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The role of the neuroendocrine system in determining genetic susceptibility to experimental allergic encephalomyelitis in the rat

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SUMMARY

Experimental allergic encephalomyelitis (EAE) can be induced in some strains of rat but not others, by the injection of guinea-pig myelin basic protein in Freund's complete adjuvant. In the susceptible Lewis strain, spontaneous recovery from paralysis occurs and previous studies have shown that this recovery is dependent on the production, during the course of the disease, of high levels of corticosterone from the adrenal glands. Adrenalectomy completely abrogates the recovery phase and the disease becomes uniformly fatal unless steroid replacement therapy is given, which reproduces the serum levels of hormone that develop in intact animals with EAE. The PVG strain is not susceptible to EAE, but here it is shown that PVG rats that had been adrenalectomized developed severe disease from which they do not recover. As in the adrenalectomized Lewis rat, steroid replacement therapy could prevent the fatal outcome and in this case the disease course resembled that seen in intact Lewis animals. By a number of parameters PVG rats appear to make a more vigorous steroid response to stress than do Lewis. A comparison of the ratio of adrenal weight to body weight between these strains indicated that this ratio is larger in PVG, and serum corticosterone levels, in response to stress, were also found to be higher in this strain. Furthermore, basal levels of corticosterone were much more labile in PVG rats and had a higher mean value than those found in the age- and sex-matched Lewis animals with which they were compared. Genetic analysis using congenic rat strains showed that a high adrenal weight to body weight ratio was not linked to the major histocompatibility complex (MHC). It appears that the resistance to EAE of PVG rats depends on an enhanced stress response that mediates its immunosuppressive effect via the adrenal glands. While this stress response plays an essential part in the recovery of Lewis strain rats from EAE, it is sufficiently potent in PVG rats to virtually completely prevent signs of disease. Resistance to the induction of EAE could not be abrogated by adrenalectomy in all strains of rats studied. In particular, congenic PVG.RT1^u rats, with the same background genes as PVG but with RT1^u rather than the RT1^c MHC genes of PVG, did not develop EAE when the adrenal glands were removed. It follows that susceptibility can be determined by both MHC and non-MHC genes and that, in some strains at least, the non-MHC genes result in variations in the degree to which the immune response is inhibited by the neuroendocrine system. The possible implications for susceptibility to autoimmune diseases in man are discussed briefly.

INTRODUCTION

Inbred strains of laboratory animals differ in their ability to make immune responses to both synthetic (Benacerraf & McDevitt, 1972) and natural antigens (Green, Inman & Benacerraf, 1970), and the hereditary nature of such responses has led to the definition of immune-response genes. In many instances these genes map to the major histocompatibility complex (MHC) (Benacerraf & McDevitt, 1972; Günther, Rüde & Stark, 1972), but genetically determined immune responses do not depend solely on genes that are directly involved in antigen presentation (Cole *et al.*, 1986; Davis *et al.*, 1981). In such cases the mechanisms responsible for genetic variation are poorly defined.

Experimental allergic encephalomyelitis (EAE) is a paralytic disease that can be induced in a number of species of laboratory animal by the injection of central nervous tissue antigens (Paterson, 1966). In the rat, using guinea-pig myelin basic protein (MBP) as the encephalitogenic antigen, the disease develops 11–14 days after immunization and results in paralysis that affects the tail and hind limbs and may progress to urinary and faecal incontinence. The animals recover after 4–5 days and are then refractory to attempts to induce further episodes of the disease (Willenborg, 1979; McFarlin, Blank & Kibler, 1974).

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Not all strains of rat show this striking response: Lewis rats develop the full range of signs of the disease while many others are virtually completely resistant. Genetic studies (Gasser, Palm & Gonatas, 1975; Moore, Singer & Michael Williams, 1980; Hughes & Stedronska, 1973) of disease susceptibility have indicated that both MHC and non-MHC genes are important, but the nature of the non-MHC gene effects remain unknown. In principle, a deficiency in the T-cell receptor repertoire could be involved but experiments with the resistant PVG strain have indicated that, in this case at least, failure to develop disease is not a consequence of the inability of the immune system to recognize the encephalitogen (Ben-Nun, Eisenstein & Cohen, 1982).

