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Influence of the Major Histocompatibility Complex (*H-2*) on Glucocorticoid-Stimulated Pulmonary Surfactant Synthesis in Two Congenic Mouse Strains[1] (41892)

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Abstract. Mice with different histocompatibility alleles in otherwise identical backgrounds (congenic inbred strains of $H\text{-}2^a$ and $H\text{-}2^b$ haplotypes), were used to study the possible influence of the major histocompatibility complex (MHC) upon glucocorticoid-stimulated pulmonary surfactant synthesis during embryonic and fetal stages of mouse lung development. Pregnant C57BL/10SnJ ($H\text{-}2^b$) and congenic B10.A/SnSgJ ($H\text{-}2^a$) mice were administered single or consecutive intraperitoneal injections of betamethasone during the period of 14 through 17 days gestation. Assays for incorporation of [^{3}H]choline into total lipid and saturated phosphatidylcholine (an index for pulmonary surfactant synthesis) were conducted, 24 hr following the last treatment, on Days 17 or 18 of gestation. Control mice received injections of phosphate-buffered saline. The *H-2* genotype appears to influence the relative responsiveness of glucocorticoid-stimulated surfactant synthesis during fetal lung development. The B10.A strain ($H\text{-}2^a$ haplotype) was more responsive to glucocorticoid-stimulated pulmonary surfactant synthesis than the $H\text{-}2^b$ haplotype. Whereas both strains were responsive to glucocorticoid-stimulated [^{3}H]choline incorporation into lung surfactant, the differences in responsiveness at specific fetal stages suggest that genes within or closely linked to the *H-2* complex might have a role in pulmonary surfactant biosynthesis during mouse lung development. The results also suggest the possibility that relative susceptibility to congenital pulmonary disorders may be influenced by the *H-2* complex.

Infant respiratory distress syndrome (RDS), also known as hyaline membrane disease, is the consequence of reduced fetal lung production of pulmonary surfactant (i.e., saturated phosphatidylcholine) resulting in amounts insufficient for neonatal respiratory adaptation (1). Despite significant advances in treatment of infants with RDS during the last two decades (2), as well as the emergence of substantial understanding of many aspects of RDS pathogenesis in human and animal model systems of lung development and maturation, very little is known about genetic controls for fetal lung development (3).

A number of studies have suggested that lung epithelial differentiation is mediated by glucocorticoids during the fetal period of embryogenesis (4–7), and that the fetal lung is a target organ for glucocorticoids (8, 9). Glucocorticoids may regulate epithelial cell differentiation into type II alveolar cells producing pulmonary surfactant (7–8). The normal or physiological role of glucocorticoids for different developmental stages of lung organogenesis (e.g., glandular, canalicular, and saccular) is not yet known. But glucocorticoids, alone or in combination with other hormones (e.g., thyroxin), may have a significant function in the regulation of lung morphogenesis and differentiation (10).

The major histocompatibility complex (MHC) has been found to influence the susceptibility to certain experimental diseases or congenital malformations in mice (11), e.g., thyroiditis (12), myasthenia gravis (13), hemolytic anemia (14), viral-induced diabetes (15), and glucocorticoid-induced cleft palate (16–22). Using congenic strains, i.e., mice with different histocompatibility loci on an identical genetic background, $H\text{-}2^a$ haplotype strain (B10.A) were found to be highly susceptible to glucocorticoid-induced cleft palate (46–81%

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clefting), whereas *H-2*^{b} haplotype (B10) were relatively resistant (5–22% clefting) (16, 17). Recently, B10.A strain adult mice lung cytosol preparations were found to have significantly higher glucocorticoid receptor levels than B10 strain (23). *H-2*-linked differences have also been reported for other hormones including estrogens (24), insulin (25), glucagon (26), and androgens (27). However, it has not been demonstrated that MHC-related genes regulate glucocorticoid influences upon pulmonary surfactant biosynthesis.

