GENETIC RESISTANCE TO THE INDUCTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN LEWIS RATS

I. Genetic Analysis of an Apparent Mutant Strain with Phenotypic Resistance to Experimental Allergic Encephalomyelitis*

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Experimental allergic encephalomyelitis (EAE)[1] is an autoimmune syndrome initiated by the injection of myelin basic protein (BP) in an appropriate adjuvant. Inbred Lewis (Le) rats develop acute hindquarter paralysis 10–14 d subsequent to challenge with guinea pig-derived BP in complete Freund's adjuvant (CFA). Spontaneous recovery from paralysis occurs within a few days. Le rats that have recovered from paralysis are resistant to a secondary challenge with BP-CFA. The manner in which this acquired resistance mechanism operates is unknown. In addition to acquired resistance manifested by EAE-recovered Le rats, other inbred strains of rats display genetic resistance. Disease susceptibility is reported to be controlled by an immune response (Ir) gene closely linked to the major histocompatibility complex (MHC) (1–4). The cellular mechanisms that mediate genetic resistance to disease are poorly understood partly as a result of difficulties encountered in the adoptive transfer of allogeneic cells.

The study reported here was prompted by an observation of resistance to clinical EAE induction in a large number of Lewis (designated Le-R) rats. Breeding experiments suggested that resistance was a dominant, autosomal genetic trait. Le-R leukocytes are apparently unable to transfer disease adoptively to naive Le recipients, however, Le leukocytes readily transfer disease into naive Le-R recipients. Because cellular exchange in other susceptible/resistant rat strain combinations is hindered by histocompatibility barriers, the Le/Le-R system presents a unique opportunity to investigate cellular and/or humoral aspects of genetic resistance to autoimmune neural tissue destruction.

[1] *Abbreviations used in this paper:* BN, Brown Norway; BP, myelin basic protein; C, complement; CFA, complete Freund's adjuvant; Con A, concanavalin A; EAE, experimental allergic encephalomyelitis; EF, encephalitogenic fragment of myelin basic protein; Ir gene, immune response gene; Le, Lewis; Le-R, Lewis-resistant; MHC, major histocompatibility complex; OA, ovalbumin.

Materials and Methods

Rats. The colony of Le-R rats was originally obtained from Simonsen Laboratories (Gilroy, Calif.) and was initially susceptible to EAE induction (5). The colony had been maintained as a closed colony for approximately 5 yr before the initiation of this study. Within this colony, rats were randomly bred. Conventional Le rats (RT1-1) were obtained from (*a*) Simonsen Laboratories, (*b*) Microbiological Associates, (Walkersville, Md.), and (*c*) The Rocky Mountain Laboratories, (Hamilton, Mont.). Le rats from each of these sources displayed comparable susceptibility to EAE induction. Buffalo rats (RT1-b) were obtained from Simonsen Laboratories and Brown Norway (BN) rats (RT1-n) were obtained from Microbiological Associates. Rats were provided access to food and water without restriction, and were hand-watered during periods of paralysis.

Preparation of BP and Encephalitogenic Peptide. BP was prepared from Hartley guinea pig brains and spinal cords by the procedure of Deibler et al. (6), and characterized as previously described (7). The encephalitogenic fragment (EF) of BP, comprising amino acid residues 68– 88, was a generous gift by Doctors R. B. Fritz and R. F. Kibler, Emory University School of Medicine, Atlanta, Ga. The methods for isolating and characterizing EF have been described in detail elsewhere (8).

Induction of EAE. Paralytic EAE was induced by a single injection of 50 μg of BP in CFA as previously described (7). In each experiment in which the EAE susceptibility of Le-R rats was assessed, Le rats were also included as positive controls to insure that emulsions had the capacity to induce paralysis in susceptible recipients.

Clinical Evaluation. Rats were assigned a daily clinical grade by the following criteria: 0, no sign of neurologic impairment; 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis.

Histologic Evaluation. Brains and spinal cords obtained at day 17 post-BP-CFA challenge were fixed in formalin and then stained with hematoxylin and eosin. The degree of severity of neural tissue lesions was evaluated by the criteria of Gasser et al. (3).

Serotype Analysis. An anti-Le antiserum was raised in BN rats by five weekly intraperitoneal injections of 3×10^7 Le spleen cells. This antiserum was diluted in complement (C) fixation diluent (9) in a 1:5 ratio, which was found to be the optimum concentration for killing Le target cells in preliminary experiments. Target spleen cells, obtained from Le, Le-R, Buffalo, and BN rats, were incubated with the anti-Le serum for 30 min at 37°C (5×10^6 leukocytes/ ml). An equal volume of freshly prepared homologous rat serum was added as a source of complement and the mixture incubated for an additional 30 min. Cytotoxicity was determined by trypan blue or nigrosin dye exclusion. The percent specific cytotoxicity was calculated according to the following formula:

$$\text{percent specific cytotoxicity} = \left[1 - \frac{\text{No. viable cells after treatment with BN anti-Le serum plus C}}{\text{No. viable cells after treatment with normal BN serum plus C}} \right] \times 100.$$

Migration Inhibition Assay. Cellular reactivity specific for BP or EF was determined by a direct migration inhibition assay described in detail previously (7, 10).

