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Host Genetic Regulation of Acute MHV-4 Viral Encephalomyelitis and Acute Experimental Autoimmune Encephalomyelitis in (BALB/cKe × SJL/J) Recombinant-Inbred Mice

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Summary

In the present report we provide the strain distribution patterns of susceptibility to acute mouse hepatitis virus type-4 (MHV-4) encephalomyelitis, acute experimental allergic encephalomyelitis (EAE) and vasoactive amine sensitivity (VAAS) for 9 (CXJ) recombinant-inbred strains between BALB/cKe (C) and SJL/J (J) mice. We confirm that susceptibility to MHV-4 is not linked to the H-2 complex, and that all strains susceptible to acute EAE have both a responder H-2 haplotype ($H\text{-}2^s$ or $H\text{-}2^d$) and induced (*B. pertussis*) VAAS. In addition, we provide evidence that susceptibility to acute EAE induction is controlled by an additional presently unmapped locus and that an EAE-like histopathological disease does not usually follow MHV-4 infection intracerebrally in animals susceptible to MHV-4, acute EAE and induced VAAS.

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Introduction

Genetic factors regulate susceptibility to viral infection and specific immune responses to a variety of antigens. Viral susceptibility genes are usually located outside of the major histocompatibility complex (MHC), the H-2 region on murine chromosome 17 (Brinton and Nathanson 1981), while many immune response genes are located within the MHC (Benacerraf and McDevitt 1972). Genetically regulated virus-induced and immune-mediated models of demyelination in the mouse illustrate the role of specific host genes.

Susceptibility to acute MHV-4 encephalomyelitis is controlled by a single autosomal dominant gene, unlinked to the H-2 complex (Knobler et al. 1981). Acute infection in susceptible strains is frequently characterized by a fatal necrotizing encephalomyelitits, but, survivors develop demyelinating disease due to direct infection of oligodendrocytes without perivascular infiltration of mononuclear cells (Bailey et al. 1949; Waksman and Adams 1962; Lampert et al. 1973; Weiner 1973). The incidence of demyelination can be enhanced by infection with an attenuated temperature-sensitive mutant of MHV-4, designated ts8 (Haspel et al. 1978). MHV-4 induced disease has been extensively studied in BALB/c mice (Knobler et al. 1982a, b), but does not usually occur in SJL/J mice which lack the gene for susceptibility (Stohlman and Frelinger 1978; Knobler et al. 1981, 1982a).

Susceptibility to acute experimental autoimmune encephalomyelitis (EAE), an acute perivascular inflammatory demyelinating disease, is controlled by at least two genes, one, H-2 linked [responder haplotype ($H\text{-}2^s$, $H\text{-}2^q$ or $H\text{-}2^d$)]; the other associated with natural vasoactive amine sensitivity (VAAS) or that induced by *Bordetella pertussis* (Linthicum and Frelinger 1982). Acute EAE is easily induced in SJL/J mice, but rarely in BALB/c mice (Levine and Sowinski 1973; Bernard 1976; Lando et al. 1979; Raine et al. 1980) which are genetically resistant to VAAS and the histamine sensitizing factor (HSF) of *B. pertussis* vaccine, although they have an $H\text{-}2^d$ responder haplotype for EAE (Linthicum and Frelinger 1982).

The development of recombinant-inbred (RI) strains allows determination of genetic linkage, characterization of multiple gene inheritance and investigation of genetic reassortments. RI strains are derived by systematic inbreeding of progenitors differing at identifiable loci, providing a set of strains containing a fixed recombination of linked alleles characteristic of either progenitor strain (Bailey 1971; Taylor 1981). The more gene loci mapped the more useful the RI strains become in detecting linkage. Once the strain distribution pattern (SDP) is established for a large enough set of markers, the SDP of any new one can be compared to establish linkage. Nine RI strains between BALB/cKe and SJL/J (CXJ) have been tested to provide the SDP of susceptibility to acute MHV-4 encephalomyelitis, acute EAE and VAAS.

Materials and Methods

Virus

A seed of MHV-4 initially provided by Dr. L. Weiner (Departments of Neurology and Microbiology, University of Southern California) was plaque-purified 3 times and grown as a stock in L-24-1 cells. The handling of virus, culture and plaque assay conditions for MHV-4 and ts8 have been reported elsewhere (Haspel et al. 1978; Knobler et al. 1981, 1982a).

