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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[1]

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Experimental autoimmune encephalomyelitis (EAE)[2] can be induced in some types of animals by immunizing them against the basic protein (BP) of myelin in a suitable adjuvant such as complete Freund's adjuvant (CFA) (1). Susceptibility to induction of EAE in rats appears to be influenced by immune response (*Ir*) genes located both within and without the major histocompatibility complex (MHC) (2, 3). We have recently succeeded in developing lines of T lymphocytes from EAE-susceptible Lewis rats that recognize BP specifically and that are functional in mediating EAE (4), or in vaccinating rats against active disease induced by immunization with BP/CFA (5). To analyze the cellular basis of the EAE phenotype we have studied the generation and behavior of anti-BP T cell lines in susceptible Lewis rats and in PVG and BN rats that are genetically resistant to active induction of EAE by immunization with BP/CFA (3, 6). We now report that an anti-BP line could be derived from BN rats, but that this line failed to recognize the encephalitogenic peptide (EP) determinant of BP (7) and did not mediate EAE. In contrast to BN rats, PVG strain rats developed EAE upon i.v. inoculation of allogeneic Lewis anti-BP line cells. Moreover, after injection with BP/CFA, an anti-EP effector cell line could be derived from clinically well PVG rats. This line mediated EAE in both PVG and Lewis rats and could also endow Lewis rats with resistance to subsequent active induction of EAE. Thus, allogeneic T cells serve both to mediate EAE and to protect against active EAE. Furthermore, the genetic resistance of PVG rats to EAE appears to be based on regulation of the functional behavior of existent anti-self effector T lymphocytes.

MATERIALS AND METHODS

Rats. Inbred strains of Lewis (RT1-l), PVG (RT1-c), and BN (Rt1-n) rats were supplied by the Animal Breeding Center of this Institute. Rats were used at 2 to 3 mo of age and matched for sex in each experiment.

Immunizations and lines. BP was prepared from the spinal cords of guinea pigs as described (8), except that the step of column chromatography was not done. Rats were immunized in both hind footpads with BP (25 μg/footpad) emulsified in CFA containing 200 μg/ml *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI), as described (4, 9). In some rats, lymph node cells from the draining popliteal nodes were removed 9 days later, and the cells were tested for their reactivity in proliferative responses or were used to generate T cell lines (4, 9). To mediate EAE, 10^7 line cells were passively transferred by i.v. inoculation into naive recipient rats, and the rats were observed for development of EAE diagnosed by overt paralysis of the tail and hind limbs and evidence of perivascular infiltration of the central nervous system (1). Before inoculation, the line cells were activated by incubation with BP (10 μg/ml) for 72 hr in the presence of irradiated (1500 R) syngeneic spleen or thymus cells as accessory cells, as described (4, 9).

RESULTS AND DISCUSSION

Active induction of EAE. Table I shows the results of experiments in which Lewis, PVG, or BN rats were immunized with BP/CFA and assayed for the appearance in the draining lymph nodes of lymphocytes that could respond in an *in vitro* proliferative assay, and for the development of EAE. Immunized lymph node cells of each of the three strains of rats responded to BP, to PPD, and to the T cell mitogen Con A *in vitro*. However, only the Lewis rats developed EAE and demonstrated a relatively high degree of reactivity to EP.

EAE mediated by T cell lines. From populations of BP/CFA-primed lymph node cells of Lewis, PVG, or BN rats, we developed lines of T lymphocytes reactive specifically against BP (4, 9). We found that the Z1a line (4) of Lewis origin responded to BP, to EP, or to Con A in the presence of Lewis or PVG accessory cells, but not in the presence of BN accessory cells (Table II-A). The response to PPD detected in the primed lymph node cells (Table I) disappeared in the line. Furthermore, the Lewis line cells mediated EAE in syngeneic Lewis and allogeneic PVG recipients.

Table II-B shows that the H2a PVG line included cells that responded to BP and to EP *in vitro*, and that this line could mediate EAE *in vivo* in syngeneic PVG or allogeneic Lewis rats. Table II-C demonstrates that a specific anti-BP line could be developed from primed BN rats, but in contrast to the Lewis and PVG lines, this line did not respond to EP and was ineffective in mediating EAE. The Z1c Lewis line selected for reactivity against PPD (4) failed to mediate EAE (Table II-D).

PVG anti-BP line endows Lewis rats with resistance to active EAE. We have observed that inoculation of Lewis rats with some anti-BP lines can render the rats resistant to subsequent induction of EAE by active immunization with BP/CFA (5). This acquired resistance can be conferred by intact line cells that mediate EAE as well as by attenuated line cells that vaccinate against active EAE without mediating any disease (5, 10).

TABLE I

Active induction of EAE: Lewis rats are susceptible; PVG and BN rats are resistant and do not respond to EP[a]

Strain	Proliferative Responses of Lymph Node Cells Primed by BP/CFA (Δ cpm × 10^{-3} ± SE and (SI))				% Incidence of EAE (No. of rats)
	BP	EP	PPD	Con A	
Lewis	96 ± 9 (6)	54 ± 4 (15)	110 ± 11 (7)	192 ± 31 (11)	100 (50)
PVG	30 ± 6 (2)	17 ± 2 (1.5)	110 ± 11 (4)	226 ± 26 (7)	0 (40)
BN	21 ± 1 (6)	0 (1)	110 ± 5 (27)	N.D.[b]	0 (10)

[a] Female rats 2 to 3 mo old were injected in the hind foot pads with BP/CFA and observed for development of clinical EAE, or were sacrificed on day 9 after immunization and their draining lymph node cells were assayed for proliferative responses to antigens.

