

Chronic obstructive pulmonary disease • 3: Experimental animal models of pulmonary emphysema

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The use of genetically manipulated mice together with traditional animal studies are steadily increasing our knowledge of the factors important in determining alveolar formation and destruction in emphysema. A review of the animal models used to study emphysema is presented.

Chronic obstructive pulmonary disease (COPD) is one of the commonest reasons for ill health worldwide, with 16 million individuals affected in the USA alone. It is ranked as the fourth and fifth highest cause of death in the USA and UK, respectively, with a mortality rate at least 14 times that of asthma.^{1,2} The single most important factor in the development of emphysema is cigarette smoke. Inhalation of cigarette smoke causes a chronic pulmonary inflammatory infiltrate of macrophages, neutrophils and CD8+ cells that persists long after smoking cessation. In susceptible individuals this ultimately leads to irreversible destruction and dilatation of the terminal airspaces of the lung, chronic disability due to respiratory failure, and premature death.

Animal studies have been critical in shaping contemporary views regarding the pathogenesis of emphysema. Almost 40 years ago Gross reported that intratracheal instillation of the plant proteinase papain into rats resulted in emphysema. This, combined with the clinical observation by Eriksson that deficiency of the antiproteinase α_1 -antitrypsin was associated with early onset panlobular emphysema in humans,^{3,4} fuelled the concept that an imbalance within the lung favouring proteinases over their inhibitors resulted in emphysema. As more sophisticated animal models have evolved, they have continued to develop fresh insights into lung biology and enabled the testing of novel treatments for emphysema. While the initial association of emphysema with α_1 -antitrypsin deficiency suggested that neutrophil derived proteinases were critical to the development of emphysema, recent studies—largely in animal models—have broadened the scope of cells and proteinases that may cause emphysema. Particular attention has been given to macrophage derived matrix metalloproteinases (MMPs).⁵ Furthermore, stemming from the study of alveogenesis in experimental animals, retinoic acid treatment has been shown to regenerate alveoli in rats both during development and following experimental emphysema.⁶ It is now clear that complex interacting pathways are involved in the initiation, progression, and the failure of correct repair in emphysema

While hypotheses and mechanisms of emphysema can be addressed in the test tube and cell culture, ultimately these ideas need to be tested in mammals to elucidate the complex pathophysiology of COPD. Rodents, dogs, guinea pigs, monkeys, and sheep have all been used to study emphysema. With the advent of genetic engineering in mice, this species offers the greatest ability to dissect pathogenetic pathways in mammals. In addition, more is known about its genome than any other animal and cDNA and antibody probes are abundant. They also offer the practical benefits of short breeding times, large litters, and relatively cheap housing. The main issue confounding the use of mice and other animals as a template for human disease is that we do not know how accurately they reflect human biology and pathology. Mouse and man clearly share many basic physiological processes, but the details of how gas exchange is achieved will determine how closely findings in mice can be applied to man. Each animal model should be viewed as one component of the process for studying human disease and not viewed in isolation or extrapolated directly to humans.

Emphysema can be modelled in many ways. Exogenous administration of proteinases, chemicals, particulates, and exposure to cigarette smoke result in features characteristic of human COPD. Genetic manipulation itself can result in airspace enlargement during development and throughout life. These different approaches each have their own merits and limitations and require individualised interpretation. The methods also often complement each other so their usefulness can be enhanced by using a combination of approaches to study the disease. The field has also not infrequently been stimulated by the inadvertent generation of emphysema in animals where experiments were initially undertaken for another reason.

EMPHYSEMA PRODUCED BY CHALLENGE WITH EXOGENOUS AGENTS

Intrapulmonary challenge with injurious proteins, chemicals, particulates, and other compounds into lungs of animals has been used to cause emphysema directly. Compounds have also been administered that inhibit protein function (loss of function models), resulting in airspace enlargement.