In earlier studies it was shown that spontaneous recovery of Lewis rats from EAE does not occur if the adrenal glands are removed before the onset of disease (MacPhee, Antoni & Mason, 1989); instead the disease becomes fatal. It has been known for some years that Lewis rats develop very high serum levels of corticosterone (approaching 1 μM) during the acute phase of EAE (Levine, Sowinski & Steinetz, 1980) and that exogenous stress would suppress the disease (Levine *et al.*, 1962).

These findings raised the question of whether some strains of rat, that are known to be virtually resistant to the induction of EAE, suppress the development of the disease by an exaggerated form of the adrenal response seen in Lewis rats. To examine this possibility a study was made of the effect of adrenalectomy on EAE induction in PVG strain animals.

MATERIALS AND METHODS

Animals

Rats of the strains PVG, PVG.RT1^u, Lewis and AO were from the specific pathogen-free facility of the MRC Cellular Immunology Unit, Oxford. Rats of PVG.RT1^l were the kind gift of Dr Geoff Butcher of the Agricultural Research Centre, Institute of Animal Physiology Babraham, Cambridge. They were housed in clean but not specific pathogen-free conditions.

Induction of EAE

Young adult rats were immunized in the hind footpads with a total of 50 μg guinea-pig myelin basic protein (MBP) in 100 μl Freund's complete adjuvant (FCA). Disease was scored on a scale of 1–5 as follows: 1, limp tail; 2, hind leg weakness; 3, unilateral hind limb paralysis; 4, bilateral hind limb paralysis; 5, incontinence or fatal disease.

Adrenalectomy and steroid replacement therapy

Adrenalectomy was performed by blunt dissection using a single dorsal incision through the skin and bilateral dorso-lateral incisions through the muscles of the flank. Corticosterone replacement therapy was achieved by the subcutaneous implantation of corticosterone/cholesterol pellets (Dallman *et al.*, 1987).

Ether stress

Rats were anaesthetized by ether inhalation and 1 ml of blood was taken via the tail vein. They were then allowed to recover and anaesthetized again 40 min later, at which time tail vein blood was taken for corticosterone determinations. The following day basal levels of corticosterone were obtained by collecting blood samples by decapitation within a few seconds of handling the animals.

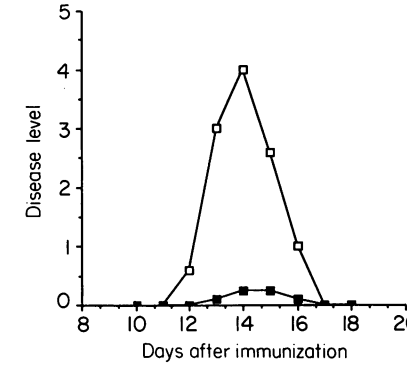

Figure 1. Comparison of the susceptibility to EAE of PVG (■) and Lewis (□) strain rats. On Day 0 of the experiment, age- and sex-matched PVG and Lewis rats were immunized in the hind footpads with 50 μg guinea-pig MBP in FCA (MacPhee *et al.*, 1989) and observed for the signs of EAE. There were six PVG rats and five Lewis: all were female since males tend to develop retention of urine with subsequent hydronephrosis.

Determination of serum corticosterone levels

These were made by radioimmunoassay (MacPhee *et al.*, 1989).

