Journal of Autoimmunity (1989) **2**, 75–86

Vaccination Against Experimental Autoimmune Encephalomyelitis Using a Subencephalitogenic Dose of Autoimmune Effector Cells (1) Characteristics of Vaccination

Evelyne Beraud,* Ofer Lider,† Ehud Baharav,† Tamara Reshef† and Irun R. Cohen†

**Laboratoire d'Immunopathologie, Universite de Medecine, 27, Bd. Jean-Moulin, 13385-Marseille, Cedex 5, France; †Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel*

We previously reported that rats could be vaccinated against EAE by inoculation with 10^7 anti-basic protein (anti-BP)-activated T cells raised as long-term lines. The activated T lines were irradiated (1,500 rads) to prevent them from causing EAE. We now report that a single inoculation of 10^4 or fewer cells of an activated anti-BP T-cell line did not cause clinical EAE but rather induced marked resistance to EAE produced by adoptive transfer of the anti-BP T cells. Resistance was less effective against EAE induced by active immunization to BP. Vaccination was immunologically specific, long lasting, and could be effected by various routes of administration.

Introduction

Experimental autoimmune encephalomyelitis (EAE) in Lewis strain rats is an acute disease of the central nervous system which can be actively induced by the injection of myelin basic protein (BP) in adjuvant, or adoptively by the injection either of lymph node cells from immunized donors [1–3] or of activated anti-BP T lymphocyte lines and clones [4]. EAE produced in either case is characterized by hind limb paralysis and by infiltration of inflammatory cells into the white matter of the central nervous system.

Rats recovering spontaneously from acute EAE usually acquire resistance to induction of a second bout of active EAE [5, 6]. The mechanisms responsible for

Correspondence to: Professor I. R. Cohen, The Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

0896–8411/89/010075+12 $03.00/0

recovery and subsequent resistance are not entirely clear but may be related to the emergence of suppressor cells; the presence of cells able to prevent the induction of EAE has been shown in lymphoid organs just before, during and after recovery [7–9]. One of the elements responsible for acquired resistance seems to be the BP antigen itself, which has been shown to induce suppression of EAE when administered in a suitable form [10]. The studies described here were undertaken to investigate resistance to EAE induced, not by the BP antigen, but by the anti-BP effector T lymphocytes, a process we have termed T-cell vaccination [11].

Vaccination of rats against EAE was first achieved by administering about 10^7 activated effector anti-BP T-line cells treated with irradiation, with mitomycin C [11, 12] or with hydrostatic pressure [13]. The specificity of the resistance induced by T-cell vaccination was found to be determined by the epitope specificity of the T-cell vaccine, suggesting the possibility that T-cell vaccination against EAE might involve immunity to the antigen receptors of the anti-BP effector T cells [14].

According to the anti-idiotypic network theory, feed-back regulation of the immune response is thought to result from anti-idiotypic lymphocytes that are activated by the lymphocyte clones responding to the antigen [15]. While vaccination with 10^7 or more T lymphocytes has been found to prevent or even treat established experimental autoimmunity [16], a major question is whether effective anti-idiotypic immunity and resistance to EAE can follow exposure to the much smaller numbers of anti-BP effector T lymphocytes that would be expected to arise in the course of disease *in vivo*. Therefore, rather than vaccinating rats with 10^7 effector T lymphocytes treated so as to render them avirulent, we undertook to vaccinate rats with subencephalitogenic doses of virulent anti-BP T lymphocyte clones. The results described here indicate that relatively small numbers of T cells, 10^2–10^4, can induce lasting resistance to EAE. The accompanying paper shows evidence that anti-idiotypic immunity functions to mediate this resistance [17].

Materials and methods

Rats

Inbred Lewis rats were supplied by the Animal Breeding Center of the Weizmann Institute. Female rats were used at 2 to 3 months of age.

Antigens and mitogens

BP was prepared as described by Hirschfeld *et al.* [18] from spinal cords of guinea pigs without purification by column chromatography. Concanavalin A (Con-A) was purchased from Miles-Yeda (Rehovet, Israel). Heat-killed *Mycobacterium tuberculosis* H37Ra (MT) was purchased from Difco (Detroit, MI).