In order to pursue this problem, we have designed studies to determine the possible influence of the MHC upon glucocorticoid-stimulated pulmonary surfactant synthesis during embryonic and fetal mouse lung development. Pregnant congenic mice, C57BL/10 and B10.A, were injected with betamethasone on Days 14–17 of gestation as single or consecutive intraperitoneal injections, and fetal lung maturation at 17 or 18 days of gestation was then evaluated by assaying [^{3}H]choline incorporation into total lipid, and specifically into saturated phosphatidylcholine (SPC). Our results indicate that at certain stages, fetal lung development was influenced by MHC-related genes as evaluated by differences in glucocorticoid-stimulation of pulmonary surfactant synthesis. The B10.A (*H-2*^{a}) strain was as much as twofold more responsive to betamethasone-stimulated [^{3}H]choline incorporation into SPC than the *H-2*^{b} haplotype strain. These findings suggest a significant genetic control for SPC synthesis during murine fetal lung morphogenesis. A preliminary abstract report has appeared (28).

Materials and Methods. *Animals.* Nonpregnant mice of congenic strains C57BL/10SnJ (B10) (*H-2*^{b} haplotype) and B10.A/SnSgJ (B10.A) (*H-2*^{a} haplotype) were purchased from Jackson Laboratories (Bar Harbor, Me.) at age 7 weeks. These congenic strains are genetically identical except for a small segment of chromosome 17 which includes the major histocompatibility complex (MHC) (29). Breeding colonies in our laboratory were maintained on a 12-hr light cycle (7 AM–7 PM). In the experiments one male and four to five virgin females (12–20 weeks; ca. 25–30 g body wt) were mated overnight. The day of vaginal plug detection was taken as Day 0 of gestation.

Administration of glucocorticoids. A sustained-action betamethasone preparation (Celestone Soluspan, Schering, N.J.) was aseptically diluted 1:2 with calcium–magnesium-free, phosphate-buffered saline (PBS) (Grand Island Biological Co., Grand Island, N.Y.), and 0.2 ml was administered by intraperitoneal injection at specific times during pregnancy (Days 14–17 of gestation). The pregnant females received glucocorticoid doses of approximately 12 mg/kg body wt. Control pregnant mice received injections of PBS. Each experiment involved injections of pregnant mice in each of four groups: B10 control (+PBS); B10 experimental (+ betamethasone); B10.A control (+PBS); and B10.A experimental (+ betamethasone). Treatments were administered as either single or as consecutive injections at 24-hr intervals as stated under Results. Removal of fetuses for subsequent lung organ culture and labeling of total lipids (TC) and saturated phosphatidylcholine (SPC) was performed 24 hr following the last injection (e.g., 17 or 18 days of gestation as shown in Table I).

Lung culture and labeling. Pregnant mice from each of the four treatment groups were sacrificed together at 17 or 18 day of gestation using cervical dislocation. The uterus was removed from each animal following laparotomy and placed in a sterile petri dish at 4°C. Subsequent procedures were performed in a horizontal laminar flow hood. Decidua were removed and fetuses placed in Hank's balanced salt solution (HBSS) (GIBCO, N.Y.) buffered with 20 m*M* Hepes containing 1% antibiotic–antimycotic solution (GIBCO, N.Y.). Fetuses were decapitated, the lungs were removed by dissection, and each lung was then separated into the five component lobes. The lobes were then randomly mixed and identical equivalents (ca. one lung equivalent per dish) were sliced (150 ± 50 μm thickness) and subsequently cultured on stainless-steel grids in Falcon organ-culture dishes.

Lung tissue slices were initially incubated for 2–6 hr at 38°C in 650 μl of BGJb medium (GIBCO, Fitton-Jackson Modification, N.Y.) in an atmosphere of 5% CO_2. Following this initial preculture, medium consisting of 2–5 μCi [*methyl*-^{3}H]choline per culture dish was introduced (sp act 80 Ci/mmole, New England Nuclear, Boston, Mass.). After 6–8 hr labeling,

tissue slices were removed from culture, washed for 10 min with cold HBSS, and then transferred by means of sharpened tungsten needles into tubes for sonication. The lung tissue was then weighed and stored at −20°C until homogenization by sonication.

Biochemical assays for DNA content, total lipid, and saturated phosphatidylcholine. Fetal lung tissues were sonicated in 300–600 μl buffer (100 m*M* NaCl, 10 m*M* Tris, pH 7.0, 10 m*M* EDTA) for 1 min at 4°C using a Bronson microprobe sonicator (100 W). Duplicate aliquots of homogenate were then removed for determinations of total radioactivity incorporated, and of DNA content. DNA was determined by a modified diphenylamine spectrophotometric method sensitive to 1.0 μg DNA/ml (30).

