Toward Therapeutic Pulmonary Alveolar Regeneration in Humans

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In humans, age results in loss of pulmonary alveoli; menopause accelerates loss of diffusing capacity, an index of alveolar surface area; and disease (e.g., chronic obstructive pulmonary disease) results in loss of alveoli. Thus, an important goal for investigators is to generate knowledge that allows induction of pulmonary alveolar regeneration in humans. Our enthusiasm for this goal and our assessment of its feasibility are based on work in several laboratories over the last decade that has disproved the notion that pulmonary alveoli are incapable of regeneration, and on the growing evidence that signals that regulate programs of alveolar turnover (loss and regeneration) are conserved from rodents to humans. We review animal models of alveolar loss and regeneration and their conservation during evolution, and hence their relevance to humans.

Keywords: age; chronic obstructive pulmonary disease; menopause

We believe an important long-term goal for investigators is to generate knowledge that allows induction of pulmonary alveolar regeneration, or that rescues failed alveologenesis, in humans. Our enthusiasm for this goal and our positive assessment of its feasibility are based on work in rodents in eight laboratories (1–6) (S. Rennard and S. Shapiro, personal communication) over the last decade that has falsified the notion that pulmonary alveoli are incapable of regeneration (7, 8), and on the growing evidence that signals that regulate programs of alveolar turnover (loss and regeneration) are conserved from rodents to humans (9–12).

CONDITIONS TARGETED

Age-related Alveolar Loss

In nonmutant animal species, whose thorax and lung volume do not increase during adulthood, an age-related loss of alveoli occurs, as indicated by an increase of distance between alveolar walls (L_m), or a decrease of alveolar surface-to-volume ratio, and most important, by a decline of alveolar surface area without a decline of lung volume (13–15). In rodents that continue to grow as adults, lung volume increases with age, as does L_m (14, 16, 17), but, more important, the volume of individual alveoli increases with age (18–20); these increases partly reflect loss of lung tissue elastic recoil with age (21) but are also due to the loss of alveolar walls, as shown by the decrease in the total number of alveoli with age (22, 23).

In humans without anatomic evidence of lung disease, morphometric studies clearly show alveoli get bigger, and alveolar

surface area and the number of alveoli decline with age (22–24). Furthermore, the loss of alveolar surface area begins in the third to fourth decade of life and proceeds more rapidly in men than in women (24); these anatomic findings, although based on few subjects, are supported by the early onset of loss of lung function and, until menopause, the more rapid loss in men than in women (25). Finally, loss of lung function in a general population is a strong predictor of mortality (26–31).

Menopause, which is age related, is an important cause of accelerated alveolar loss. Diffusing capacity, an indicator of gas-exchange surface area (32), diminishes with age in never-smokers. Men who have never smoked lose diffusing capacity at a rate of about 6%/decade; women who have never smoked lose diffusing capacity at a rate of about 2%/decade before menopause and about 6%/decade postmenopause (25). These rates of loss of diffusing capacity parallel the age-related loss of maximum O_2 consumption ($\dot{V}_{max}$) (33, 34), although, for unclear reasons, the link between alveolar loss and the associated loss of $\dot{V}_{max}$ is never made. That this menopause-related accelerated decline of diffusing capacity is due to a fall in the concentration of estrogen is supported by the observation that ovariectomy in adult mice results in alveolar loss and estrogen replacement results in alveolar regeneration (35); this observation also supports the notion that the effect of estrogen on lung function is evolutionarily conserved from mice to humans.

More recent work with women on the effect of estrogen on forced time expiratory flow rates adds additional support for evolutionary conservation of the effect of estrogen on alveolar architectural stability and regeneration. Thus, the forced time vital capacities reflect resistance to airflow in the conducting airways and lung tissue elastic recoil. Increased resistance to airflow is present in airways that are excessively narrow during expiration and can, in part, be due to the loss of the tethering effect of alveolar attachments caused by alveolar destruction (36–40). Elderly women receiving hormone replacement (estrogen plus progesterone) have a higher FEV_1 than similar-age women not receiving hormone replacement; this difference is not explained by lower rates of smoking or other health factors (41). Administration of estrogen plus progesterone (42, 43), estrogen alone (44), or an estrogen-like compound (44) to postmenopausal women increases their forced vital capacity and FEV_1 . Even in women aged 24 to 35 yr, the use of an oral contraceptive containing estradiol and a progestin increased forced expiratory flow rates, especially flow rates at low lung volumes (45). The latter is especially important because expiratory airflow at low lung volumes reflects the patency of small conducting airways, which depend substantially on the tethering effect of alveolar attachments (36–40). Hence, these findings in young women point to the alveolar-maintaining effect, and perhaps alveolar-regenerating ability, of ovarian hormones. The loss of alveoli in mice after ovariectomy and their regeneration during estradiol replacement (35) suggest estrogen is the ovarian hormone responsible for maintaining alveolar structural stability, and for inducing alveolar regeneration, in women. This evidence that the estrogen-preserving effect on alveolar architectural stability and its alveolar-regenerating effect is evolutionarily

