Ischaemia Reperfusion Injury and its Sequelae in the Peripheral Airways of the Transplanted Lung: An Experimental Study

submitted by
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Abstract.
Obliterative bronchiolitis (OB) of the peripheral airways remains the most important long-term complication following lung transplantation. In the present study, the rat unilateral lung transplant model has been used. The aims were to describe the effects of preservation at 4°C, early reperfusion and transplantation on the peripheral airway structure of the lung and to investigate whether any changes persisted and might therefore be relevant to the pathogenesis of OB. Quantitative light and electron-microscopic and cell culture techniques were used.

Preservation of the rat lung resulted in significant damage to the peripheral airways which increased after reperfusion. All intracellular damage had resolved by 48 hours after transplantation, although basal cell hyperplasia was present and persisted for up to 6 months after transplantation. This response was observed following both isogeneic and allogeneic transplants and was influenced by the strain and the maturity of the transplanted lung and recipient.

It was also shown that peripheral airway size became greater than in age-matched controls following transplantation but that airway structure and composition were otherwise normal. This response occurred in both isogeneic and allogeneic transplants, but was greatest following allogeneic transplantation and was influenced by the maturity of the transplanted lung and recipient.

The persistent basal cell hyperplasia and the increase in airway size were also observed in the recipient native non-transplanted lung but to a lesser degree, suggesting an interaction between the two lungs, possibly via humoral factors.

Cultured porcine epithelial cells and fibroblasts which had been cooled to 4°C and rewarmed exhibited a greater proliferative response to fibroblast-conditioned medium than those kept at 37°C.

In conclusion, single-lung transplantation led to long-term structural changes in the peripheral airways of both the transplanted and native lung. Early damage leading to basal cell hyperplasia may be important in the development of OB.
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Chapter 1: Introduction.
I. Pulmonary Transplantation.

A) History of pulmonary transplantation.

Pulmonary transplantation, either heart-lung, single lung or double lung has become a therapeutic option for patients with end-stage pulmonary and cardiopulmonary diseases. The first recorded interest in lung transplantation dates back to 1907 when Charles Guthrie performed a heterotopic heart-lung transplant between a kitten and an adult cat. Demikhov, a Russian physiologist performed canine heart-lung transplants in 1947. However, it was 15 years before his work was translated for the Western world. Metras in France described the technique of canine pulmonary allotransplantation in 1950.

The first recorded attempt at lung transplantation in humans was performed by Hardy and workers at the University of Missisipi in 1963. The patient survived 18 days and died of renal failure. The first reported heart-lung transplant was by Cooley and workers in 1968. Despite many attempts at isolated lung transplantation no long-term clinical success was achieved for some time. Patients survived the post-operative period only to succumb to airway complications which ultimately lead to their death. Immunosuppressive regimens at this time used high dose corticosteroids. Animal experiments at the University of Toronto demonstrated that methylprednisone hindered skin as well as bronchial anastomosis healing. A new immunosuppressive agent Cyclosporine (first known as Cyclosporine A) was discovered by Borel and workers, in 1976. This powerful immunosuppressive agent inhibits T-lymphocyte helper cells but does not impair wound-healing. This meant that high dose steroids could be avoided in the early post-operative period and thus wound healing was improved.

Using this drug in combination with the newly developed "en bloc" heart-lung transplant technique, Reitz and co-workers at Stanford were successful in the first
long-term survival of rhesus monkeys following heart-lung transplantation. Using the "en bloc" method resulted in good healing of the tracheal anastomosis. This was followed in 1981 by the first successful heart-lung transplant in a patient with end-stage primary pulmonary hypertension.

Initially, most heart-lung recipients had advanced pulmonary vascular disease caused by the Eisenmenger syndrome or primary pulmonary hypertension, but an increasing number of patients with end-stage lung disease have now been successfully treated by heart-lung transplantation.

B) Types of pulmonary transplantation.