Passive Transfer of EAE. Spleen cells were obtained at days 12–14 post-BP-CFA challenge. The cells were cultured for 3 d in the presence of 1 μg/ml of concanavalin A (Con A) or 1 μg/ ml of BP according to the procedure described previously (11, 12). At the end of the culture period, cell viability was determined by dye exclusion, and cells were infused into normal recipients by intraperitoneal injection. Data describing the specificity of the passive transfer of EAE is to be presented in detail elsewhere.[2]

Results

Susceptibility to EAE Induction by Le and Randomly Bred Le-R Rats. Le rats obtained from three independent sources were susceptible to EAE induction (Fig. 1). Out of a

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[2] Hinrichs, D. J., C. Roberts, and F. J. Waxman. Regulation of paralytic experimental allergic encephalomyelitis in rats: susceptibility to active and passive disease reinduction. Manuscript submitted for publication.
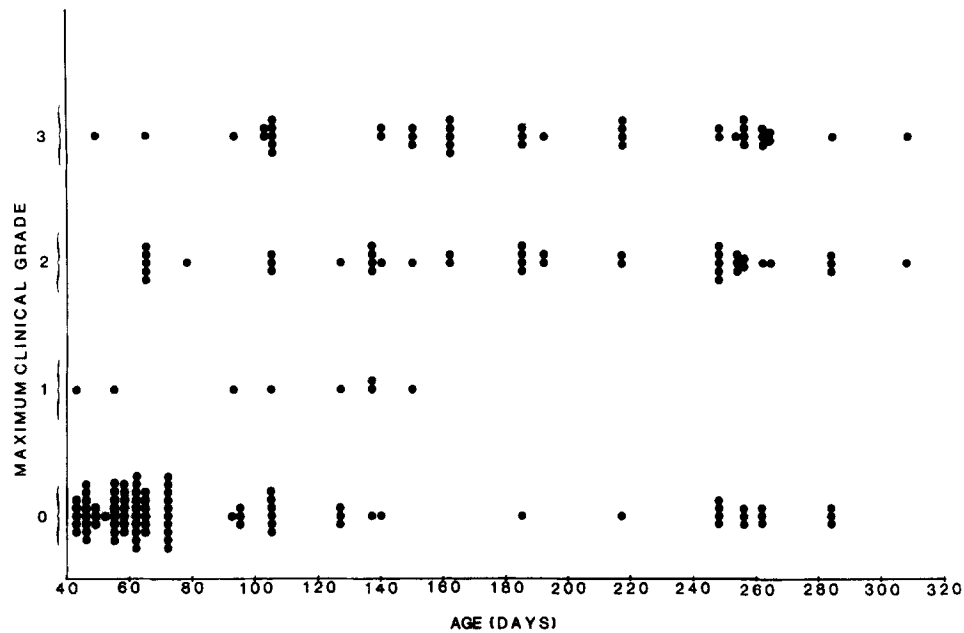
FIG. 1.   Susceptibility to EAE induction of Le rats. Rats were injected with 50 µg of BP in CFA at various ages, and subsequent clinical signs were monitored. The data are expressed as the maximum clinical response as assessed by the following criteria: 0, no symptoms; 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis. Each datum point (O) indicates the response of an individual rat.
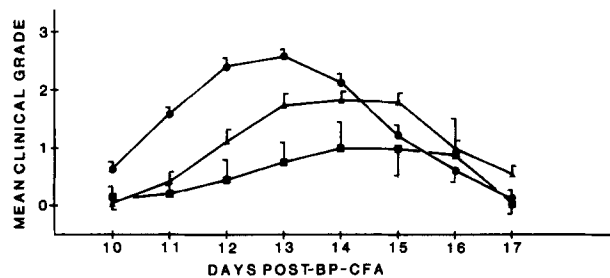
total of 161 Le rats tested, 92% developed paralysis (grade 3). EAE susceptibility was not age dependent within the period tested (43- to 263-d-old at time of BP-CFA challenge).

In contrast, only 23% of Le-R rats developed paralysis out of 180 randomly bred Le-R rats tested (Fig. 2). Resistance was most evident when Le-R rats were <100 d of age at the time of BP-CFA challenge. However, resistance was also apparent in older Le-R rats, since a majority of these animals displayed mitigated neurologic symptoms.

