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Locus controlling *Bordetella pertussis*-induced histamine sensitization (*Bphs*), an autoimmune disease-susceptibility gene, maps distal to T-cell receptor β-chain gene on mouse chromosome 6

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ABSTRACT **Pertussis toxin (PTX) is the primary component responsible for eliciting the majority of biological activities associated with *Bordetella pertussis*, including the induction of several tissue-adjuvant models of organ-specific autoimmune disease. PTX, when administered *in vivo*, enhances vascular permeability, which is made manifest by a concomitant increase in sensitivity to a variety of agents and treatments affecting the vascular bed. One such agent is histamine, and the response to PTX, as measured by hypersensitivity following vasoactive amine challenge, is genetically controlled by the *Bphs* locus. Susceptibility to the induction of both experimental allergic encephalomyelitis (EAE) and experimental allergic orchitis (EAO) in mice is associated with, and in the latter case linked to, a susceptible allele at this locus. We report here the mapping of the *Bphs* locus to mouse chromosome 6, telomeric of *Tcrb* and centromeric of *Prp* (*D6Nds8*). This region also contains a number of loci of immunologic relevance including *Igk*, *Ly-2*, *Ly-3*, *Il-5r*, *Ly-35*, *Ly-4*, and *Tnfr-2*.**

Multiple sclerosis (MS) is the major inflammatory disease of the central nervous system (CNS). Both environmental and genetic factors contribute to what is believed to be primarily an immunopathologic etiology (1). CNS vascular permeability (VP) changes have been implicated in both MS and its primary animal model, experimental allergic encephalomyelitis (EAE) (1–3). Recent magnetic resonance imaging studies indicate that increased CNS-VP is a common feature in the brains of MS patients, even during clinically stable periods, and that both the development of new lesions and the enlargement of preexisting lesions appear to be due to a breakdown in the blood–brain barrier (BBB) (4). Such studies support the concept that a pivotal event in lesion formation is an increase in CNS-VP, yet to date the initiating mechanisms that lead to such events in MS and EAE remain ill-defined.

Environmental factors including antecedent infections can alter the permeability of the BBB and even lead to the potentiation of EAE (5). In this regard, *Bordetella pertussis* is particularly important. Normal mice injected with *B. pertussis* exhibit changes in BBB permeability (6), and both pertussis vaccine and pertussis toxin (PTX) are used as ancillary adjuvants in the induction of active EAE in mice and rats (7, 8). *B. pertussis* and PTX are capable of eliciting disease alone, without the use of other adjuvants (7, 8). Disease induction is a function of both its immunopotentiating and vasoactive amine-sensitizing activities (7, 8). The latter phenotype is manifest as death due to hypotensive and hypovolemic shock following vasoactive amine challenge of PTX-treated animals and is controlled by a single autosomal dominant non-*H-2*-linked gene (9, 10), the *B. pertussis*-induced histamine-sensitization locus (*Bphs*). Susceptibility to the induction of EAE (11) and experimental allergic orchitis (EAO) in mice (12) is associated with, and in the latter case linked to, a susceptible allele at the *Bphs* locus.

The *Bphs* locus is also unique in its pleiotropic nature. PTX-treated mice with a susceptible *Bphs* allele also exhibit hypersensitivity to a variety of other agents and treatments that affect the vascular bed. These include infections, endotoxins, x-irradiation, anoxia, cold stress, peptone, bradykinin, methacholine, and serotonin (13, 14).

The association of the *Bphs* locus with organ-specific autoimmune disease and the pivotal role of VP in both CNS immunology and inflammation in general (15) make its identification of primary importance in helping to define a key pathway in the pathophysiology of such responses. With the development of simple sequence length polymorphisms (SSLP) or microsatellites (16, 17) and random amplified polymorphic DNA (RAPD) fragments (18), both of which show DNA variations between inbred strains of mice, it is now feasible to localize the genes controlling even complex traits to specific chromosomal regions (19, 20). This approach may reveal linkage to candidate genes that can be sequenced or it may provide the first step in the positional cloning of the gene of interest. Using microsatellite and RAPD marker loci distributed across the mouse genome, we have mapped the *Bphs* locus distal to *Tcrb* and proximal to *Prp* (*D6Nds8*) on murine chromosome 6.