[^{3}H]Choline incorporation into total lipid (TC) and saturated phosphatidylcholine (SPC) was determined by methods previously reported (31). An internal standard of 3000 dpm [^{14}C]dipalmitoyl phosphatidylcholine was added to lung homogenate samples before organic extraction with chloroform–methanol, thereby enabling determination of losses of SPC during extraction and assay procedures. Total lipid synthesis (TC) was determined from aliquots of the organic phase, and this phase was subsequently evaporated under nitrogen gas to dryness. Following reaction with osmium tetroxide (32), SPC was measured after separation by silica minicolumn or thin-layer chromatography. Liquid scintillation counting was performed using Aquasol II counting medium (New England Nuclear, Boston, Mass.) and the Beckman counter LS-3000 series. Efficiency and channel cross-over were corrected in each vial by use of H number. Tritium window settings of 0–317 and ^{14}C window limits of 455–655 resulted in radioactive counting efficiencies of 25–30% [^{3}H] and 52% [^{14}C].

Data analysis. Radioactive [^{3}H]choline counts incorporated into SPC were corrected for assay losses using the [^{14}C]dipalmitoyl phosphatidylcholine internal standard. All values were linearly corrected for labeling procedure variation to a standard of 5 μCi [^{3}H]choline label per organ culture dish and an 8-hr labeling period. All experimental data points used randomized single lung equivalents assayed for synthesis and DNA content each in duplicate. For ratios, standard deviations were obtained through computation of the square root of the sum of the squares of the relative standard deviations of numerator and denominator. The two-tailed Student's *t* test (with a conservative $df = 1$) was used to test significance.

Results. *Labeling of fetal lung tissue: total lipid and saturated phosphatidylcholine.* Mouse fetal lung tissues of 17 and 18 days of gestation (Theiler stages 25 and 26) (33) were found to incorporate [*methyl*-^{3}H]choline rapidly and exhibited plateau levels of aqueous-soluble precursor by 2 hr in organ culture (Fig. 1). After a slight lag period, incorporation into chloroform–methanol-extractable counts was linear over a period of 2–12 hr. The fraction assayed as SPC was constant over time for any given experimental fetal lung tissue sample. Choline incorporation, lung tissue DNA content, and incorporation on a per DNA basis all increased only slightly during this period of gestation of 17–18 days. This is in agreement with other reports (34).

Mice lungs from the B10 and B10.A strains behaved similarly in culture. Although fetal lung tissues from the two strains do not significantly differ in either average wet weight (27.4 ± 2.6 mg, B10 versus 30.0 ± 1.6 mg, B10.A) or DNA content (255 ± 22 μg, B10 versus 259 ± 23 μg, B10.A) those from the B10.A strain generally show slightly lower levels of choline incorporation (Table I).

Betamethasone effects upon lung develop-

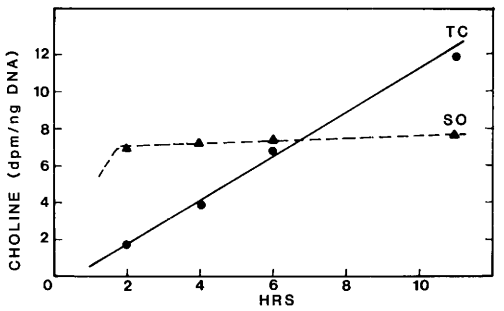

FIG. 1. Relationship between choline labeling of total lipid (TC) and aqueous-extractable, soluble pool (SO) in fetal mouse lungs. SPC (not shown) remained a constant fraction of 29 ± 2% of the total [*methyl*-^{3}H]choline incorporated (TC). Labeled lungs were sampled after 2, 4, 6, and 11 hr in culture. Protocol details are described under Materials and Methods.