RESULTS

In confirmation of published data (Hughes & Stedronska, 1973; Ben-Nun *et al.*, 1982), the results presented in Fig. 1 illustrate the resistance of PVG rats to EAE when the adrenal glands are not removed. In contrast, as the data in Table 1 show, six adrenalectomized PVG rats, given a subcutaneous corticosterone implant (Dallman *et al.*, 1987) to maintain basal serum steroid levels before being immunized with MBP, developed fatal paralysis with no survivors beyond Day 16 after injection of the encephalitogen (Group 2). (The steroid implant was necessary to prevent the acute lethal effects of immunization with FCA; MacPhee *et al.*, 1989.) A virtually identical result was obtained with rats given a second low-dose corticosterone implant 2 days after MBP injection (Group 3). The six rats in Group 4 were treated with a steroid replacement protocol that simulated the corticosterone levels that would develop in normal Lewis rats with EAE. The disease course in these PVG rats closely resembled that in non-adrenalectomized Lewis strain animals (Fig. 1) and all became long-term survivors although no further steroids were given. As expected, the six non-adrenalectomized control animals (Group 1) developed no clinical signs of EAE.

This indication that PVG rats might have corticosterone responses that are generally more vigorous that those of the Lewis strain received support from the finding that, in age- and sex-matched groups of animals, the ratio of adrenal weight to total body weight was about 60% greater in the former strain (Table 2). In this table all of the rats with PVG background genes had higher adrenal weights than any of the rats in the last two groups ($P < 10^{-6}$). Further, the corticosterone response of PVG rats to stress induced by ether anaesthesia and venesection was 41% higher than that of Lewis strain animals, and basal

Table 1. Effect of adrenalectomy and corticosterone replacement therapy on EAE in PVG strain rats

		Steroid replacement			Mean disease score on day								
Group	Treatment	25%	25%	50%	11	12	13	14	15	16	17	18	19
1	Sham	–	–	–	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
						*		**		***			
2	Adx.	+	–	–	0·0	2·2	2·6	4·0	4·0	5·0	—	—	—
							***		**	*			
3	Adx.	+	+	–	0·0	3·2	4·0	3·0	4·5	5·0	—	—	—
4	Adx.	+	+	+	0·0	0·2	1·0	2·5	2·5	1·3	0·3	0·3	0·0

Closely age-matched female PVG rats were adrenalectomized (Adx) or sham adrenalectomized on Day 0 of the experiment and immunized with 50 μg MBP in FCA. Those that were adrenalectomized were given a subcutaneous implant of 25 mg corticosterone dissolved in 75 mg cholesterol 3 days before the operation. Those in Groups 3 and 4 received a second pellet of the same composition on Day 3 and the Group 4 animals were given an additional pellet, this time of 50 mg corticosterone in 50 mg cholesterol, on Day 11. There were six rats in Groups 1, 2 and 4 and five in Group 3.

* Indicates fatal disease.

Table 2. Ratio of weights of adrenal glands to body weights in different rat strains

Strain	MHC	Adrenal wt (mg)/ body wt (g) (SE)
PVG	c	0·396 (0·02)
PVG.RT1^{l}	l	0·368 (0·02)
PVG.RT1^{u}	u	0·392 (0·03)
LEW	l	0·257 (0·03)
AO^{u}	u	0·212 (0·02)

All rats were matched for age and sex (female) and were 8–10 weeks old. They were all from the same specific pathogen-free source and were caged in groups of four to five. There were five to ten animals in each group.

Table 3. Serum corticosterone levels in PVG and Lewis strain rats subject to stress induced by ether anaesthesia

	Serum corticosterone nM (SE)	
Strain	Basal level	40-min level
PVG	166 (10)	2344 (164)
Lewis	104 (18)	1656 (132)

Groups of five to six adult PVG and Lewis rats were subjected to ether stress as described in the Materials and Methods. Blood samples were taken for corticosterone measurements at this time and again 24 hr later when the animals were not stressed. Results are shown ± SE. The steroid levels observed in the PVG rats were significantly higher (basal, $P < 0{\cdot}05$; 40 min, $P < 0{\cdot}01$) by two-tailed Student's *t*-test.

levels of the hormone in the morning (the nadir of the circadian rhythm) were 60% higher in the PVG strain rats (Table 3), approximately concordant with the higher adrenal gland weights (Table 2).