MATERIALS AND METHODS

Animals. Male and female SJL/J, C3H/HeJ, and CBA/J mice ranging in age from 4 to 6 weeks were purchased from The Jackson Laboratory. All animals were rested 2 weeks prior to testing. (SJL/J × C3H/HeJ)F_1, (SJL/J × CBA/J)F_1, (C3H/HeJ × SJL/J)F_1, (CBA/J × SJL/J)F_1, C3H/HeJ × (C3H/HeJ × SJL/J)F_1, and CBA/J × (CBA/J × SJL/J)F_1 mice were generated in the animal colony at the University of Pennsylvania School of Medicine. Animals were fed Purina mouse pellets and acidified water ad libitum.

Histamine Sensitization. Mice were injected i.p. with 10.0 μg of PTX on day 0. PTX was obtained from John Munoz, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, MT (21). In CFW mice,

Abbreviations: PTX, pertussis toxin; EAE, experimental allergic encephalomyelitis; EAO, experimental allergic orchitis; *Bphs*, *Bordetella pertussis*-induced histamine sensitization; BBB, blood–brain barrier; CNS, central nervous system; VP, vascular permeability; MS, multiple sclerosis; RAPD, random amplified polymorphic DNA; BC1, backcross 1; lod, logarithm of odds; Tnf, tumor necrosis factor; Tcr, T-cell receptor; Il-5, interleukin 5.

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the 50% histamine-sensitizing dose of the PTX preparation was found to be 51.8 ng per animal. Control mice received buffer without PTX. PTX was dissolved in 0.025 M Tris buffer containing 0.5 M NaCl and 0.017% Triton X-100 (pH 7.6) at a concentration of 0.05 mg (dry weight)/ml. Histamine sensitization was determined on day 3 by the i.p. injection of 1.0 mg of histamine (free base) in 0.2 ml of phosphate-buffered saline (PBS). Deaths were recorded 30 min after challenge; in the case of the parental strains and F_1 hybrid populations, the results are expressed as the number of deaths over the total number of animals challenged.

DNA Isolation. Genomic DNA was isolated from liver tissue as described (22). Briefly, 0.5 g of tissue, maintained in liquid nitrogen, was pulverized with a mortar and pestle. The cells were lysed and deproteinized with SDS and proteinase K followed by phenol/chloroform/isoamyl alcohol extraction(s). The DNA was then precipitated in sodium acetate with isopropyl alcohol, suspended in TE (10 mM Tris·HCl, pH 7.4/1 mM EDTA), reprecipitated with ammonium acetate and ethanol, and resuspended in TE. Working aliquots of all DNA samples were prepared by bringing them to the appropriate concentration in TE′ (10 mM Tris·HCl, pH 7.4/0.1 mM EDTA) and were stored at 4°C.

Microsatellite and RAPD Primers. Microsatellite primers were synthesized according to previously published sequences (16, 17, 19, 20). RAPD primers were purchased from Operon Technologies (Alameda, CA). An allele-specific primer set was designed to detect the presence of the SJL/J-derived allele of the T-cell receptor (Tcr) β-chain gene (*Tcrb*) called "*Tcr-Vβ10^a*" (23) in the backcross 1 (BC1) mice. The 5′ primer (GAGCAAACCCTGGACCAC), when used with the 3′ primer common to both alleles (TGGCACAGAGATACACAG), amplifies a 205-base-pair product from *Tcr-Vβ10^a* homozygotes or heterozygotes but will not produce a PCR product from *Tcr-Vβ10^b* homozygotes. Both primers were synthesized at the Hahnemann University Oligonucleotide Synthesis Facility.