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conserved supports the usefulness of this mouse model and its relevance to women.

In addition to the role of ovarian hormones, in particular estrogen, on the alveoli of healthy women, low concentrations of ovarian hormones may play a role in the development and progression of chronic obstructive pulmonary disease (COPD) (46). Women constitute 75% of never-smokers older than 55 yr with clinical and lung function evidence of COPD (26, 47). That this observation does not reflect an age bias of smoking prevalence toward men is indicated by the standardized mortality rate, which is almost twice as high in women than in men, among individuals with COPD who participated in a survey in which the average age on entry was 56.6 yr (48). Thus, evidence is growing to indicate estrogen may delay the loss of, and improve, those lung functions that reflect maintenance of alveolar structure and, as a consequence, the number of alveolar attachments to small conducting airways. If proof-of-principle testing fails to falsify these conjectures, they could result in a decrease in suffering, death, and economic loss worldwide (49, 50).

Calorie-related Alveolar Loss and Regeneration: Evidence for Evolutionary Conservation from Mouse to Human

Calorie restriction in mice (51–53), rats (54–56), and hamsters (57) and starvation in humans (10–12) cause alveolar loss through, we believe, an endogenous program that is conserved from rodents to humans. *Ad libitum* access to food after calorie restriction–induced alveolar loss results in alveolar regeneration in rodents. To our knowledge, the effect on alveolar regeneration of *ad libitum* refeeding after starvation in humans has not been tested. However, the evidence that the calorie restriction–starvation endogenous program of alveolar loss, which offers survival advantages during commonly occurring periods of starvation, is evolutionarily conserved strongly supports the notion that its opposite, alveolar regeneration in humans after refeeding following starvation, will also be conserved—hence, the importance of understanding the gene expression responsible for alveolar regeneration in this model.

MODUS OPERANDI

Identification of Very Upstream Gene Expression That Is Specific and Determinative of Alveolar Regeneration

Here we present an approach we are using with the hope it will generate discussion, criticism, and better ways to move toward alveolar regeneration in humans. The use of global gene profiling, which should be a powerful tool to identify the initial gene expression that specifies alveolar regeneration, has, we believe, been hampered by the lack of knowledge of the time when, after a regenerative stimulus, the very upstream gene expression determinative of regeneration begins. This information would narrow the period that must be studied to identify, and understand, the regulation of genes that initiate alveolar regeneration. To identify this period, we have, as a first step, enumerated cellular processes that biological knowledge indicates are required for alveolar regeneration (i.e., transforming a flat segment of alveolar wall into an elongating fold [septum] that increases gas-exchange surface area). These processes include lung cell replication, angiogenesis, remodeling of the extracellular matrix, and guided cell motion. Then, we used microarray analysis in two mouse models of alveolar regeneration (estradiol-treated ovariectomized mice and previously calorie-restricted mice with *ad libitum* access to food) killed 3 h after the regenerative stimulus. These studies revealed the presence of gene expression in lung supportive of cell replication, angiogenesis, formation of extracellular matrix, and guided cell motion within 3 h of the onset of *ad libitum* access to food and estradiol treatment. We

then used real-time polymerase chain reaction (RT-PCR) to assess expression of a subset of genes at earlier times after estradiol injection into ovariectomized mice. We identified gene expression supportive of cell replication within 1 h of estradiol treatment, and within 1 h of the onset of *ad libitum* access to food after calorie restriction. Three hours later, gene expression supportive of other processes required to form a septum was present.

We learned from this work with the ovariectomy and calorie-related models that we could go directly to using RT-PCR to identify the onset of changes in gene expression determinative of alveolar regeneration. Therefore, with our third model, alveolar regeneration produced by treating mice that have elastase-induced emphysema with all-trans-retinoic acid (5), we will study mice with elastase-induced emphysema 1 h after treatment with all-trans-retinoic acid.