A single intrapulmonary challenge with proteinases including porcine pancreatic elastase (PPE), papain, and human neutrophil elastase causes panacinar emphysema.⁷ Their effectiveness was directly related to their elastolytic activity, while instillation of bacterial collagenases did

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not cause emphysema. PPE mediated emphysema was accompanied by secretory cell metaplasia and abnormalities of pulmonary function, hypoxaemia, and right ventricular hypertrophy that are characteristic of human COPD. Following an intratracheal bolus of PPE, there is an initial loss of elastin and collagen. Over time, elastin and glycosaminoglycans return to normal and collagen is enhanced, yet intraparenchymal extracellular matrix (ECM) remains diminished and distorted and the architecture of the lung is grossly and permanently abnormal.⁸ Airspace enlargement develops immediately after elastolytic injury, followed by inflammation. The subsequent progression of emphysema over the next few months is probably caused by the destructive effect of host inflammatory proteinases. Although repair mechanisms fail to establish the normal lung structure, impairment of elastin and collagen cross linking with the addition of the lathyrogen β -aminopropionile (BAPN) worsens the emphysema,⁹ suggesting that some repair occurs. Instillation of elastases remains a useful tool, particularly to study effects downstream of proteolytic injury. Yet inflammation and host injury following instillation suggests that this model might also provide insights into upstream events such as inflammation and endogenous proteinases. However, extrapolating these findings to the slowly developing smoking induced disease in humans is not straightforward as additional mediators are likely to be involved.

Various other agents have also been used in an attempt to recreate inflammation and lung injury. For example, repeated endotoxin administration recruits neutrophils and activates macrophages with resultant airspace enlargement.¹⁰ Administration of oxidants such as nitrogen dioxide and ozone, the two major airborne pollutants, causes lung injury. Repeated long term administration of nitrogen dioxide results in mild focal emphysema, while ozone results in fibrosis.¹¹ Administration of cadmium chloride, a constituent of cigarette smoke, also results in primarily interstitial fibrosis with tethering open of airspaces simulating emphysema. While this mechanism differs from airspace enlargement secondary to matrix destruction that characterises emphysema, we now appreciate that airway fibrosis is a significant factor in centrilobular emphysema seen in human smokers.¹² Interestingly, a combination of cadmium and BAPN enhanced the emphysematous changes.¹³

Coal dust and silica result in focal emphysema, and animal models have uncovered complex inflammation and oxidant injury with connective tissue breakdown that was attributed to neutrophil mediated injury.¹⁴

Intravascular administration of a vascular endothelial cell growth factor receptor-2 (VEGFR-2) blocker has recently been used to generate a model of non-inflammatory emphysema.¹⁵ VEGF is a trophic factor required for endothelial cell survival and normal blood vessel development, and its absence results in endothelial cell apoptosis.^{16,17} There is increased septal cell death in human emphysematous lungs, which is associated with reduced lung expression of VEGF and VEGFR-2 (KDR/Flk-1).¹⁸ Chronic VEGFR-2 blockade with SU5416 caused alveolar septal cell apoptosis, pruning of the pulmonary arterial tree, and airspace enlargement. This mechanism of airspace enlargement revisits the previous “vascular” hypothesis of the disappearing alveolar cells in emphysema.¹⁹ Importantly, this also raises the concept that initial loss of epithelial cells can lead to matrix destruction and airspace enlargement, as opposed to the traditional view that inflammatory cell proteinases degrade matrix with subsequent loss of attachment and death of structural cells.

CIGARETTE SMOKE INDUCED EMPHYSEMA

Various animals have been exposed to cigarette smoke using smoking chambers. The first unequivocal animal model of smoking related emphysema was reported in 1990 by Wright

and colleagues.²⁰ Exposure of guinea pigs to the smoke of 10 cigarettes each day, 5 days per week, for 1, 3, 6, and 12 months resulted in progressive emphysema and pulmonary function abnormalities similar to those seen in humans with cigarette smoke induced COPD. Cessation of smoke exposure stabilised—but did not reverse—airspace enlargement. In response to long term cigarette smoke exposure guinea pigs exhibit marked neutrophilia and accumulation of macrophages and CD4+ cells.^{21,22} Latent adenoviral infection combined with cigarette smoke exposure potentiates the emphysema in guinea pigs and, interestingly, this infection independently increased neutrophils, macrophages, and CD8+ cells.²¹

