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Multiple loci govern the bone marrow-derived immunoregulatory mechanism controlling dominant resistance to autoimmune orchitis

(testis/autoimmune disease/linkage analysis/dominant nonresponsiveness/immunoregulation)

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ABSTRACT **The existence of immunoregulatory genes conferring dominant resistance to autoimmunity is well documented. In an effort to better understand the nature and mechanisms of action of these genes, we utilized the murine model of autoimmune orchitis as a prototype. When the orchitis-resistant strain DBA/2J is crossed with the orchitis-susceptible strain BALB/cByJ, the F_1 hybrid is completely resistant to the disease. By using reciprocal radiation bone marrow chimeras, the functional component mediating this resistance was mapped to the bone marrow-derived compartment. Resistance is not a function of either low-dose irradiation- or cyclophosphamide (20 mg/kg)-sensitive immunoregulatory cells, but can be adoptively transferred by primed splenocytes. Genome exclusion mapping identified three loci controlling the resistant phenotype. *Orch3* maps to chromosome 11, whereas *Orch4* and *Orch5* map to the telomeric and centromeric regions of chromosome 1, respectively. All three genes are linked to a number of immunologically relevant candidate loci. Most significant, however, is the linkage of *Orch3* to *Idd4* and *Orch5* to *Idd5*, two susceptibility genes which play a role in autoimmune insulin-dependent type 1 diabetes mellitus in the nonobese diabetic mouse.**

Experimental allergic orchitis (EAO) is an organ-specific autoimmune disease which can be induced in mice by active immunization with autologous or homologous mouse testicular homogenate (MTH) in conjunction with complete Freund's adjuvant (CFA) and pertussis toxin (PTX) (1). Results with a large number of independent, *H-2* congenic, and *H-2* recombinant congenic strains as well as F_1 hybrid mice demonstrate that genetic control of EAO is complex and governed by both *H-2*-linked and non-*H-2*-linked genes (2).

The *H-2*-linked immune response gene controlling susceptibility to autoimmune orchitis, *Orch1*, has been mapped to the *Hsp70.3/G7* interval within the *H-2S/H-2D* region (3, 4). Additionally, non-*H-2*-linked immunoregulatory genes exist which suppress the phenotypic expression of disease associated with a susceptible *Orch1* allele (5). Similar immune-suppression genes have been identified in other animal models of organ-specific autoimmune disease, such as experimental allergic encephalomyelitis (6). Little is known about the mechanisms of action of these genes, but it is clear that they produce dominant resistance in F_1 hybrids and multigenerational backcross progeny (5–7).

In this study, genome exclusion mapping (8, 9) was employed to map the immune suppression genes controlling the phenotypic expression of dominant resistance to autoimmune orchitis. In addition, protocols involving reciprocal radiation bone marrow (BM) chimeras and adoptive spleen cell transfer were used to identify the functional compartment(s) mediating this resistance.

MATERIALS AND METHODS

Animals. BALB/cByJ (C/ByJ) and DBA/2J (D2) mice were purchased from The Jackson Laboratory. (BALB/cByJ × DBA/2J)F_1 hybrid (CD2F_1) and (BALB/cByJ × DBA/2J) × BALB/cByJ backcross (BC1) mice were generated and maintained in the animal facilities at the University of Pennsylvania School of Medicine (Philadelphia) and Brigham Young University on a diet of Purina mouse pellets and acidified water *ad libitum*.

Induction and Evaluation of EAO. Mice were immunized with MTH plus CFA in conjunction with PTX and were sacrificed 30 days later. The testes were processed for histological examination (5). Histopathologic analysis was carried out in a double-blind manner with each testis being scored individually on a pathology index (PI) scale of 0–10 (5). The overall score for each animal was calculated as the average of both testes.

Adoptive Transfer of Disease Resistance, Immunosuppressive Treatments, and Radiation BM Chimeras. Adoptive transfer, immunosuppressive treatments, and radiation BM chimera protocols were carried out as described (10).

DNA Isolation, Microsatellite Primers, Amplification Conditions, and Detection of PCR Products. Genomic DNA was isolated from liver tissue, and working aliquots of DNA samples were prepared by bringing them to the appropriate concentration in TE (10 mM Tris·HCl, pH 7.4/0.1 mM EDTA) (9). Microsatellite primers were synthesized according to sequences obtained through the Whitehead Institute/Massachusetts Institute of Technology/Mouse Genome Database (11). PCR parameters for microsatellite typing were as described (9, 12). Microsatellite size variants were resolved by electrophoresis in either agarose (4% Metaphor; FMC) or 6% polyacrylamide gels and visualized by ethidium bromide.