Amplification Conditions and Detection of PCR Products. PCR parameters for microsatellite typing were performed as described (16, 17, 19, 20). Both the efficiency and specificity of the PCRs were optimized by titrating primer pairs against various Mg^{2+} concentrations, annealing temperatures, and amplification cycles as described (16, 17, 19, 20). PCRs were carried out on a Perkin–Elmer/Cetus thermal cycler. Two standard temperature–time cycles were used: 1 cycle at 94°C for 3 min; 35 cycles at 94°C for 15 sec, 55°C for 2 min, and 72°C for 2 min; 1 cycle at 72°C for 10 min; 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 30 sec; and 1 cycle at 72°C for 10 min. Microsatellite size variants were resolved by electrophoresis and visualized in ethidium bromide-stained TBE (16)/agarose gels (3% NuSieve and 1% ME agarose) (FMC).

RAPD reactions were carried out as described (18). Amplification was performed on a Techne model MW-1 Dri-Plate Cycler programmed for 45 cycles of 94°C for 1.5 min, 33°C for 2.0 min, and 72°C for 2.5 min. Reaction products were resolved by electrophoresis at 75 V for 4 hr in a 2.5% ME-agarose (SeaKem, FMC) gel containing 1× TAE buffer (18) and visualized with ethidium bromide.

The temperature–time cycle for the *Tcr-Vβ10* allele-specific PCR reactions was 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min for 25 cycles on a Techne MW-1 Dri-Plate cycler. PCR-amplified products were separated on 6% acrylamide gels and detected by staining with ethidium bromide.

Linkage Analysis. Genotype frequency differences were tested by χ^2 in 2 × 2 contingency tables. The maximum likelihood genetic map of chromosome 6 as well as the maximum likelihood positional estimate of the *Bphs* locus was performed with the MAPMAKER program (24).

RESULTS AND DISCUSSION

SJL/J, C3H/HeJ, CBA/J, F_1 hybrid, and BC1 populations were examined for their susceptibility to PTX-induced histamine sensitization (Table 1). Groups of male and female SJL/J, C3H/HeJ, CBA/J, and F_1 hybrid mice, ranging in age from 6 to 8 weeks, were challenged with 1.0 mg of histamine-free base 3 days after the injection of PTX; mice treated with carrier on day 0 served as controls. SJL/J mice and both F_1 hybrid populations clearly exhibited increased sensitivity to histamine after PTX treatment (Table 1). No significant difference was observed between male and female mice, indicating a lack of sex-linked influence. In contrast, neither C3H/HeJ nor CBA/J mice exhibited an increase in death due to hypotensive and hypovolemic shock after PTX pretreatment and histamine challenge. Carrier-treated parental strains and both F_1 hybrid populations also failed to exhibit sensitivity after histamine challenge, as expected.

Our results are consistent with previously published observations that SJL/J mice are highly susceptible to PTX-induced histamine sensitization. In fact, SJL, SWR, and CFW serve as the three susceptible prototype strains, while C3H/HeJ and CBA/J are their resistant counterparts (9–11, 13, 14, 25). Conflicting results have been reported for the majority of other mouse strains (9–11, 25). Factors that contribute to the varying results observed among different laboratories include the route of injection of both PTX and histamine challenge, the preparation of pertussis vaccine or PTX or both, the environmental status of the animals, infectious agents, and the age of the mice (13, 14). With regard to the latter, DBA/2J mice have been reported by Linthicum and Frelinger (11) and Teuscher (12) to be resistant to histamine sensitization, whereas Bergman and Munoz (25) and Wardlaw (9) have found them to be susceptible.

Table 1. Histamine sensitization in parental, F_1 hybrid, and BC1 mice

	Carrier-treated			PTX-treated		
Mouse strain	Male	Female	Total	Male	Female	Total
SJL/J	0/5	0/5	0/10	10/10	9/9	19/19
C3H/HeJ	0/5	0/5	0/10	0/10	0/10	0/20
(SJL/J × C3H/HeJ)F_1	0/4	0/5	0/9	5/5	6/6	11/11
(C3H/HeJ × SJL/J)F_1	0/6	0/4	0/10	7/7	4/4	11/11
CBA /J	0/5	0/5	0/10	0/10	0/10	0/20
(SJL/J × CBA/J)F_1	0/4	0/4	0/8	6/6	5/5	11/11
(CBA/J × SJL/J)F_1	0/4	0/5	0/9	5/5	4/4	9/9
BC1 (C3H/HeJ)	—	—	—	25/54	19/40	62/114
BC1 (CBA/J)	—	—	—	13/30	11/38	24/68

Histamine sensitivity was determined by i.p. injection of 1.0 mg of histamine-free base in 0.2 ml of PBS 3 days after i.p. injection of 10 μg of PTX. Control animals received carrier on day 0 and 1.0 mg of histamine-free base 3 days later. Deaths due to hypotensive and hypovolemic shock were recorded 30 min after histamine challenge.

