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Autoimmune Orchitis, Epididymitis, and Vasitis Are Immunogenetically Distinct Lesions

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Experimental allergic orchitis (EAO), the principle animal model of noninfectious testicular inflammatory disease, is a genetically determined phenotype. Classical EAO, induced by inoculation with testicular homogenate and the appropriate adjuvants, is characterized by inflammatory infiltrates in the testis (orchitis), epididymis (epididymitis), and vas deferens (vasitis). In this study, the genetic control of susceptibility and resistance to these three lesions was analyzed in the mouse. The results obtained with independent inbred strains and *H2* congenic mice show that the genetic control of all three lesions is complex and involves both *H2* and non-*H2*-linked genes. Whole-genome exclusion mapping was performed on a backcross population segregating for all three phenotypes. Permutation-derived thresholds provided experimentwise, chromosomewise, comparisonwise, and marker-specific chromosomewise thresholds for declaration of significant regions linked to marker loci. Unique loci were identified on chromosome 8 for orchitis, chromosome 16 for epididymitis, and chromosome 1 for vasitis and have been designated as *Orch6*, *Epd1*, and *Vas1*, respectively. These results show that autoimmune orchitis, epididymitis, and vasitis are immunogenetically distinct lesions. *(Am J Pathol 1998, 152:1337–1345)*

Autoimmune disease of the testis can lead to infertility in animals and likely causes some types of human infertility.[1,2] In men with noninfectious testicular inflammatory disease, one group of infertile patients develops nonspecific granulomatous orchitis presumed to be autoimmune in nature.[1–3] A second, more common group of infertile patients displays epididymitis and/or vasitis, characterized by unilateral granuloma formation, circulating antisperm antibodies, and little or no orchitis.[1–3] Similarly, classical experimental allergic orchitis (EAO), induced by inoculation with testis homogenate and the appropriate adjuvants, is characterized by inflammatory infiltrates in the testis (orchitis), epididymis (epididymitis), and vas deferens (vasitis).[4]

The appearance of autoimmune orchitis, epididymitis, and vasitis as clinically independent lesions suggests they are distinct diseases. Evidence for separate immunopathogenic mechanisms first came from experiments using different crude aspermatogenic antigens to induce EAO in the guinea pig. These antigens were designated S, P, and T. Inoculation with S resulted primarily in orchitis, P led to epididymovasitis, and T caused an early wave of aspermatogenesis.[5] In mice, $CD4^+$ T cell clones derived after *in vitro* stimulation with testicular antigens respond better to testis peptides than to epididymal sperm peptides. The reverse is also true for epididymal sperm-specific T cell clones.[6] The histopathology of passive disease induced by the adoptive transfer of such cells also significantly differs from that elicited by active immunization with adjuvants. Lesions in active disease are observed almost exclusively in association with the seminiferous tubules,[7,8] whereas in passive disease, the lesions are observed in the straight tubules, rete testes, and ductus efferentes.[8] In adjuvant independent models of EAO, severe orchitis and aspermatogenesis, without epididymitis and vasitis, are transferable to normal mice by $CD4^+$ T cell lines.[9,10] Taken together, these observations suggest that the histopathological profiles seen in murine EAO are due to different immunopathological responses elicited by unique aspermatogenic autoantigens. However, such unique aspermatogenic antigens have not yet been isolated despite numerous attempts.[11]

As an alternative approach to further clarify the pathogenetic relationship between orchitis, vasitis, and epididymitis, we analyzed the genetic control of these pathological entities in the mouse. Using various inbred and congenic strains, we identified different patterns of susceptibility for all three lesions. In addition, genome exclusion mapping[12–14] was used on a backcross population

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segregating for all three traits. Previous results have shown that quantitative and qualitative analyses can identify severity and/or susceptibility loci.[12,14] Biologically, we can differentiate between quantitative and qualitative classifications of data and uniquely test linkage between the segregating genome and each trait. The culmination of our quantitative and qualitative results demonstrate that both analyses indicate an exclusive genetic linkage for each trait and confirm that autoimmune orchitis, epididymitis, and vasitis are immunogenetically distinct.

Materials and Methods

Animals

A/J, AKR/J, A.SW/SnJ, B10.A/J, B10.BR/SgSnJ, B10.D2/nSnJ, B10.M/SnJ, B10.RIII/SnJ, B10.SM/SnJ, B10.Y/SnJ, BALB/cByJ, BALB/cJ, C3HeB/FeJ, C57BL/10J, CBA/J, DBA/1J, DBA/2J, SJL/J, and SWR/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6NCr, MRL/NCr, NFS/NCr, NZB/NCr, and NZW/NCr mice were purchased from the Animal Genetics and Production Branch, National Cancer Institute (Bethesda, MD). A.By/Sa, A.D2/Sa, B10.Q/SgDv, B10.S/DvTe, BALB.B10, BALB.C3H, (B10.S/DvTe × SJL/J) F_1, (B10.S/DvTe × SJL/J) × B10.S/DvTe backcross (BC1) mice were generated and maintained in the animal facilities at either the University of New Mexico School of Medicine (Albuquerque, NM), the University of Pennsylvania School of Medicine (Philadelphia, PA), or Brigham Young University (Provo, UT).

Induction and Evaluation of EAO

Mice were immunized as previously described with mouse testicular homogenate emulsified in complete Freund's adjuvant supplemented with *Mycobacterium tuberculosis* (H37Ra).[7,15] In addition, each animal received 10.0 μg of crude pertussis toxin at the time of inoculation and 5.0 μg 24 to 48 hours later. Control animals were inoculated with 10.0 mg of allogeneic mouse liver homogenate and adjuvants. All animals were sacrificed at 25 to 30 days, and the testes were processed for histological examination.[15] Histopathological analyses were carried out in a double-blind manner with each testis, epididymis, and vas deferens scored individually on a scale from 0 to 10.[4,15,16] The overall pathology index (PI) for each strain was calculated as the average of all of the animals within that strain. Quantitative analysis was based on the overall PI for each animal as calculated by the average PI for both testes, epididymides, and vasa deferens. For qualitative analysis, animals with an average PI of 1.0 or higher were considered affected as immunization with mouse liver homogenate and adjuvant occasionally resulted in inflammatory infiltrates in the testes, epididymides, and vasa deferens with an average PI < 1.0 (see Table 1).