Mice

The (CXJ) RI lines were initially derived in 1978 (in the laboratory of M.C., Salk Institute for Biological Studies) from the successive breeding of F_2 offspring obtained by mating F_1 hybrid mice (1 SJL/J male × 2 BALB/cKe females and 1 BALB/cKe male × 2SJL/J females), F_2 offspring were brother-sister-mated for 21 successive generations and at least 9 experimental colonies (CXJ-1, -3, -4, -5, -8, -9, -10, -11, -15) were thus established. Progenitor strains BALB/cKe and SJL/J, and SJA_{20} (congenic with SJL/J except at Igh locus; gift of Roy Riblet) were also maintained in the vivarium of the Salk Institute. Mice of both sexes at 6–10 wk of age were used for these studies.

Characterization of the H-2 haplotype

These were determined by PVP microhemagglutination (Takasugi and Hildemann 1969), with $H\text{-}2^s$ or $H\text{-}2^d$ specific antisera (from Dr. R. Hyman) and primary MLR of each parent against each RI strain, which could rule out intra-H-2 recombinants.

Preparation and infection of peritoneal elicited macrophages

Macrophages were obtained from the peritoneal cavities of no fewer than 4 mice, of each strain tested, inoculated intraperitoneally with 2 ml of 3.8% thioglycollate broth (Brewer's modified thioglycollate, Becton, Dickinson and Co, Cockeysville, MD). Five days later peritoneal exudate cells were harvested by lavage with 5–6 ml of sterile Eagle's minimal essential media which contained 20 units/ml heparin, 1% glutamine, 100 μg/ml penicillin and streptomycin, while the animal was under ether anesthesia. Cells were spun down, resuspended in fresh media and counted. Sufficient numbers of cells were plated to provide 1×10^6 adherent cells/dish. Nonadherent cells were removed 1–2 h after plating by vigorous washing, as previously reported (Brautigam et al. 1979; Knobler et al. 1981). Adherent cells were identified as macrophages by their ability to ingest zymosan particles, and by their morphology (Van Furth et al. 1978). Homogeneity was usually > 95% with a range of 95–99%.

Macrophage cultures were infected at a multiplicity of infection (MOI) of 0.1, by adsorbing virus for 1 h at 37°C and then washing the cells 3 times in phosphate-buffered saline (pH 7.4) before replacing fresh media. The cells were incubated at 37°C in a 5% CO_2 atmosphere and observed for the development of cytopathic effect 24–48 h after infection. MHV-4 permissive macrophages fuse to form multinucleated giant cells (syncytia) within 18 h after infection. Nonpermissive cells do not fuse. In addition, the 3rd wash after infection and the supernatant culture fluids at 24 h after infection were assayed to quantitate virus replication.

Infection and evaluation of viral encephalomyelitis

A minimum of 5 but usually more mice of each strain were inoculated intracerebrally with 0.05 ml of MHV-4 or ts8 while under ether anesthesia. This inoculum contained 1000 PFU MHV-4 or 10 000 PFU ts8. Mice were observed daily for signs of illness which included ruffled fur, irritability, lethargy, limb paralysis and death. Surviving animals were killed at 21 and 60 days while under ether anesthesia, by exsanguination followed by intracardiac perfusion with 10% formalin. After fixation, the brain was embedded transversely and the spinal cord was embedded longitudinally in paraffin, sectioned and stained with hematoxylin and eosin (H&E).

Induction and evaluation of EAE

Mice were immunized with mouse spinal cord homogenate (MSCH), 10 mg dry weight, suspended in saline and emulsified in an equal volume of Freund's complete adjuvant supplemented with 5 mg/ml *Mycobacterium tuberculosis* (Difco, Detroit, MI, H37RA). All four footpads were injected, the total inoculum volume being 0.1 ml. Immediately thereafter and 48 h later, *B. pertussis* vaccine, 15×10^9 organisms, was given i.v. This immunization procedure produces 95–100% incidence of EAE in (SJL × BALB/c) F_1 hybrids (Linthicum and Frelinger 1982).

