Vitamin A-Enhanced Cleft Palate Susceptibility Gene Maps between C4 and B144 within the H-2 Complex (43563)

MARVIN L. TYAN*,[1] AND DOLLY B. TYAN[†]

West Los Angeles Veterans Affairs Medical Center and Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024 and Cedars-Sinai Medical Center,[†] Los Angeles, California 90048*

Abstract. **Pregnant mice congenic with C57BL/10 in the region of H-2, the major histocompatibility complex on Chromosome 17 (B10.BR, B10.A, B10.A(1R), B10.A(2R), B10.A(15R), B10.A(18R), B10.OL, or crosses between them) were fed Purina Laboratory Chow or the same diet plus 400 IU of vitamin A daily from conception and given dexamethasone (80 or 160 mg/kg) intraperitoneally on the twelfth day of pregnancy. It was found that the added vitamin A increased the frequency of isolated cleft palate only in strains that had *b* alleles between C4 and B144. The enhancement of susceptibility to glucocorticosteroid-induced cleft palate by vitamin A appears to be a recessive trait and the locus, called *Acp*, maps centromeric to another corticosteroid-induced cleft palate gene (*Dcp-2*) that also is in the C4:B144 interval.** [P.S.E.B.M. 1993, Vol 202]

Susceptibility of mice to glucocorticosteroid-induced cleft palate (CP) is determined in part by at least three loci associated with the major histocompatibility complex, H-2. Two genes that determine susceptibility to corticosteroid-induced CP interact in *cis* in a co-dominant manner: one locus (*Dcp-1*) has been mapped to the interval between the centromere and Eβ and the second (*Dcp-2*) to the region between H-2S and H-2D (1–7). A third locus, *Acp*, determines the effect of vitamin A on the susceptibility to glucocorticosteroid-induced CP; susceptibility is increased in strains that have *b* alleles between H-2S and H-2D (2, 8).

Presented below are studies which show that (i) *Acp* and *Dcp-2* map between C4 and B144, (ii) vitamin A-enhanced glucocorticosteroid CP susceptibility is a recessive trait, and (iii) *Acp* is most likely centromeric to *Dcp-2* in the C4 to B144 interval.

Materials and Methods

The H-2 congenic strains C57BL/10 (the host strain), B10.BR, B10.A, B10.A(1R), B10.A(2R), B10.A(15R), B10.A(18R), and B10.OL have been maintained in this laboratory by brother × sister matings. The H-2 haplotypes are shown in Table I (also see Ref. 9). In the experiments, one male and two virgin 10- to 12-week-old females were placed in each cage. On the day a vaginal plug was detected (Day 0), the dams were assigned to receive the control or vitamin A-supplemented diet. On Day 12, the mice were weighed and injected intraperitoneally with 80 mg/kg or 160 mg/kg of dexamethasone (Merck, Sharp and Dohme). The mice were sacrificed on the eighteenth day of pregnancy and the number of living fetuses and resorptions was recorded. Living fetuses were weighed and examined for gross external malformations. The oral cavity was inspected for the presence of CP, and the internal organs were examined for defects and to determine sex.

The average pregnant mouse (~25 g) consumes approximately 5 g of food per day (i.e., the equivalent of one average Purina Laboratory Chow 5001 biscuit). The mice were fed Purina Laboratory Chow that contained 12 IU/g of vitamin A or Purina Laboratory Chow to which 70 IU/g of vitamin A had been added by soaking the bisquit in 0.2 ml of vitamin A palmitate (Sigma Chemical Co., St. Louis, MO) in vegetable oil (1,750 IU/ml). The mice were fed *ad libitum*, and it was assumed that mice in the control group consumed approximately 60 IU of vitamin A daily (~2,400 IU/

[1] To whom requests for reprints should be addressed at Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

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Table I. Major Histocompatibility Complex Alleles of Congenic Strains Used in These Studies[a]