***Mls1, Akp1, Pep3, Pgm1, Pgm3,* and *Es3* Genotyping.** *Mls1* typing was performed indirectly by immunohistochemical staining (13). *Akp1, Pep3, Pgm1, Pgm3,* and *Es3* isozyme typing was done by traditional methods utilizing electrophoretic variation of migrating bands (14).

Linkage Analysis. An initial screen was performed with the 44 most severely affected BC1 animals. Genotype frequency differences for marker loci were tested by *t* test (15) and by standard χ^2 test against a predicted frequency of 1:1. An additional 92 animals were then genotyped with the markers that exhibited a $\chi^2 \geq 4.0$ ($P < 0.05$) or a *t* score ≥ 1.75 ($P <$

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Abbreviations: EAO, experimental autoimmune orchitis; PTX, pertussis toxin; MTH, mouse testicular homogenate; CFA, complete Freund's adjuvant; BM, bone marrow; PI, pathology index; IDDM, insulin-dependent diabetes mellitus; cM, centimorgan(s).

0.05), and genotype frequency differences were reanalyzed. Linkage of marker loci to disease was considered significant at $P \leq 0.001$.

RESULTS

C/ByJ, D2, and $CD2F_1$ mice were studied for susceptibility to EAO. C/ByJ mice are highly susceptible to autoimmune orchitis, exhibiting an average overall PI of 3.1 ± 0.3 ($n = 95$). In contrast, both D2 and $CD2F_1$ mice are resistant, with average PIs of 0.1 ± 0.1 ($n = 35$) and 0.6 ± 0.1 ($n = 60$), respectively.

Reciprocal and control hematopoietic radiation BM chimeras between C/ByJ and D2 were constructed to map the functional component mediating dominant resistance to either the BM-derived or non-BM-derived tissue constituents (Table 1). Both autologous (C/ByJ → C/ByJ, PI = 0.4 ± 0.2, and D2 → D2, PI = 0.1 ± 0.1) and reciprocal (C/ByJ → D2, PI = 0.6 ± 0.3, and D2 → C/ByJ, PI = 0.8 ± 0.3) chimeras immunized with adjuvants alone failed to exhibit significant inflammation of the testes. When immunized with MTH for the induction of EAO, however, autologous (C/ByJ → C/ByJ, PI = 4.0 ± 1.2, and D2 → D2, PI = 0.5 ± 0.3) and reciprocal (C/ByJ → D2, PI = 5.7 ± 0.8, and D2 → C/ByJ, PI = 1.2 ± 0.3) chimeras developed testicular inflammation corresponding to the BM donor phenotype.

To ascertain whether immunoregulatory cells play a role in dominant disease resistance, D2 mice were pretreated with either low-dose whole-body irradiation or low-dose cyclophosphamide 2 days before immunization (10). Neither treatment abrogated resistance. There was no observable difference between treated animals immunized with MTH (irradiated group PI = 0.5 ± 0.2, $n = 13$; cyclophosphamide-pretreated group PI = 0.1 ± 0.1, $n = 20$) and treated animals immunized with adjuvants only (irradiated group PI = 0, $n = 5$; cyclophosphamide-pretreated group PI = 0, $n = 5$).

To further explore the role of immunoregulatory cells in resistance, spleen cells from MTH-immunized $CD2F_1$ mice were adoptively transferred to naive C/ByJ recipients 3 days before inoculation for disease induction. C/ByJ mice that received spleen cells from C/ByJ mice primed with MTH plus adjuvants served as controls. Recipients receiving cells from $CD2F_1$ donors developed significantly less severe inflammation than their counterparts (PI = 1.4 ± 0.4, $n = 5$, vs. 3.8 ± 0.7, $n = 5$, respectively).