In those Le-R rats that did develop clinical manifestations of EAE, the day of onset of symptoms was delayed, disease severity was reduced, and the duration of clinical signs was abbreviated, compared with Le rats (Fig. 3). This mitigated disease pattern was again most evident in young Le-R rats.

In addition to clinical evaluations, brains and spinal cords of selected EAE-susceptible and EAE-resistant Le-R rats were evaluated by histologic examination (Table I). Neural tissue lesions characteristic for EAE were seen in BP-CFA-challenged Le rats. Similar lesions were seen in Le-R rats, which had developed clinical EAE subsequent to BP-CFA challenge. In Le-R rats, that were resistant to clinical EAE, lesions were minimal or absent. Thus, evaluation of EAE by histopathologic criteria corresponded with clinical assessments.

Inheritance of EAE Resistance by Progeny of Le-R Rats Selected for EAE Susceptibility or Resistance.   Because there was some heterogeneity in the susceptibility of individual Le-R rats, it was possible that the Le-R colony might contain two separate populations: one being susceptible, the other resistant. In order to determine if susceptible and resistant Le-R rat populations could be selected by breeding, young randomly

Fig. 2. Age-related resistance to EAE induction of randomly bred Le-R rats. Rats were injected with 50 μg of BP in CFA at various ages, and subsequent clinical signs were monitored. The data are expressed as the maximum clinical response as assessed by the following criteria: 0, no symptoms; 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis. Each datum point (●) indicates the response of an individual rat.

Fig. 3. Reduced severity of disease in EAE-susceptible Le-R rats. Le (age 43–263 d) rats (●), young (age 43–65 d) Le-R rats (■), and older (age 98–310 d) Le-R rats (▲) were evaluated for clinical neurologic impairment on days 10–17 after BP-CFA challenge. The data are expressed as the mean clinical grade ± 1 SEM for 160 Le rats, 9 young Le-R rats, and 81 older Le-R rats (rats that displayed no signs of neurologic impairment are not included in these data). Clinical grades were assigned according to the following criteria: 0, no symptoms; 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis. The mean ± 1 SEM day of onset of clinical signs of EAE were: Le rats, 10.8 ± 0.1 d; young Le-R rats, 13.1 ± 0.6 d; older Le-R rats, 12.1 ± 0.1 d. The mean ± 1 SEM duration of clinical signs were: Le rats, 5.4 ± 0.1 d; young Le-R rats, 2.9 ± 0.4 d; older Le-R rats, 4.7 ± 0.2 d.

bred Le-R rats were challenged with BP-CFA and classified as susceptible or resistant. Breeding trios composed of rats selected for resistance, and trios of rats selected for susceptibility were established. The F$_1$ progeny from resistant parents, or susceptible parents, were likewise classified according to their susceptibility. F$_2$ progeny were then produced by brother-sister mating of susceptible F$_1$ rats, or resistant F$_1$ rats. F$_3$

TABLE I

Histologic Evaluation of Neural Tissue Lesions in EAE-susceptible or EAE-resistant Le-R Rats

Strain	Group	Rat no.	Maximum clinical grade*	Lesion severity‡
Le	EAE-susceptible§	1	3	+++
		2	3	++++
Le-R	Normal‖	3	0	0
		4	0	0
Le-R	EAE-susceptible§	5	2	++
		6	2	++++
		7	2	+
		8	2	++
		9	2	+++
Le-R	EAE-resistant§	10	0	0
		11	0	0
		12	0	++
		13	0	±
		14	0	0

* Clinical grades were assigned by the following criteria: 0, no symptoms; 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis.

‡ Spinal cords were fixed in formalin and then stained with hematoxylin and eosin. The degree of severity of neural tissue lesions was graded as described by Gasser et al. (3). 0, no perivascular, meningeal, or intraparenchymal infiltrates of mononuclear cells; ±, only one cellular infiltrate in a spinal root or in leptomeninges; +, several infiltrates in spinal roots and occasionally in spinal cord; ++, many infiltrates in the roots and at least one infiltrate in the cord at X 30 field; +++, confluent infiltrates in the roots, many infiltrates in the cord, occasional infiltrates in the cerebrum; ++++, confluent infiltrates in roots and spinal cord, several infiltrates in cerebrum.

§ Neural tissue specimens were obtained from the rats at day 17 post-BP-CFA injection.

‖ Neural tissue specimens were obtained from rats that had not been challenged with BP-CFA.

progeny of entirely resistant lineage were produced by brother-sister mating of resistant F_2 rats.

The EAE susceptibility of these Le-R rats is shown in Table II. A majority of F_1 and F_2 Le-R rats of either susceptible or resistant lineage was resistant when challenged with BP-CFA at 7-11 wk of age. Thus, the susceptibility of F_1 and F_2 progeny had no relationship to their ancestry. F_3 rats were uniformly resistant to EAE induction at all ages tested. These data suggest that a single inbred population with phenotypic resistance to EAE induction had been selected from the Le-R colony.

