Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4758–4762, May 1995
Immunology

Aod1, the immunoregulatory locus controlling abrogation of tolerance in neonatal thymectomy-induced autoimmune ovarian dysgenesis, maps to mouse chromosome 16

(immunologic tolerance/autoimmunity/linkage analysis/regulatory T cells)

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Communicated by Neal L. First, University of Wisconsin, Madison, WI, January 3, 1995 (received for review August 31, 1994)

ABSTRACT **Mice thymectomized at three days of age (D3Tx) develop during adulthood a variety of organ-specific autoimmune diseases, including autoimmune ovarian dysgenesis (AOD). The phenotypic spectrum of AOD is characterized by the development of anti-ovarian autoantibodies, oophoritis, and atrophy. The D3Tx model of AOD is unique in that disease induction depends exclusively on perturbation of the normal developing immune system, is T-cell-mediated, and is strain specific. For example, D3Tx A/J mice are highly susceptible to AOD, whereas C57BL/6J mice are resistant. After D3Tx, self ovarian antigens, expressed at physiological levels, trigger an autoimmune response capable of eliciting disease. The D3Tx model provides, therefore, the opportunity to focus on the mechanisms of self-tolerance that are relevant to disease pathogenesis. Previous studies indicate that the principal mechanisms involved in AOD susceptibility are genetically controlled and govern developmental processes associated with the induction and maintenance of peripheral tolerance. We report here the mapping of the *Aod1* locus to mouse chromosome 16 within a region encoding several loci of immunologic relevance, including *scid, Igl1, VpreB, Igll, Igl1r, Mtv6* (*Mls-3*), *Ly-7, Ifnar*, and *Ifgt*.**

Neonatal mice thymectomized at three days of age (D3Tx) develop, during adulthood, a variety of organ-specific autoimmune diseases which include prostatitis (1), orchitis and epididymovasitis (2, 3), thyroiditis (4), gastritis (3, 5, 6), and oophoritis (3, 7–9). Inbred strains that are susceptible to D3Tx-induced autoimmune ovarian dysgenesis (AOD) also develop circulating anti-ovarian autoantibodies that inhibit fertilization *in vitro* (10). Similarly, anti-ovarian autoantibodies, ovarian lymphocytic infiltrates, and abnormalities in T-cell subsets and lymphokine levels have been observed in women with premature ovarian failure (POF) (11). POF is also seen in conjunction with other organ-specific autoimmune disorders such as type 1 and type 2 polyendocrinopathies (11). Immunosuppressive therapy has also been used to restore normal ovarian function (11). In addition to autoimmunity, D3Tx in mice (12) and prenatal thymectomy in nonhuman primates (13) result in disturbances of serum levels of gonadotropin and sex steroids as well as abnormal ovarian development (14). Therefore, D3Tx-induced AOD serves as an ideal animal model for studying human POF.

The mechanism by which D3Tx abrogates tolerance to ovarian autoantigens has been investigated at the cellular level. The T-cell repertoire of both neonatal and D3Tx adult mice is enriched for self-reactive T cells (15). In the adult repertoire, $CD4^+$ $CD5^{low}$ T cells have the capacity to elicit autoimmune oophoritis by adoptive transfer (16, 17). Thus the ability to transfer disease is due, in part, to failure of the neonatal thymus to delete autoreactive cells before it is removed. In addition, D3Tx mice are deficient in $CD4^+$ $CD5^{high}$ regulatory T cells (16) required for controlling oophoritogenic T cells that mature in the adult thymus (17). These findings suggest that expansion of the autoreactive neonatal T-cell repertoire, frozen by D3Tx, results in disease. The fact that susceptibility to AOD is both genetically controlled (18, 19) and results from perturbation of ovarian antigen-specific tolerance mechanisms, including deletion of regulatory T cells, suggests that the genes controlling the phenotypic expression of disease may include those that govern the induction and maintenance of peripheral tolerance (17). In this study, we carried out genome exclusion mapping (20, 21) employing a backcross population derived from the AOD-susceptible A/J and AOD-resistant C57BL/6J strains. We report the identification and mapping to chromosome 16 of the major locus controlling the development of both the humoral and T-cell-mediated anti-ovarian autoimmune responses following D3Tx.