It was not until 1983 that the first successful unilateral lung transplantation was carried out, in Toronto. Widespread use of heart-lung transplantation is limited by the availability of suitable donors. Single-lung transplantation has several advantages. The donor heart and lungs can be used for different recipients, the risk of intra-operative and post-operative bleeding is lower since the surgical procedure is easier and it is possible to avoid using cardiopulmonary bypass. The disadvantages of single-lung transplantation include complications related to the remaining diseased lung. The most important difference between single or double lung and heart-lung transplantation is that the coronary to bronchial collaterals are not disrupted by heart-lung transplantation and therefore the healing of the tracheal anastomoses is more reliable. The problem of ischaemic disruption of the bronchial anastomosis in unilateral lung transplantation was eased by the withdrawal of corticosteroids in the early post-operative period and the omental pedicle being wrapped around the bronchial anastomosis to aid healing and bronchial revascularisation. More recent work by the Stanford group has disproved the need for the omental pedicle wrap, instead telescoping of the bronchi and continuous sutures have been advocated. Single-lung transplantation was first performed in patients with fibrotic lung diseases such as cryptogenic fibrosing alveolitis and sarcoidosis. However,
single-lung as well as double-lung transplantation has now been used successfully for transplant patients with pulmonary vascular disease.\textsuperscript{57}

The first successful "en-bloc" double-lung transplant in humans was carried out by the Toronto Lung Transplant Group in 1988 in a woman with end-stage emphysema secondary to alpha-1-antitrypsin deficiency.\textsuperscript{139} Despite initial encouraging results the "en-bloc" double-lung transplant procedure was plagued with problems related to tracheal healing.\textsuperscript{175} To address the issue of ischaemic airway complications Noirclerc and co-workers in Marseilles introduced the technique of bilateral bronchial anastomoses.\textsuperscript{133} The next step was the so-called "bi-lateral lung transplant" described by Pasque and co-workers from Washington University.\textsuperscript{138} The advantage of this latter technique which involves sequential transplantation of the lungs, is that cardiopulmonary bypass is not necessary and bleeding complications are substantially ameliorated.

As the procedure of lung transplantation has become an accepted form of treatment for patients with end-stage pulmonary parenchymal and vascular disease, lack of donor organs has become the major limitation. This shortage is even greater in the paediatric population.\textsuperscript{179} Therefore grafts of reduced size, lobes rather than whole lungs, have been used in selected patients.\textsuperscript{54} In initial animal experiments the smaller lung graft has provided adequate pulmonary function.\textsuperscript{36,66,74} In clinical work, lobes have been taken from cadavers and more controversially, from a living-related donor.\textsuperscript{31,179} Bilateral lobar transplantation for patients with cystic fibrosis has involved lobes donated from 2 relatives, the right lower lobe from one and the left lower lobe from another.\textsuperscript{31} The immunological advantages of related-donor organs has not yet been fully evaluated and so the recipients still receive triple-immunosuppressive therapy. At present, lobar transplantation seems to be best suited for children and small adults. The main concern of this type of pulmonary transplantation is uncertainty about how the mature lobe is going to cope as the young recipient grows. This question remains unanswered in humans, although at Stanford a 12 year old girl with bronchopulmonary...
dysplasia who received an adult upper lobe was still alive three years later, with no physical handicap and increased lung function along with somatic growth.\textsuperscript{179} Experimental work in rats has shown that the immature lung continues growing after transplantation, the alveoli and airways increasing in size after single-lung transplantation.\textsuperscript{74} However, other studies have demonstrated abnormal vascular and airway function in transplanted immature porcine lungs, possibly due to chronic denervation or abnormal growth.\textsuperscript{94,119} When the airway function of transplanted porcine immature whole lungs was compared with that of transplanted mature lobes, the mature lobes showed superior long-term airway function.\textsuperscript{104} It appears that mature lobar transplants in immature animal recipients are capable of supporting life,\textsuperscript{8,36,66} although functional compensatory growth of these transplanted mature lobes may not be adequate\textsuperscript{103,104} and this may limit clinical effectiveness. At worst, mature lobar transplants could be used as a bridge until the child is old enough to accept a larger adult transplant.