DNA Isolation, Microsatellite Primers, Amplification Conditions, and Detection of Polymerase Chain Reaction Products

Genomic DNA was isolated from liver tissue, and working aliquots were prepared by bringing the DNA to the appropriate concentration in Tris/EDTA (10 mmol/L Tris/HCl, pH 8.0, and 0.1 mmol/L EDTA, pH 8.0).[17] Microsatellite primers were either synthesized according to sequences obtained through the Whitehead Institute/MIT Center for Genome Research (www.genome.wi.mit.edu/cgi-bin/mouse/index)[18] or purchased from Research Genetics (Huntsville, AL). Polymerase chain reaction parameters for microsatellite typing were followed as previously outlined.[17,19] Microsatellite size variants were resolved in denaturing polyacrylamide gels (7%) and visualized by autoradiography on Kodak film.

Quantitative Analysis

Linkage maps were estimated using the Kosambi map function within the MAPMAKER/EXP computer package.[20] Likelihood ratio tests (LRTs) were used in an interval mapping[21] framework to test for linked quantitative trait loci (QTL) using QTL-Cartographer,[22] model 3.

Qualitative Analysis

A χ^2 test statistic was used to test for linkage between marker loci and susceptibility, independent of marker location.

Establishment of Critical Threshold Values

In each of the previous analyses, the specifics of the experimental situation (eg, marker number, sample size, segregation distortion, etc) as well as the multiple testing issues implicit to single-marker or interval-mapping methods were of concern. To accommodate these issues, we relied on permutation thresholds.[23,24] In short, when the data indicate significant linkage, permutation effectively destroys any association between trait values and the analysis points and supplies a random sample of the data from the null hypothesis of no linkage. On the other hand, when no real linkage with the genome exists, randomly shuffling the traits will change nothing. If the appropriate test statistic (ie, LRT or χ^2) is computed at each analysis point in the random (shuffled) data set, stored, and processed (ie, shuffling and analysis), and repeated N times (in our case, 1000 times), the distribution of the respective test statistic can be estimated and used to declare linkage. In this sense, we are essentially establishing a true P value (ie, the number of times the test statistic supplied by the actual data are greater than the critical value estimated by permutation) for each marker.

The 1000 permutations and analyses of the data supply test statistic values at each analysis point and, when collected under different scenarios, supply the appropriate critical values. Experimentwise threshold values are

Table 1. Susceptibility to Autoimmune Orchitis, Epididiymitis, and Vasitis in Inbred Strains of Mice

Phenotype	Strain	*H2* haplotype	*n*	PI ± SE		
				Orchitis	Epididymitis	Vasitis
Type I	DBA/2J	*d*	26*	0.1 ± 0.02	0.2 ± 0.1	0.6 ± 0.2
			5†	0	0.3	0.2
	CBA/J	*k*	24	0.2 ± 0.1	0.5 ± 0.2	0.4 ± 0.1
			4	0.5	0.1	0
	C57BL/10J	*b*	13	1.7 ± 0.2	1.7 ± 0.4	1.1 ± 0.4
			5	0.7	0.3	0.9
	NZB/NCr	*d*	23	1.1 ± 0.2	1.1 ± 0.2	1.5 ± 0.2
			5	0	0.5	0.9
	BALB/cJ	*d*	30	1.4 ± 0.5	0.4 ± 0.3	0.8 ± 0.3
			6	0	0	0
Type II	C57BL/6NCr	*b*	29	3.0 ± 0.3	2.7 ± 0.2	3.5 ± 0.3
			4	0.3	0.3	0.4
	SJL/J	*s*	26	3.8 ± 0.3	3.2 ± 0.1	3.3 ± 0.2
			5	0.3	0.9	0.4
	NZW/NCr	*z*	30	3.6 ± 0.3	3.9 ± 0.6	5.8 ± 0.5
			7	0.1	0.2	0
	SWR/J	*q*	23	4.5 ± 0.3	2.5 ± 0.4	3.9 ± 0.4
			4	0	0	0
	BALB/cByJ	*d*	29	5.6 ± 0.4	2.6 ± 0.4	2.1 ± 0.4
			6	0.5	0	0
Type III	NFS/NCr	*s*	26	2.6 ± 0.4	3.9 ± 0.7	0.4 ± 0.2
			7	0.1	0.1	0.4
Type IV	A/J	*a*	25	4.4 ± 0.4	1.0 ± 0.3	3.3 ± 0.5
			7	0.2	0.2	0.6
	MRL/NCr	*k*	21	2.1 ± 0.2	0.5 ± 0.2	1.6 ± 0.3
			5	0.1	0.2	0.1
Type V	DBA/1J	*q*	22	0.2 ± 0.1	3.7 ± 0.5	3.2 ± 0.3
			4	0	0.8	0
Type VI	AKR/J	*k*	19	0.3 ± 0.1	0.5 ± 0.2	3.3 ± 0.4
			4	0	0	0
	C3HeB/FeJ	*k*	17	0.2 ± 0.1	0.1 ± 0.1	1.2 ± 0.5
			5	0	0	0

Orchitis results are as previously reported with the exception that the SE for the mouse-testicular-homogenate-immunized mice have been rounded to a single significant figure and have been left off the PI for the mouse-liver-homogenate-immunized controls.[15]

*Each animal received 10.0 mg of mouse testicular homogenate emulsified in complete Freund's adjuvant. The inoculum (0.1 ml) was distributed equally in both hind footpads. Immediately thereafter, each animal received 10.0 μg of pertussis toxin dissolved in 0.1 ml of 0.025 mol/L Tris/HCl buffer containing 0.5 mol/L NaCl and 0.017% Triton X-100, pH 7.6, by intraperitoneal injection and 5.0 μg again 24 or 48 hours later. All animals were killed 25 to 30 days after inoculation and examined histologically in a double-blind fashion for testicular lesions.