Strain	Centromeric to H-2K	K	E_β	S	D	Qa-1	Telomeric to Qa-1
C57BL/10	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.A(18R)	*b*	*b*	*b*	*b*	*d*	*a*	*b*
B10.A(5R)	*b*	*b*	*b/k*	*d*	*d*	*a*	*b*
B10.A	*k* or *b*	*k*	*k*	*d*	*d*	*a*	*b*
B10.A(1R)	*k* or *b*	*k*	*k*	*d*	*b*	*b*	*b*
B10.A(2R)	*k* or *b*	*k*	*k*	*d*	*b*	*b*	*b*
B10.A(15R)	*k* or *b*	*k*	*k*	*d*	*b*	*b*	*b*
B10.0L	*d* or *b*	*d*	*d*	*k*	*k*	?	*b*
B10.BR	*k* or *b*	*k*	*k*	*k*	*k*	*a*	*b*

[a] See Ref. 9.

Table II. Effect of Added Dietary Vitamin A (400 IU/day) on the Frequency of Dexamethasone (80 mg/kg)-Induced Cleft Palate in Congenic Strains of Mice

Strain	Vitamin A added	Litters (*n*)	Total implants	Resorptions (%)	Cleft palate (%)	Arcsine[a]
B10.BR	–	14	115	19.1	19.3	27.9 ± 16.9
	+	12	109	14.6	29.0	32.5 ± 15.6
B10.A	–	9	81	16.1	54.4	49.1 ± 13.5
	+	8	67	15.9	33.9	34.8 ± 25.2
B10.A(1R)	–	18	142	23.1	26.6	29.6 ± 15.4
	+	16	131	15.2	21.6	28.4 ± 13.0
B10.A(15R)	–	15	137	17.5	15.9	23.6 ± 12.1
	+	15	135	15.3	18.4	25.7 ± 13.7
B10.0L	–	18	146	29.4	13.6	23.1 ± 13.4
	+	19	132	24.2	17.0	28.7 ± 22.0
C57BL/10	–	15	85	12.5	16.4	18.6 ± 4.9
	+	11	68	18.0	39.2	36.2 ± 8.7
B10.A(18R)	–	11	75	30.6	25.0	26.9 ± 11.4
	+	9	64	28.0	52.1	48.2 ± 19.8

[a] Data are expressed as mean ± SD. Regular diet: B10.A versus B10.BR, B10.A(1R), $P < 0.01$; B10.A versus B10.0L, B10.A(15R), B10.A(18R), C57BL/10, $P < 0.001$. Regular diet versus added vitamin A: C57BL/10, $P < 0.001$; B10.A(18R), $P < 0.02$.

kg) and the experimental group approximately 400 IU (~16,000 IU/kg).

The sampling unit in these studies was the litter. The proportion of CP in each litter was transformed to an arcsine by using the table of Freeman-Tukey arcsine transformations for small sample sizes, as calculated by Mosteller and Youtz (10). This transformation has a weighting factor for litter size and for 0% and 100%. The proportion of CP per litter was transformed so that parametric analysis could be used. Means were compared using Student's *t* test.

High molecular weight DNA, prepared from fresh spleen cells, was digested with the restriction enzymes *Eco*RI, *Hind*III, or *Pst*I, sized by electrophoresis in 0.7% agarose gels, and blotted onto nylon filters by standard methods (11). The blotted DNA was hybridized to a ^{32}P-labeled C4 cDNA probe (12), washed sequentially in 2× SSC/0.1% sodium dodecyl sulfate at room temperature and then in 0.1× SSC/0.1% sodium dodecyl sulfate at 65°C twice for 45 min each, and exposed to x-ray film for 3 to 5 days.

Results

In the group given the regular diet (Purina 5001) and dexamethasone 80 mg/kg on the twelfth day of gestation, B10.A mice had significantly higher frequencies of CP (54.4%) than did B10.BR (19.3%), B10.A(1R) (26.6%), B10.A(15R) (15.9%), B10.OL (13.6%), C57BL/10 (16.4%), and B10.A(18R) (25.0%) strains (Table II). When vitamin A (400 IU/day) was added to this diet the frequency of CP was significantly increased only in the C57BL/10 and B10.A(18R) strains.