TABLE I. COMPARISON OF MURINE FETAL LUNG TOTAL LIPID AND SATURATED PHOSPHATIDYLCHOLINE SYNTHESIS BETWEEN CONGENIC STRAINS C57BL/10 (*H-2*^{b}) AND B10.A (*H-2*^{a})[a]

Days of injection		C57BL/10			B10.A			{B10.A ratio / B10 ratio}
		+0	+BM	Ratio	+0	+BM	Ratio	
14–16	[17] TC	4.83 ± 0.55	8.60 ± 0.91	1.78 ± 0.28	3.28 ± 0.27	7.80 ± 0.75	2.38 ± 0.31	1.34*
	SPC	1.78 ± 0.07	3.08 ± 0.31	1.73 ± 0.19	1.17 ± 0.17	2.81 ± 0.24	2.40 ± 0.40	1.39*
15–16	[17] TC	4.62 ± 0.14	8.05 ± 0.75	1.74 ± 0.17	3.68 ± 0.16	7.61 ± 0.46	2.07 ± 0.15	1.19*
	SPC	1.51 ± 0.03	2.56 ± 0.42	1.70 ± 0.28	1.14 ± 0.05	2.32 ± 0.19	2.04 ± 0.20	1.20
16	[17] TC	5.68 ± 0.17	4.88 ± 0.36	0.86 ± 0.07	4.88 ± 0.72	5.95 ± 0.30	1.22 ± 0.20	1.42*
	SPC	1.86 ± 0.10	1.64 ± 0.27	0.85 ± 0.15	1.66 ± 0.22	1.76 ± 0.11	1.06 ± 0.15	1.25
15–17	[18] TC	8.90 ± 0.84	14.21 ± 1.11	1.60 ± 0.19	9.77 ± 0.42	14.3 ± 2.3	1.46 ± 0.25	0.91
	SPC	2.78 ± 0.36	4.83 ± 0.25	1.74 ± 0.24	2.63 ± 0.50	3.80 ± 0.72	1.44 ± 0.30	0.83
16–17	[18] TC	6.73 ± 0.75	7.10 ± 0.34	1.05 ± 0.13	4.38 ± 0.11	6.92 ± 0.49	1.58 ± 0.12	1.50*
	SPC	2.20 ± 0.25	2.37 ± 0.16	1.08 ± 0.14	1.20 ± 0.13	2.38 ± 0.11	1.98 ± 0.24	1.85*
17	[18] TC	6.73 ± 0.24	6.46 ± 0.24	0.96 ± 0.07	6.13 ± 0.17	5.12 ± 0.45	0.84 ± 0.08	0.88
	SPC	2.00 ± 0.17	1.71 ± 0.03	0.86 ± 0.07	1.57 ± 0.05	1.25 ± 0.02	0.80 ± 0.02	0.95

[a] Maternal intraperitoneal injections were on single or consecutive days as shown and as described in the text. Assays for incorporation of [^{3}H]choline into total lipid (TC) and saturated phosphatidylcholine (SPC) were conducted 24 hr following the last day of injection, i.e., on Day 17 or 18 of gestation shown in brackets. Values are expressed as ^{3}H dpm per nanogram fetal lung tissue DNA with standard deviations. Controls (+0) were saline-injected pregnant mice at appropriate times of treatment and experimentals (+BM) were betamethasone-treated pregnant mice. The ratios are those of hormone-treated to control mice, with standard deviations. Also shown is the comparison between *H-2*^{a} and *H-2*^{b} haplotype data expressed as the ratio of B10.A to B10 stimulation ratios. The asterisks indicate those indices which represent a B10.A response significantly different than the B10 response (at confidence level better than $P < 0.10$). The experiments at 15, 16, 17, [18], and 17, [18] days showed no significant differences between B10 and B10.A mice, but in all experiments with assay at [17] days and in the 16, 17, [18] days experiment, B10.A response was greater than B10 response to hormone treatment, with six of eight of these differences significant.

ment. Betamethasone was administered to pregnant B10 and B10.A mice during the fetal period. The synthetic glucocorticoid analogue was injected for the previous 1, 2, or 3 days prior to removal of lung tissues (which here is reported for Theiler stages 25 and 26) and subsequent assay for [^{3}H]choline incorporation into TC and SPC (which latter represents an index of pulmonary surfactant synthesis) (Table I). Repeated injections of betamethasone always caused stimulation of choline incorporation into TC and SPC (usually a 50–100% increase) compared to controls. The betamethasone stimulated increase in SPC synthesis was not usually at the expense of other lipids: the proportion of total lipid choline incorporation present as SPC remained relatively constant. Multiple betamethasone injections also resulted in up to 40% decreases in both total DNA content and fetal lung tissue wet weight as compared to PBS-injected controls. In contrast, single injections of hormone 1 day before assay at 17 or 18 days of gestation did not stimulate SPC synthesis in the fashion multiple injections did. In particular, after injection on 17 days of gestation there was actually slight decrease in SPC synthesis assayed at 18 days.