†Control animals were immunized with 10.0 mg of mouse liver homogenate plus adjuvants.

obtained by collecting the maximum test statistic from each of the 1000 random analyses of the genome, ordering them and taking the $100(1 - \alpha)$ percent. Similarly, chromosomewise threshold values are deduced by limiting the maximum test statistic from each of the 1000 random analyses to a specific chromosome. If each marker is considered uniquely, comparisonwise thresholds may be obtained by ordering the 1000 test statistics at each marker and obtaining the approximate $100(1 - \alpha)$ percent. Marker-specific chromosomewise thresholds are distinct to the markers on a particular chromosome and are derived from the total marker number and number of permutations (eg, 15 markers on a chromosome with 1000 permutations provides a distribution based on 15,000 random samples). For this application, marker-specific chromosome *P* values were derived only for qualitative analysis, as QTL-Cartographer does not provide this information.

Results

Fifteen different inbred strains of mice were examined for susceptibility to autoimmune orchitis, epididymitis, and vasitis (Table 1). The strains were classified into six different groupings according to observed disease phenotypes. Type I included strains DBA/2J, CBA/J, C57BL/10J, NZB/NCr, and BALB/cJ and were all low responders for the three diseases. Type II represented strains that were susceptible to the induction of all three lesions and consisted of the strains C57BL/6NCr, SJL/J, NZW/NCr, SWR/J, and BALB/cByJ. NFS/NCr mice were susceptible to orchitis and epididymitis but were low responders for vasitis and were designated as type III responders. Similar results were reported for C57BL/6J mice.[15,25] Type IV responding strains, A/J and MRL/NCr, exhibited susceptibility to orchitis and vasitis. Even though MRL/NCr mice were clearly susceptible to orchitis while being marginally

Table 2. Susceptibility to Autoimmune Orchitis, Epididymitis, and Vasitis in *H2* Congenic Strains of Mice

Strain	*H2* haplotype	*n*	PI ± SE		
			Orchitis	Epididymitis	Vasitis
BALB/cByJ	*d*	36	5.6 ± 0.7	1.2 ± 0.3	2.1 ± 0.4
BALB.B10	*b*	10	0.4 ± 0.2	0.4 ± 0.2	0.1 ± 0.1
BALB.C3H	*k*	10	0.4 ± 0.3	1.5 ± 0.6	3.1 ± 1.2
B10.D2/nSnJ	*d*	12	5.3 ± 1.0	3.2 ± 0.7	2.2 ± 0.4
C57BL10/J	*b*	11	1.0 ± 0.4	1.7 ± 0.4	1.1 ± 0.4
B10.BR/SgSnJ	*k*	12	0.3 ± 0.1	0.5 ± 0.2	0.1 ± 0.1
B10.A/J	*a*	17	3.6 ± 0.8	1.8 ± 0.6	3.4 ± 0.7
B10.Y/SnJ	*p*	15	3.8 ± 0.8	1.5 ± 0.3	1.9 ± 0.4
B10.Q/SgDv	*q*	15	1.4 ± 0.5	1.8 ± 0.3	0.8 ± 0.3
B10.SM/SnJ	*v*	14	1.4 ± 0.6	1.0 ± 0.4	1.4 ± 0.4
B10.S/DvTe	*s*	11	0.6 ± 0.2	0.2 ± 0.1	0.0 ± 0.0
B10.RIII/SnJ	*r*	14	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
B10.M/SnJ	*f*	6	0.3 ± 0.3	0.1 ± 0.1	0.0 ± 0.0
A.D2/Sa	*d*	26	5.7 ± 0.2	0.9 ± 0.1	1.2 ± 0.1
A.BY/Sa	*b*	7	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.2
A/J	*a*	25	4.4 ± 0.4	1.0 ± 0.3	3.3 ± 0.5
A.SW/SnJ	*s*	21	4.4 ± 0.3	0.1 ± 0.1	0.4 ± 0.2

Animals were immunized with mouse testicular homogenate plus adjuvants, and histopathological analyses were performed as described in Table 1. Orchitis results for the C57BL/10J and BALB/c congenic lines are as previously reported.[15,40]

susceptible to vasitis, they were included in the type IV classification. Only one strain, DBA/1J, was susceptible to epididymitis and vasitis while being resistant to orchitis (type V). AKR/J and C3H/HeJ mice were susceptible to only vasitis and were designated type VI responders, although inflammatory infiltrates in the vas deferens were minimal in C3HeB/FeJ.

A comparison of the *H2* haplotypes among the various strains encompassed within each response type failed to reveal a clear-cut correlation between *H2* and susceptibility to any of the three lesions. This suggests that the genetic control of autoimmune orchitis, epididymitis, and vasitis is complex and involves both *H2* and non-*H2*-linked genes. Similar results have been reported for other organ-specific autoimmune diseases.[26]

To assess the role of *H2* in the genetic control of autoimmune orchitis, epididymitis, and vasitis, three sets of *H2* congenic strains of mice were studied (Table 2). BALB/c congenic mice possessing the $H2^d$ and $H2^k$ haplotypes were susceptible to different combinations of the three diseases, whereas $H2^b$ mice were essentially resistant to all three. On the C57BL/10J background, $H2^d$ mice were susceptible to all three lesions, $H2^a$ mice were susceptible to orchitis and vasitis, and $H2^p$ mice were susceptible to only orchitis. B10 mice with the $H2^{b,v,k,q,s,r,f}$ haplotypes were low responders or resistant to all three lesions. A/J congenic mice with the $H2^a$ haplotype exhibited significant orchitis and vasitis whereas $H2^d$ and $H2^s$ mice were susceptible only to orchitis. $H2^b$ A/J congenic mice were resistant to all three diseases. Overall, susceptibility to neither orchitis, epididymitis, nor vasitis was associated with any one haplotype on the three backgrounds studied. These results suggest that epistatic interactions between *H2*-linked and background genes play a role in controlling the phenotypic expression of the three lesions. Similar results have been reported for the genetic control of nonimmunologically mediated spontaneous aspermatogenesis observed in C57BL/10J mice.[27]