EAE Susceptibility of (Le × Le-R)F_1 and Backcross Progeny. In order to determine if the EAE resistance displayed by Le-R rats was a genetic trait, 100% EAE-resistant F_3 Le-R rats were crossed with Le rats, and the EAE susceptibility of (Le × Le-R)F_1 progeny was assessed (Table III). The F_1 progeny of Le males × Le-R females, as well as the offspring of Le females × Le-R males were resistant to EAE induction. Both male and female F_1 progeny were resistant. Thus, EAE resistance in 7- to 8-wk-old (Le × Le-R)F_1 rats appeared to be inherited as a dominant, autosomal trait.

(Le × Le-R)F_1 rats were then backcrossed to Le rats, and the progeny were tested

TABLE II

Susceptibility to EAE by Progeny of Le-R Rats Selected for EAE Resistance or EAE Susceptibility

Strain	Lineage*	Generation	Age when challenged with BP-CFA	Percent resistant	Percent sensitive (maximum clinical grade attained)‡			No. of rats tested
					1	2	3	
			wk					
Le	—	—	7–11	1	2	6	91	95§
Le-R	Resistant	F₁	7–11	68	4	23	5	72
Le-R	Resistant	F₂	7–11	51	9	28	12	67
Le-R	Susceptible	F₁	7–11	60	8	30	2	40
Le-R	Susceptible	F₂	7–11	69	15	8	8	26
Le-R	Resistant	F₃	7–11	100	0	0	0	49
Le-R	Resistant	F₃	26–42	100	0	0	0	26

* Breeders were classified as EAE sensitive if they displayed a clinical grade of 2 or 3 after BP-CFA challenge, or classified as EAE resistant if they showed no signs of neurological impairment after BP-CFA challenge.

‡ Clinical grades were assigned by the following criteria: 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis.

§ Only those Le rats that were challenged at 7–11 wk of age are included in this table.

TABLE III

EAE Susceptibility of (Le × Le-R)F₁ and Backcross Rats*

Group	Percent resistant	Percent sensitive (maximum clinical grade attained)‡			No. of rats tested
		1	2	3	
Le§	0	0	0	100	19
(Le × Le-R)F₁‖, ¶	95	3	0	2	60
(Le × Le-R)F₁ × Le	25	4	25	46	71**

* Rats were injected with BP-CFA at 7–8 wk of age.

‡ Clinical grades were assigned by the following criteria: 1, flaccid tail; 2, hindquarter weakness; 3, hindquarter paralysis.

§ These Le rats were included in each experiment as positive controls for the encephalitogenicity of BP-CFA emulsions.

‖ Of the (Le × Le-R)F₁ rats tested, 36 were derived from matings between Le-R males and Le females whereas the remaining 24 were derived from matings between Le males and Le-R females. 34 of the (Le × Le-R)F₁ rats were females, and 26 were males.

¶ The Le-R rats used as breeders for the production of (Le × Le-R)F₁ and backcross rats were 100% EAE-resistant F₃ rats (Table II).

** The χ^2 value (4.68) obtained when comparing resistant vs. susceptible backcross rats was significantly different ($P < 0.05$) from the expected value for three genes.

for their susceptibility to EAE induction (Table III). A total of 46% of the backcross progeny developed paralysis, whereas 29% developed EAE with mitigated severity. 25% of the backcross progeny was completely resistant to EAE induction. These data suggest that EAE resistance is mediated by one or two genes.

RT1 Serotype of Le-R Rats. It is possible that Le-R rats manifest EAE resistance as a result of the accidental interbreeding of Le and non-Le white rats. In order to test

this possibility, an anti-Le alloantiserum was raised in BN rats. Target spleen cells from F_1 and F_2 Le-R rats of either susceptible or resistant lineage were treated with anti-Le plus C', and specific cytotoxicity was assessed by dye exclusion. In each experiment, spleen cells from Le rats were included as positive controls. Spleen cells from Buffalo or BN rats served as negative controls.

The anti-Le serum plus C' treatment was cytotoxic for Le cells (79% mean specific percent cytotoxicity) but not for Buffalo (3% mean specific percent cytotoxicity) or BN (9% mean specific cytotoxicity) cells. This antiserum was cytotoxic (68% mean specific percent cytotoxicity) for Le-R spleen cells independent of their lineage. Thus, Le-R rats shared at least some portion of the Le MHC antigen complex. This protocol would not distinguish between homozygous vs. heterozygous $RT1-1^+$ Le-R rats, however, the results of breeding experiments presented above would seem to exclude the possibility that Le-R rats were heterozygous.