Our modus operandi is as follows:

1. Provide a regenerative stimulus.
2. Use RT-PCR to determine the onset of signaling at the RNA level for cell replication, guided cell motion, extracellular matrix remodeling, and angiogenesis; our data indicate that, for each of the two animal models tested, signaling for cell replication is a very early response to a regenerative stimulus (e.g., within 1 h)
3. Establish the earliest time of appearance in each model, after injection of each regenerative reagent, of signaling at the RNA level for cellular processes supporting alveolar regeneration; this identifies the time of early gene expression determinative of alveolar regeneration

When this timing is established for all three models, or all reagents, we will

4. Perform microarray analysis over a short (1 h) period to get a global view of gene expression in lung in the models used
5. Use computational analysis of gene expression among the models or reagents

If we detect a common pattern of gene expression among two or more of the models or reagents, our proof-of-principle will be to

6. Manipulate the pattern of gene expression using small inhibitory ribonucleic acid and morphometrically determine the effect on alveolar regeneration

If we do not detect a similar pattern of gene expression among two or more of the models or reagents, it will mean the animal models relevant to human diseases must be studied individually, and candidate regenerative maneuvers must, as has so far been the case (1–7), be selected on available information and emerging insights.

ALVEOLAR REGENERATION IN RODENTS

Work over the past decade has falsified the notion that alveoli do not regenerate (1–6, 51, 52) (S. Rennard and S. Shapiro, personal communication, March 2006). During this period: (1) among seven laboratories, four reagents—all-trans-retinoic acid (Figure 1) (1–3) (S. Rennard and S. Shapiro, personal communication, March 2006), granulocyte colony-stimulating factor (58), adrenomedullin (4), and hepatocyte growth factor (5)—induced alveolar regeneration and abrogated key features of elastase- and cigarette smoke-induced emphysema in rodents; (2) two laboratories reported all-trans retinoic acid rescues failed alveologenesis in mice and rats (6, 59); and (3) one laboratory demonstrated estradiol induces alveolar regeneration after ovariectomy-induced alveolar loss in adult mice (35) and preserves alveolar

Figure 1. Histologic sections showing (A) lung of a rat not treated with elastase; (B) lung of a rat in which elastase was instilled into the trachea and, 3 wk later was injected daily with oil, the vehicle for all-trans-retinoic acid, for 2 wk; (C) lung of a rat in which elastase was instilled into the trachea and that, 3 wk later, was treated daily with all-trans-retinoic acid for 2 wk. Lungs were fixed at a transpulmonary pressure of 20 cm H₂O. Reprinted by permission from Reference 1.

formation after ovariectomy in rats at the time they are weaned (60). By contrast, studies in five laboratories have not found that all-trans-retinoic acid induces alveolar regeneration in mouse (61, 62), rat (63), guinea pig (64), or rabbit (65). The reason for this failure is unclear. Two of the studies, which were excellent, were performed at high altitude (Denver [61] and Albuquerque [63]). Because hypoxia inhibits alveologenesis (19, 36, 66, 67), it is possible this explains the failure of regeneration in Denver and Albuquerque. Our conclusion is that if nine miners strike gold, but five do not, gold is present. Finally, work in several laboratories, and the evidence that the regulation of alveolar loss and regeneration is conserved from mouse to humans, suggests we are not at the “beginning of the end,” but perhaps at

“the end of the beginning” (Winston Churchill, 1942, wartime speech given at the Lord Major’s luncheon) in the pursuit of alveolar regeneration in humans.

Conflict of Interest Statement: D.M. has stocks in several companies that have been chosen by an advisor. G.D.M. has a financial advisor who invests for her. She has not been to any meetings organized by a pharmaceutical company, nor does she plan to attend such meetings. D.M. and G.D.M. hold a patent for the use of retinoids in lung diseases.