C) Donor lung.

Lung preservation.

Good preservation of the donor lung is essential for early graft function and thus survival. Adequate and prolonged preservation prior to transplantation is one of the major problems of lung transplantation. Optimising the technique is complicated, not only due to the delicate alveolar-capillary network of the lung but because our knowledge of the pathogenic mechanisms involved in pulmonary ischaemia-reperfusion injury is incomplete. This has resulted in optimal preservation of the lung lagging behind that of other organs.\textsuperscript{134} The important factors to be considered include the method of preserving the lung, the type of preservation solution, the time and temperature of preservation, whether to store the lung inflated or deflated and donor pre-treatment. All these issues have been addressed in animal models. Clinical methods used for lung preservation have included "immediate transplantation" (donor transported to recipient hospital), hypothermic atelectasis, flushing the pulmonary
artery with cold preservation fluid followed by hypothermic storage with and without varying degrees of inflation, donor-core cooling on cardiopulmonary bypass, and autoperfused heart-lung preparations at normothermia. Currently the most widely used methods involve some form of hypothermic pulmonary flush. Donor-core cooling is used at some transplantation centres and has been used in over 50% of the world experience in heart-lung transplantation.

Despite the Toronto lung transplantation group making a point in their early experience of keeping the allograft deflated during procurement, in recent years lung inflation during hypothermic storage has become an issue in experimental lung preservation models. Locke and workers showed that preservation with lung inflation is superior to preservation after absorption atelectasis of the lung. The issue as to whether 100% oxygen, room air or 100% nitrogen should be used for this technique is still controversial and reports are conflicting.

**Preservation fluids.**

A wide variety of perfusates have been used in experimental lung preservation. Marshalls solution was described in 1976 by Ross and workers and was first used for flush perfusing kidneys for subsequent transplantation. Using Marshalls, which is a crystalloid fluid, successful kidney preservation was achieved for 72 hours. Marshalls solution has never been used clinically for lung preservation. It has however been used experimentally.

Modified EuroCollins kidney solution remains the most frequently used solution in clinical lung transplantation. In 1986 Starkey and workers reported a successful 6 hour preservation of primate heart-lung allografts using cold modified Euro-Collins solution to flush the pulmonary arteries followed by static hypothermic storage. At this time it was realised that using a larger volume of perfusate flushed through the pulmonary arteries over several minutes at a low pressure resulted in more uniform
cooling of the lungs than the lower flush volumes previously used. These advances in pulmonary preservation provided sufficient protection to enable routine distant donor procurement. Baldwin and workers reported the successful clinical application of this technique. Since that time, modified Euro-Collins solution has become the standard pulmonary flush solution for heart-lung and lung transplantation in most centres. At the same time that Starkey was developing a preservation procedure using Euro-Collins solution, the Papworth group were using an extra-cellular blood-based solution containing prostacyclin for distant heart-lung procurement. This solution gave satisfactory lung preservation for up to 4 hours.

Intra-cellular crystalloid solutions have proved to be successful for lung preservation and so in this study lungs were flush perfused and then stored in Marshalls solution at 4°C in the inflated state. The inability to preserve the lung safely for more than 4 to 6 hours still represents a major impediment to pulmonary transplantation. Better alternatives to Euro-Collins solution have been sought. Euro-Collins solution may cause pulmonary vasoconstriction due to its high potassium. Alternatives include an extra-cellular, low potassium dextran flush solution which improved lung function after 12 hours of preservation in the canine single-lung transplant, and in the rabbit isolated lung model stored for 18 hours.