To map the non-*H2* genes controlling disease susceptibility, we used $H2^s$ identical strains of mice. SJL/J mice were highly susceptible to EAO, whereas B10.S/DvTe mice were essentially resistant (Table 3). Susceptibility to all three lesions was inherited as the dominant phenotype in (B10.S/DvTe × SJL/J) F_1 hybrids. A (B10.S/DvTe × SJL/J) × B10.S/DvTe backcross (BC1) population of 109 mice was established and phenotyped for susceptibility to autoimmune orchitis, epididymitis, and vasitis. Whole genomic exclusion mapping was performed using the DNA from the BC1 mice and 200 previously mapped microsatellite markers that distinguish the parental strains. We generated a linkage map with the Kosambi mapping function in the MAPMAKER/EXP computer software.[20] In this map, mean intermarker recombination fractions ranged between 0.060 and 0.147 for different chromosomes with a maximum intermarker distance of between 14.0 and 20.4 cM. Genome coverage was verified by comparing the genetic map generated in this study with the Whitehead Institute/MIT Center for Ge-

Table 3. Incidence of Autoimmune Orchitis, Epididymitis, and Vasitis in SJL/J, B10.S/DvTe, F_1 Hybrid, and Backcross Mice

Strain/cross	Incidence (PI)		
	Orchitis	Epididymitis	Vasitis
SJL/J	25/26 (3.8)[b]	24/26 (3.2)	19/25 (3.3)
B10.S/DvTe	3/10 (0.6)	1/10 (0.2)	0/10 (0.0)
(B10.S/DvTe × SJL/J) F_1	14/17 (4.2)	10/17 (3.7)	15/17 (3.2)
F_1 × B10.S/DvTe	40/109	34/109	54/109

Animals were immunized with mouse testicular homogenate plus adjuvants as described in Table 1.

Table 4. Mouse Chromosome 8 Linkage Map and Association of Marker Loci to Autoimmune Orchitis

Locus*	MIT†	θ‡	Affected§		Unaffected		Chromosomewise¶		Comparisonwise‖	LRT**
			Ho	He	Ho	He	χ^2	*P* value	*P* value	
D8Mit95	6.6	0.064	13	27	39	30	5.86	0.0175	0.0090	7.929
D8Mit4	12.0	0.009	13	27	38	31	5.18	0.0242	0.0180	6.984
D8Mit258	13.1	0.000	12	28	38	31	6.41	0.0144	0.0090	9.311
D8Mit291	14.2	0.000	12	28	38	31	6.41	0.0144	0.0090	9.311
D8Mit224	16.4	0.064	12	28	38	31	6.41	0.0144	0.0090	9.314
D8Mit65	24.0	0.000	12	28	38	30	6.79	0.0104	0	10.926
D8Mit339	25.1	0.110	12	28	39	30	7.15	0.0084	0	10.929
D8Mit177	31.7	0.000	10	30	39	30	10.17	0.0012	0	12.865
D8Mit31	35.0	0.000	10	30	39	30	10.17	0.0012	0	12.865
D8Mit179	35.0	0.000	10	30	39	30	10.17	0.0012	0	12.865
D8Mit27	35.0	0.018	10	30	39	30	10.17	0.0012	0	12.868
D8Mit304	36.1	0.018	11	29	40	29	9.44	0.0020	0	13.171
D8Mit180	36.1	0.037	11	29	38	31	7.78	0.0062	0	11.638
D8Mit74	36.1	0.028	10	30	39	30	10.17	0.0012	0	17.457
D8Mit207	41.5	0.000	10	30	38	31	9.29	0.0023	0	16.832
D8Mit77	38.3	0.028	10	30	38	31	9.29	0.0023	0	16.817
D8Mit262	38.3	0.128	13	27	38	31	5.18	0.0242	0.009	7.903
D8Mit12	55.7	0.046	14	26	37	32	3.53	0.0632	0.0460	8.493
D8Mit271	59.0	0.083	16	24	34	35	0.88	0.3671	0.3470	6.549
D8Mit36	68.9		18	22	31	38	0.00	0.9861	0.8170	

Histopathological analysis of orchitis was performed in a double-blind fashion with each testis scored individually on a PI of 0 to 10.
*Markers arranged centromeric to telomeric.
†Positions as reported by the Whitehead Institute/MIT Center for Genomic Research (www.genome.wi.mit.edu/cgibin/mouse/index).
‡Recombination fractions calculated using MAPMAKER/EXP 3.0.
§Animals with an average PI of 1 or higher were considered affected.
¶χ^2 test statistic using 2 × 2 contingency tables. Results are based on 1000 permutations of the original data. χ^2 experimentwise cutoffs were as follows: 90% = 10.95; 95% = 12.59; 99% = 16.38. Comparisonwise χ^2 chromosomewise cutoffs: 90% = 5.78; 95% = 7.57; 99% = 10.17.
‖The *P* values are the probabilities of achieving a χ^2 value for that marker greater than the one observed from experimentation.
**LRT done using QTL-Cartographer under the model 3 specification using the estimated map distances. Experimentwise cutoffs: 90% = 11.16, 95% = 12.45, 99% = 14.83. Comparisonwise significance was at 99% for all LRT results.

nome Research map (www.genome.wi.mit.edu/cgi-bin/mouse/index)[28] (Tables 4 to 6).

Using the experimentwise (maximum statistic of each of the 1000 permutations of all markers included in this study) threshold to determine maximal linkage for each trait in the entire genome, quantitative (degree of lesion immunopathology) analysis of autoimmune orchitis under a 95% experimentwise threshold value of 12.45 indicated maximal linkage to regions on chromosome 8 (Table 4). Qualitative (comparison of animals with and without lesions) analysis of epididymitis exhibited a 90% experimentwise threshold value of 11.58 showing maximal linkage to loci on chromosome 16 (Table 5). Although experimentwise thresholds did not supply a significant result for autoimmune vasitis, maximal linkage was detected on chromosome 1 and was pursued (Table 6).