Immunization of animals

To induce EAE, rats were injected in each hind footpad with 0.05 ml of an emulsion of BP in complete Freund's adjuvant (CFA). Each rat received 25 μg of BP and

200 μg of MT. The rats were observed for development of EAE and scored according to the method of Coates *et al.* [19]: flaccid tail = 1, hind leg weakness = 2, hind leg paralysis = 3, and moribund state = 4.

To induce adjuvant arthritis, rats were inoculated intradermally at the base of the tail with 0.1 ml of CFA containing 1 mg of MT [20]. The system described by Trentham *et al.* [21] was used to assess severity of arthritis. Each of the four paws was graded from 0 to 4 based on erythema, swelling, and joint deformity. The highest achievable score was 16.

Selection and maintenance of lines

Line Z1a, reactive to BP, was isolated from rats immunized with BP in CFA [4]. Clone D9 was derived from Z1a by limiting dilution [22]. However, Z1a itself appears to be a clone; it has a single rearrangement of its antigen receptor genes which is identical to that of the D9 clone [A. Ben-Nun *et al.*, in preparation].

Clone A2b [23] was raised from line A2 [20], a line obtained from rats immunized to MT to induce adjuvant arthritis. Clone A2b was found to be arthritogenic in irradiated Lewis rats.

All cell cultures utilized Dulbecco's modification of Eagle's medium. The medium used for stimulation was supplemented with L-glutamine (1 mM; Bio-Lab, Jerusalem, Israel), antibiotics, sodium pyruvate (1 mM), 2 mercaptoethanol (5×10^{-5} M), non-essential amino acids (1% × 100; Bio-Lab) and 1% fresh autologous serum. The medium used for propagation was the stimulation medium described above minus autologous serum and supplemented with 15% (v/v) of supernatant of Con-A-stimulated lymphocytes as a source of T-cell growth factor prepared as described [24] and 10% horse serum (Gibco, Grand Island, NY).

For antigenic stimulation or activation, the cells (2×10^5 to 4×10^5/ml) were incubated for 3 d with BP (10 μg/ml), or Con-A (1.5 μg/ml) in the presence of syngeneic irradiated thymus cells (15×10^6/ml) as accessory cells. On the third day, the lymphoblasts were transferred into propagation medium until the next restimulation, or were used to mediate EAE or to vaccinate.

To mediate EAE, the lymphocytes were collected after antigenic stimulation, washed and, in phosphate buffered saline, were injected intravenously or intraperitoneally into Lewis rats. To vaccinate against EAE, 10^2–10^4 living, activated cells were injected intravenously or by other routes into recipients. In several experimental groups, cells were first treated by irradiation (2,500 rads) from a gamma source [11].

Histologic examination of EAE

Rats were sacrificed by ether anesthesia and brains and spinal columns were removed immediately. After fixation in formalin, the brains and spinal cords were sectioned, embedded in paraffin and processed for light microscopy by using hematoxylin and eosin staining to detect inflammatory lesions in the cerebrum, cerebellum, brain stem and spinal cord. The severity and the distribution of meningeal and perivascular infiltrations were graded from 0 to 4, as previously described by Richert *et al.* [3].

Table 1. *Recovery from adoptive EAE is associated with resistance to adoptive and active EAE*

Primary inoculation (3×10^6 cells)	Primary adoptive EAE (%)	Secondary EAE challenge Incidence (%) BP/CFA	Z1a
None	0	100 (5)	100 (10)
Z1a	100 (13)	0 (5)	0 (8)

Groups of Lewis rats (numbers of rats in parentheses) were or were not inoculated with BP-activated Z1a cells to induce EAE. Three weeks later the recovered and naive rats were challenged with BP/CFA or BP-activated Z1a cells to test their susceptibility to EAE.

Statistical analysis

Statistical analysis was carried out using Student's *t*-test. Mean differences were considered significant when $P < 0.05$.

Results

Recovery from adoptive EAE is associated with resistance to active and adoptive EAE

Experience with the Z1a anti-BP T-cell line since its isolation in 1980 [4] has taught that the degree of EAE it produces is roughly proportional to the numbers of activated Z1a cells with which recipient Lewis rats are inoculated: 10^7 cells can be lethal, 10^6 cells usually produce moderate to severe disease, 10^5 cells cause mild disease, and 10^4 or less cells usually produce no pathology, clinical or histological. Recovery from EAE produced by Z1a cells, similar to recovery from EAE induced by active immunization with BP/CFA [5, 6], was associated with acquisition of resistance to subsequent attempts to produce a second bout of EAE, as illustrated in Table 1. Lewis rats that recovered from severe EAE produced by 3×10^6 activated Z1a cells were found to be resistant to EAE induction after immunization with BP/CFA or after anti-BP cell administration. Hence, exposure to an encephalitogenic dose of Z1a cells vaccinated rats against EAE. Would exposure to a subencephalitogenic dose of Z1a also induce resistance to EAE?