Mice were examined daily for clinical signs of EAE, scored on a scale of 0–3: 0 (no disease), 1 (tail atonia, slight hind limb weakness), 2 (hind limb paralysis, incontinence of bladder), and 3 (moribund state or death due to EAE). Histological assessment of EAE was made on mid-sagittal paraffin sections of brain and spinal cord stained with H&E, without knowledge of the specific CXJ strain. The size and frequency of perivascular mononuclear infiltrates in the white matter of the CNS were graded on a scale of 0–3, as 0 (no lesions), 1 (few lesions, mainly leptomeningial and ependymal), 2 (numerous infiltrates in the white matter of the brain stem, cerebellum, and spinal cord), and 3 (florid lesions throughout the brain and spinal cord white matter). A minimum of 4 mice but usually more of each strain were tested. Body temperature was measured using a Bailey BAT-9 thermistor probe. The rectal temperature of mice immunized for EAE was measured each day in the afternoon. Body weights were determined by weighing mice on an Ohaus B1500D digital balance with ± 0.01 g sensitivity.

Vasoactive amine (VAA) sensitivity determinations

B. pertussis has been demonstrated to induce a 'hypersensitivity' to both VAA serotonin and histamine (Bergman and Munoz 1968) which is characterized by the onset of hypotensive and hypovolemic shock following VAA challenge. For the sake of simplicity, we chose to test 'natural' and 'induced' hypersensitivity to VAA with only histamine. To determine 'natural' histamine sensitivity, naive mice were challenged with 5 mg histamine i.p. and the deaths recorded 2 h later. 'Induced' histamine hypersensitivity due to *B. pertussis* administration was determined by intraperitoneal injection of 1 mg histamine free base (denoted as 'challenge') in 0.2 ml neutral buffered saline 4 days after the initial inoculation of 5×10^9 *B. pertussis* whole cells. Deaths (due to hypotensive and hypovolemic shock) were recorded over

the next 2 h, and the results reexpressed as number of deaths divided by the total number of animals challenged. Mice challenged with 1 mg histamine without pretreatment with *B. pertussis* served as negative controls.

Results

MHV-4 replication in macrophages

Following MHV-4 infection of permissive macrophages (BALB/cKe, CXJ-4, -6, -8, -9, -11) at an MOI of 0.1, 10^4–10^5 PFU/ml of virus was detected in the supernatant culture fluid at 24 h after infection (Table 1). This was not due to the carryover of virus inoculum, since viral content of the 3rd PBS wash after infection was below the level of detection (< 10 PFU/ml). Cytopathic effects (CPE) in permissive macrophages was fusion to form multi-nucleated giant cells (syncytia). In contrast, at 24 h after infection the viral content of the supernatant culture fluid of non-permissive macrophages (SJL/J, SJA_{20}, CXJ-1, -3, -10, -15) was below the level of detection, and there was no evidence of CPE. Further, there was neither CPE nor evidence of viral replication in the non-permissive macrophage cultures at 48 h after infection and later. Aging the non-permissive macrophages up to 4 weeks prior to infection did not alter their response to MHV-4.

Susceptibility to MHV-4-induced encephalomyelitis

The intracerebral inoculation of 1 000 PFU of MHV-4 represents an infection of approximately 1 000 LD_{50} for susceptible strains of mice, such as BALB/c. All mice of each strain with MHV-4 permissive macrophages (BALB/cKe, CXJ-4, -6, 8, -9, -11) were dead at 3 days after infection (Table 1). In contrast, all mice of each strain with macrophages non-permissive for MHV-4 (SJL, SJA_{20}, CXJ-1, -3, -10, -15) survived intracerebral challenge with 1 000 BALB/c LD_{50} (Table 1), except CXJ-3. The CXJ-3 strain had 2 fatalities (5 and 7 days after infection) and the appearance of clinical symptoms in a 3rd mouse, which persisted until killed at 3 weeks after infection. Five CXJ-3 mice challenged with 10-fold less virus (100 BALB LD_{50}) showed no clinical symptomatology and survived until killed at 3 weeks.

In the MHV-4 susceptible strains (BALB/cKe, CXJ-4, -6, -8, -9, -11) symptoms included ruffled fur, irritability which progressed to lethargy and a moribund state. Histopathologically these mice showed evidence of a severe necrotizing encephalomyelitis, enlarged ventricles, with infiltration of polymorphonuclear and mononuclear cells in the meninges and choroid plexus, hippocampus, diencephalon, pons extending into the cerebellum and the spinal cord (Knobler et al. 1982a). Similar findings were observed in the two CXJ-3 animals that died. In the symptomatic surviving CXJ-3 mouse symptoms included ruffled fur, lethargy and bilateral hind limb paralysis. Histopathologically this mouse showed evidence of demyelinating lesions, however, infiltrating mononuclear cells were not in a perivascular distribution. Similar histopathological findings were apparent at 21 and 60 days after i.c. infection with ts8 in BALB/cKe, CXJ-4, -6, -8, -9 and -11 mice. The asymptomatic CXJ-3 mice and the other asymptomatic survivors (SJL/J, SJA_{20}, CXJ-1, -10, -15),