Cellular Reactivity of Le-R Rats. It is possible that Le-R rats manifest EAE resistance because of a general impairment in their ability to develop cellular reactivity when challenged with antigen. In order to test this possibility, Le-R rats were challenged with BP-CFA and classified as susceptible or resistant. At day 21 postchallenge, rats received an injection of ovalbumin (OA) in CFA. Cellular reactivity specific for OA, as assessed by macrophage migration inhibition, was determined at day 21 after the second injection.

Both EAE susceptible and resistant Le-R rats developed cellular reactivity specific for OA after challenge with OA-CFA (Fig. 4A). These data suggest that EAE resistance is not a result of a nonspecific T cell deficit.

There is evidence that EAE-resistant BN rats fail to develop cellular reactivity specific for the critical disease inducing antigenic determinant (EF) located within amino acid residues 68–88 of the BP molecule (13, 14). In contrast, it has been established that Le rats develop cellular reactivity for BP and EF, and that BP and EF specific cellular reactivity is detectable after primary and secondary BP-CFA challenge (7, 13–15). It is possible that the resistance to EAE induction displayed by Le-R rats is a result of an impairment in their ability to develop EF-specific cellular reactivity when challenged with encephalitogenic antigen. In order to test this possibility, Le-R rats were challenged with BP-CFA and subsequently classified as susceptible or resistant. At day 21 postchallenge, rats received a second injection of BP-CFA. Cellular reactivity specific for BP and EF, as assessed by macrophage migration inhibition, was determined at day 21 after the second injection.

Susceptible and resistant Le-R rats developed both BP- and EF-specific cellular reactivity after secondary challenge with BP-CFA (Fig. 4B and C). Similar data (not shown) were obtained at day 21 after a primary BP-CFA injection. Although the remote possibility of recognition of a nonencephalitogenic determinant on the 19 amino acid EF molecule cannot be rigorously excluded with presently available reagents, these data suggest that the clinical resistance to EAE induction displayed by Le-R rats is not caused by a failure to develop BP and EF specific cellular reactivity.

Passive Transfer of EAE into Le-R Recipients. Because Le-R rats developed EF-specific cellular reactivity, it was of interest to determine if Le-R cells also had the capacity to transfer paralytic EAE. Conversely, were Le-R rats suitable recipients for the passive transfer of EAE? To address these questions, the recently described passive transfer system of Pannitch and McFarlin (11) and Richert et al. (12) was utilized.
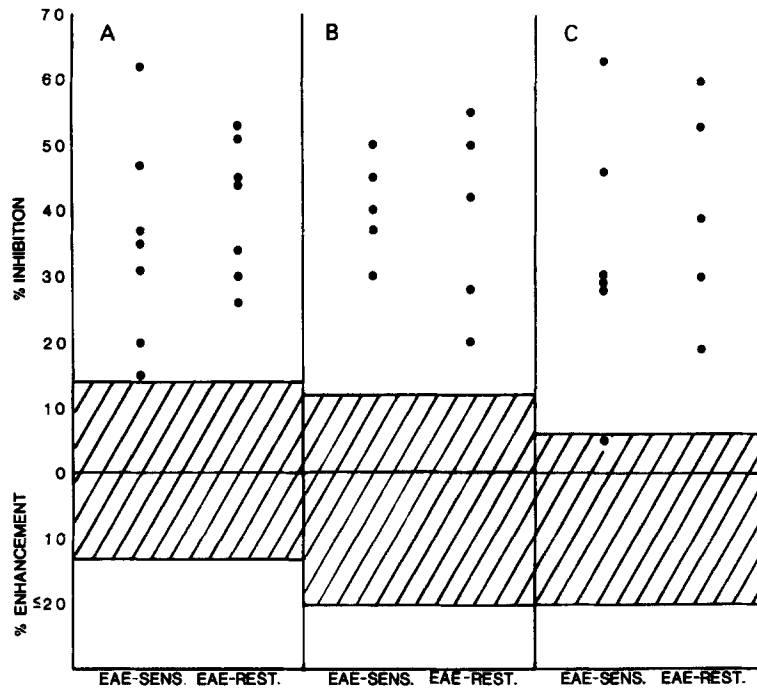
Fig. 4.   The development of cellular reactivity specific for OA (panel A), intact BP (panel B), or EF (panel C) by EAE-susceptible and EAE-resistant Le-R rats. Rats were challenged with BP-CFA at 7–8 wk of age (day 0), and classified as EAE-susceptible (EAE-SENS) or EAE-resistant (EAE-REST). At day 21, rats in both groups were injected with either 100 µg of OA in CFA or 50 µg of BP in CFA. Cellular reactivity was assessed by a direct macrophage migration inhibition assay performed at day 42. The data are expressed as percent inhibition of migration calculated by the following formula:

$$\text{per cent inhibition} = \left[1 - \frac{(\bar{x}\ \text{migration in the presence of antigen})}{(\bar{x}\ \text{migration in the absence of antigen})} \right] \times 100.$$

When $\bar{x}$ migration in the presence of antigen exceeded $\bar{x}$ migration in the absence of antigen, data are expressed as percent enhancement. Four replicate capillary tubes were used for each antigen variable. As specificity controls, naive rats were included in each assay. The values for these normal controls are expressed as a normal range (▨) reflecting the 95% confidence interval about the mean percent inhibition (or percent enhancement) measured in four experiments as described previously (7, 10). Each datum point (●) indicates the response of an individual rat.