A subencephalitogenic dose of activated Z1a cells induces resistance

In the experiment tabulated in Table 2, Lewis rats were inoculated intravenously with 10^4 Z1a cells, either activated or in a resting state, or activated and then irradiated. The treated rats, along with naive controls, were then challenged by immunization with BP/CFA or by intravenous inoculation with an encephalitogenic dose of activated Z1a cells. Although the primary inoculation of 10^4 activated Z1a cells caused neither clinical EAE nor lesions in the white matter of the brain or spinal cord (not shown), the recipient rats acquired a significant degree of resistance to EAE. The resistance to adoptive EAE was more striking than that of active EAE,

Table 2. *10^4 activated Z1a cells vaccinate against both active and adoptive EAE*

Vaccination 10^4 cells	Cell activation	EAE induction	EAE Incidence[1] (%)	EAE Mortality (%)	EAE Mean clinical score[2]	EAE Duration (days)
None	—	BP/CFA	100 (13)	8	3.5	7.1
Z1a	Yes	BP/CFA	64 (14)[3]	0	1.3[3]	4.8[3]
	No	BP/CFA	100 (4)	25	4	7.2
	Yes + irradiation	BP/CFA	100 (4)	0	3.5	6.8
None	—	Z1a	100 (8)	75	3.5	4.5
Z1a	Yes	Z1a	45 (11)[3]	9[3]	1[3]	3
	No	Z1a	100 (4)	75	4	4.5
	Yes + irradiation	Z1a	100 (4)	100	4	5

Z1a lymphocytes were activated by incubation with BP, and were or were not irradiated (2,500 rads). Non-activated Z1a cells were used after 10 d in culture in propagation medium. 10^4 living Z1a cells were then injected intravenously into naive Lewis rats. Nine days later, the recipients were challenged for EAE either with BP/CFA or with activated Z1a line cells (5×10^6, intravenously). Clinical and histological EAE scores were recorded.

[1]Number of rats in parentheses.

[2]EAE negative animals were not included in the calculation of the mean.

[3]Incidence, mortality, mean clinical score and duration were significantly lower in the vaccinated rat group than in the other groups ($P < 0.05$).

although the adoptive disease was more severe clinically. With regard to adoptive EAE, mortality of the Z1a treated rats was 9% compared to 75%; about half the rats had no detectable disease and most of those affected had only tail weakness. There was also a marked reduction in the histological score of the vaccinated rats (score = 0.1) compared to the unvaccinated controls with adoptive EAE (score = 2). With regard to actively induced EAE, about a third of the Z1a-treated rats escaped active EAE and the rest had only mild disease. Despite this resistance to clinical EAE produced by active immunization to BP/CFA, the vaccinated rats showed the same histological score (score = 3) as did the unvaccinated control rats (score = 3). Thus, vaccination with 10^4 Z1a cells was less effective in preventing active EAE than it was in preventing adoptive EAE.

The ability of 10^4 Z1a cells to vaccinate against EAE depended on the state of cell activation; non-activated Z1a cells were not effective in inducing resistance. Activated Z1a cells also did not vaccinate after irradiation, suggesting that the 10^4 activated cells had to be able to proliferate in the recipients.