Further studies have not confirmed that the low potassium dextrin solution is superior to modified Euro-Collins solution used in conjunction with prostaglandin (vasodilator) treatment. University of Wisconsin solution is a colloidal solution which has significantly prolonged the preservation of liver, kidney and pancreatic grafts. UW solution is now used as a preservation solution in clinical practice. An improved UW solution has been developed with a low potassium content which results in improved gas exchange and less oedema during reperfusion in the isolated rabbit lung than either the standard UW or Euro-Collins (high potassium) solution.
There is evidence that oxygenated fluorocarbon solution can be used as preservation fluid for lungs. These solutions have been reported to enhance oxygenation, and to improve perfusion and function of ischaemic myocardial tissue. A study by Lehtola and co-workers demonstrated better functional and morphologic preservation in the porcine lung with an oxygenated fluorocarbon solution (FC-43) compared with modified Euro-Collins solution.

Storage temperature.

Storage temperature is an important factor in lung preservation. Hypothermia prolongs lung preservation and 4°C has been thought to be an acceptable temperature and was used in the present preservation study. Some studies have suggested that 10°C appears to be the optimal temperature for the long term preservation of the lung. The traditional technique of lung storage after procurement has been immersion in cold saline or iced slush. The problems with this method are uneven cooling of the lung due to its buoyancy and portions of the graft reaching a temperature below freezing point in iced slush, which results in cryogenic lung injury. Studies into topical lung cooling by cold compressed air seemed to suggest better oxygen transfer and less extra-vascular lung water than in grafts immersed in an iced slush bath. More research into this type of storage is needed before it can be applied clinically.

Structural studies of preservation and reperfusion injury.

Detailed structural and ultra-structural studies on the effect of preservation and reperfusion on the lung are sparse and tend to concentrate on the blood vessels and the blood-gas barrier. Rat studies have shown that during preservation of the donor lung, capillary morphology changes rapidly. Both endothelial cells and type 1 pneumocytes become thinner. Blebbing of endothelial cells can occur. Peri-capillary oedema develops which involves the blood-gas barrier and basement membrane thickness increases. This damage resolves on reperfusion. A study by Lehtola and
workers compared porcine lung tissue preservation with modified Euro-Collins and fluorocarbon solutions. The study, using light microscopy, showed that preservation induced dilation of the perivascular lymphatics and pleural oedema, while reperfusion was followed by intra-alveolar bleeding, vascular congestion and a pleural inflammatory reaction. These alterations were more pronounced in the Euro-Collins group than in the fluorocarbon group. By electron-microscopy, the general appearance was of well preserved lung tissue in both groups, although moderate oedema and mitochondrial damage occurred in both groups. After reperfusion, the appearance of the alveolar epithelial cells was significantly better following fluorocarbon rather than Euro-collins preservation. The alveolar epithelial cells were slightly swollen and the alveolar septae were also thickened. In poorly preserved areas of lungs in both groups, alveoli totally denuded of epithelial cells were seen. After reperfusion, the alveolar walls became more swollen and there was reduction and deformation of alveolar spaces. Intra-alveolar fibrin nets, blood corpuscles, exfoliated epithelial cells, desquamated or totally occluded alveoli and thrombosed vessels could be seen in poorly preserved areas. In conclusion, the general appearance was that of well preserved lung structures in both groups but preservation was thought to be better in lungs preserved in fluorocarbon.

A study by Hidalgo and co-workers examined the morphological changes in rat single-lung isografts after 24 and 48 hours preservation in modified University of Wisconsin solution which either a) mimicked the intra-cellular medium, high potassium and low sodium or b) mimicked the extra-cellular medium, low potassium and high sodium. Female inbred rats were used and lungs were examined by light and electron-microscopy. Four weeks after transplantation, both lungs stored in a) and b) for 24 hours had the general appearance of well preserved pulmonary tissue although some lungs presented areas of scarring, limited principally to the apical part of the lobes. Smaller perivascular fibrotic areas were found but neither oedema nor haemorrhage were observed. Macrophages laden with haemosiderin were found in scarred areas.
The structure of the lungs which had been stored for 48 hours in either solution were considerably different from normal 4 weeks later. The lungs presented fibrotic organisation with limited chronic inflammation of lymphocytes and plasma cells. Small foci of scarring with focal mild inflammation in the pleura were observed. Macrophages full of haemosiderin were occasionally seen in unscarred parenchymal tissue. They concluded that extended preservation of the lungs was possible but that the concentration of potassium and sodium in the preservation solution did not have a significant impact in the long term.