The frequency of CP was essentially the same when pregnant females of reciprocal crosses between vitamin A-resistant (B10.A(1R) or B10.A(15R)) and -susceptible (B10.A(18R) or B10.A(2R)) strains were fed the control diet or the diet supplemented with 400 IU/day of vitamin A and given 160 mg/kg of dexamethasone on the twelfth day of gestation; however, the frequency increased significantly under the same conditions when the crosses were between two vitamin A-susceptible

Table III. Frequency of Dexamethasone (80 mg/kg)-Induced Cleft Palate among Progeny of Crosses between Vitamin A-Susceptible and -Resistant Strains Fed Purina Laboratory Chow with or without Added Vitamin A (400 IU/day)

Strain[a]	Vitamin A added	Litters (*n*)	Total implants	Resorptions (%)	Cleft palate (%)	Arcsine (mean ± SD)
B10.A(15R) × B10.A(18R)	−	13	107	10.2	11.4	19.7 ± 10.6
	+	14	95	20.0	26.3	28.8 ± 12.4
B10.A(18R) × B10.A(15R)	−	7	43	20.9	14.7	20.5 ± 12.0
	+	8	64	17.1	16.9	26.8 ± 13.4
B10.A(1R) × B10.A(2R)	−	17	128	17.9	29.5	34.3 ± 17.4
	+	17	136	16.2	33.3	33.9 ± 22.4
B10.A(2R) × B10.A(1R)	−	8	63	17.5	25.0	26.7 ± 22.9
	+	7	58	15.5	22.4	31.5 ± 13.1
B10.A(18R) × B10.A(1R)	−	10	77	18.1	22.2	25.1 ± 14.2
	+	14	103	13.6	22.4	27.6 ± 16.5
B10.A(2R) × C57BL/10	−	11	65	10.7	13.7	15.6 ± 16.2[b]
	+	13	79	18.9	39.0	41.8 ± 14.8
C57BL/10 × B10.A(2R)	−	6	54	25.9	20.0	19.6 ± 10.1[b]
	+	6	54	20.3	52.4	49.7 ± 8.9

[a] The female is listed first and the male second, i.e., female strains × male strain.
[b] Regular diet versus added vitamin A: $P < 0.001$, Student's *t* test.

Table IV. Frequency of Dexamethasone (160 mg/kg)-Induced Cleft Palate among Crosses between Susceptible and Resistant Strains Fed Laboratory Chow

Strain[a]	Litters (*n*)	Total implants	Resorptions (%)	Cleft palate (%)	Arcsine[b]
B10.BR	15	78	23.1	25.0	27.2 ± 18.5
B10.BR × B10.A	14	106	16.0	57.3	54.4 ± 13.6
B10.A × B10.BR	14	102	10.0	51.6	48.7 ± 16.3
B10.A	18	126	7.9	68.1	58.4 ± 11.6
B10.A × C57BL/10	10	91	22.1	64.8	54.5 ± 10.4
C57BL/10 × B10.A	19	157	21.0	50.8	44.2 ± 9.6
C57BL/10	17	124	14.5	24.5	29.5 ± 12.1

[a] The female is listed first and the male second.
[b] Data are expressed as mean ± SD. B10.BR versus B10.A and B10.BR × B10.A, $P < 0.001$; B10.BR versus B10.A × B10.BR, $P < 0.01$; B10.A versus C57BL/10 and C57BL/10 × B10.A, $P < 0.001$. C57BL/10 versus C57BL/10 × B10.A and B10.A × C57BL/10, $P < 0.001$.

strains (B10.A(2R) and C57BL/10) and the dams were fed the vitamin A-enhanced diet (Table III). The progeny of reciprocal crosses between glucocorticosteroid-induced, CP-sensitive (B10.A) and -resistant (C57BL/10 [in absence of added vitamin A] and B10.BR) strains had CP frequencies very close to that of the corticosteroid-sensitive parental strain (Table IV).