Subsequent studies in our lab indicate that young DBA/2J mice—i.e., 6–8 weeks old—are resistant, whereas old DBA/2J mice—i.e., 20–24 weeks old—are susceptible (unpublished observations). Similar results are seen with AKR/J and ST/bJ but not with C3H/HeJ and CBA/J mice (9).

BC1 populations were generated by mating (C3H/HeJ × SJL/J)F_1 and (CBA/J × SJL/J)F_1 hybrids with their respective recessive, resistant parental strains. C3H/HeJ ($n = 114$) and CBA/J ($n = 68$) BC1 mice were studied for susceptibility to histamine sensitization (Table 1). If histamine sensitization is controlled by a dominant allele at a single locus, the mortality is expected to be 50% in a BC1 generation. χ^2 goodness of fit tests of mortality in the two populations ($\chi^2 = 0.281$ for C3H/HeJ and $\chi^2 = 2.44$ for CBA/J) indicated that neither significantly deviates from the expected frequency for a single locus. These results are consistent with genetic control by a single dominant locus and are in agreement with the results of previously published genetic analyses (9, 10).

To map the *Bphs* locus within the murine genome, we generated a molecular-based linkage map using both the C3H/HeJ and CBA/J BC1 populations. Previously mapped microsatellites (16, 17, 19, 20) and RAPD markers (18) that distinguish the two sets of parental strains were used. The linkage of marker loci to susceptibility to PTX-induced histamine sensitization was evaluated by a χ^2 test of genotype frequencies in both susceptible and resistant populations (Table 2). Linkage was judged to be significant when χ^2 values exceeded 13.8, the value numerically equivalent to a lod (logarithm of odds) score of 3. Such values assure that the proportion of true linkages determined among significant ones is greater than 95% when testing randomly chosen genetic loci in humans (19). This approach revealed that four markers on chromosome 6 exhibit significant linkage to the susceptible phenotype in the C3H/HeJ BC1 population: *D6Nds3* (*Ly-3*) ($\chi^2 = 45.2$), *D6Mit8* ($\chi^2 = 46.8$), *D6Nds2* ($\chi^2 = 46.1$), and *Prp* (*D6Nds8*) ($\chi^2 = 25.4$). A significant linkage was also found with *Prp* (*D6Nds8*) ($\chi^2 = 19.7$) in the CBA/J BC1 population, although significant linkages with the other three markers were not observed. However, when we combined both BC1 populations, thereby effectively increasing the total number of animals studied to 165, highly significant associations of *Bphs* with *D6Nds3* (*Ly-3*) ($\chi^2 = 50.3$), *D6Mit8* ($\chi^2 = 55.4$), *D6Nds2* ($\chi^2 = 58.2$), and *Prp* (*D6Nds8*) ($\chi^2 = 46.6$) resulted.

Estimates for the location of *Bphs* on chromosome 6 were carried out by multilocus maximum likelihood linkage analysis with the MAPMAKER program. Composite linkage maps of chromosome 6 for both the individual BC1 populations and the combined BC1 populations were generated (Fig. 1). The results of the C3H/HeJ BC1 population localize *Bphs* to the interval bordered by *D6Nds3* and *D6Nds2* (Fig. 1*A*). In contrast, the CBA/J BC1 data place *Bphs* more distal, with the maximum lod score (12.3) at *Prp* (*D6Nds8*). However, the interval can be extended centromeric to also include *D6Nds2* (Lod = 11.2), since little difference exists between the two lod scores. The C3H/HeJ BC1 results are supported by the composite map generated from the combined BC1 data, which clearly localize *Bphs* between *D6Nds3* and *D6Nds2*. Although the maximum likelihood estimate localizes *Bphs* between *D6Nds3* and *D6Nds2*, the data are also consistent with placement within an expanded interval between *Tcrb* and *Prp* (*D6Nds8*). Additional animals and markers will be required to refine the support interval.