MATERIALS AND METHODS

Animals. Female (C57BL/6J × A/J) F_1 hybrid and male C57BL/6J mice were purchased from The Jackson Laboratory. One hundred and forty-four D3Tx (C57BL/6J × A/J) F_1 × C57BL/6J backcross (BC_1) mice were generated in the animal colony at the State University of New York at Binghamton. D3Tx was performed while the animals were under ether anesthesia, using a suction pipette technique at 3 days of age (22). Animals were maintained on Purina mouse pellets and acidified water ad lib. D3Tx females were sacrificed at 60 days of age.

Indirect Immunofluorescence Analysis of Anti-Ovarian Autoantibodies. (See Fig. 1.) The most common anti-ovarian autoantibodies found in D3Tx mice are directed against cytoplasmic antigens of oocytes in growing and antral follicles (3, 8). Less common are autoantibodies to the zona pellucida and luteinized cells in the interstitial space and the corpus luteum. Antinuclear and anti-smooth muscle autoantibodies are seen occasionally. Anti-ovarian autoantibodies present in the sera of the D3Tx BC_1 animals were studied by indirect immunofluorescence (3). The location and intensity of antibody binding for serial dilutions of each serum were scored in a

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Abbreviations: D3Tx, day three thymectomy; AOD, autoimmune ovarian dysgenesis; cM, centimorgan.

double-blind fashion with the antibody titer being the greatest dilution with positive reactivity. High-titered sera with known anti-oocyte autoantibody activity were used as a positive control and normal mouse serum served as the negative control.

Histopathologic Analysis of Autoimmune Oophoritis and Atrophy. (See Fig. 2.) The earliest histopathologic change (3–4 weeks after thymectomy) observed in the ovaries of D3Tx mice is oophoritis, represented by lymphocyte infiltration in the hilar region and, later, their extension into the interfollicular areas as well as within growing and antral follicles (3). Subsequently, at about 6 weeks, the infiltrating cells may regress from the ovaries, which then become atrophic. Atrophic areas are devoid of ovarian follicles of all stages of development and are replaced by interstitial cells that appeared luteinized. The ovaries of the BC_1 mice were fixed in Bouin's fixative and embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin. Multiple-step sections were evaluated in a double-blind fashion and scored for oophoritis and atrophy.

DNA Isolation. Genomic DNA was isolated from liver tissue as described (21). Working aliquots of all DNA samples were prepared by bringing them to the appropriate concentrations in TE′ (10 mM Tris·HCl, pH 7.4/0.1 mM EDTA).

Microsatellite Primers, Amplification Conditions, and Detection of PCR Products. Microsatellite primers were synthesized according to sequences obtained through the Whitehead Institute/MIT Mouse Genome Database. PCRs for microsatellite typing were performed as described (20, 21, 23, 24). Microsatellite size variants were resolved by electrophoresis in 40 mM Tris acetate, pH 8.5/2 mM EDTA/agarose gels (3% NuSeive and 1% ME agarose; FMC) and visualized with ethidium bromide or were resolved by electrophoresis on large-format denaturing polyacrylamide gels and visualized by autoradiography on Kodak film.

Linkage Analysis. Only affected animals were employed in the analysis to avoid phenotyping errors due to residual thymic tissue. The phenotypes of AOD, anti-ovarian autoantibody, oophoritis, or atrophy were scored as detailed above, with the following criteria: mice were considered positive for autoantibody if antibody was detectable in a greater than 1:10 dilution of serum; oophoritis and atrophy were scored in a plus/minus fashion; and AOD was considered present in mice that developed a constellation of any or all of these traits. Linkage of marker loci to disease phenotypes was evaluated by χ^2 with linkage being significant at $P \leq 0.001$. Genotype frequency differences were tested within the affected population against a predicted frequency of 1:1 for marker loci. Segregation distortion was examined by determining the genotype frequency of randomly selected marker loci distributed throughout the genome in 144 D3Tx BC_1 mice. In no case was significant distortion from the predicted frequency of 1:1 observed.