This procedure, which involves the incubation in vitro of BP-sensitized cells in the presence of Con A or BP, facilitates the induction of EAE of paralytic severity with a relatively small number of sensitized cells. Spleen cells were collected from paralyzed Le and from asymptomatic Le-R rats at day 12–14 after BP-CFA challenge. After a 72-h in vitro culture period in the presence of Con A, the cells were infused into naive Le and Le-R recipients and the subsequent development of clinical EAE was monitored.

These data are shown in Table IV. Spleen cells obtained from BP-CFA-injected Le-R rats did not transfer EAE into either Le or Le-R recipients. In contrast, cells obtained from BP-CFA-injected Le rats readily transferred EAE into both Le-R and Le recipients. These results indicate that there is no physiologic barrier to EAE induction in Le-R rats. In addition, these data suggest that the blockade in the

TABLE IV

*Passive Transfer of EAE into Le-R Recipients**

Cell donor	Recipient	No. of viable cells transferred	No. of recipients with EAE/no. of rats tested
Le-R	Le	$3 \times 10^7\text{--}10 \times 10^7$	0/12
Le-R	Le-R	$5 \times 10^7\text{--}10 \times 10^7$	0/6
Le	Le	3×10^7	12/14
Le	Le-R	3×10^7	14/16

* Donor spleen cells were obtained from Le or Le-R rats at day 12–14 post-
BP-CFA challenge. The cells were then conditioned in vitro for 3 d and
infused into normal recipients as described in Materials and Methods. The
data for the transfer of EAE into Le-R recipients are compiled from three
independent experiments in which the donor Le cells were incubated in vitro
in the presence of Con A; similar data (not shown) were obtained in a fourth
experiment in which the donor Le cells were cultured in vitro in the presence
of BP.

induction of EAE in Le-R rats occurs at some point during the differentiation of
antigen-reactive cells into EAE-inducing effector cells.

Discussion

The Le-R rat is apparently a mutant Le rat. This genetic change is expressed as
resistance to clinical EAE induction. Resistance was evident not only when Le-R rats
were challenged with the standard encephalitogenic dose for Le rats (50 μg of BP),
but also when Le-R rats were challenged with up to 10 encephalitogenic doses of BP,
or with an extremely potent mixture of spinal cord homogenate plus *Bordetella pertussis*
extract (not shown). In addition, the histologic neural tissue lesions that typically
accompany EAE are minimal or absent in EAE-resistant Le-R rats.

Population susceptibility to EAE induction appeared to increase with age in
randomly bred Le-R rats. Nevertheless, EAE rsistance was evident in both young and
older Le-R rats since the clinical signs of neurologic dysfunction were mitigated in
older animals. The phenomenon of increasing susceptibility with age was no longer
apparent by the F_3 generation of Le-R rats, which was resistant at all ages tested.
Although (Le × Le-R)F_1 rats were resistant to EAE induction at 7–8 wk of age,
preliminary observations suggest that (Le × Le-R)F_1 rats are susceptible to EAE
induction at 36–40 wk of age. This suggests that the age-related susceptibility initially
observed in randomly bred Le-R rats reflected the presence of rats with mixed Le ×
Le-R ancestry. The pattern of increasing susceptibility to disease with age may reflect
an age-related loss in suppressor cell function as has been reported in autoimmune
disease-prone mice (16). Alternatively, age-related susceptibility may be caused by
age-related modulation of the specific effector cell populations involved in neural
tissue destruction.

Because some persistent viral infections have been reported to suppress immunologic
function, the possibility that the resistance of Le-R rats was of viral etiology was
considered. No evidence of depressed immunologic function was evident in Le-R rats
by the criteria of their blastogenic response to mitogens in vitro (not shown), or their
ability to develop OA-specific hypersensitivity after challenge with OA-CFA. In
addition, Le rats could be housed and bred in the same room as Le-R rats without

acquiring resistance. Finally, the F_1 progeny resulting from matings between Le-R males and Le females were resistant to EAE induction. Because the pregnant Le females were separated from the Le-R males before the birth of litters, these rats manifested EAE resistance in the absence of direct contact with any Le-R rat. The Le mothers of these progeny retained their EAE susceptibility in spite of their sexual contact with Le-R males (not shown). Although the remote possibilities of the Le females acquiring carrier status through contact with Le-R males, or a seminal fluid route of transmission, or that EAE induction in Le rats may be the result of activation of an encephalitogenic virus absent in Le-R rats cannot be excluded, it seems unlikely that the EAE resistance of Le-R rats involves persistent viral infection.