TABLE 1

SUSCEPTIBILITY TO MHV-4 EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND TO NATURAL AND *B. PERTUSSIS*-INDUCED HISTAMINE HYPERSENSITIVITY IN (CXJ) RI MICE AND RELATED STRAINS

Strain	H-2	In vitro macrophage MHV-4 response		In vivo MHV-4 response 1000 BALB/c LD_{50}		Acute EAE indices		% Histamine sensitivity	
						Clinical	Histological	Natural	*B. pertussis*
		CPE	PFU/ml	Symptomatic	Survival				
BALB/c	d	+ + +	$>10^4$	5/5	0/5	0.0 (0/6)	0.0 (0/6)	0 (0/4)	0 (0/4)
SJL	s	nil	<10	0/5	5/5	ND	ND	ND	ND
SJL/J × BALB/c)F_1	s/d	+ + +	$>10^5$	5/5	0/5	2.3 (11/12)	2.4 (11/11)	0 (0/6)	100 (6/6)
CXJ-1	d	nil	<10	0/5	5/5	1.8 (8/10)	2.3 (9/10)	83 (5/6)	88 (14/16)
CXJ-3	d	nil	<10	3/5	2/5	0.0 (0/6)	0.0 (0/6)	100 (6/6)	74 (14/19)
CXJ-4	s	+ + +	$>10^5$	5/5	0/5	2.5 (4/4)	2.5 (2/5)	0 (0/4)	50 (9/18)
CXJ-6	s	+ + +	$>10^4$	5/5	0/5	1.9 (6/8)	1.9 (6/8)	0 (0/6)	100 (10/10)
CXJ-8	d	+ + +	$>10^5$	5/5	0/5	1.1 (4/8)	1.1 (6/8)	0 (0/20)	91 (21/22)
CXJ-9	d	+ + +	$>10^5$	5/5	0/5	0.1 (1/16)	0.1 (1/16)	0 (0/7)	89 (16/18)
CXJ-10	d	nil	<10	0/5	5/5	0.0 (0/7)	0.1 (1/7)	0 (0/4)	80 (4/5)
CXJ-11	d	+ + +	$>10^5$	5/5	0/5	2.5 (4/4)	2.5 (4/4)	0 (0/5)	100 (5/5)
CXJ-15	s	nil	<10	0/5	5/5	0.0 (0/8)	0.1 (1/8)	0 (0/4)	100 (10/10)
SJA_{20}	s	nil	<10	0/5	5/5	2.8 (5/5)	2.6 (5/5)	100 (5/5)	100 (5/5)

and the 5 CXJ-3 challenged with 100 PFU had histopathological findings confined to the inoculation track.

EAE susceptibility

Of the 9 RI strains examined, 5 strains (CXJ-1, -4, -6, -8, -11) developed clinical and histopathological signs of EAE and 4 strains (CXJ-3, -9, -10, -15) were not susceptible (Table 1). Parental SJL/J, congenic SJA_{20} and (SJL × BALB/c)F_1 hybrid mice were all susceptible to EAE, while BALB/c mice were not. Susceptibility to EAE did not require 'natural' histamine sensitivity, but all strains which developed EAE were susceptible to *B. pertussis*-induced VAA hypersensitivity (Table 1).

Using body weight and rectal thermometer probe temperature readings we followed several strains (Fig. 1) during the course of EAE development and found that a dramatic drop in body weight and temperature was an indicator of disease intensity and duration.