RESULTS

The phenotypic spectrum of AOD in D3Tx mice is characterized by the development of anti-ovarian autoantibodies, oophoritis, and atrophy (3, 7–9). Each of these three characteristics was treated as an independent disease phenotype. To map the genes controlling susceptibility to AOD within the murine genome, we generated a genetic linkage map using the 104 affected BC_1 mice. These mice had any or all of the autoimmune phenotypes. Previously mapped microsatellites ($n = 100$) that distinguish C57BL/6J (AOD-resistant) and A/J (AOD-susceptible) mice were used (20, 23, 25).

Linkage analysis carried out on the AOD-affected population as a whole revealed significant linkage of AOD to *D16Mit58* ($\chi^2 = 11.1$, $P = 0.00086$) and *D16Mit60* ($\chi^2 = 11.1$, $P = 0.00086$) on chromosome 16. The locus controlling AOD and mapping to this region has been designated *Aod1*.

Linkage analysis was also performed for each of the major phenotypes. The BC_1 mice were divided into groups as follows. Of 144 BC_1 mice studied, 89 had significant titers of anti-ovarian autoantibodies (Fig. 1). Of these 89 animals, 78 BC_1 mice had autoantibodies against cytoplasmic antigens of oocytes in growing and antral follicles. In addition, 4 mice exhibited anti-zona pellucida autoantibodies, 3 had autoantibodies to the luteinized cells in the interstitial space and the corpus luteum, 3 had autoantibodies to smooth muscle, and 16 had anti-nuclear antibody activity. The anti-ovarian autoantibody response exhibited maximal linkage to *D16Mit29* ($\chi^2 = 11.9$, $P = 0.00056$) at ≈16 centimorgans (cM) from the centromere (Table 1). Therefore, as expected, the anti-ovarian autoantibody response and AOD susceptibility map to the same region.

The earliest evidence of histopathology, at 3–4 weeks after thymectomy, is oophoritis (Fig. 2). Histopathologic studies revealed that 74 of the 144 D3Tx BC_1 animals developed autoimmune oophoritis. Oophoritis exhibited linkage to the following: *D16Mit57* ($\chi^2 = 11.5$, $P = 0.00070$), *D16Mit58* ($\chi^2 = 13.2$, $P = 0.00028$), *D16Mit60* ($\chi^2 = 13.2$, $P = 0.00028$), and *D16Mit4* through *D16Nds2* (all at $\chi^2 = 11.5$ with $P = 0.00070$) (Table 1). Again, as with the anti-ovarian autoantibody response, autoimmune oophoritis and AOD cosegregated.

In some animals, after oophoritis is established, the infiltrating cells may regress from the ovaries, which then become atrophic (3). Of the 74 with oophoritis, 24 developed atrophy. In an attempt to identify the gene or genes involved in atrophy, linkage analysis was performed on this subpopulation. Markers on chromosome 16 failed to exhibit significant linkage to atrophy. Rather, atrophy exhibited an association with *D3Mit6* ($\chi^2 = 7.6$, $P = 0.00584$) and *D3Mit65* ($\chi^2 = 7.1$, $P = 0.00771$) at 19 and 20 cM from the centromere (Table 1). In addition, the tendency to develop ovarian atrophy also exhibited an association with *D9Mit12* ($\chi^2 = 7.6$, $P = 0.00584$) on chromosome 9. When linkage analysis for atrophy was carried out using the most severely affected population ($n = 44$), significant increases in the χ^2 values were observed with both chromosomes 3 and 9 (Table 1). However, in neither case did the level of significance exceed 0.001. Larger numbers of animals with the atrophic phenotype will be required to determine the significance of these potential linkages.

