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AUTOIMMUNE ENCEPHALOMYELITIS (EAE) MEDIATED OR PREVENTED BY T LYMPHOCYTE LINES DIRECTED AGAINST DIVERSE ANTIGENIC DETERMINANTS OF MYELIN BASIC PROTEIN. VACCINATION IS DETERMINANT SPECIFIC[1]

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Lines of T lymphocytes reactive against the basic protein of myelin (BP) were found in previous studies to mediate experimental autoimmune encephalomyelitis (EAE) in rats. Moreover, inoculation of rats with attenuated anti-BP line cells vaccinated them against subsequent attempts to induce active EAE by injection of BP in adjuvant. In the present study, we investigated the effects of T lymphocyte lines reactive to different antigenic determinants on the BP molecule, they are the major encephalitogenic peptide (EP) determinant present on guinea pig BP (G-BP), and minor, non-EP determinants present on bovine BP (B-BP). We found that both lines of T lymphocytes could mediate EAE. Resistance to active EAE acquired by spontaneous recovery from line mediated EAE or by vaccination with attenuated cells, however, was found to be specific for the particular BP determinant. Thus, EAE may be mediated by lines of T lymphocytes reactive to different determinants on the BP molecule, but the resistance to EAE acquired by exposure to line cells is determinant specific. This suggests that acquired resistance to EAE is directed by the receptor specificity of the autoimmune anti-BP T cells.

Experimental autoimmune encephalomyelitis (EAE)[3] is a disease that can be induced actively in Lewis rats (1) by a single injection of myelin basic protein (BP) emulsified in complete Freund's adjuvant (CFA), or produced passively by i.v. inoculation of activated T lymphocyte line cells (2, 3) or lymphocytes from primed rats (4). EAE produced in either manner is characterized by acute onset of paralysis chiefly of the hind limbs. Spontaneous recovery from clinical paralysis usually occurs after 4 to 6 days. Rats that have recovered from an episode of active EAE or of line-mediated EAE (5) acquire resistance to additional attempts to induce EAE by inoculation with BP/CFA. A similar degree of resistance to active EAE can be induced by inoculating Lewis rats with anti-BP T lymphocyte line cells that have been attenuated by treatment with irradiation or mitomycin C (5, 6).

Studies (7–9) have shown that the severity of active EAE depends upon the species origin of the BP used. Although BP antigens derived from rats (R-BP) or guinea pigs (G-BP) are strong encephalitogens, bovine BP (B-BP) is a relatively weak encephalitogen in Lewis rats.

Attempts have been made to define specific encephalitogenic determinants in BP molecules (reviewed in Reference 10). It was found that G-BP has a major encephalitogenic peptide (EP) determinant formed by residues 68 to 88 (11). Location of the B-BP encephalitogenic determinant(s) is less clear, but some evidence suggests the presence of two distinct determinants located at residues 37 to 42 and 108 to 113 (12). The sequence 68 to 88 of B-BP differs from that of G-BP and R-BP and is not encephalitogenic (13).

The anti-BP T lymphocyte lines that we have selected for their responsiveness to R-BP, G-BP, or EP have shown reactivity to the major EP determinant (14). The goal of the experiments reported here was to develop and study the properties of a T lymphocyte line raised against non-EP determinants present on B-BP. We wished to learn whether such T lymphocytes would be functionally active in producing or preventing EAE and whether line-mediated protection was immunologically restricted by the receptor specifity of the T lymphocyte line.

MATERIALS AND METHODS

Rats. Inbred Lewis rats were obtained from the Animal Breeding Center of this Institute. Rats were used at 2 to 3 mo of age and were matched for age and sex in each experiment.

Antigens. BP was prepared as described (15) from the spinal cords of guinea pigs (G-BP), rats (R-BP), or cattle (B-BP), with (purified) or without (crude) the step of purification by column chromatography. The experiments described were done with crude BP unless indicated as being done with purified BP. The purification of BP was monitored by gel electrophoresis. EP composed of residues 68 to 88 of G-BP was prepared, purified, and kindly donated by Dr. F. C.-H. Chou of Emory University, Atlanta, GA. (11). Concanavalin A (Con A) was purchased from Miles-Yeda (Rehovot, Israel).

Immunization of animals. To induce EAE, rats were inoculated in each rear footpad with 0.05 ml containing 12.5 μg G-BP or 12.5 to 100 μg B-BP in phosphate-buffered saline (PBS) emulsified in equal volumes of CFA containing 200 μg/ml of *Mycobacterium tuberculosis* H_{37} Ra (Difco, Detroit, MI). Inoculated rats were observed for development of EAE or were used on day 9 after immunization as donors of lymph nodes from which cells were grown as lines. Rats were observed daily for clinical signs of EAE.