Natural and B. pertussis-induced sensitivity to histamine

Of the 9 RI strains of mice tested only 2 (CXJ-1 and -3) revealed a 'natural' sensitivity to histamine (Table 1); this trait was also observed in the original SJL/J parental and congenic SJA_{20} strains. The other RI strains tested (CXJ-4, -6, -8, -9, -10, -11, -15) were naturally resistant to histamine, a characteristic which is shared by the BALB/cKe parental strain and (SJL/J × BALB/c)F_1 hybrids. Inoculation with *B. pertussis* caused an increased sensitivity to histamine in most individuals of all RI strains, and parental SJL/J and the congenic SJA_{20} mice (Table 1). This was

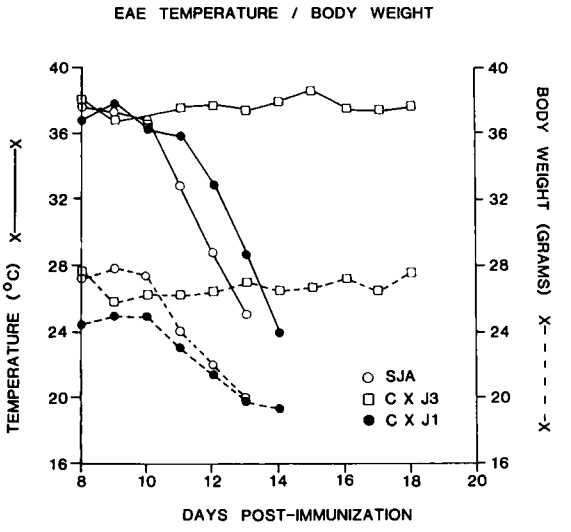

Fig. 1. Temperature and body weight as an indication of acute EAE intensity and duration. Animals sensitive to acute EAE (SJA, CXJ-1) show a drop in temperature and body weight, while animals resistant to EAE (CXJ-3) show no such change.

not observed in the BALB/c strain although (SJL/J × BALB/c)F_1 hybrids are susceptible to *B. pertussis*-induced histamine hypersensitivity.

Discussion

In the present report we have provided the strain distribution pattern (SDP) of susceptibility to acute MHV-4 encephalomyelitis, acute EAE and VAAS for 9 (CXJ) RI strains between BALB/cKe and SJL/J mice. We confirm that susceptibility to MHV-4 is unlinked to the H-2 complex and is consistent with control by a single gene locus (Knobler et al. 1981). We demonstrate that despite all 9 CXJ strains having both a required responder H-2 haplotype (H-2^s or H-2^d) and induced (*B. pertussis*) VAAS for susceptibility to acute EAE induction (Linthicum and Frelinger 1982), 4 strains (CXJ-3, -9, -10 and -15) are resistant, indicating control by at least an additional presently unmapped, genetic locus. Finally, although some (CXJ) RI strains are susceptible to acute EAE, induced VAAS and acute MHV-4 encephalomyelitis EAE-like histopathological disease does not occur in these animals following MHV-4 intracerebral infection.

RI strains are derived by systematic inbreeding of 2 pre-existing inbred progenitor strains that are unrelated (Bailey 1971; Taylor 1981). For a trait that differentiates the progenitor strains, one or the other progenitor phenotypes will be recovered in association with each derived RI strain if it is controlled by a single locus. The appearance of novel phenotypes indicates a more complex pattern of inheritance. The probability of control by one or more loci may be calculated from the distribution of responses amongst the RI strains tested (Taylor 1976; Bailey 1981). The difference between BALB/c and SJL/J for susceptibility to MHV-4 (Table 2) is

TABLE 2

SUMMARY OF FINDINGS IN (CXJ) RECOMBINANT INBRED MICE

Strain designation	H-2 type	MHV-4 macrophage response	MHV-4 in vivo response	EAE	Histamine sensitivity	
					Natural histamine	Pertussis challenge
BALB/cKe (C)	H-2^d	Sensitive	Sensitive	Resistant	Resistant	Resistant
SJL/J (J)	H-2^s	Resistant	Resistant	Sensitive	Sensitive	Sensitive
(SJL/J × BALB/c)F_1	C/J	C	C	J	C	J
CXJ-1	C	J	J	J	J	J
CXJ-3	C	J	J	C	J	J
CXJ-4	J	C	C	J	C	J
CXJ-6	J	C	C	J	C	J
CXJ-8	C	C	C	J	C	J
CXJ-9	C	C	C	C	C	J
CXJ-10	C	J	J	C	C	J
CXJ-11	C	C	C	J	C	J
CXJ-15	J	J	J	C	C	J

TABLE 3

CALCULATION OF THE NUMBER OF GENES DIFFERING BETWEEN BALB/c AND SJL/J REGULATING DIFFERENT PHENOTYPES

Probabilities of occurrence were obtained from mathematical tables comparing the frequencies found with the expected frequency for a one- or more-gene difference regulating the phenotypes characterized.

