line cells. A group of rats was inoculated with 5×10^8 thymus cells obtained from rats that had been vaccinated against EAE by inoculation with Z1a anti-BP T lymphocyte line cells (9). Resistance to AA was evident in rats that had

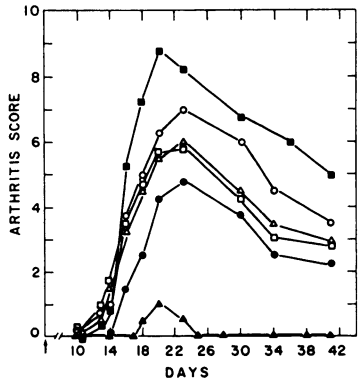

Figure 1. Transfer of resistance to adjuvant arthritis by thymus cells of vaccinated donors. Groups of 10 rats each were irradiated with 150 rad and then inoculated intravenously with 1.5 ml of serum (closed squares) or with 10^9 spleen (closed circles) or 10^9 thymus cells (closed triangles) obtained from rats that had been vaccinated 1 mo early with A2 line cells. Control rats received no cells (open squares) or were inoculated with 10^9 spleen cells (open circles) or 10^9 thymus cells (open triangles) obtained from naive donor rats. The arthritis score was recorded over time.

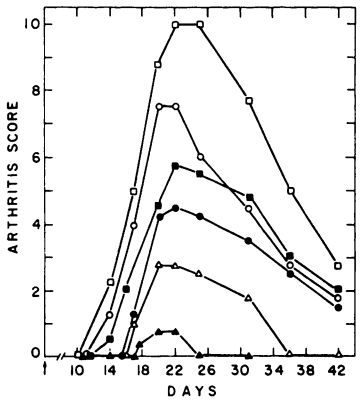

Figure 2. Dose response of transfer of resistance to adjuvant arthritis. Groups of 10 rats were irradiated with 150 rad and then not inoculated (open circles) or inoculated with 5×10^8 thymus cells obtained from donor rats that had been vaccinated 1 mo earlier with Z1a anti-BP line cells (open squares). Other groups of rats received 10^9 (closed triangles), 5×10^8 (open triangles), 2.5×10^8 (closed circles), or 10^8 (closed squares) thymus cells obtained from rats that had been vaccinated 1 mo earlier with A2 line cells. The arthritis score was recorded over time.

received 5×10^8 or 1×10^9 thymus cells obtained from donors that had been vaccinated against AA. Thymus cells (5×10^8) from rats that had been vaccinated against EAE rather than protect, actually seemed to enhance the development of AA. Thus, the degree of protection appeared to be related to the number of thymus cells transferred from rats that had been specifically vaccinated with A2 line cells.

Presence of thymus not required for successful vaccination. The above results suggested that the thymus gland contained cells that could transfer resistance to AA. It was therefore important to learn whether the presence of the thymus was essential for successful vaccination against AA. Accordingly, rats were either thymectomized, splenectomized, or sham operated at the age of 4 wk, and some rats were inoculated with 2×10^7 A2 cells 45 d later. To assay susceptibility to AA, all the rats were challenged with CFA 80 days after surgery. Table IV tabulates the results which illustrate two findings. First, regarding rats that had not been vaccinated with A2 line cells, splenectomy had no influence on the susceptibility of the rats to AA, while adult thymectomy either alone or in combination with splenectomy hastened the onset

Table IV. Presence of Thymus or Spleen Is Not Required for Induction of Vaccination Against AA

		AA induced by CFA at day 80		
Treatment at day 0	Line A2 at day 45	Percentage incidence (*n* rats)	Mean day of onset	Mean arthritis score
	2×10^7		±*SD*	±*SD*
Sham surgery	Yes	0 (15)	—	—
	No	94 (16)	13.5±1.4	9.0±1.6
Thymectomy	Yes	15 (13)	16.5±0.7	2.5±0.7
	No	100 (9)	10.9±1.2	13.7±2.4
Splenectomy	Yes	20 (5)	14.0	4.0
	No	100 (5)	14.2±0.4	9.4±1.8
Thymectomy plus splenectomy	Yes	20 (10)	15.0±0	2.0±0
	No	100 (10)	12.0±0.7	11.6±1.7

Lewis rats were surgically treated at 4 wk of age (day 0), and some were inoculated with A2 line cells 45 d later. At day 80, after surgery, the rats were immunized with CFA and scored for development of AA. Parentheses indicate number of rats in each group.

of disease (day 10.9 compared to day 14.4) and increased the severity of AA (arthritis score 13.7 compared to 9.2). Second, rats that had undergone either thymectomy, splenectomy, or both procedures acquired resistance to AA as a result of vaccination with A2 line cells. Thus, although the induction of AA seemed to be enhanced in adult thymectomized rats, such rats could still be vaccinated successfully against AA by inoculation of A2 line cells. Hence, adult thymectomy performed before vaccination did not interfere with acquisition of resistance. Therefore, the presence of the thymus and/or spleen was not essential for the induction of the protective mechanism.