DISCUSSION

D3Tx-induced AOD is characterized by the formation of circulating anti-ovarian autoantibodies, the development of

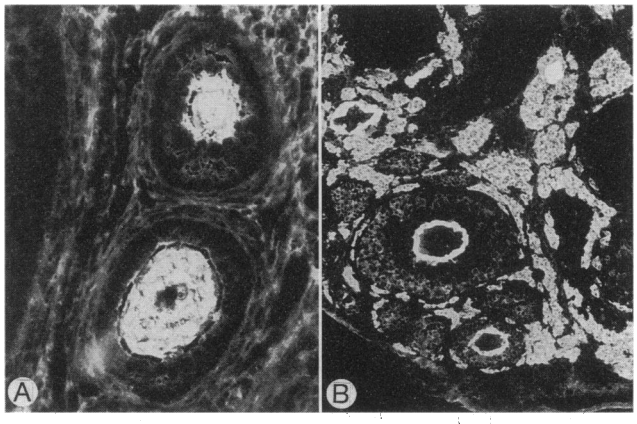

FIG. 1. Immunofluorescent autoantibodies produced by D3Tx mice. Most common are antibodies against cytoplasmic antigens of oocytes in growing and antral follicles (*A*). (×200.) Less common are antibodies to the zona pellucida and luteinized cells in the interstitial space and the corpus luteum (*B*). (×100.)

Table 1. Linkage map of the mouse genome and linkage of marker loci with AOD, anti-ovarian autoantibody response, oophoritis, and atrophy