Onset and duration of resistance

To study the time of onset and duration of resistance induced by 10^4 activated cells, treated rats and age-matched naive rats were challenged with an encephalitogenic dose of activated Z1a cells at various times after vaccination. Table 3 shows that rats

Table 3. *Onset and duration of resistance induced by vaccination with 10^4 activated Z1a cells*

Day of EAE challenge after vaccination	EAE in vaccinated rats				EAE in control rats			
	Incidence (%)	Mortality (%)	Mean clinical score	Duration (days)	Incidence (%)	Mortality (%)	Mean clinical score	Duration (days)
2	5/5	2/5	3.2±0.65	6.8±1.5	5/5	2/5	2.6±0.4	7.3±1.15
4	4/5	1/5	1.6±0.4	4.2±1.0	5/5	5/5	4.0±0	3[1]
7	1/5	0/5	1.0±0.5	1	5/5	2/5	2.8±0.6	6.2±1.8
12	0/3	0/3	—	—	3/3	2/3	3.2±0.3	7.2±0.6
31	0/5	0/5	—	—	4/5	1/3	3.4±0.6	7.0±0.5
125	0/3	0/3	—	—	3/3	1/3	1.8±0.5	5.7±1.2

Lewis rats were vaccinated with 10^4 activated Z1a cells. At various intervals, groups of vaccinated rats and unvaccinated age-matched control rats were inoculated with activated Z1a cells (5×10^6) and observed for the development of EAE. Means are shown ± standard deviations.

[1]Until death.

Table 4. *10^4 Z1a cells vaccinate via various routes of administration*

Route of vaccination	Number of rats	Adoptive EAE	
		% Incidence	% Mortality
—	15	100	40
Intravenous	10	10	0
Intraperitoneal	10	20	10
Subcutaneous (dorsal)	14	7	0
Subcutaneous (footpads)	8	0	0

Lewis rats were vaccinated with 10^4 Con-A-activated Z1a cells administered by various routes. The rats were challenged 9 d later by an intravenous inoculation of 3×10^6 activated Z1a cells to induce adoptive EAE.

Table 5. *Specificity of vaccination against active EAE*

T lymphocyte vaccination	Disease	Active EAE or adjuvant arthritis				
		Incidence (%)	Mortality (%)	Mean score	Mean day of onset	Duration (days)
None	Arthritis	100	0	12.4	13.2	over 60
Z1a	Arthritis	100	0	13.4	13.4	over 60
A2b	Arthritis	100	0	12.2	13.0	over 60
None	EAE	100	40	3.4	10.2	8.8
Z1a	EAE	100	0	2.4	12.4	4.4
A2b	EAE	100	20	3.6	10.0	8.2

Groups of 10 rats were vaccinated with Con-A-activated Z1a or A2b cells and challenged 10 d later with BP/CFA to induce EAE, or with MT to induce adjuvant arthritis. EAE developed after vaccination with Z1a T lymphocytes was significantly less severe than that which developed in the control groups ($P < 0.05$).

challenged 2 d after vaccination had no protection. Challenge at 4 d was moderately resisted, while at 7 and 12 d there was marked resistance. Resistance to an encephalitogenic challenge of Z1a was evident for at least 125 d. Thus, inoculation with a subencephalitogenic dose of activated Z1a cells provided recipient rats with protection against adoptive EAE, this resistance appeared about a week later and lasted for a considerable part of the rats' life span.

Routes of administration

The above results showed that 10^4 activated Z1a induced resistance to EAE when administered intravenously. Table 4 shows that vaccination against adoptive EAE was effective using other routes of administration of the Z1a cells: intraperitoneal, subcutaneously into the back, or hind footpads, as well as intravenously.

Table 6. *Dose response and specificity of vaccination against adoptive EAE*

T lymphocytes	Cell number	Adoptive EAE		
		Incidence (%)	Mortality (%)	Mean clinical score
None	—	100	100	4
D9	10^2	60	0	1.6
	10^3	0	0	0
	10^4	0	0	0
A2b	10^2	100	80	4
	10^3	100	100	4
	10^4	100	80	4

Groups of five rats were vaccinated intravenously with Con-A-activated D9 or A2b cells. The rats were challenged intraperitoneally 9 d later to induce adoptive EAE with 2×10^6 BP activated Z1a cells.

Vaccination is immunologically specific

Table 5 shows the results of an experiment in which rats were treated with 10^4 activated Z1a cells or A2b cells. (A2b is a clone that has been found to recognize a cartilage antigen cross-reactive with MT [25, 26] and to produce adjuvant arthritis in heavily irradiated rats [23]). The rats were then actively challenged with MT in oil to induce active adjuvant arthritis or with BP/CFA to induce EAE. No resistance to adjuvant arthritis was evident after treatment with either Z1a or A2b. However, Z1a did induce partial resistance to active EAE, caused no mortality, postponed the day of onset, and induced shorter and milder disease. A2b was not effective in vaccinating against active EAE.