H-2 haplotype differences in betamethasone-stimulation of pulmonary surfactant synthesis. B10.A (*H-2*^{a}) mice responded significantly greater to betamethasone treatment than did B10 (*H-2*^{b}). The difference between these two haplotype strains was more evident upon assay at Theiler stage 25 (17 days) than at stage 26 (18 days of gestation). Upon assay at stage 25, betamethasone stimulation of pulmonary surfactant was 20–40% greater with B10.A (*H-2*^{a}) mice than with B10 (*H-2*^{b}). However, upon assay at stage 26 (18 days), B10.A showed differentially higher hormone-stimulation ratios than B10 mice only in those experiments in which hormone injections were at 16 and 17 days of gestation. No hormone stimulation was detected following a single injection of betamethasone at 17 days of gestation in either mouse strain. The anomalous result among multiple injection regimes was the 15-, 16-, and 17-days of gestation experiment after which B10 and B10.A mice did not show

markedly different responses to hormone treatment.

Discussion. This study provides evidence that glucocorticoid-stimulated pulmonary surfactant synthesis during critical stages of fetal lung development is influenced by genes within or closely linked to the murine major histocompatibility complex. Betamethasone-stimulation of SPC synthesis was 20–40% greater in B10.A ($H\text{-}2^a$) than with B10 ($H\text{-}2^b$) mice when assayed at Theiler stage 25 (17 days of gestation). The responsiveness of specific MHC haplotypes to glucocorticoid-stimulated pulmonary surfactant synthesis during fetal lung morphogenesis may benefit studies attempting to identify putative mechanisms of genetic susceptibility toward congenital malformations.

Previous studies comparing inbred mouse strains reported that during midgestation (ca. 12 days), the concentration of glucocorticoid cytoplasmic receptors was twofold higher in A/Jax ($H\text{-}2^a$) compared to C57Bl/6SnJ ($H\text{-}2^b$) strain mice embryos (35). In other studies designed to investigate the content of glucocorticoid receptors in fetal ectomesenchyme cells of the secondary palate from inbred strains, $H\text{-}2^a$ strain cells had a twofold increase in receptor sites per cell compared with $H\text{-}2^b$ strain ectomesenchyme cells (36). More recently, congenic mouse strains were used to evaluate the influence of the *H-2* histocompatibility locus on glucocorticoid receptor number and strength in adult male and female mouse thymus and lung (23). The B10.A ($H\text{-}2^a$) strain of mice was found to have significantly higher glucocorticoid receptor levels, and B10.A thymocytes showed greater response to dexamethasone in a prostaglandin production assay *in vivo* than the B10 ($H\text{-}2^b$) strain (23). These findings are complementary to those obtained with glucocorticoid-induced cleft palate using congenic mouse strains (16, 17, 20–22).

The present study was undertaken to determine whether glucocorticoid stimulation of pulmonary surfactant during critical stages of fetal mouse lung development is influenced by *H-2* haplotype. Our results illustrate that whereas both B10 and B10.A untreated, control fetal lungs at Theiler stages 25 or 26 have comparable wet weights and DNA content, each strain responded to hormone treatment, with subsequent TC and SPC synthesis according to its *H-2* haplotype; B10.A strain was more responsive than the B10 strain. However, demonstration of *H-2* associated influences between hormone stimulation and subsequent pulmonary surfactant synthesis were only identified when consecutive injections of hormone were administered prior to Theiler stage 25 (Table I). Glucocorticoid receptors have been detected during the embryonic period of mouse development (12 days of gestation) (35), and glucocorticoids seem to be able to regulate fetal lung development in animal and human systems (7–9, 34–40) prior to birth (e.g., 17–19 days of gestation in mice). Administration of large doses of exogenous glucocorticoids during late fetal stages may also inhibit tissue growth. The significant decreases in fetal lung wet weight as well as DNA content observed during our late gestation hormone treatments are consistent with reports describing the growth inhibiting effects of glucocorticoids on near-term rabbit (38) and monkey (39) fetal lungs. Whereas understanding how glucocorticoids affect stimulation or depression of biochemical processes is not known, several possible mechanisms for such hormone modulation of embryonic organogenesis have been suggested (10, 40, 41).