Because the experimentwise thresholds demonstrated maximal linkage to chromosomes 1, 8, and 16 for vasitis, orchitis, and epididymitis, respectively, we chose to examine these three chromosomes individually as evidence of independent genetic control of the three distinct lesions. We used chromosomewise (maximum statistic from 1000 permutations of the markers on a distinct chromosome), comparisonwise (based on 1000 permutations at a single marker), and marker-specific chromosomewise (sum of all 1000 permutations for every marker on a specific chromosome) thresholds for confirmation of significance using quantitative or qualitative analysis for each trait. Given the two methods of analysis (quantitative and qualitative) performed on each trait and that experimentwise thresholds or maximal linkage determined our course of analysis, we confirmed significant experimentwise results for the three traits using the alternative analysis (ie, for orchitis, qualitative analysis; for epididymitis, quantitative analysis; for vasitis, qualitative analysis).

Qualitative analysis of orchitis on chromosome 8 demonstrated chromosomewise significance at a 95% (7.57) threshold (Table 4). Quantitative analysis of epididymitis on chromosome 16 showed comparisonwise significance at 95% (5.39; Table 5). Maximal association of vasitis was detected on chromosome 1 by qualitative analysis (Table 6).

To pinpoint a trait-specific susceptibility locus, we examined experimentwise and chromosomewise, comparisonwise, and marker-specific chromosomewise linkage using quantitative and qualitative analysis. We reasoned that the most likely region for susceptibility to each of the three lesions would be the locus with maximal linkage using experimentwise and chromosomal/marker-specific-based analyses. Therefore, the most likely region for susceptibility to autoimmune orchitis was on chromosome 8 at *D8Mit74*. This marker explained 14.8% of the QTL variance (no other marker accounted for more variance) and had a marker-specific chromosomewise *P* value of 0.0012. We designated this orchitis susceptibility locus *Orch6*.[14] Likewise, susceptibility loci for autoimmune epididymitis and vasitis were located on chromosomes 16 and 1, respectively. Markers on the centromeric end of chromosome 16, including *D16Mit130*, *D16Mit79*, *D16Mit154*, and *D16Mit182*, identified a locus

Table 5. Mouse Chromosome 16 Linkage Map and Association of Marker Loci to Autoimmune Epididymitis

Locus	MIT	θ	Affected		Unaffected		Chromosomewise		Comparisonwise *P* value	LRT†
			Ho	He	Ho	He	χ^{2*}	*P* Value		
D16Mit32	0	0.028	9	25	46	29	11.37	0.0010	0	6.215¶
D16Mit130	3.3	0.000	8	26	44	31	11.58	0.0008	0	5.939¶
D16Mit79	3.3	0.000	8	26	44	31	11.58	0.0008	0	5.939¶
D16Mit154	3.3	0.000	8	26	44	31	11.58	0.0008	0	5.939¶
D16Mit182	3.3	0.009	8	26	44	31	11.58	0.0008	0	5.935¶
D16Mit129	3.3	0.000	9	25	44	31	9.71	0.0019	0	5.516§
D16Mit55	3.3	0.000	9	25	44	31	9.71	0.0019	0	5.516§
D16Mit160	4.4	0.000	9	25	44	31	9.71	0.0019	0	5.516§
D16Mit9	4.4	0.000	9	24	44	31	9.04	0.0031	0	5.516§
D16Mit131	6.6	0.000	9	25	44	31	9.71	0.0019	0	5.516§
D16Mit122	6.6	0.000	9	25	44	31	9.71	0.0019	0	5.516§
D16Mit163	6.6	0.009	9	25	44	31	9.71	0.0019	0	5.511§
D16Mit181	3.3	0.009	9	25	43	32	8.93	0.0033	0	4.957§
D16Mit72	7.7	0.046	9	24	44	31	9.04	0.0031	0	5.524§
D16Mit164	7.7	0.156	8	26	42	33	9.93	0.0017	0	6.851¶
D16Mit166	23.0	0.000	12	22	41	34	3.51	0.0569	0	3.377‡
D16Mit58	24.0	0.046	12	22	40	33	3.53	0.0563	0.0450	3.372‡
D16Mit12	25.1	0.174	12	22	40	35	3.05	0.0811	0.0360	3.648§
D16Mit50	39.3	0.083	12	22	40	34	3.28	0.0723	0.0270	5.020§
D16Mit153	45.9		14	20	36	39	0.44	0.4980	0.3040	

Histopathological analysis of epididymitis was performed in a double-blind fashion with each testis scored individually on a PI of 0 to 10. See Table 4 footnotes for explanations of headings.

* χ^2 experimentwise cutoffs were as follows: 90% = 11.58; 95% = 13.12; 99% = 16.39. Comparisonwise χ^2 chromosomewise cutoffs: 90% = 4.57; 95% = 5.39; 99% = 8.93.

†Experimentwise cutoffs: 90% = 10.64; 95% = 11.76; 99% = 14.63.

‡Comparisonwise significance at 90%.

§Comparisonwise significance at 95%.

¶Comparisonwise significance at 99%.

for epididymitis susceptibility. *D16Mit130* accounted for 5.5% of the quantitative variance and had a marker-specific chromosomewise *P* value of 0.0008. This locus has been designated *Epd1*. Even though other loci accounted for a slightly larger QTL variance, the strength of the experimentwise qualitative analysis along with the quantitative result warranted designation of this locus as *Epd1*. The vasitis locus identified by *D1Mit76* at 32.8 cM maximally accounted for 9.4% of the variance and had a marker-specific chromosomewise *P* value of 0.0113. We have designated this locus *Vas1*, although we realize that significance was not established at an acceptable level. As our data showed no shared regions of significance in common (experimentwise, chromosomewise, comparisonwise, and marker-specific chromosomewise), this investigation showed maximal association to distinct loci for autoimmune orchitis, epididymitis, and vasitis. This further supports the independent genetic control of the three lesions as seen in the inbred and congenic strains. We have not, however, conclusively ruled out interaction between susceptibility loci for the three different lesions.