Chromosome (cM)*	Locus	AOD			Anti-ovarian antibody			Oophoritis			Oophoritis†				
											With atrophy		Without atrophy		
		Ho	He	$\chi^2 \geq 6.0$	Ho	He	$\chi^2 \geq 6.0$	Ho	He	$\chi^2 \geq 6.0$	Ho	He	Ho	He	$\chi^2 \geq 6.0$
1 (9)	*D1Mit3*	37	35		27	24		37	34		13	11	24	24	
1 (50)	*D1Mit46*	55	46		50	34		36	35		13	11	23	25	
1 (71)	*D1Nds2*	56	45		47	36		34	37		11	13	24	24	
1 (91)	*D1Mit15*	42	30		34	17		41	41		16	8	26	22	
1 (104)	*Crp* (119)	46	53		39	43		34	37		14	10	20	28	
1 (114)	*D1Mit210*	38	56		28	47		30	36		12	9	18	27	
1 (115)	*D1Mit17*	35	37		25	26		35	36		16	8	19	29	
2 (4)	*D2Mit5*	51	50		39	45		35	36		11	13	25	23	
2 (49)	*D2Mit14*	33	37		20	29		32	37		11	12	22	25	
2 (76)	*D2Mit51*	53	47		43	40		39	31		12	12	28	19	
3 (5)	*D3Mit62*	45	56		40	43		28	42		6	17	22	26	
3 (13)	*D3Mit55*	45	56		38	45		30	42		7	17	24	25	
3 (16)	*D3Mit21*	43	60		38	47		27	45		5	19	23	26	(7.8)
3 (19)	*D3Mit6*	43	61		38	48		28	45		4	20	25	25	7.6 (9.7)
3 (20)	*D3Mit65*	42	61		37	48		27	45		4	20	24	25	7.1 (9.7)
3 (27)	*D3Mit22*	45	59		41	45		29	44		6	18	24	26	(7.4)
3 (31)	*D3Mit40*	47	53		45	38		30	41		7	17	24	24	
3 (46)	*D3Mit14*	53	51		48	38		36	37		9	15	28	22	
3 (51)	*D3Mit38*	50	44		42	33		35	31		8	13	27	18	
3 (60)	*D3Mit44*	56	39		50	27	6.9	36	33		11	12	26	21	
3 (62)	*D3Mit32*	53	41		44	31		37	29		9	12	28	17	
4 (7)	*D4Mit2*	36	35		27	24		35	35		13	10	23	25	
4 (32)	*D4Mit15*	50	49		42	40		38	32		14	9	24	24	
4 (51)	*D4Mit16*	50	50		43	40		38	33		14	10	24	24	
4 (68)	*D4Mit14*	48	47		39	39		38	31		12	10	26	22	
5 (18)	*D5Mit80*	42	50		32	42		30	34		12	8	18	26	
5 (19)	*D5Mit11*	28	44		17	34		28	43		12	12	16	32	
5 (46)	*D5Nds3*	38	34		28	23		38	33		12	12	26	22	
5 (50)	*D5Mit24*	42	59		32	51		30	41		11	13	19	29	
5 (53)	*D5Mit188*	41	53		30	45		28	38		10	11	18	27	
5 (70)	*D5Mit30*	51	42		41	36		40	29		14	8	26	22	
6 (27)	*D6Mit8*	50	51		41	43		35	36		13	11	23	25	
6 (43)	*D6Mit36*	36	36		23	28		35	36		12	12	24	24	
6 (69)	*D6Mit15*	48	48		37	42		38	33		13	11	25	23	
7 (20)	*D7Mit27*	45	51		38	42		30	41		12	12	19	29	
7 (30)	*D7Nds1*	36	34		26	23		36	33		13	10	23	24	
7 (57)	*D7Mit71*	54	47		46	38		41	30		15	9	26	22	
8 (8)	*D8Mit4*	47	54		39	45		33	38		13	11	21	27	
8 (33)	*D8Mit31*	35	35		23	26		34	35		11	12	24	23	
8 (66)	*D8Mit14*	57	42		45	38		38	31		11	13	28	18	
9 (12)	*D9Mit2*	52	48		42	40		35	35		14	10	22	25	
9 (33)	*D9Mit105*	45	49		39	36		29	37		13	8	16	29	(7.2)
9 (43)	*D9Mit11*	33	39		27	24		32	39		16	8	17	31	6.3 (9.5)
9 (51)	*D9Mit12*	40	54		35	40		25	41		13	8	12	33	7.6 (8.2)
9 (68)	*D9Mit18*	44	56		40	43		33	38		14	10	20	28	
10 (0)	*D10Nds1*	38	33		27	23		37	33		11	13	27	20	
10 (8)	*D10Mit2*	40	32		26	25		39	32		14	10	26	22	
10 (41)	*D10Mit42*	50	54		43	43		36	37		13	11	23	27	
10 (51)	*D10Mit10*	46	58		38	48		34	39		13	11	21	29	
10 (69)	*D10Mit14*	38	34		27	24		38	34		14	10	24	24	
11 (2)	*D11Mit2*	41	31		29	22		40	31		12	12	29	19	
11 (25)	*D11Mit86*	21	31		16	23		16	16		2	8	15	8	
11 (37)	*D11Mit4*	56	45		47	36		41	29		11	13	31	16	
11 (49)	*D11Mit41*	53	51		46	40		39	34		10	14	30	20	
12 (1)	*D12Mit1*	39	31		27	23		38	31		13	11	26	20	
12 (4)	*D12Mit12*	44	57		39	45		32	39		12	12	20	28	
12 (32)	*D12Mit5*	45	56		36	48		34	37		11	13	24	24	
12 (60)	*D12Nds10*	35	37		24	27		34	37		10	14	25	23	
13 (1)	*D13Mit3*	42	30		29	22		41	30		14	10	28	20	
13 (21)	*D13Mit21*	48	52		39	44		32	38		9	14	23	25	
13 (39)	*D13Mit45*	46	44		38	35		36	35		9	15	27	21	
14 (14)	*D14Mit14*	45	55		39	44		34	36		10	13	25	23	
14 (34)	*D14Mit37*	52	47		44	38		40	31		13	11	28	20	
14 (52)	*D14Mit7*	40	32		30	21		39	32		13	11	27	21	