Culture medium. All cell cultures utilized Dulbecco's modification of Eagle's medium (Grand Island Biological Co., Denver, CO). The medium used for proliferation assays and for selective cultures (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 and propagate antigen-specific cell lines in long-term culture (propagation medium) was the proliferation medium supplemented with 15% (v/v) of supernatants of Con A-stimulated lymphocytes as a source of T cell growth factor (3), 10% horse serum (GIBCO), 1 mM sodium pyruvate, and nonessential amino acids (Bio-Lab).

Lines of T lymphocytes. The Z1a anti-G-BP line was developed and maintained as described (2). The B1 anti-B-BP line was developed without the step of gradient density separation (3) from a cell suspension of popliteal lymph nodes removed from rats inoculated with 25 μg of B-BP in CFA. Lymph node cells (5.10^6/ml) were incubated in the presence of 60 μg/ml of B-BP in

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[2] *Correspondence to be addressed to*: Irun R. Cohen, M. D., Department of Cell Biology, The Weizmann Institute of Science, P.O. Box 26, Rehovot, 76100, ISRAEL.
[3] Abbreviations used in this paper: BP, basic protein of myelin; B-BP, bovine BP; CFA, complete Freund's adjuvant; EAE, experimental autoimmune encephalomyelitis; EP, encephalitogenic peptide; G-BP, guinea pig BP; R-BP, rat BP.

proliferation medium for 72 hr at 37°C in humidified air plus 7.5% CO_2. After 72 hr of incubation the cells were collected, washed twice with PBS, and transferred at a concentration of 2 to 4 × 10^5/ml into propagation medium. The cultures were transferred every 3 to 4 days for 2 mo before the study of their proliferative response to antigens or ability to mediate EAE. Both lines bear the markers of helper/delayed hypersensitivity T lymphocytes (3).

Proliferative responses of T cell lines. Line cells were incubated in flat-bottomed microtiter plates (Costar, Cambridge, MA) in quadriplicate wells. Each well contained 2.5 × 10^4 line cells plus 2 × 10^6 accessory cells in the form of irradiated (1500 R) syngeneic thymus cells in 0.2 ml of proliferation medium, with antigens in the optimal concentrations determined by dose response experiments: G-BP, 50 μg/ml; EP, 5 μg/ml; B-BP, 50 μg/ml; R-BP, 100 μg/ml; and Con A, 2.5 μg/ml. After 24 hr of incubation, each well was pulsed with 1 μCi of [^{3}H]thymidine (specific activity 10 Ci/mmol; Nuclear Research, Negev, Israel) for the last 18 hr. The cultures were then harvested on fiberglass filters and the incorporation of thymidine was measured in a liquid scintillation counter. The proliferative response was expressed as the mean cpm.

EAE mediated by cell lines. Cells (2 × 10^5/ml) of lines were restimulated by incubation for 3 days with 10 μg/ml of either G-BP or B-BP in the presence of syngeneic, irradiated (1500 R) thymus cells (15 × 10^6/ml) as accessory cells. The lymphoblasts were collected, washed twice in PBS, and 5 × 10^6 or 2 × 10^7 cells were injected either untreated or irradiated with 1500 R into the veins of the tails of Lewis rats, in 1 ml of PBS. The recipient rats were observed daily for the development of EAE, as judged by paralysis, at least of the hind limbs. At the termination of each experiment, the rats were studied histologically and paralyzed rats showed perivascular infiltration in the white matter characteristic of EAE (1).

RESULTS

Active EAE induced by B-BP/CFA. We compared the incidence and severity of EAE actively induced by 25, 100, or 200 μg B-BP/CFA with that induced by G-BP/CFA at an optimal dose of 25 μg per rat. Table I shows that B-BP/CFA was not encephalitogenic at a dose of 25 μg and that encephalitogenicity comparable to that of 25 μg G-BP/CFA required immunization with 200 μg B-BP/CFA.

Specificity of an anti-B-BP T lymphocyte line. An anti-B-BP T lymphocyte line, designated B1 was raised from the draining lymph node cells of Lewis rats that had been primed with 25 μg B-BP/CFA. Table II shows the proliferative responses of the B1 line, compared with those of the Z1a anti-G-BP line. As can be seen, line Z1a responded well to G-BP, to R-BP, and to EP, but only very weakly to B-BP. In contrast, line B1 responded well to B-BP, weakly to G-BP, and not at all to R-BP or EP. The B1 and Z1a lines responded to the same extent to the T cell mitogen Con A. Although there was very little if any cross-reactivity between the Z1a and B1 lines, to rule out the possibility that contaminants in the preparation influenced the results, we investigated the proliferative responses with the use of purified BP.