EAE resistance was not a universal phenomenon in the randomly bred Le-R rat colony since a small percentage of Le-R rats developed paralysis subsequent to BP-CFA challenge. This suggested the possibility that the colony might contain two distinct populations: one susceptible, the other resistant. However, breeding experiments indicated that F_1 progeny of Le-R rats, selected either for resistance or susceptibility, were largely EAE resistant. Further, the F_2 progeny derived from susceptible parents and grandparents were as resistant to EAE induction as F_2 rats of entirely resistant lineage. Thus, susceptibility could not be selected for by breeding. Although it was not possible to produce an F_3 generation of entirely susceptible lineage because of the absence of brother-sister littermates in the very small group of F_2 rats that were susceptible, the F_3 generation of EAE-resistant lineage was uniformly resistant suggesting that a homogeneous population with phenotypic resistance had been selected.

In order to determine if the EAE resistance of Le-R rats was a genetic trait, Le-R rats were mated with Le rats and the EAE susceptibility of the F_1 progeny was assessed. (Le × Le-R)F_1 rats challenged at 7–8 wk of age displayed phenotypic EAE resistance comparable with Le-R rats. Because susceptibility was not sex-linked, resistance appeared to be inherited as a dominant, autosomal trait.

The (Le × Le-R)F_1 × Le backcross progeny's susceptibility to EAE induction segregated in a ratio of 25% entirely resistant to 75% susceptible. The susceptible group fell into two categories: approximately one-half (46%) of the backcross population developed paralytic EAE whereas the remaining 29% developed EAE with mitigated neurologic symptoms. The simplest explanation of these data is that resistance is mediated by a single gene. Variability in the expression of this gene caused by some unknown extrinsic factor might account for the mitigated symptoms seen in some backcross animals. These data are also compatible with the hypothesis that complete EAE resistance is dependent upon the inheritance of two unlinked genes. It is possible to speculate that one of these genes may be a strong resistance gene, the other a relatively weak resistance gene. Assuming that the genes involved segregate in a Mendelian fashion, one-fourth of the backcross progeny should inherit both genes thus conferring complete EAE resistance. Another one-fourth of the backcross rats would be expected to inherit only the strong resistance gene, which theoretically might have the capacity to mitigate but not completely abrogate the clinical signs of EAE in the absence of the weak resistance gene. The remaining one-half of the backcross population, which inherited either the weak resistance gene alone or which inherited neither of these resistance genes, would comprise the entirely susceptible group which developed paralytic EAE. Proof for these models will require

precise mapping of the resistance gene(s), and further delineation of the biological expression of the gene products.

There is evidence that EAE susceptibility is dependent on the inheritance of an Ir gene (Ir-EAE) closely linked to the rat MHC (1–4). It seems likely that Le-R rats possess Ir-EAE because of the following observations: (a) they develop EF-specific hypersensitivity, and (b) they share at least some common serologically detectable MHC antigens with Le rats. However, the possibility of a genetic recombination occurring between the loci controlling the production of serologically detectable MHC antigens and the putative Ir-EAE locus is not precluded.

Based on observations reported here and elsewhere, it is possible to design a theoretical model of the events leading to the induction of EAE (Fig. 5). The injection of BP-CFA initiates the development of antigen-reactive T lymphocytes with specificity for the encephalitogenic determinant(s) on the BP molecule. The failure of BN rats to develop EAE is apparently a result of a deficit at this step because BN rats do not develop detectable EF-specific hypersensitivity (13, 14). In contrast, Le-R rats evidently develop specific antigen-reactive cells, as assessed by macrophage migration inhibition in the presence of BP or EF. However, these data do not exclude the possibility that Le-R rats may develop smaller numbers of specific antigen-reactive cells than Le rats. Subsequent steps in the induction of EAE require the in vivo differentiation (step 2) of antigen-reactive cells into EAE effector precursor cells, which can be educated (step 3) by incubation in vitro in the presence of either polyclonal (Con A) or specific (BP or EF) culture stimulants to develop into educated EAE effector cells. There is evidence[2] that both steps 2 and 3 are required for the adoptive transfer of EAE by rat spleen cells since of the following criteria: (a) large numbers of guinea pig BP-sensitized Le cells fail to transfer EAE unless they are incubated in vitro in the presence of either Con A or encephalitogenic antigen, and (b) cells from Le rats, which had not been sensitized with encephalitogenic antigen, fail to transfer EAE in spite of in vitro conditioning in the presence of Con A or encephalitogenic antigen. However, it is possible that the in vitro culture period may involve the clonal expansion of EAE effector cells or reduction in the activity of suppressor cells, rather than differentiation of effector cells from effector precursor cells. The educated EAE effector cells have the capacity to induce clinical EAE (step 4) after adoptive transfer into naive recipients. Le-R rats are not blocked at step 4 in this process since they develop paralytic EAE after the adoptive transfer of Con A- or BP-stimulated effector cells obtained from BP-CFA-sensitized Le rats. The adoptive
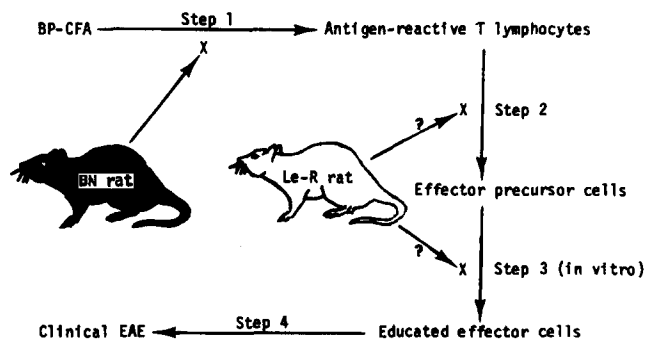