Discussion

Histologically, actively induced murine EAO is characterized by orchitis, aspermatogenesis, epididymitis, and vasitis.[4,7] Orchitis is first seen as inflammatory infiltrates around blood vessels within the interstitium. Subsequently, lymphocytes, macrophages, neutrophils, and eosinophils invade the seminiferous tubules through disrupted Sertoli cell tight junctions. Desquamation of spermatocytes and spermatids occurs adjacent to inflammatory foci, ie, aspermatogenesis. Aspermatogenesis associated with the formation of giant spermatids can also be observed independent of orchitis. In severe orchitis, complete aspermatogenesis and necrosis are found.[4,7] Epididymitis occurs primarily in the cauda and corpus epididymides and less frequently in the caput epididymis.[4,7,16] Lymphoid infiltrates are found between and within the ducts, and sperm disappears from the lumen. Similarly, the early lesions of vasitis are characterized by small clusters of submucosal inflammatory infiltrates. The infiltrating cells then spread into the muscularis mucosae and invade the ductal lumen. Large abscesses or sperm granulomas are seen in severe cases of both epididymitis and vasitis.[4,7,16]

Our results using inbred strains of mice showed different inheritance patterns for autoimmune orchitis, epididymitis, and vasitis. Evidences of three distinct lesions include the following: different inbred strains of mice were grouped according to immunopathological lesions at specific anatomical locations; no definitive *H2* association accounted for susceptibility to differential combinations of the three lesions; and unique genetic regions of the genome associated with distinct disease pathology were based on segregation of disease with microsatellite markers.

The marked differences in disease susceptibility to the three lesions between C57BL/10 and C57BL/6 as well as BALB/cJ and BALB/cByJ mice are of interest. The fact that C57BL/10 and C57BL/6 mice are both $H2^b$ and BALB/cJ and BALB/cByJ are $H2^d$ suggests that differen-

Table 6. Mouse Chromosome 1 Linkage Map and Association of Marker Loci to Autoimmune Vasitis

Locus	MIT	θ	Affected		Unaffected		Chromosomewise		Comparisonwise	
			Ho	He	Ho	He	χ^{2*}	P value	P value	LRT†
D1Mit67	5.5	0.084	29	24	22	32	2.09	0.1400	0.0810	0.983
D1Mit278	17.5	0.037	27	26	19	36	2.97	0.0865	0.0540	1.554
D1Mit322	23.0	0.046	28	26	19	36	3.33	0.0666	0.0630	2.227
D1Mit375	25.1	0.055	28	26	20	35	2.65	0.1063	0.0630	4.093§
D1Mit161	26.2	0.000	30	24	20	35	4.04	0.0483	0.0270	9.310¶
D1Mit75	31.7	0.000	30	24	20	35	4.04	0.0483	0.0270	9.310¶
D1Mit377	31.7	0.018	30	24	20	35	4.04	0.0483	0.0270	9.321¶
D1Mit5	32.8	0.009	32	22	20	35	5.73	0.0197	0.0090	10.480¶
D1Mit76	32.8	0.009	33	21	20	34	6.26	0.0133	0	10.743¶
D1Mit328	33.9	0.000	32	22	20	35	5.73	0.0197	0.0090	8.298¶
D1Mit178	35.0	0.018	32	22	20	35	5.73	0.0197	0.0090	8.295¶
D1Mit128	37.2	0.018	31	23	21	34	4.04	0.0497	0.0540	7.737¶
D1Mit24	41.5	0.018	32	22	22	33	4.04	0.0466	0.0270	8.499¶
D1Mit46	43.7	0.064	31	23	23	32	2.65	0.1087	0.1170	6.332§
D1Mit51	52.5	0.029	31	23	22	33	3.31	0.0733	0.0540	6.355¶
D1Mit157	59.0	0.136	29	25	23	31	1.34	0.2524	0.2360	3.859‡
D1Mit30	73.2	0.119	27	27	24	31	0.44	0.5259	0.5110	2.266
D1Mit33	82.0	0.092	25	28	26	29	0.00	0.9888	0.9280	0.000
D1Mit15	86.3	0.009	29	24	24	31	1.33	0.2542	0.2910	0.383
D1Mit36	91.8	0.073	29	25	24	31	1.11	0.2946	0.2820	0.061
D1Mit406	102.7	0.046	26	28	23	32	0.44	0.5360	0.4820	0.100
D1Mit17	110.4		25	29	21	34	0.74	0.4154	0.3390	

Histopathological analysis of vasitis was performed in a double-blind fashion with each testis scored individually on a PI of 0 to 10. See Table 4 for explanations of headings.

*χ^2 experimentwise cutoffs were as follows: 90% = 11.25; 95% = 12.66; 99% = 15.66. Comparisonwise χ^2 chromosomewise cutoffs: 90% = 6.69; 95% = 7.78; 99% = 8.81.

†Experimentwise cutoffs: 90% = 11.36; 95% = 12.94; 99% = 15.58.

‡Comparisonwise significance at 90%.

§Comparisonwise significance at 95%.

¶Comparisonwise significance at 99%.

tial susceptibility is more than likely a function of non-*H2*-linked genes. C57BL/10J and C57BL/6J mice were derived as sublines of the C57BL strain,[29] whereas BALB/cJ and BALB/cByJ were derived from the BALB/c Snell line.[30] Extensive analysis has revealed limited genetic diversity between both sets of substrains.[29–31] This implies that allelic variation in a gene involved in all three lesions may be responsible for the differential susceptibility to autoimmune orchitis, epididymitis, and vasitis in these two sets of co-isogenic lines.[32]

The results from linkage analyses adds to current knowledge concerning EAO and autoimmune diseases. The susceptibility locus for vasitis co-localizes to the proximal end of chromosome 1 with *Orch5*, a previously identified autoimmune orchitis susceptibility locus.[14] Therefore, it is possible that *Vas1* may represent the same gene as *Orch5*. However, the most significantly linked marker to *Orch5* is at 17.5 cM[14] whereas the most significantly linked marker to autoimmune vasitis is at 32.8 cM. This suggests that *Vas1* and *Orch5* are distinct. Congenic strains will aid in determining whether or not the two loci may be the same gene.