Table 1. (*Continued.*)

Chromosome (cM)*	Locus	AOD Ho	AOD He	AOD $\chi^2 \geq 6.0$	Anti-ovarian antibody Ho	Anti-ovarian antibody He	Anti-ovarian antibody $\chi^2 \geq 6.0$	Oophoritis Ho	Oophoritis He	Oophoritis $\chi^2 \geq 6.0$	Oophoritis† With atrophy Ho	Oophoritis† With atrophy He	Oophoritis† Without atrophy Ho	Oophoritis† Without atrophy He	Oophoritis† $\chi^2 \geq 6.0$
15 (6)	*D15Mit11*	58	43		43	41		44	27		14	10	31	17	
15 (8)	*D15Mit53*	45	44		34	37		36	35		10	14	27	21	
15 (34)	*D15Mit5*	34	38		23	28		33	38		8	16	26	22	
15 (34)	*D15Mit28*	51	50		40	44		34	37		12	12	23	25	
15 (63)	*D15Mit16*	40	32		28	23		39	32		14	10	26	22	
16 (0)	*D16Mit32*	39	65	6.5	33	53		25	48	7.2	7	17	18	32	
16 (7)	*D16Mit122*	36	66	8.8	30	55	7.4	24	48	8.0	8	16	16	33	
16 (8)	*D16Mit87*	40	64		33	53		26	47	6.0	9	15	18	32	
16 (16)	*D16Mit29*	37	67	8.7	27	59	11.9	24	49	8.6	8	16	16	34	
16 (24)	*D16Mit57*	36	68	9.8	31	55	6.7	22	51	11.5	8	16	14	36	
16 (27)	*D16Mit58*	35	69	11.1	30	56	7.9	21	52	13.2	8	16	13	37	
16 (28)	*D16Mit60*	35	69	11.1	29	57	9.1	21	52	13.2	7	17	14	36	
16 (28)	*D16Mit4*	36	68	9.8	29	57	9.1	22	51	11.5	7	17	15	35	
16 (28)	*D16Mit59*	38	66	7.5	31	55	6.7	22	51	11.5	7	17	16	34	
16 (28)	*D16Mit12*	38	66	7.5	31	55	6.7	22	51	11.5	7	17	16	34	
16 (28)	*D16Nds2*	38	66	7.5	31	55	6.7	22	51	11.5	7	17	16	34	
16 (36)	*D16Mit5*	43	61		36	50		26	47	6.0	8	16	19	31	
16 (44)	*D16Mit19*	44	58		37	47		30	41		12	12	19	29	
17 (10)	*D17Nds3*	41	31		26	25		40	31		10	14	31	17	
17 (19)	*D17Mit10*	54	45		44	38		37	33		11	13	27	20	
17 (30)	*D17Mit20*	53	41		42	33		36	30		11	10	25	20	
17 (45)	*D17Mit2*	45	51		43	37		31	37		14	9	17	29	
18 (2)	*D18Mit20*	34	36		25	24		34	35		11	12	23	24	
18 (15)	*D18Mit24*	55	47		46	38		38	34		12	12	27	22	
18 (26)	*D18Mit9*	50	51		41	43		34	37		11	13	24	24	
18 (38)	*D18Mit4*	33	39		23	28		32	39		11	13	22	26	
19 (14)	*D19Mit16*	41	31		30	21		40	31		14	10	27	21	
19 (25)	*D19Mit19*	59	42		51	33		39	31		16	8	23	25	
19 (43)	*D19Mit1*	56	45		51	33		39	31		14	10	25	23	
X (0)	*DXMit55*	49	49		40	45		31	40		11	12	21	28	
X (27)	*DXMit22*	44	60		41	45		30	43		12	12	18	32	
X (30)	*DXNds3*	46	56		38	47		30	41		10	14	20	28	
X (32)	*DXMit25*	42	60		39	45		28	43		11	13	17	31	
X (32)	*DXMit1*	27	45		22	29		27	44		11	13	16	32	
X (39)	*DXMit16*	28	44		22	29		28	43		10	14	18	30	
X (60)	*DXMit36*	44	58		38	47		30	41		9	15	21	27	