Our aim is to identify and characterize the non-major-histocompatibility complex genes that influence susceptibility to a number of tissue-adjuvant models of organ-specific autoimmune disease. The *Bphs* locus is a prime candidate because it has been associated with susceptibility to EAE (11) and linked to EAO (12). In addition to its utility in inducing organ-specific autoimmune disease, *B. pertussis* exhibits a broad range of immunopotentiating activities that make it a useful adjuvant (8). PTX is capable of markedly stimulating antibody production, particularly IgE (14, 33) and also stimulating, augmenting, and prolonging antigen-specific delayed-type hypersensitivity reactions (8, 34). This effect is antigen specific and mediated by $CD4^+$ T cells. PTX also has been shown to greatly enhance the inflammatory and granulomatous response in animals receiving complete Freund's adjuvant (8, 34). Enhancement of inflammation correlates with increased lymphokine/cytokine production by T cells after antigenic stimulation (8, 34).

The exact relationship among the various immunopotentiating activities of PTX and its role in eliciting EAE and EAO is unknown, but the results of our mapping studies raise several intriguing possibilities, particularly as they relate to candidate genes located in the same region. These genes include *Igk*, *Ly-2*, *Ly-3*, *Il-5r* [gene encoding interleukin 5 (Il-5) receptor], *Ly-35*, *Ly-4*, and *Tnfr-2* [gene encoding tumor necrosis factor (Tnf) receptor 2] (35, 36). At present, our mapping data are inadequate to identify which, if any, of these candidate genes is related to the *Bphs* locus. The likelihood that any of these genes are important in susceptibility to EAE and MS as well as EAO is related to its potential role in the immunopathology of the disease process. Preliminary data indicate that the *Bphs* locus may control the sensitivity of SJL/J mice to lymphokines/cytokines such as Tnf (unpublished observation from this laboratory). Thus, the mapping of both *Tnfr-2* and *Il-5r* within the interval encoding *Bphs* and the apparent requirement for lymphokine/cytokine secretion (particularly Tnf) by encephalitogenic (37) and orchitogenic (K. S. K. Tung, personal communication) T-cell lines and clones may be more than mere coincidence. It is conceivable that T cells gain access to the CNS through a combination of events involving lymphokine/cytokine secretion and the expression of a susceptible allele at the *Bphs* locus. The recent demonstration that entry into the CNS is a function of the activation state—i.e., lymphokine/cytokine secretion—of T cells rather than the nature of either the Tcr or major histocompatibility complex-encoded gene products supports this possibility (38).

The importance of CD8 in the EAE process is shown by studies using transgenic mice bred to have two chromosomes bearing a null allele at the *CD8* locus ("homozygous *CD8* knockouts"). Such mice display less mortality but more relapses during the course of EAE (39). Similarly, reduction of circulating CD8 cells by anti-CD8 antibody also increases the frequency of EAE relapse (26). Finally, a statistically significant excess of an allotype at the *Igk* locus [Km(1)] was found among patients with the most severe form of MS (27). It is striking that MS severity is associated with this marker; however, similar results were observed in a study on EAE using a rat BC1 population (28). Formulating a hypothesis on the basis of action of *Bphs* in autoimmune disease susceptibility will be difficult unless we know its precise location and identity. It is clear that further investigation of these loci is needed to assess their individual impact on EAE and MS as well as EAO.