Mice are able to tolerate at least two cigarettes per day for a year with non-toxic carboxyhaemoglobin levels and minimal effects on body weight. Despite the fact that they are obligate nose breathers, they do not have extensive cilia and inefficiently filter tobacco smoke. Other anatomical differences from man include a paucity of submucosal glands (limited to the trachea) and fewer Clara cells. They also have less extensive airway branching and lack respiratory bronchioles. The development of emphysema in mice is dependent on the strain of mouse. Neutrophil recruitment occurs following the first cigarette and is followed by a more gradual accumulation of macrophages. Early neutrophil influx in the lungs is accompanied by a measurable increase in collagen and elastin degradation products. At this very early stage both the BAL neutrophilia and the connective tissue breakdown is preventable by pretreating the mice with an antibody to neutrophils or α_1 -antitrypsin.²³ Epithelial changes including loss of cilia are observed after a couple of months of cigarette exposure and Clara cell hyperplasia after about 6 months (SD Shapiro, unpublished observations). Increased alveolar duct area and enlarged alveolar spaces are clearly seen after 3–6 months of exposure to cigarette smoke in susceptible strains,²⁴ and macrophage mediated destruction appears to be prominent at these time points.

NATURALLY OCCURRING GENETIC MUTANT MOUSE STRAINS WITH AIRSPACE ENLARGEMENT

Several inbred strains of mice develop emphysema spontaneously due to genetic abnormalities (table 1). A number of mutations, many of which are not obviously related to lung structure, result in multisystem effects including abnormal airspace size. The effect of fibrillin on elastogenesis and lung development is obvious in tight skin mice, yet patients with Marfan's syndrome do not appear to have emphysema as a major problem. While the gene defects in blotchy, pallid, and beige mice have been determined, the pathophysiological basis for abnormal lung development and increasing airspace enlargement with age is not clearly understood. Emphysema in some of these strains may in part be attributed to reduced α_1 -antitrypsin levels.

GENETIC ENGINEERING IN MICE

Transgenic and gene targeted mice provide powerful techniques to determine protein function in vivo. Gain of function models may be achieved by overexpression of proteins in transgenic mice and loss of function models achieved by targeted mutagenesis.²⁵ Techniques to achieve germline transmission of genetic material in mice have drastically changed our ability to approach biological questions by allowing investigators to change single variables and perform controlled experiments in mammals. These models may help to determine both physiological functions of proteins as well as dissect mechanisms of disease.

Transgenic mice are derived by injecting DNA constructs—often with a tissue specific promoter driving the gene of interest—into the pronucleus of individual egg cells soon after

Table 1 Naturally occurring mice strains that develop airspace enlargement and emphysema

Mouse	Genetic/physiological defect	Phenotype	Lung phenotype	Reference
Tight skin (Tsk+/-)	<ul style="list-style-type: none"> Duplication of fibrillin 1 (Fbn-1), main component of 10–12 nm extracellular microfibrils (causes Marfan's syndrome in man) Possible dominant negative effect of Tsk Fbn-1 by incorporation into microfibrils, rendering them susceptible to proteolysis 	<ul style="list-style-type: none"> Tight skins, hyperplasia of subcutaneous connective tissues, ↑ growth of cartilage and bone, small tendons, hyperplasia of tendon sheaths 	<ul style="list-style-type: none"> Panlobular emphysema develops after 4 days of life Emphysema is preceded by a low level macrophage-neutrophil alveolitis 	58–63
Beige (Bg)	<ul style="list-style-type: none"> 5 kb deletion in <i>Lyst</i> (causes Chediak-Higashi syndrome in man) Lysosomal missorting of proteins such as elastase, glucuronidase and cathepsin G Intracellular vesicle transport defect and generalised immunodeficiency in mice and humans (abnormal function of NK cells, CTL) 	<ul style="list-style-type: none"> Dilution of coat colour, recurrent infections, giant granules 	<ul style="list-style-type: none"> Normal at birth, abnormal neonatal alveolarisation Adult lungs: fewer alveoli, smooth terminal air ducts lacking the normal complement of alveolar struts 	60, 63–65
Blotchy	<ul style="list-style-type: none"> X-linked defective copper transport Abnormal RNA splicing of the mouse homologue of copper ion transporting ATPase, <i>Atp7A</i> (Menke gene; Menkes disease in humans) Impaired lysyl oxidase, abnormal collagen and elastin cross linking 	<ul style="list-style-type: none"> Aortic aneurysms, reduced skin tensile, develop osteoarthritis 	<ul style="list-style-type: none"> Enlarged airspaces Disruption of elastic fibres 	66–68
Pallid (Pa)	<ul style="list-style-type: none"> 1 of 13 platelet storage pool deficiency mouse mutants Nonsense mutation at codon 69 in <i>Pallidin</i> which interacts with syntaxin 13: important in vesicle docking and fusion Defect in organelle biosynthesis 	<ul style="list-style-type: none"> Prolonged bleeding time, pigment dilution, kidney lysosomal enzyme elevation Serum α_1-antitrypsin deficiency, abnormal otolith formation 	<ul style="list-style-type: none"> Progressive emphysema from 1 month ↑ elastase, ↓ lung elastin ↑ intracytoplasmic crystalloid inclusions related to collagen degradation in pulmonary macrophages 	60, 69–71
Osteopetrotic (Op/Op)	<ul style="list-style-type: none"> Deficient in macrophage colony stimulating factor 	<ul style="list-style-type: none"> Osteopetrosis 	<ul style="list-style-type: none"> Young mice have reduced numbers of lung macrophages (which return to normal as they age) and emphysema Macrophages release more MMPs BAL IL-3 is increased 	72