	MHV-4			EAE			Natural histamine sensitivity				Pertussis enhanced histamine sensitivity			
	Gene difference			Gene difference			Gene difference				Gene difference			
	1	2	3	1	2	3	1	2	3	4	1	2	3	4
Expected frequency	0.5	0.25	0.125	0.5	0.25	0.125	0.5	0.25	0.125	0.0624	0.5	0.25	0.125	0.0024
Probability of occurrence	[0.2461]	0.1168	0.018	[0.2461]	0.0389	0.0029	0.0703	[0.3003]	[0.2159]	0.07	0.002	0.0751	[0.03095]	[0.70]
Frequency found	4/9 (0.444)			5/9 (0/555)			2/9 (0.222)				0/9			

[ ] Compatible with a gene difference of the indicated number of genes.

_____ Ruled out as incompatible with a gene difference of the indicated number of genes.

- - - - - - Ruled out borderline as incompatible with a gene difference of the indicated number of genes.

compatible with control by one locus (Table 3), marginally compatible with two ($P = 0.1$) and incompatible with three ($P = 0.02$). This gene is unlinked to the other markers analyzed here, i.e., EAE and VAAS, and unlinked to other markers established in the (CXJ) RI strains on chromosomes 1, 5, 12, 16 and 17 (data not shown). Recent work maps this gene to the proximal end of murine chromosome 7 where it is linked to the *Svp-2* locus (Knobler et al., in press). Control of resistance to acute MHV infection by a single recessive gene has also been reported for MHV-2 (Weiser et al. 1976) and MHV-3 (Levy-Leblond et al. 1979). Our results indicating a single gene controlling MHV-4 differ, however, from a proposed two-gene model (Stohlman and Frelinger 1978). The cause for these differences is not apparent.

The difference between BALB/c and SJL/J for susceptibility (sensitivity) to EAE (Table 2) is compatible with a one-gene difference (Table 3) and incompatible with two or more genes ($P = \leq 0.04$); for histamine sensitivity (Table 2) the data is marginally compatible with one gene ($P = 0.07$), compatible with two or three genes (Table 3) and marginally compatible with four genes ($P = 0.07$); for *B. pertussis* enhancement of histamine sensitivity (Table 2), the data is incompatible with one gene ($P = 0.002$), marginally compatible with two genes ($P = 0.07$) and compatible with three or more genes (Table 3). We will discuss the difference between BALB/c and SJL/J for susceptibility to EAE in terms of one gene locus; to histamine, two-gene loci; and to *B. pertussis* enhancement, three loci.

Sensitivity to acute EAE had previously been demonstrated to be inherited in a dominant fashion and dependent upon a background of both an H-2 responder haplotype and sensitivity to histamine inherited in a recessive fashion or dominantly inherited responsiveness to *B. pertussis* enhancement of histamine sensitivity (Linthicum and Frelinger 1982). In the present report, these relevant background genes were present in all 9 (CXJ) strains, which thus unmasked the newly found locus. The distribution of sensitives and resistants based upon a single gene difference would predict a ratio of sensitives to resistants of 1.0. Five sensitives and 4 resistants were found, giving a ratio of 1.25, which is compatible with a single gene difference. Possibly this EAE locus is an IR-gene present in the non-H-2-linked background which controls responsiveness to myelin basic protein (MBP) or another encephalitogenic component of the inoculum used to induce acute EAE. Alternatively, this locus controls a suppressor response to the relevant antigen(s). These hypotheses may be tested by determining immune-responsiveness to MBP or other components in the (CXJ) RI strains to determine if they have a correlation with sensitivity to EAE induction.

The difference between BALB/c (R) and SJL/J (S) as regards histamine sensitivity and its enhancement by *B. pertussis* is a quantitative not qualitative property. Histamine sensitivity was assayed at 5 mg/animal under conditions where the LD_{50} for BALB/c is 17 mg/animal and SJL/J is 0.3 mg/animal (Linthicum and Frelinger 1982). The proposed two-gene difference between the parents and the dominance of the resistant phenotype in the (SJL/J × BALB/c)F_1 hybrid, might be explained as follows:

One gene (H_D) determines a histamine destroying activity, e.g., histaminase, the activity of which is presumed higher in BALB/c (hi) than in SJL/J (lo). The other

gene (H_R) controls the histamine receptor which is proesumed to be of higher affinity in SJL/J (hi) than in BALB/c (lo). The (BALB/c × SJL/J)F_1, under the conditions of our assay (5 mg/animal) would score as resistant (R), protected by the histaminase activity. The four projected phenotypes would be BALB/c ($H_D^{hi}H_R^{lo}$) resistant, SJL/J ($H_D^{lo}H_R^{hi}$) sensitive, (CXJ) RI ($H_D^{hi}H_R^{hi}$) resistant, (CXJ)RI ($H_D^{lo}H_R^{lo}$) resistant. The expected proportion of sensitives in the RI strains would be 1/4 (0.25) and 2/9 (0.29) were found.