To determine whether there was a critical time at which the adrenal glands acted to prevent the development of EAE, PVG rats were given a low-dose corticosterone implant subcutaneously and immunized with MBP 3 days later. The rats were divided into three groups, defined by the time interval between immunization and subsequent adrenalectomy. As Table 4 shows, delaying adrenalectomy by 8 days produced disease with a time of onset and severity in distinguishable from that obtained in rats that were adrenalectomized at the time of immunization, indicating that the essential immunosuppressive effect of the adrenal gland occurred later than this. However, delaying adrenalectomy to Day 14 allowed long-term survival of the rats with no signs of disease. This latter finding is consistent with the earlier conclusion, using Lewis rats, that the long-term refractory phase that develops in rats that have recovered from EAE does not depend on corticosterone-mediated suppression (MacPhee *et al.*, 1989). In a second experiment adrenalectomy was delayed until Day 9: the three rats in this group also developed fatal paralysis but rats adrenalectomized on Days 12 or 15 showed no signs of disease (data not shown).

It appears from these kinetic studies that the adrenal gland starts its immunoregulatory role in EAE in the short time interval between Day 9 after immunization and the appearance of signs of disease, usually on Day 11 or 12: it seems that the critical time is 24–48 hr before paralysis occurs. By Day 12–14 the adrenal glands could be removed without the subsequent development of disease, indicating that the long-term refractory phase (MacPhee *et al.*, 1989) is sufficiently established by this time to prevent detectable disturbance of central nervous system (CNS) function.

Table 4. Effect of delayed adrenalectomy on the development of EAE in PVG rats

Day of Adx.	Rat no.	Day after immunization								
		10	11	12	13	14	15	16	17	18
0	1	0	0	1	2	4	2	ND	*	—
	2	0	3	*	—	—	—	—	—	—
	3	0	0	4	*	—	—	—	—	—
	4	3	*	—	—	—	—	—	—	—
	5	0	1	4	4	*	—	—	—	—
8	6	0	0	0	2	2	2	ND	*	—
	7	0	0	0	1	4	*	—	—	—
	8	0	1	4	*	—	—	—	—	—
	9	0	0	1	4	*	—	—	—	—
	10	0	0	0	1	2	*	—	—	—
14	11	0	0	0	1	1	0	ND	0	0
	12	0	0	0	0	0	0	ND	0	0
	13	0	0	0	0	0	0	ND	0	0
	14	0	0	0	0	0	0	ND	0	0

Fourteen female PVG rats were given a 25 mg corticosterone/75 mg cholesterol implant subcutaneously on Day −3. Three days later, on Day 0 of the experiment, five animals were adrenalectomized and all 14 were immunized with MBP/FCA. Of the remaining nine animals, five were adrenalectomized on Day 8 and the rest on Day 14. The tabulated numbers are the disease scored for each rat.

* All rats in the first two groups developed fatal disease.

ND, not done.

As Table 2 shows, a high ratio of adrenal weight/body weight appears to be associated with the PVG background genes rather than the RT1^c MHC genes. If these background genes were responsible for the refractoriness of the PVG strain to EAE then one would predict that the congenic strain PVG.RT1^l, which possesses the MHC of Lewis but the non-MHC genes of PVG, would be non-susceptible to EAE unless the adrenal glands were removed. This prediction was verified: of 10 adrenalectomized PVG.RT1^l rats, nine developed EAE. In contrast, only one of nine control rats developed EAE and this was very mild ($P<0{\cdot}01$).

In addition to the role played by non-MHC PVG genes in preventing EAE in PVG and PVG.RT1^l rats, it is evident that MHC genes can also determine susceptibility to this disease (Gasser *et al.*, 1975; Moore *et al.*, 1980; Hughes & Stedronska, 1973). In the present experiments adrenalectomy of AO and PVG.RT1^u rats did not change their normal non-susceptible status (data not shown).

