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Genetics

Genes in mice that affect susceptibility to cortisone-induced cleft palate are closely linked to *Ir* genes on chromosomes 2 and 17

(major histocompatibility complex/*H-3* locus)

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ABSTRACT **Inbred and congeneic strains of mice have been examined for susceptibility to cortisone-induced cleft palate, and the role of genes linked to *H-2* on chromosome 17 has been confirmed. Increasing degrees of susceptibility were associated with the $H\text{-}2^d$, $H\text{-}2^b$, $H\text{-}2^k$, and $H\text{-}2^a$ haplotypes, respectively, with $H\text{-}2^q$ and $H\text{-}2^s$ also being associated with fairly high levels of susceptibility. Evidence was obtained that suggests that one gene maps within the *B* region of *H-2*, and that a second *H-2*-linked gene which acts by complementation maps to the right of *E*. Another gene affecting this trait is closely linked to the *H-3* and *Ir-2* loci on the second chromosome.**

Injection of cortisone into pregnant mice at a sensitive period is known to induce varying frequencies of cleft palate among the fetuses of various inbred strains (1–4). At least part of the high susceptibility of the A/J strain can be accounted for by genes linked to the *H-2* histocompatibility complex on chromosome 17 (5–7). This discovery is of special interest because it is known that the major histocompatibility complex (MHC) is associated in some way with susceptibility to various diseases (8–11). Most of these associations could be explained by the effects of immune response genes (*Ir* genes) which are known to be included in the MHC. In addition to these *Ir* genes, the MHC also includes genes which regulate effector–target cell interactions and various components of the complement system. However, a disorder such as cortisone-induced cleft palate is difficult to explain on the basis of regulation of the immune system, and would not be expected to be associated with *Ir* genes.

We now report data on the genetics of susceptibility of mice to cortisone-induced cleft palate which have demonstrated close linkage between this trait and *Ir* genes on chromosomes 2 and 17.

MATERIALS AND METHODS

Animals. The B10.Q strain was obtained from Chella David (Mayo Clinic, Rochester, MN), The B6.*T1a*^{a} strain was from E. A. Boyse (Sloan–Kettering Institute, New York, NY), and the B10.S strain was from Barbara Knowles (Wistar Institute, Philadelphia, PA). All other mice were obtained from The Jackson Laboratory.

Drug Injections. Female mice were weighed and mated overnight with the appropriate males. Eleven days later they were weighed again, and those that had gained at least 3 g were injected, on days 11 through 14, with cortisone at either 50 or 100 mg/kg, as indicated.

Test of Significance. It has been argued convincingly that the litter rather than the fetus is the proper unit of measurement in teratology (12), so all tests of significance were done by using the Mann–Whitney *U* test.

RESULTS

In order to determine the rate of spontaneous appearance of cleft palate, the fetuses from mothers that had received no drug injection were examined (Table 1). In a total of 535 fetuses examined, 27 cleft palates were observed, and 26 of these were associated with cleft lip. This agrees with the observation by others that spontaneous cleft palate is usually associated with cleft lip. It is known that the lip forms before palatal closure, and before day 11, when drug injections were begun (13). In subsequent experiments, all cases of cleft palate associated with cleft lip were deleted from the data because it was assumed that these abnormalities were not induced by the injected drug.

The data on cortisone-induced cleft palate among *H-2* congeneic strains are shown in Table 2. These results confirm the observation by Miller (14) that the type of mouse chow used affects the frequency of abnormal fetuses and also confirm the observations by others (5, 7) that B10.A is significantly more susceptible than B10.

We have also observed that the B10.Q congeneic strain is significantly more susceptible than B10 ($P < 0.002$ for B10.Q). This suggests that the high susceptibilities of two $H\text{-}2^q$ strains, DBA/1 and SWR (15), may be related to their *H-2* haplotypes. Our data suggest that the $H\text{-}2^s$ haplotype is also associated with high susceptibility.

The B10 strain has been the least susceptible line studied in the previous experiments of this type (5–7), but the data shown in Table 2 demonstrate that B10.D2 was significantly less susceptible than B10 when either type of mouse chow was used ($P < 0.02$). The importance of non-*H-2*-linked genes is apparent from the results with the A.BY strain, which was significantly more susceptible than B10 ($P < 0.002$). An effect of the $H\text{-}2^b$ haplotype could also be demonstrated with this strain because A.BY was significantly less susceptible than A/J when the dosage was reduced to 50 mg/kg ($P = 0.002$).

In order to determine which specific region of the *H-2* complex accounts for susceptibility, various strains with recombinant *H-2* haplotypes were examined. The frequency of affected fetuses in B10.A(2R) was significantly greater than that in either B10.A(4R) or B10.A(5R) when either type of chow was used, which suggests that the *k* allele in the *B* region is associated with high susceptibility. However, the *B* region alone does not account for all of the data because B10.BR was significantly less susceptible than B10.A ($P < 0.002$), suggesting that a gene to the right of *E* is also involved.