TABLE I
Encephalitogenicity of B-BP and G-BP[a]

Immunization	BP (μg)	Incidence	Mean Day of Onset	Clinical Score[b]
G-BP/CFA	25	10/10	12	2.8
B-BP/CFA	25	0/5		
	100	4/5	15	1.5
	200	5/5	14	2.2

[a] Lewis rats were immunized with G-BP/CFA or B-BP/CFA and studied for EAE.
[b] Mean clinical score was determined based on paralysis observed in individual rats, as follows. No paralysis, 0; paralysis of tail, 1; paralysis of tail and rear limbs, 2; and paralysis of four limbs; 3.

TABLE II
Proliferative responses of Z1a anti-G-BP and B1 anti-B-BP T cell lines to various antigenic determinants

Cell Line	Proliferative Response (cpm × 10^{-3})					
	No antigen	B-BP	R-BP	G-BP	EP	Con A
Z1a	0.3	2.9	20.0	21.7	22.3	50.0
B1	1.9	24.4	2.2	5.7	2.0	48.3

Table III shows that essentially the same results were obtained; line Z1a responded strongly to purified G-BP but hardly at all to purified B-BP, whereas the opposite result was seen with line B1. Thus, line B1 was directed largely against determinants specific for B-BP and very little against the major EP determinant. In contrast, line Z1a recognized the EP determinant present on G-BP.

Mediation of EAE by line cells. Table IV shows that i.v. injection of 5 × 10^6 cells of line B1 or line Z1a resulted in EAE about 4 to 5 days after injection. Thus, line B1 was functional in producing EAE despite the fact that it was derived from rats primed with a weakly encephalitogenic dose of B-BP and did not respond to the major EP determinant.

Line B1 induces determinant-specific resistance to EAE. It was found that rats that recovered from EAE induced by line Z1a acquired resistance to subsequent attempts to induce EAE by active immunization with G-BP/CFA (5). We therefore tested whether line B1 could also protect rats against active EAE. Rats that had recovered from EAE mediated by the B1 line were challenged 30 days later with either G-BP/CFA (25 μg per rat) or B-BP/CFA (200 μg per rat). The results are shown in Table V.

TABLE III
Proliferative responses of Z1a and B1 T cell lines to purified BP preparations

Cell Line	Proliferative Response (cpm × 10^{-3})		
	No antigen	Purified B-BP	Purified G-BP
Z1a	0.6	1.8	72.7
B1	10.1	140.7	17.8

TABLE IV
EAE mediated by T lymphocyte lines B1 and Z1a[a]

Line	Antigen Specificity	Line-Mediated EAE			
		Incidence	Mean day of onset	Clinical score[b]	Mean duration (days)
B1	B-BP	18/18	4.7	2.3	3.6
Z1a	G-BP	10/10	4.2	3.0	4.1

[a] Lewis rats were inoculated i.v. with 5 × 10^6 B1 or Z1a line cells and were observed for development of EAE.
[b] See legend to Table I.

TABLE V
Recovery from EAE mediated by anti-B-BP line induces resistance to B-BP/CFA but not to G-BP/CFA[a]

Cell Line	Incidence of Line-Mediated EAE	Percent Inhibition (no. of rats) of active EAE[b] Subsequently Induced by:	
		G-BP/CFA (25 μg)	B-BP/CFA (200 μg)
None	—	0 (20)	0 (18)
B1	18/18	0 (8)	90[c] (10)

[a] Lewis rats were inoculated i.v. with 5 × 10^6 B1 line cells and 3 wk after spontaneous recovery from EAE they were challenged by immunization with G-BP/CFA or B-BP/CFA. Control rats did not receive line cells.
[b] Determined by the incidence of active EAE developing in each group of rats.
[c] $p < 0.05$.

TABLE VI
Immunospecificity of vaccination against EAE[a]

Irradiated Line Cells	Incidence of Line-Mediated EAE	Percent Inhibition (no. of rats) of Active EAE Subsequently Induced by:	
		G-BP/CFA (25 μg)	B-BP/CFA (200 μg)
None	—	0 (20)	0 (18)
B1	0/20	0 (10)	80[b] (10)
Z1a	0/24	78[b] (14)	20 (10)

[a] Lewis rats were inoculated i.v. with 2 × 10^7 line cells that had been irradiated (1500 R), and the rats were observed for the appearance of line-mediated EAE. Three weeks later, the rats were challenged with G-BP/CFA or B-BP/CFA and the percent of inhibition of active EAE was determined by comparing the incidence of EAE with the 100% incidence observed in control rats that had not been vaccinated with irradiated line cells.
[b] $p < 0.05$.