To map the genes controlling dominant resistance, a large (BALB/cByJ × DBA/2J) × BALB/cByJ BC1 population consisting of 197 mice was established. Using this BC1 population and microsatellite DNAs that distinguish C/ByJ and D2 mice, we then generated a linkage map. In the initial screen, the 44 most severely affected animals (PI ≥ 2.5) were genotyped at 88 loci (Table 2). Of these loci, 22 exhibited an association ($\chi^2 \geq 4.0$ and/or t score ≥ 1.75) with markers on seven different chromosomes (nos. 1, 2, 3, 10, 11, 14, and 19). To verify or rule out linkage, we then genotyped 92 resistant animals at each of these loci. This analysis revealed significant linkage to chromosomes 1 and 11. Maximal linkage was seen on chromosome 11 to *D11Mit219* ($\chi^2 = 18.2$, $P = 0.00002$; t score = 3.72, $P = 0.00016$) and *D11Mit8* ($\chi^2 = 17.2$, $P = 0.00003$; t score = 4.12, $P = 0.00004$) at 39.2 and 41.4 centimorgans (cM) from the telomere, respectively. Significant linkage to the following regional markers was also observed: *D11Mit86* ($\chi^2 = 15.3, P = 0.00009$; t score = 3.36, $P = 0.00056$), *D11Mit29* ($\chi^2 = 14.3, P = 0.00016$; t score = 3.42, $P = 0.00046$), *D11Mit90* ($\chi^2 = 14.3, P = 0.00016$; t score = 3.42, $P = 0.00046$), *D11Mit118* ($\chi^2 = 17.2$, $P = 0.00003$; t score = 4.08, $P = 0.00005$), *D11Mit36* ($\chi^2 = 12.5$, $P = 0.00041$; t score = 3.18, $P = 0.00096$), and *D11Mit41* ($\chi^2 = 11.6$, $P = 0.00066$; t score = 3.11, $P = 0.00119$). Resistance on chromosome 1 exhibited maximal linkage to *D1Mit210* ($\chi^2 = 11.6, P = 0.00066$; t score = 3.59, $P = 0.00026$) at 115.6 cM from the centromere.

We hypothesized that in addition to susceptibility/resistance genes, loci that mediate the severity of lesions in affected animals may exist. To identify such loci, we modified our statistical analysis: only diseased animals were included, and the highest individual testis PI score was used as the response variable instead of the average. As severity of inflammation is a semi-quantitative trait, we evaluated linkage by using only the t test method (15). With this modified analysis, a locus associated with lesion severity linked to *D1Mit170* (t score = 3.44, $P = 0.00046$) at 18.2 cM from the centromere of chromosome 1 (Table 3).

Table 1. Summary of autoimmune orchitis in BM radiation chimeras

Group	n	Immunogen	Orchitis PI
C/ByJ → C/ByJ	7	CFA + PTX	0.4 ± 0.2
	11	MTH	4.0 ± 1.2
C/ByJ → D2	8	CFA + PTX	0.6 ± 0.3
	21	MTH	5.7 ± 0.8
D2 → D2	8	CFA + PTX	0.1 ± 0.1
	5	MTH	0.5 ± 0.3
D2 → C/ByJ	5	CFA + PTX	0.8 ± 0.3
	12	MTH	1.2 ± 0.3

Host mice were lethally irradiated (850 rads; 1 rad = 0.01 Gy) at 6–10 weeks of age with a Gammacell ^{137}Cs source at a dose rate of 134 rads/min. They were then reconstituted by intravenous injections of 10^7 T-cell-depleted (by treatment with anti-Thy-1.2 monoclonal antibody plus complement) donor bone marrow cells. For immunization with MTH, animals received 10.0 mg of MTH in 0.1 ml of CFA emulsion distributed equally in both hind footpads. Immediately thereafter each animal received 10.0 μg of PTX dissolved in 0.1 ml of 0.025 M Tris·HCl, pH 7.6/0.5 M NaCl/0.017% Triton X-100 by intraperitoneal injection. All animals were killed 30 days after immunization and examined histologically in a double-blind fashion for testicular lesions.

DISCUSSION

Actively induced autoimmune orchitis in mice is characterized histologically by inflammatory infiltrates around blood vessels and in the interstitium. Lymphocytes, macrophages, polymorphonuclear neutrophils, and eosinophils invade the seminiferous tubules through disrupted Sertoli cell tight junctions. This inflammation leads to aspermatogenesis (1). Because of the immune privilege of the testes and the sequestering of aspermatogenic autoantigens behind the blood–testes barrier (16), we hypothesized that differential susceptibility to autoimmune orchitis may be due to strain specific differences in the target organ. However, our results indicate that dominant resistance to autoimmune orchitis is instead controlled by an active immunoregulatory mechanism independent of the target organ (16).

