Effects of H-2 on Neural Tube Defects in Congenic Mice (43458)

MARVIN L. TYAN[1]

West Los Angeles Veterans Affairs Medical Center and Department of Medicine, University of California, Los Angeles, School of Medicine, Los Angeles, California 90024

Abstract. **Pregnant mice congenic with C57BL/10 (B10.A, B10.BR, B10.D2, B10.A[2R], B10.A[5R], B10.A[15R], B10.A[1R], B10.A[18R], and B10.0L) were fed Purina Mouse Chow or the same diet plus 200 IU of vitamin A daily. The pregnant dams were sacrificed on the eighteenth day of gestation, and the fetuses were sexed and examined for defects in neural tube development.**

The frequency of neural tube defects was low (mean frequency of all strains, 0.36%) and was not affected by the addition of vitamin A (200 IU/day) to the diet. Twenty-seven of the 29 defects observed occurred in the anterior tube (exencephaly); fourteen were identified in female fetuses, but the sex could not be determined in the other 15 cases because of fetal death and early autolysis. Variations in frequency among the strains suggest that a locus between E_β and H-2D has a moderate influence on the occurrence of neural tube defects. Strains that had H-2^d alleles in this segment of the H-2 complex had relatively high frequencies, and those with H-2^b or H-2^k alleles had significantly lower frequencies. [P.S.E.B.M. 1992, Vol 200]

Approximately one of every 1000 babies born in the United States has a neural tube defect, anencephaly, exencephaly, or meningomyelocele (1). In reviewing studies on cleft palate in congenic strains of mice conducted in this Laboratory since 1976 (2), it was apparent that the frequency of these neural tube defects varied moderately among strains which should differ only in the region of the major histocompatibility complex (H-2) on Chromosome 17 (3).

Presented below are the data gathered from these studies which suggest that a locus which maps between E_β and H-2D within the H-2 complex has a modest effect on the frequency of these defects. Vitamin A at the dose given did not influence the frequency of these defects.

Materials and Methods

The data reported here have been gathered from noninjected or sham injected groups that were controls for studies on glucocorticosteroid-induced cleft palate conducted between 1976 and 1988. Studies performed since 1988 have been for the sole purpose of investigating the roles of the major histocompatibility complex and vitamin A on the frequency of eye abnormalities and to extend and/or confirm the older observations. The May 1988 fire at the Jackson Laboratory has prevented confirmation of earlier work in B10.D2 and B10.A mice.

The congenic strains B10.D2, B10.0L, C57BL/10, B10.A(18R), B10.A(5R), B10.A(1R), B10.A(15R), B10.A(2R), B10.BR, and B10.A were maintained in this laboratory by brother × sister matings. The differences in H-2 haplotypes are shown in Table I (see also Ref. 3). In the experiments, one male and two virgin 10–12-week-old females were placed in each cage. The day a vaginal plug was detected was considered to be Day 0 of pregnancy. On the eighteenth day of gestation, the pregnant mice were sacrificed. Fetuses were weighed and examined for gross external abnormalities, including neural tube defects. The oral cavity was inspected for the presence of cleft palate, and the internal organs were examined for defects and to determine the sex. Between 1976 and 1984, five individuals performed fetal examinations. Since 1985, I have conducted all studies.

The average pregnant female mouse consumes approximately 5 g of food per day (i.e., the equivalent of

[1] To whom requests for reprints should be addressed at West Los Angeles VA Medical Center, W 111M, Los Angeles, CA 90073.

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one average Purina Mouse Laboratory Chow [5001] biscuit, which contains 12 IU/g of vitamin A). In indicated experiments, 200 IU of vitamin A were added to each biscuit by soaking the biscuit in 0.2 ml of vitamin A palmitate (Sigma Chemical Co., St. Louis, MO) in vegetable oil (1,000 IU/ml). The biscuits fed to the control groups in the studies on the effects of vitamin A were soaked in 0.2 ml of vegetable oil only. The mice in the control groups consumed approximately 60 IU of vitamin A daily (2,400 IU/kg) and the experimental group consumed approximately 260 IU (10,400 IU/kg).

The sampling unit in these studies was the individual fetus. Frequencies were compared by means of Fisher's exact test and by loglinear analyses (4) using the NCSS statistical program (NCSS, Kaysville, UT).

Results

Nineteen anterior neural tube defects (exencephaly) and two posterior tube defects were observed among 5849 fetuses from the 10 congenic strains fed Laboratory Chow (0.36%). The frequency of exencephaly was 0.40% among the 1975 fetuses from dams given the diet with added vitamin A (Table I); no posterior tube defects were noted.

The sex of 15 of the 29 with neural tube defects could not be determined because the fetuses were dead and abdominal autolysis had begun. In no case was a fetus with a neural tube defect identified as being a male.