Southern blot analysis using a C4 cDNA probe revealed that B10.A, B10.A(1R), B10.A(2R), and B10.A(15R) had similar restriction fragment length polymorphism patterns with *Eco*RI and *Hind*III that clearly differed from those observed with C57BL/10 and B10.A(18R) (Fig. 1).

Discussion

In previously reported studies, pregnant mice from the H-2 congenic strains C57BL/10, B10.BR, B10.A, B10.A(5R), B10.A(2R), and B10.A(18R) were fed Purina Laboratory Chow or the same diet plus 400 IU of vitamin A daily and given 80 mg/kg of dexamethasone intraperitoneally on the twelfth day of gestation (8). It was found that only strains with *b* alleles between H-2S and H-2D had significantly higher frequencies of isolated cleft palate among their progeny when fed the supplemental vitamin A (C57BL/10, B10.A(2R), and B10.A(18R)). In the present studies, this effect was confirmed in strains C57BL/10 and B10.A(18R), but it was not observed in B10.A(1R) and B10.A(15R) strains, which, like B10.A(2R), are recombinants between a C57BL/10 host ($H\text{-}2^b$) and an $H\text{-}2^a$ donor with host *b* alleles distal to the recombination site in the H-2S:H-2D interval (9). From these observations, one would conclude that the locus that modulates this effect (*ACP*) is (i) proximal to the recombination site of B10.A(18R) in the H-2S:H-2D interval because this strain has the *d* allele at H-2D; (ii) distal to Eβ because B10.A(5R),

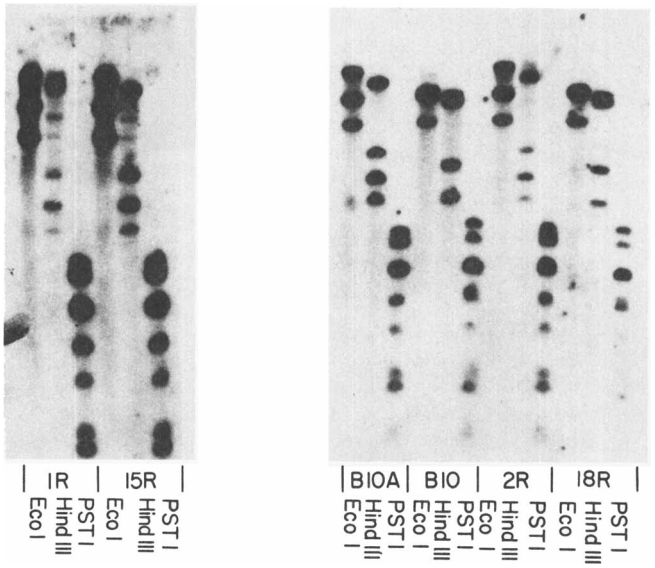

Figure 1. Southern blot hybridization of spleen cell DNA of H-2 recombinant congenic strains with C4 cDNA probe indicates that *Acp* and *Dcp-2* are distal to C4. See text for details.

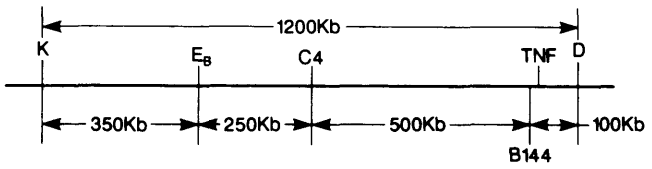

Figure 2. Approximate locations of E_B, C4, and B144 within the H-2 complex.

which is resistant to the vitamin A effect, has *d* alleles telomeric to this point; and (iii) between the recombination sites in the H-2S:H-2D interval of B10.A(1R) and B10.A(15R) (both resistant to the vitamin A effect) and B10.A(2R) (vitamin A sensitive).