The occurrence and severity of inflammation seen in the reciprocal radiation BM chimeras map the functional component mediating resistance to the D2 BM-derived compartment and not the non-BM-derived components of the testes. This conclusion is supported by the results demonstrating that resistance can be transferred by primed spleen cells from resistant donors. However, resistance in D2 mice is not abrogated by either low-dose irradiation or cyclophosphamide, suggesting that the immunoregulatory cells mediating this phenotype are fully resistant to both treatments.

Immunoregulatory cells are known to mediate resistance in a number of animal models of organ-specific autoimmune disease. In experimental allergic encephalomyelitis, both recovery-associated immunoregulatory cells isolated from spontaneously recovering animals (17, 18) and those induced by oral tolerance to myelin basic protein are capable of adoptively transferring the resistant phenotype (19). At the cellular level, immunoregulatory activity has been ascribed to both $CD4^+$, type 2 helper T cell-like cells secreting transforming growth factor β, interleukin 4, and interleukin 10 (19) and $CD8^+$ cells secreting transforming growth factor β (20–22). In this regard,

Table 2. Linkage map of the mouse genome and linkage of marker loci with autoimmune orchitis

Chromosome (cM)*	Locus	Affected			Unaffected				*t* test§				
		Ho, no.	He, no.	$\chi^2 \geq 4.0$†	Ho, no.	He, no.	$\chi^2 \geq 4.0$‡	*P*	Ho PI	He PI	*t* score	df	$P \leq 0.005$
1 (5.8)	*D1Mit67*	28	16		39	53	5.4	0.02014	2.8 ± 0.4	1.7 ± 0.3	2.38	120	
1 (8.1)	*D1Mit3*	29	15	4.5	39	52	6.3	0.01207	2.9 ± 0.4	1.6 ± 0.3	2.69	119	0.00409
1 (9.2)	*D1Mit52*	30	14	5.8	39	53	7.9	0.00494	2.9 ± 0.4	1.5 ± 0.3	2.94	121	0.00197
1 (12.6)	*D1Mit120*	29	14	5.2	41	51	6.1	0.01352	2.8 ± 0.4	1.6 ± 0.3	2.61	123	
1 (18.2)	*D1Mit170*	29	15	4.5	40	52	6.0	0.01431	2.9 ± 0.4	1.6 ± 0.3	2.72	120	0.00375
1 (26.1)	*D1Mit213*	27	17		42	50			2.7 ± 0.4	1.8 ± 0.3	1.96	126	
1 (34.0)	*D1Mit76*	23	21		43	49			2.5 ± 0.4	2.0 ± 0.3	1.06	128	
1 (67.0)	*D1Nds2*	20	24		42	50			2.3 ± 0.4	2.2 ± 0.3	0.14	127	
1 (69.0)	*Pep3*	14	9		19	30			2.6 ± 0.5	1.4 ± 0.4	2.00	63	
1 (98.0)	*Crp*	23	20		36	53			2.8 ± 0.4	2.8 ± 0.3	2.06	100	
1 (99.0)	*Mls*	9	5		6	16	4.8	0.02846	3.3 ± 0.9	1.3 ± 0.4	2.00	19	
1 (107.0)	*Akp1*	27	17		32	44	4.1	0.04288	3.1 ± 0.4	2.0 ± 0.3	2.00	108	
1 (112.2)	*D1Mit17*	27	17		32	60	8.6	0.00336	3.0 ± 0.4	1.6 ± 0.3	2.78	103	0.00323
1 (115.6)	*D1Mit210*	29	14	5.2	34	60	11.6	0.00066	3.2 ± 0.4	1.4 ± 0.2	3.59	97	0.00026
2 (25.9)	*D2Mit7*	25	19						5.4 ± 0.5	6.4 ± 0.5	1.37	40	
2 (41.7)	*D2Mit11*	21	23						5.4 ± 0.6	6.2 ± 0.5	1.11	41	
2 (60.6)	*D2Nds3*	24	19		41	46			2.8 ± 0.4	1.7 ± 0.3	2.09	114	
2 (65.1)	*D2Mit21*	28	15						5.8 ± 0.5	5.6 ± 0.6	0.27	33	
2 (72.9)	*D2Mit48*	28	15		45	47			2.6 ± 0.4	1.8 ± 0.3	1.61	132	
2 (78.9)	*D2Mit51*	31	12	8.4	45	45	5.8	0.01603	2.6 ± 0.4	1.7 ± 0.4	1.81	127	
2 (83.0)	*D2Mit52*	30	14	5.8	38	42	4.9	0.02686	2.7 ± 0.4	2.2 ± 0.4	1.05	116	