Late thymectomy of vaccinated rats abolishes resistance to AA. Despite successful vaccination of thymectomized rats, the finding that thymus cells of vaccinated rats could transfer resistance to AA suggested that the thymus might house the cells responsible for resistance after they were induced. We therefore vaccinated 1-mo-old rats by inoculating them with A2 or control C1a (anti-ovalbumin) line cells, and then removed the thymus 3 or 35 d later. 45 d after the thymectomy, the rats were challenged with CFA to test their resistance to AA. Table V shows that recipients of C1a line cells were susceptible to induction of AA whether they were or were not thymectomized. In contrast, the rats that had been inoculated with A2 line cells were resistant to AA provided that they had been sham thymectomized or thymectomized only 3 d after vaccination. However, rats that had been thymectomized 35 d after vaccination with A2 line cells manifested a high incidence of severe arthritis. Therefore, late thymectomy led to loss of acquired resistance to AA. Hence, the cells responsible for acquired resistance could be generated in the absence of the thymus, but, given a thymus, they seemed to be housed there.

Discussion

Resistance to active induction of autoimmune disease is achievable by inoculating experimental subjects with cells of long term lines of T lymphocytes specifically reactive against the relevant antigens. We have called this phenomenon vaccination by its analogy to the use of attenuated microbial pathogens to induce specific resistance (10). Vaccination has been successful in three experimental diseases: EAE, EAT, and AA.

For example, inoculation of rats with anti-BP lines produced EAE, and inoculation of mice with anti-thyroglobulin lines produced EAT. However, upon irradiation (1,500 rad), the line cells no longer mediated disease, but animals receiving a single inoculation of cells acquired resistance to induction of EAE by immunization with BP (9) or to induction of EAT by immunization with thyroglobulin (7). In the case of line-mediated AA, arthritis was produced by A2 line cells only in recipients that had been irradiated (750 rad), and therefore to avoid disease it was not necessary to irradiate the A2 line, but only to desist from irradiating the recipient rats (3). Why line A2 mediates arthritis in irradiated but not in intact rats is not known. It has been shown that irradiation augments the severity of actively induced AA (11) and that some rats subjected to total lymphoid irradiation spontaneously develop arthritis (12). Therefore, it is conceivable that natural mechanisms of resistance to AA and to line-induced arthritis exist in intact rats, and that these mechanisms can be neutralized by irradiation. Hence, vaccination might work by strengthening these mechanisms. The point of this report, however, is that induced resistance is associated with the thymus and can be transferred.

Whether or not AA is an autoimmune disease has been a controversial question. Several hypotheses have been proposed: that the arthritis results from an immune response to Mycobacterial antigens settling in the joints after immunization with CFA (13); that the physical injection of CFA sensitizes the rat to endogenous connective tissue antigens in the skin (14); or that there exists cross-reactivity between antigenic determinants of *Mycobacteria* and antigens in the joints (15). The induction of arthritis using anti-MT line cells in the absence of CFA injection supports the latter hypothesis (4). We are presently characterizing the self-antigen that is the target of the immune attack on the joints, but as yet can only say that it is probably not collagen type II (4).

In the present study we investigated factors involved in the

Table V. Thymectomy 35 D After Inoculation of Line A2 Eliminates Line-induced Protection

	Treatment of recipient rats		AA induced by CFA 45 d after treatment		
Inoculation of T cell line	Days after line inoculation	Treatment	Percentage incidence (*n* rats)	Mean day of onset	Mean arthritis score
				±*SD*	±*SD*
A2	3	Thymectomy	13 (8)	17.0	2.0
		Sham thymectomy	0 (13)	—	—
	35	Thymectomy	83 (6)	13.4±1.1	9.4±1.7
		Sham thymectomy	0 (5)	—	—
C1a	3	Thymectomy	100 (5)	12.0±1.6	10.9±1.8
		Sham thymectomy	100 (6)	14.2±1.5	8.7±1.4
	35	Thymectomy	100 (6)	11.2±0.8	12.5±1.6
		Sham thymectomy	100 (6)	14.0±2.3	9.0±1.9