References

1. Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat Med* 1997;3:675–677.
2. Ofulie AF, Xiong Y, Yang N, Belloni PN. Retinoic acid reverses cigarette smoke-induced emphysema in rats. *Am J Respir Crit Care Med* 2002;165:137.
3. Ishizawa K, Kubo H, Yamada M, Kobayashi S, Numasaki M, Ueda S, Suzuki T, Sasaki H. Bone marrow derived cells contribute to lung regeneration after elastase-induced pulmonary emphysema. *FEBS Lett* 2004;556:249–252.
4. Murakami S, Nagaya N, Itoh T, Iwase T, Fujisato T, Nishioka K, Hamada K, Kamigawa K, Kimuro H. Adrenomedullin regenerates alveoli and vasculature in elastase-induced pulmonary emphysema in mice. *Am J Respir Crit Care Med* 2005;172:581–589.
5. Shigemura N, Sawa Y, Mizuno S, Ono M, Ohta M, Nakamura T, Kaneda Y, Matsuda H. Amelioration of pulmonary emphysema by in vivo gene transfection with hepatocyte growth factor in rats. *Circulation* 2005;111:1407–1414.
6. Hind M, Maden M. Retinoic acid induces alveolar regeneration in the adult mouse lung. *Eur Respir J* 2004;23:20–27.
7. Cagle PT, Thurlbeck WM. State of the art: postpneumonectomy compensatory lung growth. *Am Rev Respir Dis* 1988;138:1314–1326.
8. Holmes C, Thurlbeck WM. Normal lung growth and response after pneumonectomy in rats at various ages. *Am Rev Respir Dis* 1979;120:1125–1136.
9. Massaro GD, Radaeva S, Clerch LB, Massaro D. Lung alveoli endogenous programmed destruction and regeneration. *Am J Physiol* 2002;293:L305–L309.
10. Fliederbaum J. Clinical aspects of hunger diseases in adults. In: Winick M, editor. *Hunger disease: studies by the Jewish physicians in the Warsaw ghetto*. New York: John Wiley and Sons; 1979. pp. 11–36.
11. Cook VJ, Coxson H, Mason AG, Bar TR. Bullae, bronchiectasis and nutritional emphysema in severe anorexia nervosa. *Am J Respir Crit Care Med* 2004;170:748–752.
12. Coxson HO, Chan IH, Mayo JR, Hlynsky J, Nakano Y, Birmingham CL. Early emphysema in patients with anorexia nervosa. *Am J Respir Crit Care Med* 2004;170:748–752.
13. Maudlerly J, Hahn FF. The effects on lung function and structure of adult animals. *Adv Vet Sci Comp Med* 1982;26:35–77.
14. Robinson NE, Gillespie JR. Lung volumes in aging beagle dogs. *J Appl Physiol* 1973;35:317–321.
15. Hyde DM, Robinson NE, Gillespie JR, Tyler WS. Morphometry of the distal air spaces in lungs of aging dogs. *J Appl Physiol* 1977;43:86–91.
16. Massaro D, Teich N, Maxwell S, Massaro GD, Whitney D. Postnatal development of alveoli: regulation and evidence for a “critical period” in rats. *J Clin Invest* 1985;76:1297–1305.
17. Massaro D, Teich N, Massaro GD. Postnatal development of pulmonary alveoli: modulation in rats by thyroid hormones. *Am J Physiol* 1985;250:R50–R55.
18. Blanco LM, Massaro GD, Massaro D. Alveolar dimensions and numbers: developmental and hormonal regulation. *Am J Physiol* 1989;257:L240–L247.
19. Blanco L, Massaro D, Massaro GD. Alveolar size, number, and surface area: developmentally dependent response to 13% O₂. *Am J Physiol* 1991;261:L370–L377.
20. Massaro GD, Massaro D. Formation of alveoli in rats: postnatal effect of prenatal dexamethasone. *Am J Physiol* 1992;263:L37–L41.
21. Sahebajani H. Lung tissue elasticity during the lifespan of Fischer 344 rats. *Exp Lung Res* 1991;17:887–902.
22. Stupina AS, Chermyi IM. Sterologic analysis of the respiratory zone of the lungs of the laboratory rat and man during aging. *Arkiv Anat Gistol Embriol* 1985;88:65–69.
23. Massaro D, Massaro G. Pulmonary alveoli: formation, the “call for oxygen,” and other regulators. *Am J Physiol* 2002;282:L345–L358.
24. Thurlbeck WM. The internal surface area of nonemphysematous lungs. *Am Rev Respir Dis* 1967;95:765–773.