Structural studies of lung preservation injury are obviously more scarce in human transplantation. However, Fehrenbach and workers studied the preserved contra-lateral donor lung in clinical single-lung transplantation. This study used Euro-Collins as a preservation solution, and good to excellent preservation of the lung tissue was observed using light microscopy. Transmission electron microscopy however, revealed some changes in the blood-gas barrier. In places, the capillary endothelium showed small apical vacuoles, tentacle-like protrusions and double-membrane-bound blisters. Most of the cells of the alveolar epithelium showed a normal appearance, but swelling of both type I and II pneumocytes was observed. The surfactant storing multi-lamellar bodies were normal in appearance.

D) Reimplantation response.

The reimplantation response has been defined as "the morphologic, roentgenographic and functional changes that occur in a lung transplant in the early post-operative period as a result of surgical trauma, ischaemia, denervation, lymphatic interruption and other injurious processes (exclusive of rejection)." Functionally, the pulmonary reimplantation response impairs ventilation-perfusion relationships in the transplanted lung. Siegelmann and workers showed that the pulmonary reimplantation response consists of alveolar oedema which is evident on histologic examination and alveolar infiltrates which could be seen on chest roentgenograms. In mongrel dogs
these changes remained for three days after autotransplantation and then regressed over one to three weeks. Radiographically, these changes were manifested by perihilar air space disease with air bronchograms. These changes were maximal on the third post-operative day and cleared between days 7 and 21 in dogs, but persisted for as long as 4-6 weeks in baboons. Bishop and colleagues have also shown that canine unilateral lung ischaemia using a balloon occluder followed by reperfusion produces bilateral lung injury which is greatest in the ischaemic lung. Histologically, marked oedema and inflammatory infiltrates were observed in the reperfused lung using light microscopy and to a lesser extent in the native lung. Electron microscopy demonstrated lysis of both capillary endothelial and alveolar epithelial cells bilaterally with the frequency of cell injury greater on the reperfused side. Since that work, a study by Bryan and workers showed that both right and left lungs were affected by the pulmonary reimplantation response following canine left lung allografts. Prop has studied the reimplantation response in isogeneically transplanted rat lungs. On the day of transplantation, light microscopy showed interstitial oedema throughout the lung, mainly along the vessels and to a lesser degree along the bronchi. Foci of alveolar oedema and haemorrhages were scattered throughout the lung. One day post transplantation, the oedema around vessels and bronchi was still present but the alveolar oedema now involved larger areas. Erythrocytes were present in many alveolar spaces but not to an extent that indicated gross bleeding. On the fourth day after transplantation these abnormalities were disappearing. From day seven the transplanted lung looked almost normal. The native right lungs were normal except for some perivascular oedema and alveolar haemorrhages during the first days after transplantation.

Clinically, the reimplantation response is an early transient process which varies in its manifestation on x-rays from a subtle perihilar haze to dense consolidation with air bronchograms. These findings are non-specific and can be mistaken for those of fluid-overload, atelectasis, mucous plugging and pneumonia.
Herman and co-workers encountered the reimplantation response in 13 patients. Herman also observed that the process was milder in patients who underwent heart-lung transplantation than in those who underwent single-lung transplantation. O'Donovan also showed that the re-implantation response was more impressive radiographically in patients who received single-lung transplants for primary pulmonary hypertension than in those who underwent lung transplantation for other conditions. 

Greater understanding of the reimplantation response and more appropriate pre- and post- transplantation treatment will hopefully lead to superior early pulmonary function and morphology in the future. The cause of early reperfusion lung damage is unclear but is thought to be as a result of leukocyte and platelet activation, oxygen free radical formation, the complement cascade, generation of inflammatory mediators and arachidonic acid metabolites.