The frequency of neural tube defects varied moderately among the strains. Loglinear analyses indicated that B10.A, B10.A(2R), B10.A(15R), B10.D2, and B10.A(5R) strains did not differ significantly from each other, differed questionably from the B10.BR (P = 0.082) strain, but differed clearly from C57BL/10 and B10.A(18R) (P = 0.0013) strains. These results suggested the presence of a locus between E_β and H-2D which determines the frequency of neural tube defects to some degree (e.g., B10.A vs B10.A[5R], P = 0.539; B10.A vs B10.A[18R], P = 0.003; B10.A vs B10.D2, P = 0.746). When all strains that bear *d* alleles between E_β and H-2D (B10.A, B10.A[1R], B10.A[2R], B10.A[15R], B10.A[5R], and B10.D2) were compared with those that do not (B10.0L, B10.BR, C57BL/10, and B10.A[18R]), the difference was highly significant (P = 0.00046).

The addition of vitamin A to the diet had no effect on litter size or resorption rate (data not shown; see Ref. 2). No correlation was found between neural tube defects and microphthalmia or cleft palate.

Discussion

Neural tube birth defects are common in humans and are noted more frequently in females (5). In many cases, these defects appear to be multifactorial threshold traits (5), and in others, they appear to be components of syndromes affecting several organs or tissues (5, 6) or the result of environmental factors (e.g., anticonvulsant agents [7]). An association between neural tube defects and HLA, the major histocompatibility complex of man, has been noted by some (8–10), but not by others (11, 12).

Studies carried out at term or later in the mothers of affected children have suggested that low folate or high vitamin A intake occurred more frequently than average during pregnancy in these women (13). The administration of multivitamins/folate early in gesta-

Table I. Frequency of Neural Tube Defects in Male and Female 18-Day-Old Congenic Mouse Fetuses from Mothers Fed Laboratory Chow with and without Added Vitamin A (200 IU/day)[a]

Strain	H-2 haplotype				Male		Female[b]	
	K	E_β	S	D	Regular diet (*n*/total) (%)	Added vitamin A (*n*/total) (%)	Regular diet (*n*/total) (%)	Added vitamin A (*n*/total) (%)
B10.OL	*d*	*d*	*k*	*k*	0/49 0.0	0/50 0.0	0/54 0.0	0/50 0.0
B10.A(1R)	*k*	*k*	*d*	*b*	0/49 0.0	0/65 0.0	0/60 0.0	0/46 0.0
B10.A(18R)	*b*	*b*	*b*	*d*	0/682 0.0	0/159 0.0	1/672 0.1	1/164 0.6
C57BL/10	*b*	*b*	*b*	*b*	0/419 0.0	0/132 0.0	1/438 0.2	0/137 0.0
B10.BR	*k*	*k*	*k*	*k*	0/360 0.0	0/127 0.0	0–1/355 0.3	0/154 0.0
B10.A(2R)	*k*	*k*	*d*	*b*	0/282 0.0	0/81 0.0	2/293 0.7	0–1/100 1.0
B10.A(5R)	*b*	*b/k*	*d*	*d*	0/166 0.0	0/90 0.0	1–1/173 1.2	0–1/123 0.8
B10.D2	*d*	*d*	*d*	*d*	0/209 0.0		0–2/155 1.3	
B10.A(15R)	*k*	*k*	*d*	*b*	0/73 0.0	0/66 0.0	0–1/98 1.0	2/80 2.5
B10.A	*k*	*k*	*d*	*d*	0/695 0.0	0/183 0.0	4–7/567 1.9	2–1/168 1.8
Total					0/2984 0.0	0/953 0.0	9–12/2865 0.7	5–3/1022 0.8

[a] All neural tube defects were anterior, with the exception of two posterior defects in B10.A fetuses.
[b] In some cases, sex could not be determined because of fetal death and early resorption: 1–2/100 = 1 female, 2 unknown/100. Percentages have been calculated on the assumption that all defects occurred in female fetuses.

tion reduced the incidence of these defects in two studies (14, 15), but the results were not confirmed by a third study (16).

When retinoic acid was given to mutant strains of mice that have increased incidences of neural tube defects, the frequency was increased in the strains given high doses (17, 18) but was unchanged in another given a lower dose (19). In these mutant strains, the frequency of defects was higher in females. In another mutant strain with a high incidence of posterior neural tube defects, curtailed, the mutation mapped to the T locus on Chromosome 17 (20).

The results reported here suggest that in the congenic strains of mice studied, a locus that tentatively maps to the interval between E_β and H-2D within the major histocompatibility complex contributes to some degree to the occurrence of neural tube defects, primarily exencephaly. Strains with *d* alleles in this region had relatively high frequencies and those with *k* or *b* alleles had low frequencies of these defects. Despite the fact these defects were noted only in females, it is not possible to state with certainty that females are more susceptible because the sex of the affected fetuses could not be established in over 50% of the cases.

The addition of a low dose of vitamin A to the diet had no effect on the occurrence of these defects in contrast to the case with cleft palate (2), microphthalmia (21), and micrognathia (unpublished data).

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