Chromosome 6 is of particular significance to autoimmune disease. The role of *Tcrb* as a genetic factor in susceptibility to EAE is currently surrounded in controversy (29, 30). The same is true for *Tcra* (31, 32, 40). Experimental protocols aimed at modifying the function of autoreactive T cells in both EAE and MS and other autoimmune diseases have clearly shown the role of *Tcrb* gene products in the disease process (41). However, as far as definitive genetic linkage studies are concerned, a consensus is clearly lacking. Our results show that the *Bphs* locus maps telomeric of *Tcrb*, thereby indicating that at least one additional gene important in controlling EAE susceptibility resides on chromosome 6. Similar results have been observed with Theiler's murine

Table 2. Linkage map of the mouse genome and linkage of marker loci with *B. pertussis*-induced histamine sensitization

Chromosome location, cM	Locus*	Sensitive Ho	Sensitive He	Resistant Ho	Resistant He	$\chi^2 > 4$†
		Chromosomes 1–5				
1 (62)	*D1Nds2*	22	19	23	21	
3 (55)	*D3Byu7*	21	27	15	30	
3 (62)	*D3Mit9*	17	28	20	25	
3 (86)	*D3Nds5*	24	21	21	24	
4 (26)	*D4Mit1*	24	21	21	23	
4 (38)	*D4Nds12 (Orm-1)*	24	21	19	26	
5 (46)	*D5Nds4*	22	23	—	—	
		C3H/HeJ × (C3H/HeJ × SJL/J)				
6 (9)	*D6Mit1*	19	35	26	22	
6 (20)	*Tcrb*	19	35	34	15	10.7 (0.18)
6 (32)	*D6Nds3 (Ly-3)*	8	46	40	8	45.2 (0.08)
6 (36)	*D6Mit8*	8	48	40	8	46.8 (0.08)
6 (43)	*D6Nds2*	8	46	41	8	46.1 (0.08)
6 (60)	*D6Nds8 (Prp)*	15	41	35	9	25.4 (0.12)
6 (75)	*D6Mit15*	20	35	25	24	
		CBA/J × (CBA/J × SJL/J)				
6 (9)	*D6Mit1*	14	7	21	21	
6 (20)	*Tcrb*	7	14	28	14	5.0 (0.18)
6 (32)	*D6Nds3 (Ly-3)*	7	14	29	13	5.9 (0.17)
6 (36)	*D6Mit8*	5	16	27	13	8.9 (0.16)
6 (43)	*D6Nds2*	5	16	30	12	11.0 (0.14)
6 (60)	*D6Nds8 (Prp)*	3	18	27	7	19.7 (0.10)
6 (75)	*D6Mit15*	3	17	19	22	4.5 (0.23)
		Combined data chromosome 6				
6 (9)	*D6Mit1*	33	42	47	43	
6 (20)	*Tcrb*	26	49	62	29	17.1 (0.18)
6 (32)	*D6Nds3 (Ly-3)*	15	60	69	21	50.3 (0.12)
6 (36)	*D6Mit8*	13	64	67	21	55.4 (0.11)
6 (43)	*D6Nds2*	13	62	71	20	58.2 (0.11)
6 (60)	*D6Nds8 (Prp)*	18	59	62	16	46.6 (0.12)
6 (75)	*D6Mit15*	23	52	44	46	4.9 (0.24)
		Chromosomes 7–19 and Y				
7 (49)	*D7Nds7 (Hbb)*	20	25	25	20	
8 (33)	*D8Byu4*	23	22	21	24	
8 (33)	*D8Byu5*	23	23	22	23	
9 (68)	*D9Mit18*	26	18	21	24	
10 (21)	*D10Mit3*	23	22	21	24	
10 (50)	*D10Mit12*	23	22	21	24	
11 (59)	*D11Nds8*	22	23	15	30	
12 (18)	*D12Mit2*	30	15	25	20	
12 (65)	*D12Nds7*	25	20	21	24	
14 (3)	*D14Byu1*	20	23	21	23	
15 (14)	*D15Mit5*	27	18	22	23	
15 (45)	*Int-1*	25	20	18	25	
16 (31)	*D16Bvu2*	19	27	26	19	
17 (30)	*D17Mit10*	25	20	15	23	
18 (29)	*D18Mit9*	22	23	24	21	
18 (57)	*D18Mit3*	17	28	26	19	
19 (4)	*D19Nds1*	21	24	15	30	
Y	*DYByu9*‡	22	23	23	21	

Histamine sensitivity was determined by i.p. injection of 1.0 mg of histamine-free base in 0.2 ml of PBS 3 days after i.p. injection of 10.0 μg of PTX. Deaths due to hypotensive and hypovolemic shock were recorded 30 min after histamine challenge.