[b] N.D., not done.

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[1] This work was supported by the National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda, MD, Grant No. NS 18168.
[2] Abbreviations used in this paper: BP, basic protein of myelin; EAE, experimental autoimmune encephalomyelitis; EP, encephalitogenic peptide; *Ir*, immune response; MHC, major histocompatibility gene complex; RTI, rat MHC.

TABLE II

Immunospecificity of T cell lines and their ability to mediate EAE and to confer protection against induction of active EAE[a]

Origin of Anti-BP Cell Lines	Proliferative Response to Antigens					Mediation of EAE by Cell Lines		Subsequent Induction of Active EAE by BP/CFA[c] (% Incidence)
	Accessory cells	cpm × 10^{-3} ± SD and (SI)				Recipients	% Incidence of EAE[b]	
		BP	EP	PPD	Con A			
A. Lewis (Z1a)	Lewis	58 ± 4 (73)	59 ± 3 (67	0 (1)	72 ± 8 (90)	Lewis	92 (40)	36[d]
	PVG	51 ± 7 (65)	34 ± 5 (44)	0 (1)	60 ± 8 (75)	PVG	80 (15)	
	BN	0 (1)	0 (1)	0 (1)	9 ± 2 (11)	BN	0 (10)	
B. PVG (H2a)	PVG	118 ± 10 (91)	37 ± 6 (26)	0 (1)	107 ± 6 (82)	PVG	62 (24)	
	Lewis	112 ± 8 (74)	25 ± 3 (16)	0 (1)	93 ± 11 (52)	Lewis	83 (12)	0[d]
						BN	0 (10)	
C. BN (B1a)	BN	63 ± 4 (80)	0 (1)	0 (1)	69 ± 7 (85)	BN	0 (10)	
D. Lewis (Z1b, anti-PPD)	Lewis	0 (1)	0 (1)	85 ±6 (78)	90 ± 7 (82)	Lewis	0 (20)	100
E. None	—	—	—	—	—	—	— (50)	100

[a] Anti-Bp cell lines selected from susceptible Lewis (Z1a) or from resistant PVG (H2a) or BN (B1a) rats were assayed (2.5 × 10^4/well) for their proliferative response *in vitro* to antigens in the presence of various genotypes of irradiated accessory thymus cells (2 × 10^6/well) or were restimulated *in vitro* with BP in the presence of irradiated syngeneic accessory thymus cells, and 10^7 activated lymphoblasts were injected i.v. into syngeneic or allogeneic rats. The recipient rats were observed for development of EAE.

[b] Number of rats.

[c] Twenty-five days after recovery from line-mediated EAE, the Lewis rats were challenged with BP/CFA to induce active EAE.

[d] $P < 0.01$ compared with control groups D and E.

Table II shows that Lewis rats that had been inoculated with either the Lewis Z1a or the PVG H2a anti-BP lines and had developed EAE, acquired resistance to active EAE induced by injection of BP/CFA. The Lewis Z1c line reactive to PPD but not to BP (4, 5, 10) failed to produce resistance.

Although the Lewis, PVG, and BN rats used in these studies were derived from separate breeding stocks and are considered to carry independent MHC haplotypes (11), we found that cooperative interactions between allogeneic Lewis and PVG cells were possible both *in vivo* and *in vitro*. The failure of BN rats or their cells to participate in these cooperative interactions suggests that the successful cooperation between Lewis and PVG was not due to some nonspecific allogeneic effect (12). Moreover, the difference between the generation and behavior of anti-BP line cells in PVG and BN rats suggests the possibility that these rat strains might enjoy an EAE-resistant phenotype based on different genetically controlled regulatory mechanisms.

Mutual presentation of BP and EP by Lewis and PVG cells might be related to some homology in the MHC I region between these strains (13). Whether or not this turns out to be true, it is evident that PVG antigen-presenting cells can process and present BP and EP to T lymphocytes in an immunogenic form *in vitro*, resulting in the generation and proliferation of lines of EAE T effector cells. Moreover, BP in an encephalitogenic form must also be available in PVG rats, because these rats could be primed to produce anti-BP and anti-EP effector cells and could suffer EAE mediated by such cells grown as lines. Hence, the EAE-resistant *Ir* phenotype of PVG rats cannot be attributed to a failure of their antigen-presenting cells to present the critical EP determinant of BP (13, 14) or to a failure of their T cells to recognize EP (15), but may be explained by reversible inhibitory or suppressive mechanisms (16, 17). In support of this idea, we have recently found that suppressed EAE effector T lymphocytes can be rescued as cell lines from Lewis rats that have recovered from active EAE and have acquired resistance to reinduction of active disease (9).

It was interesting that injection of Lewis rats with PVG anti-BP line cells led to acquired resistance to induction of active EAE (Table II). The results of preliminary experiments indicate that this form of acquired resistance involves immunity to anti-BP receptors (manuscript in preparation). This suggests that allogeneic PVG and Lewis rats might share V region genes that encode anti-BP receptors on T cells.

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