More important, *Vas1* co-localizes with *Idd5*, a disease susceptibility locus involved in autoimmune insulin-dependent type I diabetes mellitus (IDDM).[33,34] These results suggest that autoimmune vasitis and IDDM may share a common autoimmune disease susceptibility locus. Similar co-localization results have been observed for other disease susceptibility loci, including autoimmune orchitis and autoimmune ovarian dysgenesis with IDDM.[14,35] These findings led us to hypothesize that two classes of non-MHC-linked disease susceptibility loci may exist: those that play a role in multiple autoimmune diseases and those that are disease or cross specific.[14,34] The vasitis mapping data reported in this study are consistent with the possible existence of common autoimmune disease susceptibility loci.

Epd1 co-localizes with *Prm1* and *Prm2* on chromosome 16.[36] Protamines 1 and 2 are two genes of particular relevance in autoimmune responses to sperm. They are arginine-rich proteins that replace histones during sperm head condensation[37,38] and are known to be autoantigenic.[39] Anti-protamine autoantibodies are frequently found in the sera of infertile men and develop after vasectomy.[39] Thus, allelic variations in either of the protamine gene products may play a role in the genetic control of susceptibility and resistance to autoimmune epididymitis. Alternatively, this region may encode additional genes that are of particular relevance to the immunobiology of the epididymis.

In conclusion, we have demonstrated that experimental autoimmune orchitis, epididymitis, and vasitis are immunogenetically distinct diseases controlled by both MHC-linked and non-MHC-linked genes. These results are of particular clinical relevance, especially as they relate to the differential diagnosis and treatment of human testicular inflammatory disease. Preliminary comparative mapping results suggest that the major gene encoding for susceptibility to autoimmune vasitis may be of significance in IDDM, another organ-specific autoimmune dis-

ease. Additionally, susceptibility to autoimmune epididymitis is linked to *Prm1* and *Prm2*, two sperm proteins that are known to be autoantigenic. Although the information for autoimmune vasitis and epididymitis confirms existing hypotheses for EAO and autoimmune disease, our results for orchitis add new information. The molecular characterization of the loci identified in this study will aid in defining the pathogenetic mechanisms involved in EAO and provide insight into organ-specific immunoregulatory processes.