CTL=cytotoxic T lymphocytes; NK=natural killer cells.

fertilisation. Inducible transgenic systems, often using tetracycline, have been generated whereby the gene of interest may be expressed at specific times. Null mutant or gene targeted mice (“knockout mice”) are generated by electroporation and homologous recombination of a targeted construct in embryonic stem (ES) cells. One can essentially place (“knock in”) any gene under the control of any other using ES technology.

GENE TARGETED MICE AND AIRSPACE ENLARGEMENT

Emphysema and airspace enlargement in gene targeted mice can occur as a result of abnormal lung development or can develop spontaneously with age and following challenge with cigarette smoke (table 2).

Gene targeted mice with abnormal alveogenesis

A dynamic coordinated interaction between transcription factors, growth factors, extracellular matrix components, and integrin signalling pathways directs cell migration and lineage determination during lung morphogenesis.²⁶ Lung embryogenesis begins in mice at embryonic day (E) 9.5 with the outpouching of two epithelial buds from the ventral foregut into the surrounding mesoderm. Subsequent airway development is divided into several well characterised stages culminating with alveogenesis that occurs largely by septation of the saccules from postnatal day 4 through to 14 (in the mouse).²⁶ Developmental airspace enlargement with gene targeting is elucidating pathways of alveogenesis (table 2).

Transcription factor null mutant mice have resulted in various developmental abnormalities described in detail elsewhere.^{26–27} Disruption of elastic fibres would be expected to impair the structural integrity of the lung. In support of this concept, a deficiency of elastin, platelet derived growth factor A (PDGF-A), and fibulin-5 (a microfibrillar component) leads

to airspace enlargement.^{28–29} PDGF-A null mice lack myofibroblasts which is a key source of tropoelastin and is required for alveolar septation.^{30–31}

Members of the large family of fibroblast growth factors (FGF) are essential for several stages of mammalian lung development. Lungs of gene targeted mice lacking receptors for both FGFR-3 and FGFR-4 (but not single knockouts) have markedly impaired alveogenesis with increased synthesis of collagen.³² Retinoic acid signalling is essential for normal embryonic development and deletions of retinoic acid receptor isoforms (nuclear transcription factors) have varied effects on morphogenesis.³³ Retinoic acid receptor- γ deficient mice display airspace enlargement that is potentiated by co-deletion of retinoid X receptor- α .³⁴ Null mice for the transcription factors POD-1 and Forkhead Box F1 (Foxf1) and the transmembrane metalloprotease-disintegrin tumour necrosis factor- α converting enzyme (TACE/ADAM-17) all cause more generalised abnormalities of lung development which include impaired alveogenesis.^{35–37} Developmental airspace enlargement should be distinguished from emphysema defined as destruction of mature alveoli. Nevertheless, understanding the process of normal lung development will be crucial for devising strategies to restore normal lung architecture and function in emphysema.