Table 6 shows the results of an experiment in which the rats were challenged by adoptive EAE produced by intravenous inoculation of 2×10^6 activated Z1a cells. Treatment consisted of 10^4, 10^3, and 10^2 activated A2b or D9 cells (D9 is a clone of Z1a). It can be seen that treatment with the A2b cells had no effect on lethal adoptive EAE. In contrast, 10^4 and 10^3 D9 cells completely protected the rats against adoptive EAE, while exposure to 10^2 D9 cells prevented mortality entirely and reduced the incidence and severity of adoptive EAE.

Discussion

The experiments described here were done to learn whether untreated, autoimmune effector lymphocytes capable of causing EAE can also induce a heightened state of resistance to EAE. Recovery from actively induced EAE was observed to be associated with resistance to a second encephalitogenic immunization with BP/CFA [5, 6], and as we demonstrated here, recovery from EAE adoptively produced by the anti-BP Z1a cells was also marked by resistance to both active and adoptive EAE. To test whether small numbers of Z1a cells could vaccinate, we administered an amount of the anti-BP cells below the threshold number required to produce adoptive EAE. We

found that 10^4 or fewer Z1a cells could induce resistance in the absence of clinical or histological EAE.

Despite their ability to induce resistance to EAE, Z1a cells are clearly T-effector cells which bear the W3/25 (CD4) marker and are negative for the OX8 (CD8) marker [24]. Z1a cells injected at numbers of 10^6 or more were found to produce severe EAE [4] and delayed type hypersensitivity reactions [27]. A fraction of the injected cells was able to penetrate the blood–brain barrier and accumulate in the central nervous system shortly before the onset of EAE [28]. Z1a was also found to be pathogenic *in vitro*; it specifically inhibited the action potential in an isolated Lewis rat optic nerve preparation [29], and was shown to mediate cytotoxicity [30].

On the basis of the rearrangement of its antigen receptor, Z1a can be considered a clone [A. Ben-Nun *et al.* in preparation]. However, to ensure that its ability to induce both protection and disease were not the properties of a mixture of separate clones, we tested D9, a cloned isolate of Z1a able to produce adoptive EAE [22]. D9 was able to induce measurable resistance to EAE produced by Z1a after vaccination with as few as 10^2 cells. Thus, we may conclude that a subencephalitogenic dose of an effector T clone can vaccinate against EAE.

It is not likely that resistance was caused by BP antigen inadvertently carried along with the anti-BP cells; Z1a cells cultured with BP and then irradiated did not induce resistance, and activation of Z1a for vaccination could be done as effectively with the mitogen Con-A as with the BP antigen. How activation contributes to vaccination is as yet unknown. Activation, however, was also found to be necessary for the effector functions of Z1a; cell migration [28], delayed type hypersensitivity [27], and disease production *in vivo*, and for inhibition of nerve conduction *in vitro* [29]. Activation leads to changes in cell membrane markers and presumably certain of these changes are critical to T-cell behavior. The accompanying paper provides evidence that the signal for resistance involves the anti-BP receptor [17] and it is conceivable that activation enhances the immunogenicity of the receptor.

Vaccination with 10^4 Z1a cells induced more effective resistance to adoptive EAE than it did to active EAE produced by immunization with BP/CFA. Paradoxically, in earlier studies of vaccination using 10^7 irradiated Z1a cells, resistance to active EAE was more marked than it was to adoptive EAE produced by the Z1a cells [12]. The reasons for this difference have yet to be clarified. Nevertheless, it is evident that a relatively small number of Z1a cells administered into a variety of sites can influence the state of susceptibility of the recipient to EAE. This change in state became evident within a week of vaccination and lasted for a considerable period of time.

Suppression of EAE in guinea pigs was induced by transfer of several million lymph node cells from BP-primed animals, a number 5 to 10 times less than the dose needed to transfer lethal EAE [31]. Thus, vaccination against EAE with a subencephalitogenic dose of lymphocytes is not limited to the rat.

What maintains the state of resistance is as yet unknown. We have detected the persistence of anti-BP effector T cells in the thymuses of rats that recovered from adoptive EAE [32] and in various lymphoid organs of rats that recovered from active EAE [8]. It is possible that these persisting anti-BP T cells are important in maintaining resistance [33].