3 (25.9)	*D3Mit22*	18	25						6.1 ± 0.5	5.4 ± 0.5	0.98	39	
3 (37.6)	*D3Mit11*	18	25						6.4 ± 0.5	5.5 ± 0.5	1.18	40	
3 (56.1)	*D3Mit18*	12	17						6.3 ± 0.7	6.3 ± 0.6	0.02	22	
3 (59.5)	*D3Mit44*	15	29	4.5	34	57			2.5 ± 0.5	2.1 ± 0.3	0.60	85	
4 (6.9)	*D4Mit2*	11	19						6.6 ± 0.7	6.2 ± 0.6	0.44	21	
4 (20.0)	*D4Nds6*	18	25						5.9 ± 0.6	5.6 ± 0.5	0.32	35	
4 (31.5)	*D4Mit15*	22	19						5.5 ± 0.5	5.8 ± 0.6	0.45	37	
4 (62.2)	*D4Mit13*	23	20						5.4 ± 0.5	6.1 ± 0.6	1.00	36	
5 (3.4)	*D5Mit1*	17	12						5.7 ± 0.5	7.0 ± 0.7	1.54	22	
5 (18.0)	*D5Mit80*	21	23						6.5 ± 0.5	5.2 ± 0.6	1.73	41	
5 (26.0)	*Pgm1*	8	10		19	15			2.1 ± 0.5	2.4 ± 0.6	0.41	49	
5 (28.0)	*D5Nds2*	20	23						6.2 ± 0.5	5.4 ± 0.5	1.09	40	
5 (64.2)	*D5Mit30*	21	20						5.3 ± 0.5	6.0 ± 0.6	0.86	37	
6 (3.4)	*D6Mit1*	15	28		44	48			1.8 ± 0.3	2.6 ± 0.4	1.52	131	
6 (21.2)	*D6Mit16*	19	23						6.0 ± 0.6	5.7 ± 0.5	0.33	38	
6 (27.9)	*D6Mit9*	15	26						5.9 ± 0.7	5.6 ± 0.4	0.36	26	
6 (39.7)	*D6Mit36*	17	26						6.7 ± 0.6	5.4 ± 0.5	1.73	32	
6 (60.7)	*D6Mit14*	21	23						6.1 ± 0.6	5.6 ± 0.5	0.64	40	
7 (13.4)	*D7Mit25*	26	17						5.7 ± 0.5	6.1 ± 0.6	0.42	35	
7 (29.3)	*D7Nds1*	15	25		44	48			1.9 ± 0.4	2.3 ± 0.3	0.91	122	
7 (32.6)	*D7Mit62*	17	25						6.0 ± 0.6	5.8 ± 0.5	0.24	33	
7 (69.3)	*D7Mit15*	26	16						5.7 ± 0.6	5.9 ± 0.5	0.30	38	
8 (7.9)	*D8Mit4*	17	26						6.5 ± 0.6	5.2 ± 0.5	1.69	36	
8 (37.2)	*D8Mit45*	22	22						5.1 ± 0.5	5.4 ± 0.6	0.00	47	
8 (64.1)	*D8Mit14*	22	22						5.7 ± 0.5	6.0 ± 0.6	0.36	41	
9 (21.3)	*D9Mit22*	19	24						5.7 ± 0.6	5.8 ± 0.6	0.09	37	
9 (41.0)	*Pgm3*	8	10		18	16			2.2 ± 0.6	2.3 ± 0.6	0.19	49	
9 (55.8)	*D9Mit16*	22	21						5.8 ± 0.5	5.7 ± 0.6	0.14	38	
10 (2.2)	*D10Nds1*	18	25		45	47			2.3 ± 0.4	3.0 ± 0.3	0.44	119	
10 (40.6)	*D10Mit42*	21	22						6.3 ± 0.5	5.2 ± 0.5	1.58	40	
10 (72.1)	*D10Mit35*	23	21						5.6 ± 0.5	6.1 ± 0.6	0.70	39	
11 (2.2)	*D11Mit2*	25	19		35	55			2.6 ± 0.4	2.0 ± 0.3	1.34	117	
11 (23.5)	*D11Mit86*	30	14	5.8	30	62	15.3	0.00009	3.2 ± 0.4	1.5 ± 0.3	3.36	96	0.00056
11 (35.8)	*D11Mit29*	30	14	5.8	31	61	14.3	0.00016	3.2 ± 0.4	1.5 ± 0.3	3.42	98	0.00046
11 (36.9)	*D11Mit90*	30	14	5.8	31	61	14.3	0.00016	3.2 ± 0.4	1.5 ± 0.3	3.42	98	0.00046
11 (39.2)	*D11Mit219*	32	12	9.1	31	61	18.2	0.00002	3.2 ± 0.4	1.4 ± 0.3	3.72	103	0.00016
11 (41.4)	*D11Mit8*	32	12	9.1	32	60	17.2	0.00003	3.3 ± 0.4	1.3 ± 0.2	4.12	100	0.00004
11 (41.4)	*D11Mit118*	32	12	9.1	32	60	17.2	0.00003	3.3 ± 0.4	1.3 ± 0.2	4.08	100	0.00005
11 (42.5)	*D11Mit36*	30	14	5.8	33	59	12.5	0.00041	3.1 ± 0.4	1.5 ± 0.3	3.18	109	0.00096
11 (47.0)	*D11Mit41*	30	14	5.8	34	58	11.6	0.00066	3.1 ± 0.4	1.5 ± 0.3	3.11	112	0.00119
11 (59.7)	*D11Mit67*	30	14	5.8	36	56	10.1	0.00148	2.9 ± 0.4	1.6 ± 0.3	2.73	123	0.00363
11 (75.5)	*D11Mit12*	28	16		40	49	4.1	0.04288	2.7 ± 0.4	1.9 ± 0.3	1.65	130	
11 (80.0)	*Es3*	11	7		18	16			2.5 ± 0.6	1.9 ± 0.6	0.77	48	
12 (12.3)	*D12Mit46*	21	23						5.6 ± 0.6	6.0 ± 0.4	0.56	36	
12 (27.9)	*D12Mit4*	19	22						6.3 ± 0.6	5.6 ± 0.4	0.94	32	
12 (57.4)	*D12Mit8*	17	24						6.2 ± 0.7	5.6 ± 0.5	0.80	29	