4-wk-old Lewis rats were inoculated with 2×10^7 line cells, thymectomized or sham thymectomized 3 or 35 d later, and then immunized with CFA 45 d after surgical treatment. Parentheses indicate number of rats in each group.

acquisition of resistance to AA induced by vaccination with A2 line cells. Vaccination against AA was found to be a function of the number of A2 line cells (Table I), and required time to become established (Table II). We have observed that resistance to AA becomes solid by ~10 d after inoculation of A2 line cells. Once it sets in, resistance seemed to persist (Table II). As illustrated in Table III, vaccination could not be explained by possible carryover of MT antigens. Line A2 did not need to be incubated with MT to induce resistance, and incubation of clone A2b or of thymus accessory cells alone with MT did not produce vaccination. Moreover, A2 line cells were as effective after incubation with water soluble MT as they were after incubation with particulate MT.

The importance of the thymus in vaccination was indicated by the transfer of resistance by thymus cells and not by spleen cells or serum obtained from vaccinated rats (Figs. 1 and 2). In this study, transfer of vaccination was successful when the recipient rats were irradiated with 150 rad. Transfer was unsuccessful when the recipients were not irradiated (not shown). Why lymphocyte transfer is enhanced by mild irradiation is unknown, but several mechanisms have been proposed including the creation of "space" in lymphoid organs by depletion of recipient lymphocytes (16).

Vaccination has been shown to be antigen-specific in AA (3), EAE (17), and EAT (7), and its transfer by thymus cells, probably T lymphocytes, suggests that resistance is mediated by an immunological mechanism. We have found that the specificity of vaccination was directed by the fine specificity of the line cells used for vaccination, which suggests the possibility that the antigen-specific line cells immunized the recipients against the receptors needed to recognize the self-antigens critical for the autoimmune disease (17). Although anti-receptor or anti-idiotypic immunity might serve to regulate autoimmunity (18, 19), other mechanisms are conceivable and the question remains to be resolved. For example, clone A2b, although arthritogenic, could not vaccinate against AA (4), and several encephalitogenic clones have been isolated that do not vaccinate against EAE (in preparation). Be that as it may, the present study shows that the thymus can be involved in acquired resistance to autoimmunity. However, the role of the thymus in this resistance, although major, was not exclusive. Vaccination against AA could be obtained in rats that had been thymectomized, even though adult thymectomy seemed to increase the severity of AA in this study (Table III) and in others (11). Nevertheless, by 35 d after vaccination, resistance could be abolished by removing the thymus. This implies that resistance can be induced and maintained outside of the thymus, but in the presence of the thymus the resistance mechanism may settle there and hence becomes vulnerable to thymectomy.

The thymus has been looked upon as an organ devoted primarily to the differentiation of T lymphocytes and disconnected from immune responses which take place in the periphery. However, the results of investigations carried out in this laboratory indicate that the thymus receives and houses antigen-activated T lymphocytes from the periphery and may perform important functions in regulating autoimmune responses. The return to the thymus of potential effector T lymphocytes was demonstrated by inoculating rats with radiolabeled lines of T lymphocytes specifically reactive against BP or against foreign antigens. About 1% of the inoculated line cells accumulated in the thymus and persisted there for months (6, 20). Anti-BP line cells recovered from the thymus were found to be capable of mediating EAE in normal recipient rats. Thus, autoimmune, potential effector lymphocytes persisted in clinically well rats.

Another study involved rats that had acquired resistance to induction of a second attack of EAE subsequent to recovery from primary EAE induced by immunization to BP (21). It was found that this form of acquired resistance too was associated with the appearance in the thymus, and less so in the spleen, of cells that specifically inhibited the proliferative responses in vitro of anti-BP effector T lymphocyte line cells. A function for the thymus in regulating EAE was also suggested by the finding that adult thymectomy of rats increased their susceptibility to progressive or relapsing EAE (22). Whatever the cellular mechanisms of resistance turn out to be, the present findings indicate that it is feasible using T lymphocyte line cells to manipulate the state of resistance to induction of what appears to be autoimmune arthritis. The clinical implication of these studies is clear (10, 19).

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