Platelet-activating factor (PAF) is synthesised and released by platelets, white blood cells, macrophages and endothelial cells. PAF binds to platelets, causing activation and aggregation. Cytotoxic oxygen-free radicals are produced after both warm and cold lung ischaemia. Oxygen free radicals may originate from several sources during ischaemia including activated neutrophils, dissociation of the intra-mitochondrial electron-transport chain and the xanthine oxidase reaction.

E) Acute lung rejection.

The studies of Gibson and Medawar in the 1940s showed that rejection of skin grafts had features of an immune response. Transplanted organs contain antigen foreign to the organ recipient: human lymphocyte antigen (HLA); minor histocompatibility complexes and tissue-specific antigens. Donor antigen is recognised directly and indirectly. Indirect recognition occurs by processed donor antigen being presented by the host antigen-presenting cells (APC) bound to self-major histocompatibility complex (MHC) molecules. Direct recognition occurs when the donor antigen is
recognised by the T-cell receptor (TCR) of the host's lymphocytes without the intercession of APC.

HLA class II donor antigens are recognised by T helper/inducer cells (CD4 bearing lymphocytes). HLA class I donor antigens are recognised by T suppressor/cytotoxic cells (CD8 bearing lymphocytes). Dendritic cells within the transplanted organ constitutively bear HLA I and II class molecules and may function in antigen presentation. Endothelial and epithelial cells within the transplanted organ can be induced to express class II molecules under stress or cytokine exposure and may therefore participate in antigen presentation.

Acute rejection of the lung is manifest on biopsy by a lymphocytic infiltrate which is mainly perivascular but can involve other areas of the lung. The details of the histopathological stages have been well described in the rat and human tissue obtained at open lung biopsy or on trans-bronchial biopsy has shown similar features. Using isolated rat lung allografts, the same experimental model as that used in the present study, Prop described four phases of unmodified acute rejection. In the latent phase, there is no evidence of acute rejection (immune response) although a reimplantation response may well be seen. This is followed by the vascular phase, characterised by the presence of first, perivenous and then peribronchial and periarterial infiltrates of lymphocytes. Bronchus-associated lymphoid tissue (BALT) also shows an increase in immunologic activity. The alveolar phase is defined by the appearance of lymphocytes and increased numbers of macrophages in the alveolar walls and spaces. Originally Prop described the alveolar phase ending with the appearance of intra-alveolar oedema. This description defined the onset of the destructive phase. However, as intra-alveolar oedema has been seen prior to parenchymal necrosis in Prop's combined heart-lung transplantation rat study and clinically, the alveolar phase has now been divided into early (cellular) and late
(oedematous) phase. The term **destructive phase**, is now reserved for actual alveolar wall necrosis and, if it occurs, intra-alveolar haemorrhage.

Acute lung rejection may be an important factor in the subsequent development of chronic rejection and obliterative bronchiolitis. The incidence and severity of acute rejection episodes may make the transplanted lung more vulnerable to chronic disease later on, possibly by priming lymphocytes. Much of our knowledge of the pathology of lung transplantation comes from studying biopsy material. In 1990, the lung rejection study group of the Registry of the International Study of Heart and Lung Transplantation proposed a classification of lung rejection. This means that data can be compared from different centres, knowledge shared and improvements made.

**F) Chronic rejection of the lung.**

Chronic rejection of the lungs with its principle features of obliterative bronchiolitis (OB) and vascular occlusive disease occurs in 20-50% of adult lung transplant patients who have survived the first year. OB following adult pulmonary transplantation was first reported by Burke and workers in 1984 and remains the single most important complication in long term survivors of heart-lung and lung transplantation. OB is also the most significant problem after heart-lung transplantation in children.

Symptoms of OB are those of obstructive lung disease and the radiographs reveal predominantly basal honey-combed lesions. The flow-volume curve becomes curvilinear, with the forced expiratory volume (FEV₁) value falling by a greater degree than the vital capacity (VC). Diffuse bronchomalacia also occurs frequently after pulmonary transplantation and worsens functional airflow obstruction, aiding decline. Chronic lung rejection is characterised histologically by OB with variable pulmonary vascular disease. It contrasts with chronic rejection of other transplanted organs in which occlusive vascular disease leads to fibrosis and organ failure.
Pathology of obliterative bronchiolitis.