Table 2. (Continued)

Chromosome (cM)*	Locus	Affected Ho, no.	Affected He, no.	Affected $\chi^2 \geq 4.0$†	Unaffected Ho, no.	Unaffected He, no.	Unaffected $\chi^2 \geq 4.0$‡	Unaffected P	t test§ Ho PI	t test§ He PI	t score	df	$P \leq 0.005$
13 (16.6)	*D13Mit10*	14	14						5.9 ± 0.6	6.9 ± 0.6	1.28	25	
13 (21.0)	*D13Mit21*	28	15		42	41			2.6 ± 0.4	1.9 ± 0.4	1.29	120	
13 (60.8)	*D13Mit78*	25	19						5.9 ± 0.5	5.7 ± 0.6	0.19	38	
14 (8.9)	*D14Mit14*	14	29	5.2	45	47			1.8 ± 0.3	2.5 ± 0.3	1.49	130	
14 (24.5)	*D14Mit5*	17	26		48	43			1.8 ± 0.3	2.7 ± 0.4	1.72	128	
14 (58.0)	*D14Mit9*	22	21						5.8 ± 0.6	5.7 ± 0.5	0.08	40	
15 (18.2)	*D15Mit5*	17	27						6.1 ± 0.5	5.7 ± 0.5	0.59	38	
15 (41.5)	*D15Mit72*	22	21						5.6 ± 0.5	5.9 ± 0.6	0.41	39	
15 (58.0)	*D15Mit42*	19	23						6.0 ± 0.6	5.4 ± 0.5	0.86	37	
16 (10.0)	*D16Mit31*	20	23						5.5 ± 0.6	5.9 ± 0.5	0.55	38	
16 (26.9)	*D16Mit4*	17	27						5.9 ± 0.6	5.7 ± 0.5	0.26	35	
16 (41.9)	*D16Mit50*	17	26						6.2 ± 0.6	5.6 ± 0.5	0.72	38	
17 (20.2)	*D17Mit10*	19	22						5.8 ± 0.5	5.7 ± 0.6	0.11	38	
17 (35.6)	*D17Mit3*	19	22						6.0 ± 0.5	5.3 ± 0.5	0.95	38	
18 (9.0)	*D18Mit34*	21	22						6.0 ± 0.6	5.4 ± 0.5	0.79	40	
18 (25.9)	*D18Mit40*	20	23						5.4 ± 0.5	6.0 ± 0.5	0.75	40	
18 (36.1)	*D18Mit80*	20	24						5.2 ± 0.5	6.3 ± 0.5	1.49	41	
19 (13.8)	*D19Mit16*	25	16		44	48			2.6 ± 0.4	1.7 ± 0.3	1.79	126	
19 (43.6)	*D19Mit55*	24	20		37	55			2.8 ± 0.4	1.8 ± 0.3	1.90	107	
19 (45.3)	*D19Mit33*	25	18		39	52			2.8 ± 0.4	1.7 ± 0.3	2.26	107	
19 (59.1)	*D19Mit6*	24	18		36	50			2.8 ± 0.4	1.7 ± 0.3	2.13	100	