25. Neas LM, Schwartz J. The determinants of pulmonary diffusing capacity in a national sample of U.S. adults. *Am J Respir Crit Care Med* 1996; 153:656–664.
26. Knuiman MW, James AL, Divitin M, Ryan G, Bartholomew HC, Musk AW. Lung function, respiratory symptoms, and mortality; results from the Busselton Health Study. *Am J Epidemiol* 1999;9:297–306.
27. Tockman MS, Pearson JD, Fleg JL, Metter EJ, Kao SY, Rampal KG, Cruise LJ, Fozard JL. Rapid decline in FEV₁: a new risk factor for coronary heart disease mortality. *Am J Respir Crit Care Med* 1995;151: 390–398.
28. Sorlie PD, Kamnel WB, O'Connor G. Mortality associated with respiratory functions and symptoms in advanced age. *Am J Respir Crit Care Med* 1989;140:379–384.
29. Zureik M, Benetos A, Neukirch C, Courbon D, Bean K, Thomas F, Ducimetie P. Reduced pulmonary function is associated with arterial stiffness in men. *Am J Respir Crit Care Med* 2001;164:2181–2185.
30. Truelsen T, Prescott E, Lange P, Schnohr P, Boysen G. Lung function and risk of fatal and non-fatal stroke: the Copenhagen City Heart Study. *Int J Epidemiol* 2001;30:145–151.
31. Wannamethee SG, Shaper AG, Elrahim S. Respiratory function and risk of stroke. *Stroke* 1995;26:2004–2010.
32. Powell FL, Hopkins SR. Comparative physiology of lung complexity: implications for gas exchange. *News Physiol Sci* 2004;19:55–60.
33. Woo JS, Derleth C, Stratton JR, Levy WC. The influence of age, gender, and training on exercise efficiency. *J Am Coll Cardiol* 2006;47:1049–1057.
34. Kasch FW, Boyer JL, Schmidt PK, Wells RH, Wallace JP, Verity LS, Guy H, Schneider D. Ageing of the cardiovascular system during 33 years of aerobic exercise. *Age Ageing* 1999;28:531–536.
35. Massaro D, Massaro GD. Estrogen regulates pulmonary alveolar formation, loss, and regeneration in mice. *Am J Physiol* 2004;287:L1154–L1159.
36. Massaro GD, Olivier J, Dzikiowski C, Massaro D. Postnatal development of lung alveoli: suppression by 13% O₂ and a critical period. *Am J Physiol* 1990;258:L321–L327.
37. Macklem PT, Mead J. Resistance of central and peripheral airways measured by a retrograde catheter. *J Appl Physiol* 1967;22:395–401.
38. Vincent NJ, Knudson R, Leith DE, Macklem P, Mead J. Factors influencing pulmonary resistance. *J Appl Physiol* 1970;29:236–243.
39. Saetta M, Ghezzi H, Kim WD, King M, Angus GE, Wang NS, Cosio MG. Loss of alveolar attachments in smokers: a morphometric correlate of lung function impairment. *Am Rev Respir Dis* 1985;132:894–900.
40. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, et al. The nature of small airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
41. Carlson CL, Cushman M, Enright PL, Cauley JA, Newman AB; Cardiovascular Health Study Research Group. Hormone replacement therapy is associated with higher FEV₁ in elderly women. *Am J Respir Crit Care Med* 2001;163:423–428.
42. Cervirolm AS, Fidan F, Unln M, Yihmazer M, Orman A, Fenkei IV, Serteser M. The effects of hormone therapy on pulmonary function tests in postmenopausal women. *Maturitas* 2004;49:221–227.
43. Koksai N, Gaven A, Celik O, Kiran G, Kiran H, Eberlicer HC. The effects of hormone replacement therapy on pulmonary functions in postmenopausal women. *Tuberk Toraks* 2004;52:237–242.
44. Pata O, Atis S, Utbu OzA, Yazici G, Tok E, Pata C, Kilic F, Camdeviren, H., Aban, M. The effects of hormone replacement therapy type on pulmonary functions in postmenopausal women. *Maturitas* 2003;46: 213–218.
45. Strinic T, Eterovic D. Oral contraceptives improve lung mechanics. *Fertil Steril* 2003;79:1070–1073.