The possible involvement of a gene to the right of *D* was investigated by comparing B6 with B6.*T1a*^{a}, which received its

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Abbreviation: MHC, major histocompatibility complex.

Table 1. Frequencies of spontaneous cleft palate in inbred and congeneic strains of mice

Strain	*H-2*	Litters, no.	Resorptions		Fetuses				
			No.	%	Total, no.	Isolated cleft palate		Cleft lip*	
						No.	%	No.	%
C57BL/6	b	5	4	10	45	0	0	0	0
B10.D2	d	6	0	0	47	0	0	0	0
B10.A	a	8	3	4	76	0	0	0	0
B10.A(5R)	i^5	10	3	4	79	0	0	0	0
B10.Q	q	1	0	0	4	0	0	0	0
A.BY	b	14	4	4	87	0	0	19	18
B10.A(2R)	h^2	10	8	13	62	1	2	0	0
B10.BR	k	3	4	20	21	0	0	0	0
B6.*T1a*^{a}	b	9	1	1	66	0	0	0	0
A/J	a	10	13	16	70	0	0	7	10

All mice in these experiments were maintained on Purina Mouse Chow.
* With or without cleft palate.

T1a region from A/J (16). If a gene in the *T1a* region controlled this trait, the B6.*T1a*^{a} strain would be expected to have higher susceptibility than B6, but this did not occur.

Because genetic complementation of the *cis* type seems to be involved in the high susceptibility of B10.A, we examined the fetuses from a number of hybrid combinations in the hope of observing *trans* complementation. Among 11 litters of female (5R × 4R)F_1 hybrids mated with B10 males, we observed 16 affected fetuses of a total of 70 (23%). These animals were fed Purina Mouse Chow, so the frequency observed did not differ significantly from that observed among B10 mice given the same food.

An immune response gene, designated *Ir-2*, which is linked to the *agouti* coat color locus and the *H-3* histocompatibility locus on chromosome 2 has been described (17–19). When strains of mice congeneic for the *H-3* region were tested, the data shown in Table 3 were obtained. Both the B10.LP and B10.LP-*H-3*^{b} strains were significantly more sensitive than the B10 ($P < 0.05$ in each case).

DISCUSSION

In these experiments, data were obtained dealing with both *H-2*-linked and non-*H-2*-linked genes which affect the susceptibility of mice to cortisone-induced cleft palate. The non-*H-2* genetic background of the A/J strain includes genes which cause high susceptibility to both spontaneous and cortisone-induced abnormalities, as demonstrated by results shown in Table 1 and 2. Even with this high degree of susceptibility, however, the effects of the *H-2* haplotype could be demonstrated because A/J mice were significantly more susceptible than A.BY after injections of 50 mg of cortisone per kg of body weight.

Experiments designed to map the relevant genes within the *H-2* complex have demonstrated that at least two loci are involved. One of these appears to be in the *B* region because

Table 2. Frequencies of cortisone-induced cleft palate in inbred and *H-2* congeneic strains of mice

Strain	*H-2* alleles									Food	Litters, no.	Resorptions		Fetuses		
	K	*A*	*B*	*J*	*E*	*C*	*S*	*D*	*T1a*			No.	%	Total, no.	With cleft palate No.	%
B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	Wayne	15	5	4	112	11	10
										Purina	15	21	17	105	23	22
B6	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	Purina	23	19	12	146	30	21
B6.*T1a*^{a}	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*a*	Purina	10	6	8	73	19	26
B10.A	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*a*	Wayne	9	6	9	60	32	53
															149	
										Purina	26	47	21	179		83
B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*a*	Purina	14	23	20	92	38	41
B10.D2	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*c*	Wayne	7	2	5	38	0	0
										Purina	26	22	13	145	6	4
B10.A(2R)	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*b*	*b*	Wayne	16	44	37	74	24	32
										Purina	15	44	28	113	79	70
B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	Wayne	8	2	3	57	4	7
										Purina	2	0	0	16	1	6
B10.A(5R)	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*	*a*	Wayne	18	30	20	119	7	6
										Purina	31	32	13	223	47	21
B10.Q	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	?	Purina	9	11	17	53	41	77
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*b*	Purina	3	9	32	19	13	68
A.BY	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*c*	Purina	9	11	21	42	42	100
A.BY	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*c*	Purina	8	6	16	36	14	36
A/J	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*a*	Purina	11	28	33	61	45	74

The cortisone dosage was 100 mg/kg in all experiments except those shown in the last two lines; in the last two lines it was 50 mg/kg.