Finally, not all autoimmune T cells induce resistance to disease when administered at a dose of 10^4 cells. As shown here, 10^4 cells of clone A2b did not vaccinate against

adjuvant arthritis. Elsewhere we have reported that activated A2b did not vaccinate at doses of 10^7 cells unless the cells were treated with hydrostatic pressure or with a chemical cross linker before administration [16]. Administration to mice of 10^4 anti-thyroglobulin T cells was observed to induce partial resistance to experimental autoimmune thyroiditis but the resistance to thyroiditis induced by 10^6 irradiated cells was stronger [in preparation]. Some newly isolated anti-BP T cells, like Z1a, induced resistance, while others at doses of 10^4 cells did not [in preparation]. The accompanying paper demonstrates that successful vaccination with Z1a was associated with the development of anti-idiotypic T-cell immunity [17]. It is conceivable therefore that differences in the capacity of T cells to vaccinate might be related to the inherent immunogenicity of their antigen receptors. Be that as it may, the fact that some clones of autoimmune effector T lymphocytes can vaccinate at doses of 10^2–10^4 cells highlights the exquisite sensitivity of the immune system to its own components.

Acknowledgements

We thank Ms Pamela Rubinstein and Ms Lisa Hoze for preparing this manuscript. I. R. Cohen is the incumbent of the Mauerberger Chair in Immunology. This work was supported by INSERM grant No. 846001 (E.B.), by the National Institutes of Health Grant NS 23372, and by a grant from the National Multiple Sclerosis Society (I.R.C.).

References

1. Paterson, P. Y. 1960. Transfer of allergic encephalomyelitis in rats by means of lymph node cells. *J. Exp. Med.* **111:** 119–136
2. Panitch, H. S. and D. E. McFarlin. 1977. Experimental allergic encephalomyelitis: Enhancement of cell-mediated transfer by Concanavalin A. *J. Immunol.* **119:** 1134–1137
3. Richert, J. R., B. F. Driscoll, M. W. Kies, and E. C. Alvord, Jr. 1979. Adoptive transfer of experimental allergic encephalomyelitis: Incubation of rat spleen cells with specific antigen. *J. Immunol.* **122:** 494–496
4. 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:** 195–199
5. Willenborg, D. O. 1979. Experimental allergic encephalomyelitis in the Lewis rat: Studies on the mechanism of recovery from disease and acquired resistance to reinduction. *J. Immunol.* **123:** 1145–1150
6. Welch, A. M., J. H. Holda, and R. H. Swanborg. 1980. Regulation of experimental allergic encephalomyelitis. II. Appearance of suppressor cells during the remission phase of the disease. *J. Immunol.* **125:** 186–189
7. Beraud, E., S. Varriale, C. Farnarier, and D. Bernard. 1982. Suppressor cells in Lewis rats with Experimental Allergic Encephalomyelitis: Prevention of the disease and inhibition of lymphocyte proliferation by the suppressor cells or their products. *Eur. J. Immunol.* **12:** 926–930
8. 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 T cells recovered as T-cell lines. *J. Immunol.* **128:** 1450–1457
9. Ellerman, K. E., J. M. Powers, and S. W. Brostoff. 1988. A Suppressor T lymphocyte cell line for autoimmune encephalomyelitis. *Nature* **331:** 265–267
10. Swierkosz, J. E. and R. H. Swanborg. 1975. Suppressor cell control of unresponsiveness to experimental allergic encephalomyelitis. *J. Immunol.* **115:** 631–633