FIG. 5.   See text for explanation.

transfer of paralytic EAE into Le-R rats by sensitized Le leukocytes is striking in view of previously established genetic restrictions which preclude the transfer of EAE from Le into allogeneic or even MHC-compatible rat strains.[2] In the reciprocal transfer, Le rats did not develop EAE after adoptive transfer of Le-R cells. This may reflect a one-way allogeneic recognition of Le-R cells by Le rats; however, preliminary evidence suggests that Le and Le-R leukocytes are mutually nonreactive in one-way mixed leukocyte reactions, and that Le but not Le-R cells have the capacity to transfer EAE into (Le X Le-R)F_1 recipients. Thus, the resistance to EAE induction displayed by Le-R rats may be a result of a deficit in the differentiation of antigen-reactive cells to effector precursor cells in vivo (step 2), and/or the further development of active effector cells during the in vitro culture period (step 3).

The apparent feasibility of nonrestricted cellular exchange between Le and Le-R rats presents a unique opportunity to investigate cellular resistance mechanisms in EAE. Disease resistance in the Le-R rat may reflect a deficiency in a leukocyte population required for EAE induction. Because 7- to 8-wk-old (Le X Le-R)F_1 rats were resistant to EAE induction, gene complementation evidently does not occur when Le and Le-R genes are juxtaposed in a trans configuration. Alternatively, EAE resistance in the Le-R rat may be caused by an excessive production of suppressor cells and/or regulatory macromolecules. There is evidence suggesting that both antigen-specific and nonspecific humoral factors may mediate EAE resistance (17–19). However, preliminary evidence suggests that the infusion of Le-R serum into Le rats does not alter their susceptibility to EAE induction. Disease resistance in the Le-R rat may reflect an imbalance in a nonspecific suppressor cell population as has been reported in association with a canine demyelinating disease (20, 21), or from hyperreactivity in populations of BP-specific suppressor cells, which have been identified in both protected and EAE-recovered Le rats (22–26). Further efforts to delineate more precisely the nature of EAE resistance in Le-R rats are currently in progress.

Summary

Clinical resistance to the induction of experimental allergic encephalomyelitis was observed in a closed colony of Lewis (designated Le-R) rats. Disease susceptibility in randomly bred animals appeared to increase with increasing age. In the small group of young Le-R rats, which were susceptible, disease onset was delayed, severity of symptoms was reduced, and duration of clinical signs was abbreviated compared to conventional Lewis rats. The severity of histologic neural tissue lesions correlated with clinical observations. Breeding experiments indicated that most Le-R rats were resistant to disease induction regardless of whether their ancestors had been selected for susceptibility or resistance. The F_3 generation of resistant lineage was uniformly resistant at all ages tested. Virtually all (Lewis X Le-R)F_1 rats of either sex were resistant when challenged at 7–8 wk of age indicating that resistance was a dominant autosomal trait. Approximately half of (F_1 X Lewis) backcross rats developed paralytic EAE whereas one-fourth were entirely resistant, suggesting that disease resistance may be mediated by one or two genes. Le-R rats shared at least some of the Lewis rat major histocompatibility antigens. Resistance apparently did not reflect a nonspecific impairment of cellular immune responsiveness. Le-R rats, which had been challenged with myelin basic protein, developed antigen-reactive cells specific for basic protein

or its encephalitogenic fragment. Spleen cells obtained from basic protein-sensitized Le-R rats did not adoptively transfer disease into Lewis rats. In contrast, spleen cells obtained from basic protein-sensitized Lewis rats readily transferred disease into both Lewis and Le-R recipients. These data suggest that disease resistance may be a result of an immunologic deficit (or suppressor cell activity) expressed during the differentiation of antigen-reactive cells into disease-inducing effector cells.

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