Ho, homozygous. He, heterozygous.

*Markers are arranged centromeric to telomeric. Locations are as reported by the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research (11) or as best estimates based on comparisons of existing maps.

†Genotype frequency differences were tested by χ^2 against a predicted frequency of 1:1 for marker loci (df = 1).

‡Genotype frequency differences were tested by χ^2, using 2 × 2 contingency tables (df = 1).

§The response variable is the average PI of both testes for each animal. Reported means are the mean PIs for the entire population homozygous (Ho) or heterozygous (He) for each marker.

a $CD8^+$, T-cell receptor $\alpha\beta^+$ suppressor T-cell line capable of preventing orchitis in the C3H/HeN adjuvant-independent model of EAO has been described (23). In the D3Tx model of autoimmune disease, $CD4^+$, $CD5^{high}$ immunoregulatory cells capable of adoptively transferring resistance have been described (24). Similarly, in diabetes induced by adult thymectomy and sublethal γ irradiation in normal nonautoimmune rat strains, diabetes and insulitis can be completely prevented by transfer of $CD4^+$, α/β^+ T cells (25). The existence of immunoregulatory cells in spontaneous insulin-dependent diabetes mellitus (IDDM) (26, 27) and uveitis (28) is also well documented.

In addition to active suppression by immunoregulatory cells, several other mechanisms can account for dominant resistance (7). The nonresponder or resistant phenotype can arise as a result of tolerance to self antigens, particularly when the self antigens are codominantly expressed. Such a mechanism is best exemplified by the clonal elimination of potentially autoreactive T cells expressing receptors which recognize self Mls determinants (29). Although resistance was not linked to *Mls1*, it is possible that *Orch3* and/or *Orch4* encode other Mls-like elements capable of functioning in clonal deletion of orchitogenic T cells in D2 and $CD2F_1$ mice. Gene dosage can also

Table 3. Linkage analysis identifying a severity locus mapping to chromosome 1

Chromosome (cM)*	Locus	t test† Ho PI	t test† He PI	t score	df	$P \leq 0.005$
1 (5.8)	*D1Mit67*	5.3 ± 0.5	3.5 ± 0.4	2.70	83	0.00420
1 (8.1)	*D1Mit3*	5.4 ± 0.5	3.3 ± 0.4	3.05	83	0.00154
1 (9.2)	*D1Mit52*	5.3 ± 0.5	3.3 ± 0.4	3.10	85	0.00131
1 (12.6)	*D1Mit120*	5.3 ± 0.5	3.3 ± 0.4	3.04	84	0.00158
1 (18.2)	*D1Mit170*	5.5 ± 0.5	3.2 ± 0.4	3.44	82	0.00046
1 (26.1)	*D1Mit213*	5.3 ± 0.5	3.5 ± 0.4	2.55	82	0.00632
1 (34.0)	*D1Mit76*	4.7 ± 0.5	4.0 ± 0.5	1.02	85	
1 (67.0)	*D1Nds2*	4.3 ± 0.5	4.5 ± 0.5	0.26	84	
1 (69.0)	*Pep3*	3.9 ± 0.5	3.2 ± 0.7	0.74	41	
1 (98.0)	*Crp*	4.9 ± 0.5	3.9 ± 0.5	1.32	80	
1 (99.0)	*Mls*	5.5 ± 1.0	3.2 ± 0.9	1.83	19	
1 (107.0)	*Akp1*	5.0 ± 0.5	4.2 ± 0.5	1.12	79	
1 (112.2)	*D1Mit17*	5.5 ± 0.5	3.4 ± 0.4	2.97	79	0.00197
1 (115.6)	*D1Mit210*	5.3 ± 0.4	3.2 ± 0.4	3.28	84	0.00076