11. Ben-Nun, A., H. Wekerle, and I. R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis with T lymphocyte line cells reactive against myelin basic protein. *Nature* **292:** 60–61
12. Ben-Nun, A. and I. R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis: Attenuated autoimmune T lymphocytes confer resistance to induction of active EAE but not to EAE mediated by the intact T lymphocyte line. *Eur. J. Immunol.* **11:** 949–952
13. Lider, O., M. Shinitzky, and I. R. Cohen. 1986. Vaccination against experimental autoimmune diseases using T lymphocytes treated with hydrostatic pressure. *Ann. N.Y. Acad. Sci.* **475:** 267–273
14. Holoshitz, J., A. Frenkel, A. Ben-Nun, and I. R. Cohen. 1983. Autoimmune encephalomyelitis mediated or prevented by T. lymphocyte lines directed against diverse antigenic determinants of Myelin Basic Protein. Vaccination is determinant specific. *J. Immunol.* **131:** 2810–2813
15. Jerne, N. Y. 1974. Towards a network theory of the immune system. *Ann. Immunol.* (Paris) **124C:** 373–389
16. Lider, O., N. Karin, M. Shinitzky, and I. R. Cohen. 1987. Therapeutic vaccination against adjuvant arthritis using autoimmune T cells treated with hydrostatic pressure. *Proc. Natl. Acad. Sci. USA* **84:** 4577–4580
17. Lider, O., E. Beraud, T. Reshef, A. Friedman, and I. R. Cohen. Vaccination against experimental autoimmune encephalomyelitis using a subencephalitogenic dose of autoimmune effector T cells: 2. Induction of a protective anti-idiotypic response. *J. autoimmunity* **2:** 87–99
18. Hirschfeld, D., D. Teitelbaum, R. Arnon, and M. Sela. 1970. Basic encephalitogenic protein: A simplified purification on sulfoethyl sephadex. *FEBS Lett.* **7:** 317–320
19. Coates, A., I. R. MacKay, and M. Crawford. 1974. Immune protection against EAE: Optimal conditions and analysis of mechanism. *Cell. Immunol.* **12:** 370–380
20. Holoshitz, J., Y. Naparstek, A. Ben-Nun, and I. R. Cohen. 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science* **219:** 56–58
21. Trentham, D. E., A. S. Towens, and A. H. Kang. 1977. Autoimmunity to type II collagen: An experimental model of arthritis. *J. Exp. Med.* **146:** 857–868
22. Vandenbark, A. A., H. Offner, T. Reshef, R. Fritz, C. H. J. Chou, and I. R. Cohen. 1985. Specificity of T lymphocyte lines for peptides of myelin basic protein. *J. Immunol.* **135:** 229–233
23. Holoshitz, J., A. Matitiau, and I. R. Cohen. 1984. Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. *J. Clin. Invest.* **73:** 211–215
24. 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:** 303–308
25. van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman, and I. R. Cohen. 1985. Arthritis induced by a T lymphocyte clone that responds to mycobacterium tuberculosis and to cartilage proteoglycans. *Proc. Natl. Acad. Sci. USA* **82:** 5117–5120
26. van Eden, W., J. E. R. Thole, R. Van der Zee, A. Noordzij, J. D. A. van Embden, E. J. Jensen, and I. R. Cohen. 1988. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* **331:** 171–173
27. Holoshitz, J., A. Naparstek, A. Ben-Nun, P. Marquardt, and I. R. Cohen. 1984. T lymphocyte lines induce autoimmune encephalomyelitis, delayed hypersensitivity and bystander encephalomyelitis or arthritis. *Eur. J. Immunol.* **14:** 729–732
28. Naparstek, Y., A. Ben-Nun, J. Holoshitz, T. Reshef, A. Frenkel, M. Rosenberg, and I. R. Cohen. 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:** 418–423
29. Yarom, Y., Y. Naparstek, J. Holoshitz, A. Ben-Nun, and I. R. Cohen. 1983. Immunospecific inhibition of nerve conduction in isolated rat optic nerve exerted by a line of T lymphocytes reactive to basic protein of myelin. *Nature* **303:** 246–247

30. Sun, D. and H. Wekerle. 1986. Ia-restricted encephalitogenic T lymphocytes mediating EAE lyse auto-antigen-presenting astrocytes. *Nature* **320:** 70–72
31. Driscoll, B., M. Kies, and E. C. Alvord. 1982. Suppression of acute Experimental Allergic Encephalomyelitis in guinea pigs by prior transfer of suboptimal numbers of EAE-effector cells. Induction of chronic EAE in whole tissue-sensitized guinea pigs. *J. Immunol.* **128:** 635–638
32. Naparstek, Y., J. Holoshitz, S. Eisenstein, T. Reshef, S. Rappaport, J. Chemke, A. Ben-Nun, and I. R. Cohen. 1982. Effector T lymphocyte line cells migrate to the thymus and persist there. *Nature* **300:** 262–264
33. Cohen, I. R. 1986. Regulation of autoimmune disease: Physiological and therapeutic. *Immunol. Rev.* **94:** 5–21