As can be seen, recipients of line B1 were protected against EAE induced by B-BP/CFA but were susceptible to EAE induced by G-BP/CFA. Thus, recovery from EAE mediated by the B1 anti-B-BP line endowed rats with resistance that was specific for the B-BP determinants.

To learn if attenuated B1 line cells could serve as agents of immunospecific vaccination, we carried out experiments such as the one illustrated in Table VI. Lewis rats were injected with 2×10^7 cells of lines B1 or Z1a that had been attenuated by irradiation with 1500 R. Thirty days later, we challenged the recipients with either B-BP/CFA or G-BP/CFA. As can be seen, recipients of the irradiated B1 line were significantly protected against EAE induced by B-BP/CFA but not by G-BP/CFA. In contrast, recipients of the irradiated Z1a line were significantly protected against EAE induced by G-BP/CFA but not by B-BP/CFA. Thus, vaccination against active EAE induced by attenuated anti-BP lines was directed against the specific BP to which the T lymphocytes were reactive.

DISCUSSION

The results of the experiments described here illustrate two points. The first point is that two lines of anti-BP T lymphocytes that were not cross-reactive in proliferative assays could each induce EAE. The second point is that vaccination with these noncross-reactive line cells was immunospecific; that is, the specificity of vaccination was related to the specificity of the lines *in vitro*. The validity of these points does not rest on the molecular identity of the antigenic determinants involved, for which the evidence is not complete. We observed that one line, Z1a, responded to EP whereas the other line, B1, did not. We do not know to which additional antigenic fractions of BP they could have responded, but this cannot change the empirical observations that the lines were not cross-reactive and that B1 showed no reactivity to the major EP fraction of BP. Moreover, the specificity of the responses of each line was directed by the origin of the BP used for selection of the lines, B-BP, R-BP, or G-BP, and did not appear to be affected by whether the particular BP was crude or purified (Tables II and III). In any case, the results demonstrated that EAE may be mediated by T lymphocytes responsive *in vitro* to diverse determinants on the BP molecule, including determinants not present on the major EP portion.

It is noteworthy that line B1 was isolated from rats that had been primed with a subencephalitogenic dose (25 μg) of B-BP. Thus, potential EAE effector T lymphocytes developed in rats that showed no clinical disease. We have also succeeded in rescuing EAE effector T lymphocytes as lines from rats that were resistant to the induction of EAE for a variety of reasons. EAE effector T lymphocytes were obtained from PVG rats that were genetically resistant to EAE (16) or from Lewis rats that had been inoculated with suppressogenic G-BP emulsified in incomplete Freunds adjuvant (14), or that had acquired resistance to EAE after spontaneous recovery from active (14) or line-mediated EAE (17). The presence of potential EAE effector T lymphocytes in rats free of EAE or resistant to its induction indicates that clinical autoimmunity is not an inevitable consequence of the appearance of autoreactive T lymphocytes. The clinical expression of such cells would seem to be subordinate to physiologic regulatory mechanisms (18).

Immunologically specific suppressors of at least two types could be imagined to regulate the expression of EAE effector T lymphocytes: suppressor T lymphocytes that recognize suppressor determinants (19) on BP (20) and anti-idiotypic lymphocytes or antibodies that recognize the BP receptors of the EAE effector T lymphocytes (14).

The immunospecificity of the resistance to EAE induced by line B1 seems to argue for the existence of a regulatory mechanism directed against the T lymphocyte receptor. After recovery from EAE mediated by line B1, the recipient rats acquired resistance to the induction of EAE by active immunization to B-BP/CFA, but not to EAE induced by G-BP/CFA (Table V). Vaccination with attenuated line cells showed similar immunospecificity for the determinants on BP recognized by the particular B1 or Z1a line cells (Table VI). Resistance to EAE that rests on a particular suppressor determinant on BP (20) could not easily explain the specificity apparently dictated by the EAE effector T cells themselves. It is likely that potentially autoreactive lymphocytes, however, are controlled by more than one process and that different experimental approaches can be expected to highlight various agents of self-tolerance. In any case, the resistance induced by vaccination with anti-BP T lymphocytes would appear to be determinant specific.

In addition to providing a tool for the study and manipulation of EAE, T lymphocyte lines can be used to advantage in other autoimmune states. We have recently isolated T lymphocyte lines and clones that can mediate and/or protect against autoimmune arthritis in rats (21) and thyroiditis in mice (22).

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