The B144 gene is selectively transcribed by B cells and macrophages and maps proximal to tumor necrosis factor approximately 85 kb proximal to D in the BALB/c mouse (13; Fig. 2). Southern blots (13) hybridized with a B144 probe revealed that B10.A and B10.A(18R) have similar restriction length fragments (2.2 kb) that differ from C57BL/10 and B10.A(2R) (2.0 kb); this suggests that *Acp* is proximal to B144 because B10.A(18R) is vitamin A susceptible but has a B144 restriction fragment length polymorphism similar to B10.A, a resistant strain.

Southern blots of spleen cell DNA digested with restriction enzymes and hybridized with a C4 cDNA probe (Fig. 1) revealed that B10.A, B10.A(1R), B10.A(2R), and B10.A(15R) have similar restriction fragment length polymorphism patterns with *Eco*RI and *Hind*III that differ from those of C57BL/10 and B10.A(18R). This indicates that *Acp* is distal to C4 in the C4:B144 interval and between the recombinant sites of B10.A(2R) (vitamin A sensitive) and B10.A(1R) and B10.A(15R) (both vitamin A resistant).

From these studies, it is apparent (Table V) that *Dcp-2* (cf., B10.A(1R) and B10.A(15R) versus B10.A(2R)) and *Acp* map between C4 and B144 and that they are not the same locus (cf. B10.A [glucocorticosteroid sensitive, vitamin A resistant] versus B10.A(2R) [glucocorticosteroid sensitive, vitamin A sensitive]). In addition, the evidence presented here lends support to the suggestion that *Acp* is centromeric to *Dcp-2* (8), i.e., the order is *Acp, Dcp-2*. In this model, B10.A(1R) and B10.A(15R) (both ACP^d $DCP\text{-}2^b$ phenotypes) would result from a recombination with the C4:B144 interval break occurring between *Acp* and *Dcp-2*, and B10.A(2R) would be the product of an unequal recombination with inheritance of *Acp* and *Dcp-2* from both $H\text{-}2^a$ donor and $H\text{-}2^b$ host. On the other hand, with the order reversed (i.e., *Dcp-2, Acp*), B10.A(2R) would result from a recombination with break sites between *Dcp-2* and *Acp* with inheritance of donor ($H\text{-}2^a$) *Dcp-2* and host ($H\text{-}2^b$) *Acp*; however, in this model, there does not appear to be a simple explanation for the phenotype of the B10.A(1R) and B10.A(15R) strains. Mapping studies of the H-2-associated CP susceptibility genes performed with the glucocorticosteroid cortisol (2), cortisone (3), or dexamethasone (5) have yielded very similar results, i.e., one locus centromeric to $E\beta$ that interacts in *cis* with a second gene that maps in the H-2S:H-2D interval. The evidence suggests that *Dcp-2* (5) and *Cps-1* (6, 7), which both map between C4 and B144, are the same gene.

The studies of crosses between resistant and susceptible strains reveal that sensitivity to CP induced by glucocorticosteroids alone is a dominant trait and that

Table V. Proposed Order of CP-Inducible Loci and Allelic Composition in Strains Studied

Strains	S	C4	*Acp*	*Dcp-2*	B144	D	Vitamin A sensitive	Glucocorticoid sensitive
B10.A	*d*	*d*	*d*	*d*	*d*	*d*	–	+
B10.18R	*b*	*b*	*b*	*b*	*d*	*d*	+	–
B10	*b*	*b*	*b*	*b*	*b*	*b*	+	–
B10.2R	*d*	*d*	*d/b*	*d/b*	*b*	*b*	+	+
B10.1R	*d*	*d*	*d*	*b*	?	*b*	–	–
B10.15R	*d*	*d*	*d*	*b*	?	*b*	–	–

the enhancement of sensitivity produced by vitamin A is recessive.

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