The results of this study suggest that hormone stimulation of pulmonary surfactant synthesis during mouse fetal lung development is influenced by genes within or closely linked to the *H-2* complex. This suggests that the *H-2* complex may be involved in the epithelial differentiation of type II cells, and the production rates of saturated phosphatidylcholine. How *H-2*-associated gene products might control glucocorticoid acceleration of normal developmental patterns for pulmonary surfactant synthesis remains to be elucidated in further studies.

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1. Farrell PM, Avery ME. Hyaline membrane disease. Amer Rev Respir Dis **111**:657–688, 1975.
2. Farrell PM. The regulation of saturated phosphatidylcholine synthesis in the developing lung. In: Farrell

PM, ed. Lung Development: Biological and Clinical Perspectives. New York, Academic Press, pp391–397, 1982.
3. Gail DB, Lenfant CJM. Cell of the lung: Biology and clinical implications. Amer Rev Respir Dis **127**:366–387, 1983.
4. Ekelund L, Arvidson G, Astedt B. Cortisol-induced accumulation of phospholipid in organ culture of human fetal lung. Scand J Clin Lab Invest **35**:419–423, 1975.
5. Giannopoulos G. Variations in the levels of cytoplasmic glucocorticoid receptors in lungs of various species at different development stages. Endocrinology **94**:450–458, 1974.
6. Kikkawa Y, Kaibara M, Motoyama EK, Orzalesi MM. Morphologic development of fetal rabbit lung and its acceleration with cortisol. Amer J Pathol **64**:423–442, 1971.
7. Smith BT, Giroud CJP. Effects of cortisol on serially propogated fibroblast cell cultures derived from the rabbit lung and skin. Canad J Physiol Pharmacol **53**:1037–1040, 1975.
8. Ballard PL. Hormonal influences during fetal lung development. Ciba Found Symp **78**:251–274, 1980.
9. Torday JS, Smith BT, Giroud CJP. The rabbit fetal lung as a glucocorticoid target tissue. Endocrinology **96**:1462–1467, 1975.
10. Smith BT, Sabry K. Glucocorticoid–thyroid synergism in lung maturation: A mechanism involving epithelial–mesenchymal interaction. Proc Natl Acad Sci USA **80**:1951–1954, 1983.
11. Klein J. Biology of the Mouse Histocompatibility-2 Complex. New York, Springer-Verlag, 1975.
12. Vladutiu AO, Rose NR. Autoimmune murine thyroiditis. Relation to histocompatibility (H-2) type. Science **174**:1137–1139, 1971.
13. Fuchs S, Nevo D, Tarrab-Hazdai R, Yaar I. Strain differences in the autoimmune response of mice to acetylcholine receptors. Nature (London) **263**:329–330, 1976.
14. Warner NL. Genetic control of spontaneous and induced antierythrocyte autoantibody production in mice. Clin Immunol Immunopathol **1**:353–363, 1973.
15. Kronmann H, Lernmark A, Vestergaard BF, Egeberg J, Nerup J. The influence of the major histocompatibility complex (H-2) on experimental diabetes in mice. Diabetologia **16**:107–114, 1979.
16. Bonner JJ, Slavkin HC. Cleft palate susceptibility linked to histocompatibility-2 (H-2) in the mouse. Immunogenetics **2**:213–218, 1975.
17. Melnick M, Jaskoll T, Slavkin HC. Corticosteroid-induced cleft palate in mice and H-2 haplotype: maternal and embryonic effects. Immunogenetics **13**:443–450, 1981.
18. Tyan ML, Miller KK. Genetics and environmental factors in cortisone induced cleft palate. Proc Soc Exp Biol Med **158**:618–621, 1978.
19. Bonner JJ. The H-2 genetic complex, dexamethasone-induced cleft palate, and other craniofacial anomalies. Curr Top Dev Biol, in press, 1984.
20. Katsumata M, Baker MK, Goldman AS, Gasser DL. Influence of *H-2*-linked genes on glucocorticoid receptors in the fetal mouse palate. Immunogenetics **13**:319–325, 1981.
21. Gasser DL, Mele L, Lees DD, Goldman AS. 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). Proc Natl Acad Sci USA **78**:3147–3150, 1981.
22. Gasser DL, Goldman AS. Genetic and biochemical studies on glucocorticoid-induced cleft palate. In: Litwack G, ed. Biochemical Actions of Hormones. New York, Academic Press, Vol 10: pp357–382, 1983.
23. Gupta C, Goldman A. H-2 histocompatibility region: Influences on the murine glucocorticoid receptor and its response. Science **216**:994–996, 1982.
24. Palumbo DA, Vladutiu AO. Estrogen reduced in uteri of mice of different H-2 genotypes. Experientia **35**:1103–1104, 1979.
25. Moruelo D, Edidin M. The biological function of the major histocompatibility complex: Hypotheses. In: Marchalonis JJ, Cohen N, eds. Contemporary Topics in Immunobiology. New York, Plenum, pp231–253, 1980.
26. Lafuse W, Edidin M. Influence of the mouse major histocompatibility complex, H-2, on liver adenylate cyclase activity and on glucagon binding to liver cell membrane. Biochemistry **19**:49–54, 1980.
27. Ivanyi P. Some aspects of the H-2 system, the major histocompatibility system in the mouse. Proc R Soc Lond [Biol] **202**:117–158, 1978.
28. Honig LS, Donahue HG, Smith BT, Slavkin HC. Differentiation of mouse lungs of two congenic strains. J Cell Biol **99**:55a, 1983.
29. Snell GD. Methods for the study of histocompatibility genes. J Genet **49**:87–108, 1948.
30. Patterson MK Jr. Measurement of growth and viability of cells in culture. In: Jakoby WB, Pastan IH, eds. Methods in Enzymology. New York, Academic Press, Vol 58:pp141–152, 1978.
31. Smith BT. Cell line A549: A model system for the study of alveolar type II cell function. Amer Rev Resp Dis **115**:285–293, 1977.
32. Mason RJ, Nellenbogen J, Clements JA. Isolation of disaturated phosphatidylcholine with osmium tetroxide. J Lipid Res **17**:281–284, 1976.
33. Theiler K. The House Mouse. New York, Springer-Verlag, 1972.
34. Brehier A, Rooney SA. Phosphatidylcholine synthesis and glycogen depletion in fetal mouse lung: Developmental changes and the effects of dexamethasone. Exp Lung Res **2**:273–287, 1981.
35. Salomon DS, Zubairi Y, Thompson EB. Ontogeny