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References

1. Tung KSK, Lu CY: Immunologic basis of reproductive failure. Pathology of Reproductive Failure. Edited by Kraus FT, Damjanov I, Kaufmanm N. New York, Williams and Wilkins, 1991, pp 308–333
2. Tung KSK, Teuscher C: Mechanisms of autoimmune disease in the testis and ovary. Hum Reprod Update 1995, 1:35–50
3. Nistal M, Paniagua R: Testicular and Epididymal Pathology. New York, Thieme-Stratton, 1984
4. Tung KSK, Taguchi O, Teuscher C: Testicular and ovarian autoimmune diseases. Autoimmune Disease Models: A Guidebook. Edited by Cohen IR, Miller A. New York, Academic Press, 1995, pp 267–290
5. Voisin GA, Toullet F: Autoimmune aspermatogenic orchitis (A.I.A.O.): a model for three possible mechanisms of autoimmune lesions. Folia Allergol 1971, 18:310–316
6. Yule TD, Tung KSK: Experimental autoimmune orchitis induced by testis and sperm antigen-specific T cell clones: an important pathogenic cytokine is tumor necrosis factor. Endocrinology 1993, 133: 1098–1107
7. Kohno S, Munoz JA, Williams TM, Teuscher C, Bernard CCA, Tung KSK: Immunopathology of murine experimental allergic orchitis. J Immunol 1983, 130:2575–2582
8. Tung KSK, Yule TD, Mahi-Brown CA, Listrom MB: Distribution of histopathology and Ia positive cells in actively induced and passively transferred experimental autoimmune orchitis. J Immunol 1987, 138: 752–759
9. Sakamoto Y, Himeno K, Sanui H, Yoshida S, Nomoto, K: Experimental allergic orchitis in mice. I. A new model induced by immunization without adjuvants. Clin Immunol Immunopathol 1985, 37:360–368
10. Itoh M, Hiramine D, Hojo K: A new murine model of autoimmune orchitis induced by immunization with viable syngeneic germ cells alone. I. Immunological and histological studies. Clin Exp Immunol 1991, 83:137–142
11. Teuscher C, Meeker ND, Livingstone KD, Sudweeks JD, Griffith JS, Wardell BB, Hickey WF: Experimental allergic orchitis in mice. VII. Preliminary characterization of the aspermatogenic autoantigens responsible for eliciting actively and passively induced disease. J Reprod Immunol 1994, 26:233–249
12. Todd JA, Aitman TJ, Cornall RJ, Ghosh S, Hall JRS, Hearne CM, Knight AM, Love JM, McAleer MA, Prins JB, Rodrigues N, Lanthrop M, Pressey A, DeLarato NH, Peterson LB, Wicker LS: Genetic analysis of autoimmune type 1 diabetes in mice. Nature 1991, 351:542–547
13. Wardell BB, Michael SD, Tung KSK, Todd JA, Blankenhorn EP, McEntee K, Sudweeks JD, Hansen WK, Meeker ND, Griffith JS, Livingstone KD, Teuscher C: *Aod1*, the immunoregulatory locus controlling abrogation of tolerance in neonatal thymectomy-induced autoimmune ovarian dysgenesis, maps to mouse chromosome 16. Proc Natl Acad Sci USA 1995, 92:4758–4762
14. Meeker ND, Hickey WF, Korngold R, Hansen WK, Sudweeks JD, Wardell BB, Griffith JS, Teuscher C: Multiple loci govern the bone marrow-derived immunoregulatory mechanism controlling dominant resistance to autoimmune orchitis. Proc Natl Acad Sci USA 1995, 92:5684–5688
15. Teuscher C, Smith SM, Goldberg EH, Shearer GM, Tung KSK: Experimental allergic orchitis in mice. I. Genetic control of susceptibility and resistance to induction of autoimmune orchitis. Immunogenetics 1985, 22:323–333
16. Teuscher C, Smith SM, Tung KSK: Experimental allergic orchitis in mice. III. Differential susceptibility and resistance among BALB/c sublines. J Reprod Immunol 1987, 10:219–230
17. Sudweeks JD, Todd JA, Blankenhorn EP, Wardell BB, Woodward SR, Meeker ND, Estes SS, Teuscher C: The locus controlling *Bordatella pertussis* induced histamine sensitization (*Bphs*), an autoimmune disease susceptibility gene, maps distal of T-cell receptor β-chain gene on mouse chromosome 6. Proc Natl Acad Sci USA 1993, 90:3700–3704
18. Dietrich W, Miller JC, Steen RG, Merchant M, Damron D, Nahf R, Gross A, Joyce DC, Wessel M, Dredge RD, Marquis A, Stein LD, Goodman N, Page DC, Lander ES: A genetic map of the mouse with 4,006 simple sequence length polymorphisms. Nature Genet 1994, 7:220–245
19. Dietrich W, Katz H, Lincoln SE, Shin SH, Friedman J, Dracopoli NC, Lander ES: A genetic map of the mouse suitable for typing interspecific crosses. Genetics 1992, 131:423–447
20. Lander ES, Green P, Abrahamson J, Barlow A, Daley MJ, Lincoln SE, Newberg L: MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1987, 1:174–181
21. Lander ES, Botstein D: Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 1989, 121:185–199
22. Basten CJ, Weir BS, Zeng Z-B: QTL CARTOGRAPHER: A Reference Manual and Tutorial for QTL Mapping. Raleigh, NC, North Carolina State University, 1996
23. Churchill GA, Doerge RW: Empirical threshold values for quantitative trait mapping. Genetics 1994, 138:963–971
24. Good P: Permutation Tests: A Practical Guide to Re-Sampling for Testing Hypotheses. New York, Springer-Verlag, 1994
25. Person PL, Snoek M, Demant P, Woodward SR, Teuscher C: The immunogenetics of susceptibility and resistance to murine experimental allergic orchitis. Regional Immunol 1992, 4:284–297
26. Blankenhorn EP, Stranford SA: Genetic factors in demyelinating diseases: genes that control demyelination due to experimental allergic encephalomyelitis (EAE) and Theiler's murine encephalitis virus. Regional Immunol 1992, 4:331–343
27. Robison R, Tung KSK, Meeker ND, Monson FG, Teuscher C: A murine model of spontaneous aspermatogenesis: linkage to *H-2*. J Reprod Immunol 1994, 26:251–260
28. Dietrich WF, Miller J, Steen R, Merchant MA, Damron-Boles D, Husain Z, Dredge R, Daly MJ, Ingalls KA, O'Connor TJ, Evans CA, DeAngelis MM, Levinson DM, Kruglyak L, Goodman N, Copeland NG, Jenkins NA, Hawkins TL, Stein L, Page DC, Lander ES: A comprehensive genetic map of the mouse genome. Nature 1996, 380:149–152
29. Festing MFW: Origins and characteristics of inbred strains of mice, 14th listing. Mouse Genome 1992, 90:231–352
30. Potter M: The BALB/c Mouse: Genetics and Immunology. New York, Springer-Verlag, 1985
31. McClive PJ, Huang D, Morahan G: C57BL/6 and C57BL/10 inbred mouse strains differ at multiple loci on chromosome 4. Immunogenetics 1994, 39:286–288
32. Teuscher C, Blankenhorn EP, Hickey WF: Differential susceptibility to actively induced experimental allergic encephalomyelitis and experimental allergic orchitis among BALB/c substrains. Cell Immunol 1987, 122:294–304
33. Wicker LS, Todd JA, Peterson LB: Genetic control of autoimmune diabetes in the NOD mouse. Annu Rev Immunol 1995, 13:179–200
34. Vyse TJ, Todd JA: Identification of checkpoints in immunity by genetic analysis of autoimmune diseases. Cell 1996, 85:311–318
35. Teuscher C, Wardell BB, Lunceford JK, Michael SD, Tung KSK: *Aod2*, the locus controlling development of atrophy in neonatal thymectomy-induced autoimmune ovarian dysgenesis, co-localizes with *Il2*, *Fgfb*, and *Idd3*. J Exp Med 1996, 183:631–638
36. Reeves RH, Gearhart JD, Hecht NB, Yelick P, Johnson P, O'Brian SJ: Mapping of *PRM1* to human chromosome 16 and tight linkage of

Prm-1 and *Prm-2* on mouse chromosome 16. J Hered 1989, 80:442–446
37. Balhorn R: Mammalian protamines: structure and molecular interactions. Molecular Biology of Chromosome Function. Edited by Adolph KW. New York, Springer-Verlag, 1989, p 366
38. Hecht NB: Molecular biology of structural chromosomal proteins of the mammalian testis. Molecular Biology of Chromosome Function. Edited by Adolph KW. New York, Springer-Verlag, 1989, p 396
39. Samuel T, Kolk A: Auto-antigenicity of human protamines. Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man. Edited by Lepow IH, Crozier R. New York, Academic Press, 1979, pp 203–220
40. Teuscher C, Gasser DL, Woodward SR, Hickey WF: Experimental allergic orchitis in mice. VI. Recombinations within the *H-2S/H-2D* interval define the map position of the *H-2*-associated locus controlling disease susceptibility. Immunogenetics 1990, 32:337–344.