Table 3. Frequencies of cortisone-induced cleft palate in inbred and *H-3* congeneic mouse strains

Strain	Genotype in *H-3* region				Litters, no.	Resorptions		Fetuses		
	H-3	*Ir-2*	*H-13*	*A*		No.	%	Total	With cleft palate No.	%
C57BL/10*	a	a	a	a	15	21	17	105	23	22
B10.LP	b	b	b	A^w	15	39	28	103	58	56
B10.LP-*H-3*^{b}	b	b	a	a	7	7	12	52	26	50

All mice in this experiment were fed Purina Mouse Chow.
* Data from Table 2.

B10.A(2R) has high susceptibility and B10.A(4R) and B10.A(5R) have low susceptibility. It has been questioned, however, whether the *B* locus exists because no Ia antigens have been identified which map in this region (20). According to this hypothesis, the *H-2* chromosome of B10.A(4R) might have a deletion (21), and the immune responses which seem to be controlled by *B*-region genes could result from complementation. If this interpretation is correct, responses to IgG (22), LDH_B (20, 23), and trinitrophenylalbumin (24) would need to be explained by this type of mechanism. If the *B* region does exist and these immune responses are controlled by it, this control is expressed without the participation of the currently defined Ia antigens. In any case, susceptibility to cortisone-induced cleft palate involves a gene that maps to the left of *C* because B10.A is significantly more susceptible than B10.D2. This gene seems not to be in either the *K* or *A* region because B10.A(4R) appears to be less susceptible than B10.BR.

A second *H-2*-linked gene maps to the right of *E* because B10.A is significantly more susceptible than B10.BR ($P < 0.002$). This particular gene is apparently associated with high susceptibility only when in combination with an appropriate complementary gene on the left-hand side of the complex because B10.D2 (which has the same right-hand genotype as B10.A) is the least susceptible strain examined so far. The *k* gene on the left-hand side of the complex does not require the complementation of a *d* gene on the right because B10.BR was significantly more susceptible than B10 ($P < 0.002$).

The possibility that a susceptibility gene maps in the *T1a* region was examined by comparing B6 with B6.*T1a*^{a}, which did not differ significantly. Therefore, a high susceptibility gene that can act independently does not appear to map in the *T1a* region, but it has not been ruled out that a gene might map in this region which requires the complementation of an appropriate allele (such as *k*) in the left-hand side of *H-2*.

In spite of strong evidence in favor of *cis* complementation for susceptibility, no evidence has yet been obtained for complementation in the *trans* configuration.

The biochemical mechanism that would account for these data remains to be elucidated. It has been reported that B10.A mice have a higher level of palatal cortisol-binding cytosolic receptors than do B10, suggesting that an *H-2*-linked gene affects the structure or quantity of this receptor (25). More recently, this difference has been confirmed by the use of dexamethasone, which has a greater specificity of binding than cortisol, and it was also observed that B10.A(2R) mice have a higher level of cytosolic receptor than do B10.A(4R) or B10.A(5R) (26). It has also been shown that this strain difference could not be demonstrated in liver (27), which is in agreement with observations of tissue-specific variation in cortisol receptors in rats (28). However, the biochemical events that control palatal closure could be very complex, and it is by no means clear whether an *H-2*-linked effect on glucocorticoid receptors will account for the genetic differences in cleft palate susceptibility.

Levels of cyclic AMP in the liver are known to be influenced by *H-2*-linked genes (29), and this influence is now known to be the result of an *H-2*-linked effect on glucagon receptors (30). An effect of *H-2* on the quantity of estrogen receptors in the uterus has also been reported (31), and *H-2* is known to affect various other hormone-associated phenomena (32). It would not seem likely that these effects could result from the modifications in the immune response that the *H-2* region is known to control, but it has now been shown that the levels of cyclic AMP and presumably glucagon receptors (30, 33), as well as susceptibility to cortisone-induced cleft palate, are controlled by genes that map within the *I* region and involve genetic complementation. Because the only gene products that have been identified so far as having these properties are Ia antigens, the possibility should be considered that Ia antigens might be involved in the structure or regulation of hormone receptors (34). If this is not correct, then there seem to be other genes within the *I* region that play this role.

The B10.LP and B10.LP-*H-3*^{b} congeneic strains have provided the most specific information yet obtained with regard to any non-*H-2*-linked genes which affect this phenotype. These strains have obtained a short segment of chromosome 2 from the LP/J strain. The only genes known to be present so far on this segment in both strains are *H-3* and *Ir-2* (19). Susceptibility to leishmaniasis is also controlled by a gene in this region, although this may be an effect of *Ir-2* (35). The mechanism by which immune responses are controlled by *H-3*-linked *Ir* genes has not been fully elucidated, but we find it most interesting that susceptibility to cortisone-induced cleft palate should be controlled by genes closely linked to *Ir* genes in both chromosomes 17 and 2.

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