Spontaneous emphysema in gene targeted mice

The gradual appearance of emphysema with age has been described following targeted ablation of the surfactant protein D (SP-D) gene and in mice lacking tissue inhibitor of metalloproteinase-3 (TIMP-3) (but not in null mice for TIMP-1 or 2) In both cases matrix metalloproteinases were implicated as mediators of tissue destruction. The SP-D null mice had progressive pulmonary emphysema from 3 weeks of age associated with pulmonary infiltration by lipid laden alveolar macrophages. SP-D-/- macrophages have increased

Table 2 Examples of mouse models where “knockout” of a protein leads to the development of airspace enlargement due to a failure of alveogenesis or emphysema

Gene	Phenotype	Reference
<i>Gene deletions that interfere with alveogenesis</i>		
Platelet derived growth factor A (PDGF-A) $-/-$	<ul style="list-style-type: none"> • Prenatal block in the spreading of PDGF receptor-α+ cells resulting in a lack of myofibroblasts, an absence of tropoelastin expression, and failed alveolar septation • Postnatally mice are half the size of wild type litter mates and do not survive beyond 6 weeks 	30, 31
Fibroblast growth factor receptor (FGFR) 3 and 4 $-/-$	<ul style="list-style-type: none"> • Mice lacking both FGFR 3 and 4 (but not FGFR 4 alone) are normal at birth but do not form secondary septae or alveoli • Increased elastin deposition subsequent to alveogenesis, growth retardation 	32
Fibulin-5/DANCE $-/-$	<ul style="list-style-type: none"> • Integrin ligand for αvβ3, αvβ5 and α9β1, probably acts as an organising anchor between cells and elastic fibres • Abnormal distal airway development and alveogenesis due to defective development of elastic fibres. 	29
Elastin $-/-$	<ul style="list-style-type: none"> • Tortuous aorta and cutis laxa • Fewer and dilated distal air sacs at birth and arrested terminal airway development (the mice die from obstructive arterial disease due to subendothelial cell proliferation and a reorganisation of smooth muscle) 	28
Retinoic acid receptor (RAR) γ $-/-$	<ul style="list-style-type: none"> • Increased alveolar size is worsened by co-deletion of retinoid X receptor-α 	34
Forkhead Box F1 (Foxf1) transcription factor $+/-$	<ul style="list-style-type: none"> • Decreased tropoelastin mRNA and whole lung elastic tissue • Severity of the pulmonary abnormalities correlates with the levels of Foxf1 mRNA, those with lowest levels have defects in alveolarisation and vasculogenesis • Lung haemorrhage due to disruption of the mesenchymal-epithelial cell interfaces in the alveolar and bronchiolar regions 	35
Tumour necrosis factor- α converting enzyme (TACE/ADAM-17) $-/-$	<ul style="list-style-type: none"> • Transmembrane metalloprotease-disintegrin cleaves cell surface proteins including cytokines and growth factors • Impaired branching morphogenesis, defects in epithelial cell proliferation and differentiation, and delayed vasculogenesis • Lungs fail to form normal saccular structures, fewer peripheral epithelial sacs, deficient septation and thick walled mesenchyme 	37
POD-1 (Tcf21, capsulin, epicardin) $-/-$	<ul style="list-style-type: none"> • Basic-helix-loop-helix transcription factor expressed at sites of mesenchymal-epithelial interaction in the lung, kidney, intestine and pancreas • Hypoplastic lungs, abnormal lung branching, lacking alveoli and type II pneumocytes. Mice die in perinatal period 	36
<i>Gene deletions that cause airspace enlargement after birth</i>		
Tissue inhibitor of metalloproteinases (TIMP)-3 $-/-$	<ul style="list-style-type: none"> • Progressive air space enlargement evident at 2 weeks • Aged animals have reduced collagen, enhanced degradation of collagen in the peribronchiolar space, and disorganisation of collagen fibrils in the alveolar interstitium • Mice are moribund at 13 months • Increased MMP activity without an increase in inflammatory cell infiltration 	39
Surfactant protein D $-/-$	<ul style="list-style-type: none"> • Progressive pulmonary emphysema from 3 weeks of age • Lipid-laden alveolar macrophages, increased oxidant production and reactive oxidant activate NF-kappa B and MMP expression in alveolar macrophages • SP-D plays an inhibitory role in the regulation of NF-kappa B in alveolar macrophages 	38
<i>Gene deletions protecting against emphysema after challenge</i>		
Macrophage elastase (MMP-12) $-/-$	<ul style="list-style-type: none"> • Normal lung development • Protection from cigarette induced emphysema 	5
Interleukin 1 β type 1 receptor and type 1 and 2 TNF- α receptors	<ul style="list-style-type: none"> • Protected from porcine pancreatic elastase-induced emphysema • Individual gene deleted mice are not protected 	42

$-/-$ = null mice.