The phenotypes of oophoritis and atrophy were determined histologically, whereas the anti-ovarian autoantibody response was by indirect immunofluorescence. The overall AOD phenotype was considered present in mice that develop a constellation of any or all of the above traits. Genotype frequency differences for AOD, anti-ovarian antibody response, and oophoritis were tested within the affected populations by χ^2 against a predicted frequency of 1:1 for marker loci. Only $\chi^2 \geq 6.0$ are shown. He, number of mice heterozygous; Ho, number of mice homozygous.

*Markers are arranged centromeric to telomeric. Locations are as reported or as best estimates based on comparisons of existing maps. All are PCR-based microsatellites which distinguish C57BL/6J and A/J.

†Atrophy was evaluated by χ^2 in 2 × 2 contingency tables employing all mice exhibiting oophoritis. The values in parentheses are from linkage analysis using severely affected population (n = 44).

autoimmune T cells capable of infiltrating the ovary (oophoritis), and ovarian atrophy (3, 7–9, 16). Our genetic analysis of AOD has led to the identification of a single major susceptibility locus on chromosome 16 which we have designated *Aod1*. The data presented also indicate that this gene controls both the humoral and T-cell-mediated anti-ovarian autoimmune responses observed after D3Tx. The temporal appearance of ovarian autoantibody follows rather than precedes oophoritis (3), and adoptive transfer experiments have demonstrated that oophoritis is mediated by $CD4^+$ $CD5^{low}$ T cells (16, 17). Thus, our finding that a single locus controls the humoral and cellular anti-ovarian immune responses is consistent with these previous studies on AOD immunopathology as well as the requirement for T cells in the generation of IgG autoantibodies. In addition, the two phenotypes linked to *Aod1* correspond to the two major autoimmune abnormalities observed in POF, that is, the presence of circulating anti-ovarian autoantibodies and inflammatory infiltrates in the ovary (11).

The study of susceptibility loci in the AOD model has the potential to open the way for a detailed examination of genes that participate in the loss of tolerance to self-antigens. We previously demonstrated that pathogenic self-reactive T cells, relevant in autoimmune oophoritis, are not deleted in either the neonatal or adult thymus (15, 17). In neonatal mice, these T cells readily elicit autoimmune disease when transferred into syngeneic athymic (*nu/nu*) (or *scid*) recipients (17). In addition, these cells are also present in the adult spleen; however, they are normally controlled by a second population of $CD4^+$ $CD5^{high}$ T cells (17). These data collectively suggest that immunoregulatory T cells in adult mice regulate pathogenic self-reactive T cells in this model. The balance of the two cell populations ensures the maintenance of tolerance. However, when the balance tips in favor of effector T-cell activity as in mice expressing a susceptible *Aod1* allele, autoimmune disease ensues. The autoimmunity found in D3Tx mice best illustrates this loss of immunoregulatory T cells, since injection of normal adult $CD4^+$ $CD5^{high}$ T cells into D3Tx mice prevents disease