and biochemical properties of glucocorticoid receptors in midgestation mouse embryos. J Steroid Biochem **9**:95–107, 1978.

36. Salomon DS, Pratt RM. Involvement of glucocorticoids in the development of the secondary palate. Differentiation **13**:141–154, 1979.
37. Velasquez BJ, Sepulveda J. Influence of transplacental NA-872 and dexamethasone therapy on fetal and newborn mouse lung. Respiration **43**:389–400, 1982.
38. Smith BT, Torday JS, Giroud DJP. Evidence for different gestation-dependent effects of cortisol on cultured fetal lung cells. J Clin Invest **53**:1518–1526, 1974.
39. Mitzner W, Johnson JWC, Beck J, London W, Sly D. Influence of betamethasone on the development of mechanical properties in the fetal rhesus monkey lung. Amer Rev Resp Dis **125**:233–238, 1982.
40. Rall L, Pictet R, Githens S, Rutter W. Glucocorticoids modulate the in vitro development of the embryonic rat pancreas. J Cell Biol **75**:398–409, 1977.
41. Cunha GR, Shannon JM, Taguchi O, Fujii H, Meloy BA. Epithelial–mesenchymal interactions in hormone-induced development. In: Sawyer RH, Fallon JF, eds. Epithelial–Mesenchymal Interactions in Development. New York, Praeger, pp51–74, 1983.

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