For a detailed review of developmental phenotypes see references 26 and 27.

reactive oxidant species activating NF-kappa-B with consequent MMP expression.³⁸ TIMP-3 $-/-$ mice develop progressive airspace enlargement first detected at 2 weeks of age. In addition to likely developmental abnormalities, these mice display inflammation and increased MMP activity with further airspace enlargement over time.³⁹ It is interesting to speculate that in humans subtly abnormal alveogenesis followed by years of mechanical stress may be an important mechanism of emphysema.

Emphysema in gene targeted mice following exogenous challenge

The effect of exposing developmentally normal mice lacking a specific protein to cigarette smoke allows one to probe disease pathogenesis. Macrophage elastase (MMP-12) is expressed in macrophages of human smokers and in patients with emphysema. MMP-12 null mutant mice develop normally. In contrast to wild type mice, the lung structure of MMP-12 $-/-$ mice remains normal following long term exposure to

Table 3 Examples of mouse models where overexpression of a protein leads to emphysema

Mediator	Promotor	Lung pathology	Mechanism	Reference
Interleukin 13	CC10-rTA	Emphysema with enhanced lung volumes and compliance, mucus metaplasia, and inflammation	<ul style="list-style-type: none"> Increased MMP-2, -9, -12, -13, and -14 and cathepsins B, S, L, H, and K Treatment with MMP or cysteine proteinase antagonists significantly decreased the emphysema and inflammation but not the mucus 	49
Interferon- γ	CC10-rTA	Emphysema with alveolar enlargement, enhanced lung volumes, enhanced pulmonary compliance	<ul style="list-style-type: none"> Increased macrophage and neutrophil in BAL fluid Decreased secretory leucoproteinase inhibitor Increased MMP-12, MMP-9, cathepsins B, H, D, S, and L ? degradation of collagen by collagenase 	48
Human interstitial collagenase (MMP-1)	Haptoglobulin	Disruption of alveolar walls and coalescence of alveolar spaces	<ul style="list-style-type: none"> ? degradation of collagen by collagenase 	50

rTa=reverse tetracycline transactivator; CC-10=Clara cell 10 kD promotor; SP-C=surfactant apoprotein C.

cigarette smoke. MMP-12 $^{-/-}$ mice also failed to develop macrophage accumulation in response to cigarette smoke.³ This effect may be related to MMP-12 generation of elastin fragments that are chemotactic for monocytes.⁴⁰⁻⁴¹ Neutrophil elastase deficient mice are partially protected against smoking induced emphysema, while gelatinase B (MMP-9) and uPA (an MMP activator) null mutants had no demonstrable effect (unpublished observations).²⁴ MMPs can also degrade α_1 -antitrypsin, and neutrophil elastase can degrade TIMPs. These findings reinforce the concept that many cells and proteinases are likely to be important in the development of emphysema. Subsequent studies have supported MMP involvement in emphysema (tables 2 and 3).

It has recently been shown that mice deficient in both IL-1 β type 1 receptor and types 1 and 2 TNF- α receptors are protected from the development of emphysema following intratracheal challenge with PPE. They exhibited reduced inflammation and increased apoptosis. However, there was no such protection in the individual null mutant strains.⁴²

TRANSGENIC MICE WITH EMPHYSEMA

Data from transgenic mice must be interpreted cautiously. Firstly, because of the random nature of transgene integration, several founder lines should be shown to have similar phenotypes. Secondly, aberrant expression of a gene may have the capacity to cause a phenotype yet not be involved in actual disease. This is particularly important in the interpretation of airspace enlargement, since introduction of a protein not naturally associated with lung development could alter this but have no biological relevance. Use of inducible transgenic mice diminishes this concern.