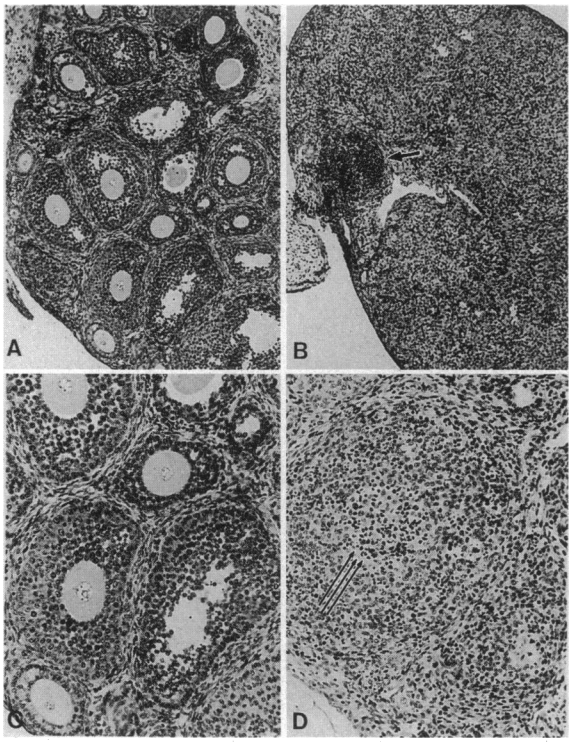

FIG. 2. Histologic appearance of ovarian atrophy and oophoritis in D3Tx mice. Compared with the normal ovary (*A*; ×50), atrophic ovary (*B*; ×50) lacks ovarian follicles; it is replaced by infiltration of interstitial cells and clusters of residual inflammation near the ovarian hilum (arrow). Inflamed ovary (*D*; ×100), or oophoritis, is typified by infiltration of inflammatory cells within ovarian follicle replacing the oocyte (three arrows). Inflammation is absent from normal ovary (*C*; ×100).

(17). Similar results are seen with diabetes in normal nonautoimmune rat strains in which disease is induced by adult thymectomy and sublethal γ-irradiation. In this model, autoimmune diabetes and insulitis can be completely prevented by transfer of $CD4^+$ T-cell receptor α/β^+ cells isolated from healthy syngeneic donors (24).

The mapping data presented place *Aod1* in a region encoding a number of immunologically relevant genes. These include *scid* (severe combined immunodeficiency), *Igl1* (immunoglobulin λ five), *VpreB* (immunoglobulin λ VpreB chain), *Igll* (immunoglobulin λ light chain), *Igl1r* (immunoglobulin λ light chain regulator), *Mtv6* (*Mls-3*) (minor lymphocyte stimulator 3), *Ly-7* (lymphocyte antigen 7), *Ifnar* (interferon α and β receptor), and *Ifgt* (interferon γ response element) (26). However, at present our mapping data are inadequate to directly identify which, if any, of these candidate genes play a role in AOD. Nevertheless, it is noteworthy that *Aod1* does not correspond to any of the previously mapped loci involved in autoimmune type 1 diabetes mellitus (20) and lupus-like autoimmune disease in mice (27, 28).

The data indicating that ovarian atrophy is not linked to *Aod1* are of potential significance endocrinologically. In D3Tx mice which develop AOD, alterations in the plasma levels of gonadotrophin hormones are observed prior to the development of autoimmune oophoritis (12). This has led to the hypothesis that in addition to ovarian autoimmunity, endocrinologic aberrations may also be involved in D3Tx-induced AOD, perhaps through deprivation of thymic hormones and/or defective thymic–hypothalamic–gonadal regulation. Genes on chromosome 3 and 9, exhibiting an association with ovarian atrophy, may control susceptibility to the aforementioned endocrinologic defects observed in D3Tx mice.

In conclusion, the abrogation of tolerance that culminates in the development of AOD in D3Tx mice appears to be controlled by a single major immunoregulatory gene which we have designated *Aod1*. Whether this locus is of general significance in other D3Tx-induced models of autoimmune disease awaits further analyses. Nevertheless, our results and the subsequent characterization of this immunoregulatory locus may provide new insight into the molecular mechanisms associated with the generation and maintenance of tolerance (29).

We thank Julie Teuscher and William J. Griffin for their expert technical assistance. This research was supported by National Institutes of Health Grants HD-21926 (to C.T.), HD-27275 (to C.T.), NS-25519 (to E.P.B.), HD-27500 (to K.S.K.T.), and HD-27506 (to S.D.M.), National Multiple Sclerosis Society Grants PP0324 (to C.T.) and RG2120 (to E.P.B.), and the Medical Research Council as part of the U.K. Human Genome Mapping Project (to J.A.T.).

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