*Markers are arranged centrometric to telomeric as reported (26–32). Locations are as reported or are best estimates based on comparisons of existing maps. All are PCR-based microsatellites or RAPDs with the exception of *Tcrb*, which was typed by PCR with a *Tcr-Vβ10* allele-specific set or primers distinguishing SJL/J from both C3H/HeJ and CBA/J.

†Genotype frequency differences among susceptible and resistant BC1 mice were evaluated by χ^2 in 2 × 2 contingency tables. Only $\chi^2 > 4$ are shown. He, heterozygous; Ho, homozygous. The number in parentheses is the recombination fraction.

‡*DYByu9* is a 680-base-pair RAPD fragment amplified by using the following 10-mer primer: 5′-GGACAACGAG-3′ [primer R15 according to Operon Technologies (Alameda, CA) designation].

encephalomyelitis virus (TMEV)-induced demyelination. Melvold *et al.* (42) observed 1 of 10 discordances between *Tcrb* and disease susceptibility among the CXJ series of recombinant inbred lines. These observations raise the pos-

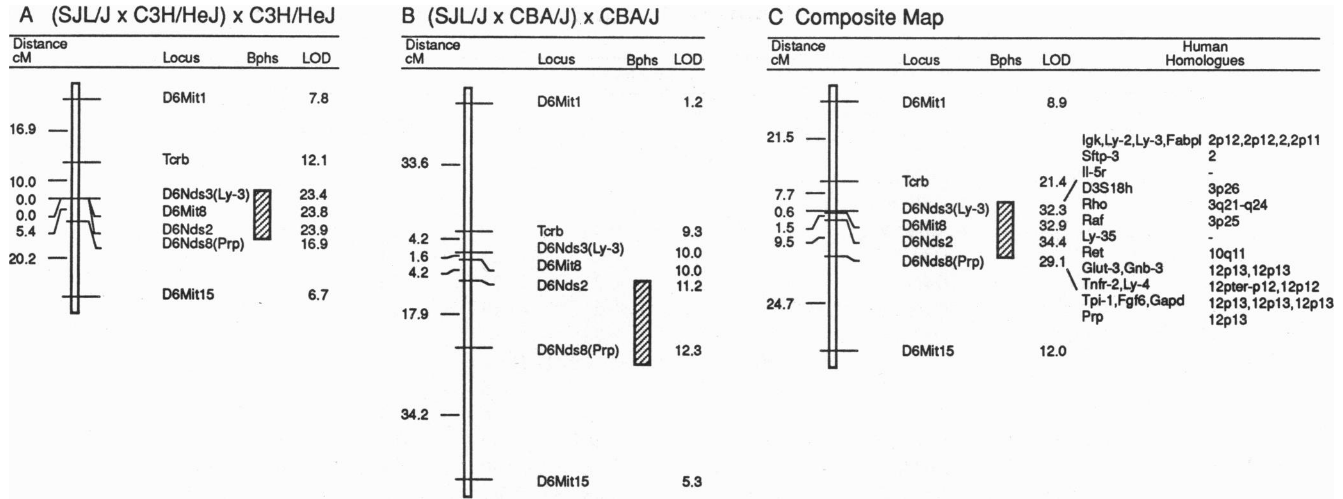

FIG. 1. Genetic map of mouse chromosome 6. The markers used and mapped in this study are detailed in Table 2. The maximum likelihood position estimates for the location of the *Bphs* locus on chromosome 6 are given for both BC1 populations (*A* and *B*) as well as the combined data (*C*). Human homologues corresponding to loci encoded within the support interval are presented relative to the composite map results. cM, Centimorgan.

sibility that several genes on chromosome 6 may be involved in EAE and EAO susceptibility. Appropriate BC1 populations are currently being studied.

Homologous human loci for those residing within the interval including the *Bphs* locus reside on different chromosomes within appropriate syntenic groups (Fig. 1*C*). These human chromosomal regions can now be considered as candidate gene regions for MS. Linkage analysis with available markers corresponding to the mouse loci should be carried out in MS multiplex families for which the *Tcrb* and *Tcra* genes have been typed (40, 43).

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