DISCUSSION

The foregoing experiments show that genetic variations in the neuroendocrine regulation of the immune response can determine the susceptibility of different rat strains to EAE. The effect is a potent one in that resistance to disease may be virtually complete, as in the PVG strain, while the susceptible Lewis develops hind limb paralysis and incontinence. There can be little doubt that the mediator of the resistance to EAE is corticosterone: not only does the PVG strain have larger adrenal glands than the susceptible Lewis and produce higher levels of corticosterone in response to stress but it could be rendered susceptible to EAE by adrenalectomy and this effect could be reversed by replacement steroid therapy. (The ability of exogenous corticosterone to produce such a reversal indicates that any part played by hormones of the adrenal medulla was of secondary importance in determining the resistance of PVG strain rats to EAE.) Studies of the susceptibility of different rat strains to streptococcal cell wall arthritis have also concluded that the neuroendocrine regulation of the immune response in Lewis rats is relatively deficient (Sternberg *et al.*, 1989) in comparison to disease-resistant strains. As in the present study, the authors were unable to precisely define the site of the defect. Table 3 shows that basal levels of corticosterone in PVG rats were significantly higher than those in Lewis. Furthermore, basal levels in the former strain proved much more labile and the levels recorded in the table could be obtained only by caging animals singly and reducing environmental and handling stresses to a minimum. In more normal conditions corticosterone levels in PVG rats, unlike those in Lewis strain animals, fluctuated between 2 and 4 times the basal levels (data not shown).

Several investigators have proposed that cytokines, produced in the immune response, induce endocrine-mediated suppression of the immune system (Munck, Guyre & Holbrook, 1984; Besedovsky *et al.*, 1986; Kroemer *et al.*, 1988; Berkenbosch *et al.*, 1987; Sapolsky *et al.*, 1987) and there is evidence that lymphokines can act on the neuroendocrine system at several levels (Munck *et al.*, 1984; Besedovsky *et al.*, 1986; Kroemer *et al.*, 1988; Berkenbosch *et al.*, 1987; Sapolsky *et al.*, 1987; Oppenheim *et al.*, 1986; Brown, Smith & Blalock, 1987; Weigent & Blalock, 1987; Smith, Meyer & Blalock, 1982). It is unclear to what extent such a feedback loop is involved in the present experiments, where disease-induced (MacPhee *et al.*, 1989; Levine *et al.*, 1980) and environmental (Levine *et al.*, 1962) stress may be expected to lead to corticosterone release. It has been noted that, in Lewis rats, serum corticosterone levels rise just before signs of EAE appear, i.e. at about the time that inflammatory cells begin to invade the CNS (MacPhee *et al.*, 1989). It may be that inflammation within the CNS evokes the release of corticosterone in EAE and that PVG rats are hypersensitive in this regard. However, given the environmentally induced changes in serum corticosterone level in this strain it is possible that environmental factors alone ensure protection from EAE.

Genetically determined variations in the neuroendocrine regulation of the immune response have also been recorded in chickens, where there is some evidence that they play a role in determining susceptibility to the development of autoimmune thyroidiotis, a disease that develops spontaneously in some strains (Kroemer *et al.*, 1988). In an outbred population like man it is to be anticipated that similar variations in the potency of neuroendocrine regulation of the immune response will occur and these could affect disease susceptibility. It would be of interest, therefore, to know whether, for example, individuals that develop multiple sclerosis produce lower levels of serum cortisol in response to stress than normal controls.

Finally, it should be noted that not all rat strains are resistant to EAE by virtue of their corticosterone response to stress. In two strains studied, both of RT1^u MHC haplotype, adrenalectomy failed to make these normally resistant strains susceptible

to EAE. Since one of these strains had the background genes of PVG, its failure to develop EAE on adrenalectomy clearly maps the immune-response gene effect to the MHC.

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