Lung specific overexpression of platelet derived growth factor B (PDGF-B), transforming growth factor α , TNF- α , interleukin (IL)-11 and IL-6 all interfere with normal lung development.⁴³⁻⁴⁷ The impact of altering the timing of protein production has been elegantly illustrated with IL-11 where inducible overexpression showed that delaying IL-11 expression until adulthood abrogated the effects on lung structure.⁴⁷

In contrast, inducible overexpression in adult mice of either the Th2 cytokine IL-13 or Th1 cytokine interferon γ (IFN- γ) produced airspace enlargement consistent with destruction of mature lung characteristic of pulmonary emphysema. With both mice transgene expression caused inflammation, activation of metallo- and cysteine proteinases. Emphysema associated with IL-13 was partially prevented by treatment with either MMP or cysteine proteinase inhibitors.⁴⁸⁻⁴⁹ The important relationship between collagen turnover and emphysema was supported by transgenic mice that constitutively overexpressed human interstitial collagenase (MMP-1) resulting in airspace enlargement.⁵⁰ Use of inducible systems would strengthen interpretation of this finding being the result of purely collagen degradation in mature lung tissue.

The finding of airspace enlargement in "Klotho" mice highlights the importance of transgene integration effects. The

Klotho mouse was developed to be a model of hypertension by overexpressing a sodium channel. The mice were normotensive but displayed abnormal premature aging in several organs and also had airspace enlargement. Further analysis revealed insertion of several copies of the transgene in the 5' region of a membrane protein with sequence homology to β -glucosidase enzymes, with disruption of function of this gene termed "Klotho". It is not settled whether the mice have abnormal development and/or premature aging, but clearly this gene affects lung structure.⁵¹

USE OF EMPHYSEMA MODELS TO TEST THERAPEUTIC AGENTS

Intratracheal instillation of elastase is a relatively simple and quick method for generating emphysema, and so lends itself well to assessing the effect of any intervention on emphysema. Early studies showed that chloromethylketone elastase inhibitors, eglin-C from the medicinal leech *Hirudo medicinalis*, and heparin fragments (inhibits neutrophil elastase) could prevent elastase induced emphysema.⁷⁻⁵² Cantor *et al*⁵³ have shown that a single instillation of hyaluronidase into hamster lungs given with PPE results in a decrease in airspace size compared with elastase treated controls. Since hyaluronic acid is not an elastase inhibitor, an effect on "shielding" elastin from elastases has been proposed and supported experimentally.⁵⁴

More recently, the elastase model has been used to demonstrate the capacity of retinoic acid to augment septation and alveolar regeneration. It has been noted that, when septa are being formed to subdivide the primitive lung sacculles, fibroblasts in the alveolar wall contain storage granules of vitamin A. Furthermore, retinoid binding proteins and nuclear retinoic acid receptor isoforms are upregulated in alveolar septal regions.⁵⁵ Administration of all-*trans* retinoic acid (tRA) to rats abrogated PPE induced emphysema by promoting the formation of new alveoli.⁶ tRA also partially rescued the failure of septation seen in "tight skin" mice.⁵⁶ Subsequent to this study, tRA treatment using a different protocol failed to reverse cigarette smoke induced emphysema in the guinea pig.²¹ tRA binds to two forms of nuclear receptors which have multiple isoforms (retinoic acid receptors (RARs) α , β and γ , and retinoid X receptors (RXRs)). RARs seem to have disparate effects. RAR β prevents septation while RAR α enhances it.³⁴⁻⁵⁷ Thus, RAR γ specific agonists might have a greater effect than the general activator RA.

FUTURE OBJECTIVES

Use of genetically manipulated mice in concert with traditional animal studies are steadily increasing our knowledge of the factors that are important in determining alveolar formation and destruction. Further progress will require addressing fundamental questions such as: how does smoking cause pulmonary inflammation, why do only a minority of

cigarette smokers get emphysema, what are the factors that control normal lung development, and how can we restore normal alveolar tissue? We now have the exciting opportunity to combine proteomics and gene microarray technology with animal models. Therefore, rather than focusing solely on individual mediators, we can begin to dissect both the cellular interactions that lead to emphysema and the factors that govern normal alveolar development. It is hoped that this will identify novel strategies to achieve the ultimate goal of a cure for this devastating disease.

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