46. Miravittles M, Ferrer M, Pont A, Viejo JL, Masa JF, Gabriel R, Jimenez-Ruiz CA, Villasant C, Fernandez-Fau L, SobradilloV. Characteristics of a population of COPD patients identified from a population based study. Focus on previous diagnosis and never smokers. *Respir Med* 2005;99:985–995.
47. Von Hertzen L, Reunamen A, Impivaara O, Malkia E, Aromaa A. Airway obstruction in relation to symptoms in chronic respiratory disease: a nationally representative population study. *Respir Med* 2000;94:356–363.
48. Ringbaek T, Seersholm N, Viskum K. Standardized mortality rates in females and males with COPD. *Eur Respir J* 2005;25:891–895.
49. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease study. *Lancet* 1997;349:1498–1504.
50. Sullivan SD, Ramsey SD, Lee TA. The economic burden of COPD. *Chest* 2000;117:55–95.
51. Massaro GD, Radaeva S, Clerch LB, Massaro D. Lung alveoli: endogenous programmed destruction and regeneration. *Am J Physiol* 2002; 283:L305–L309.
52. Massaro D, Massaro GD, Bards A, Hoffman EP, Clerch LB. Calorie-related rapid onset of alveolar loss regeneration, and changes in mouse lung gene expression. *Am Rev Respir Dis* 2004;287:L896–L906.
53. Massaro D, Massaro GD. Hunger disease and pulmonary alveoli. 2004; 170:748–752.
54. Sahebajami H, Wirman JA. Emphysema like changes in lungs of starved rats. *Am Rev Respir Dis* 1981;124:619–624.
55. Kerr JS, Riley DJ, Lanza-Jacoby RA, Berg HC, Spilker HC, Yu SC, Edelman NH. Nutritional emphysema in the rat: influence of protein depletion and impaired lung growth. *Am Rev Respir Dis* 1985;131:644–650.
56. Harkema JR, Mauderly JL, Gregory RE, Pickrell JA. A comparison of starvation and elastase models of emphysema in the rat. *Am Rev Respir Dis* 1984;129:584–591.
57. Karlinsky JB, Goldstein RH, Ojsekis B, Snider GL. Lung mechanics and connection tissue levels in starvation-induced emphysema in hamster. *Am J Phys* 1986;251:R282–R288.
58. Ishizawa K, Kubo H, Yamada M, Kobayashi S, Numasaki M, Ueda S, Suzuki T, Sasaki H. Bone marrow derived cells contribute to lung regeneration after elastase induced pulmonary emphysema. *FEBS Lett* 2004;556:249–252.
59. Massaro GD, Massaro D. Retinoic acid treatment partially rescues failed septation in rats and mice. *Am J Physiol* 2000;278:L955–L960.
60. Massaro GD, Morlota JP, Massaro D. Estrogen modulates the dimensions of the lung's gas-exchange surface area and alveoli in female rats. *Am J Physiol* 1996;270:L110–L114.
61. Fujita M, Ye Q, Ouchi H, Nakashima N, Hamada N, Hagimoto N, Kuwan K, Mason RJ, Nkanishi Y. Retinoic acid fails to reverse emphysema in adult mouse models. *Thorax* 2004;59:224–230.
62. Lucey EC, Goldstein RH, Breuer R, Rexer BN, Ong DE, Snider GL. Retinoic acid does not affect alveolar septation in adult FVB mice with elastase-induced emphysema. *Respiration (Herrlisheim)* 2003;70: 200–205.
63. March TH, Cossey PY, Esparza DC, Dix KJ, McDonald JD, Bowen LE. Inhalation of all-trans-retinoic acid for treatment of elastase induced pulmonary emphysema in Fischer 344 rats. *Exp Lung Res* 2004;30:385–404.
64. Meshi B, Vitalis TZ, Ionescu D, Elliott WM, Liu C, Wang XD, Hayashi S, Hogg JC. Emphysematous lung destruction by cigarette smoke: the effects of latent adenoviral infection on the lung inflammatory response. *Am J Respir Cell Mol Biol* 2002;26:52–57.
65. Nishi Y, Boswell V, Ansari T, Piprawa F, Satchi S, Page CP. Elastase-induced changes in lung function: relationship to morphometry and effect of drugs. *Pulm Pharmacol Ther* 2003;16:221–229.
66. Mortola JP, Morgan CA, Virgona V. Respiratory adaptation to chronic hypoxia in newborn rats. *J Appl Physiol* 1986;61:1329–1336.
67. Mortola JP, Xu LJ, Lauzon AM. Body growth, lung and heart weight, and DNA content of newborn rats exposed to different levels of chronic hypoxia. *Can J Physiol Pharmacol* 1990;68:1590–1594.