The *B. pertussis* enhancement of histamine sensitivity was assayed at 1 mg/animal. Under these conditions, BALB/c ($H_D^{hi}H_R^{lo}$) remains resistant but all of the other genotypes, SJL/J ($H_D^{lo}H_R^{hi}$), (CXJ)RI ($H_D^{lo}H_R^{lo}$) and ($H_D^{hi}H_R^{hi}$) as well as the (SJL/J × BALB/c)F_1 ($H_D^{lo/hi}H_R^{hi/lo}$) score as sensitive. The expected proportion of RI strains scoring as resistant under this assumption would be 1/4, whereas 0.9 were found. This difference is marginally compatible with two genes ($P = 0.07$). If it is assumed that BALB/c and SJL/J differ by a 3rd gene (E) controlling degree of enhancibility [BALB/c(E^{lo}), SJL/J(E^{hi})] then the expected proportion of strains scoring as resistant would become 1/8 versus 0/9 (i.e., $\leq 1/9$) found, a better fit (Table 4). Furthermore, this hypothesis suggests that the RI strains would differ quantitatively in response to *B. pertussis*, which is testable by titration of the enhancement of sensitivity.

Acute EAE is considered by many to be an immune reaction to myelin basic protein (MBP) and other myelin antigens, and has been studied as a model system of multiple sclerosis (Hashim 1978). It has been suggested that a prior viral encounter may lead to development of an EAE-like response in man (Maugh 1977). Recent evidence demonstrates cellular sensitization to MBP may follow acute measles infection in man (Johnson et al. 1984) or MHV-4 infection in rats (Watanabe et al.

TABLE 4

POSSIBLE GENOTYPES AND PREDICTED PHENOTYPES FOR GENETIC CONTROL OF NATURAL AND INDUCED HISTAMINE SENSITIVITY

	Natural sensitivity			*B. pertussis*-induced sensitivity	
	Genes		Phenotype	Gene	Phenotype
	H_D	H_R		E	
BALB/cKe (C)	hi	lo	R	lo	R
	hi	lo	R	hi	S
	lo	hi	S	lo	S
SJL/J (J)	lo	hi	S	hi	S
	hi	hi	R	lo	S
	hi	hi	R	hi	S
	lo	lo	R	lo	S
	lo	lo	R	hi	S

H_D = histamine destroying activity; H_R = histamine receptor; E = enhancing effect of histamine-sensitizing factor from *B. pertussis*; hi = high activity; lo = low activity; R = resistance, while S = sensitivity, both relative to the quantity of histamine challenge.

1983), where clinical disease is associated with central nervous system perivascular infiltration of mononuclear cells. In the present study, 4 RI strains (CXJ-4, -6, -8 and -11) sensitive to both acute MHV-4 encephalomyelitis and acute EAE did not develop perivascular infiltration of mononuclear cells following MHV-4 infection i.c., possibly because death from necrotizing encephalomyelitis occurred prior to the time for such lesions to develop. However, these same strains did not develop perivascular infiltration of mononuclear cells following ts8 infection i.c., although surviving with histopathological evidence of demyelination at 21 and 60 days after infection (data not shown). The lack of such 'chronic' lesions following MHV-4 infection in these mice may reflect absence of natural VAAS characteristic of SJL/J mice (Linthicum and Frelinger 1982), which can develop chronic EAE (Lublin et al. 1981), or a direct or indirect effect of MHV-4 or ts8 infection on cells of the murine immune systems (Knobler et al., unpublished observations) limiting their usefulness as effectors. These possibilities may now be addressed by cell transfer experiments between (CXJ) RI strains.

Finally, these (CXJ) RI strains highlight the usefulness and value for determining the SDP and pattern of gene inheritance for other neurological disorders which differ between BALB and SJL mice such as sensitivity to Theiler's murine encephalomyelitis virus (Lipton and Dal Canto 1979) and measles encephalomyelitis (Rammohan et al. 1981) amongst others.

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