*Markers are arranged centromeric to telomeric. Locations are as reported by the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research (11) or as best estimates based on comparisons of existing maps.

†The response variable is the PI for the most severely affected testis for each animal. Reported means are the mean PIs for the entire population homozygous (Ho) or heterozygous (He) for each marker.

influence the magnitude of an immune response. For example, T-cell responsiveness depends, to some extent, on the amount of relevant class II antigen expressed by antigen-presenting cells (30, 31). In this cross we eliminated this effect by utilizing *H-2*-matched parents.

Genome exclusion mapping was used to map the genes controlling resistance in D2 mice. We report here the identification of three separate loci: *Orch3* maps centrally on chromosome 11; *Orch4* maps to the telomeric end of chromosome 1; and *Orch5* maps near the centromeric end of chromosome 1. Both *Orch3* and *Orch4* are resistance loci, whereas *Orch5* appears to be a severity gene governing the extent of the inflammatory lesions observed in susceptible mice. All three of these loci map to regions encoding immunologically relevant candidate genes (32). *Orch3* is linked to genes encoding T-cell activation family 3 (*Tca3*), macrophage inflammatory proteins α and β (*Mip1*α and *Mip1*β), and inducible nitric oxide synthase (*Nos*). *Orch4* is linked to genes for two complement receptors (*Cr2, Crry*), lymphocyte antigen 33 (*Ly33*), and cluster designation 34 (*Cd34*). *Orch5* is linked to genes encoding interleukin 1 receptors type I and type II (*Il1r1* and *Il1r2*). Further analysis is required to determine which, if any, of these candidate loci play a role in resistance to autoimmune orchitis.

Most importantly, however, both *Orch3* and *Orch5* map closely to the IDDM susceptibility loci *Idd4* and *Idd5*, respectively (8, 33, 34). In fact, *Orch3* maps within 1 cM of *Idd4*. This result suggests that autoimmune orchitis and IDDM may share susceptibility loci. Furthermore, an association between *Idd4* and *Idd5* and loci which control susceptibility to systemic lupus erythematosus in (NZB × NZW) mice has recently been reported (35). If indeed, under further analysis, it is verified that *Orch3* and *Orch5* are identical to *Idd4* and *Idd5*, then the following conclusion is evident. Non-major histocompatibility complex (MHC)-linked disease susceptibility genes can be divided into two distinct classes: those that play a role in multiple autoimmune diseases and those that are disease specific. MHC-linked immune response genes clearly establish a precedent for the former.

Animal models maintain specific importance when they provide insight of clinical relevance. In this regard, it is anticipated that genes identified in animal models will lead to the identification of their human homologues. We have hypothesized that the best candidate genes for human studies are those common either to multiple species, such as mice and rats, or multiple diseases, such as autoimmune orchitis, IDDM, and systemic lupus erythematosus. In this regard, in a recent genome-wide search for human IDDM susceptibility genes, 18 different chromosomal regions showed possible linkage to disease (36). Of these 18 regions, 2 contained possible homologues of previously mapped murine IDDM genes. Those genes are *Idd4* and *Idd5*. This is particularly intriguing in light of our findings.

In conclusion, we have identified the loci controlling the BM-derived immunoregulatory mechanisms responsible for dominant resistance to autoimmune orchitis. Preliminary comparative mapping results suggest that these loci may be of significance to other organ-specific autoimmune diseases such as IDDM. Characterization of these loci will further elucidate the resistance mechanisms involved and provide insight into the molecular mechanisms associated with the generation and maintenance of immunoregulation.

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