OB in humans develops through a sequence of epithelial injury that is initiated by an active cellular phase of lymphocytic bronchiolitis with ulceration and denudation of the mucosa. This may result in luminal ingrowth from the submucosa with organisation of intralumenal plaques and the formation of a polyploid massive granulation tissue (a Masson body). A submucosal infiltration of lymphocytes and histiocytes is often observed. Eccentric submucosal scars may form with luminal re-epithelialisation and luminal narrowing, or the entire airway wall may become completely replaced by dense scar tissue when the airway is identified only by its location next to a pulmonary artery.

Many variations of bronchiolitis with subsequent scarring and obliteration are seen during the course of the disease. The net result is bronchiectatic widening of the peripheral bronchioli and mucous plugging. Diffuse interstitial fibrosis around the damaged bronchioli can also be seen. Both the arteries and the veins of the pulmonary vasculature may show an accelerated form of arteriosclerosis consisting of concentric intimal proliferation, often with an intact internal elastica.

Pathogenesis of obliterative bronchiolitis.

Suggested aetiologies for OB include rejection, infection, bronchial artery ligation and denervation. Presently, it is thought that the incidence and severity of episodes of acute rejection and infection, especially CMV infections are the main determinants of the development of OB.

Rejection: Scott and co-workers noted that chronic lung rejection is a likely outcome in patients with early poorly controlled severe rejection. Yousem and workers in 1991, reported that the intensity and persistence of early acute rejection episodes associated with injury to bronchioles correlated with the presence of histological changes thought to be characteristic of OB. They postulated that acute rejection may
prime lymphocytes for subsequent respiratory epithelial injury and airway fibrosis late in the post-operative period.\textsuperscript{215}

Many immunological studies have been carried out in an attempt to discover whether OB is a manifestation of chronic rejection. Both Uyama\textsuperscript{194} and Sakiyama\textsuperscript{165} have shown that late airway changes in long-term lung allografts are immunologically mediated. Romanik and workers in 1990 showed that expression of Class II antigens on the bronchial epithelium in rats is induced during early rejection and that this could be suppressed by Cyclosporine treatment.\textsuperscript{161} Class II positive epithelium might serve as a rejection target\textsuperscript{161} and indeed, heart-lung transplantation patients show enhanced expression of certain class II MHC antigens on airway epithelial cells.\textsuperscript{53} However this phenomenon may not be specific to transplanted organs.\textsuperscript{5} Both Yousem and co-workers\textsuperscript{212} and Uyama\textsuperscript{193,195} reported a significant increase in dendritic cells around the airways in lung allografts. Dendritic cells are antigen-presenting cells and donor dendritic cells are thought to play a role in acute rejection. Recipient dendritic cells can also present graft antigens and it has been suggested that this might stimulate T helper cells to release cytokines and thus cause local tissue damage by inducing a vicious circle of increased MHC expression and attraction of dendritic cells, leading to recruitment and activation of lymphocytes, which leads to relentless tissue damage and OB.\textsuperscript{195}

The lymphocyte subsets associated with OB have been studied. Holland and workers\textsuperscript{77} used immunohistochemistry to illustrate an increased presence of CD8+ cells as compared with CD4+ cells in the lung tissue and BAL. The CD8+ cells were predominantly located in the peri-bronchial, peri-vascular and interstitial areas of the tissue. A detailed study of the phenotypic profile of blood lymphocytes during OB has also been described.\textsuperscript{48} However, it must be noted that lymphocyte profiles may be different in the actual graft compared with those in the blood.
**Infection:** An experimental study in rats by Winter\(^{204}\) showed that a respiratory viral infection aggravates the airway damage in rat lung allografts with chronic rejection, suggesting a synergistic role for chronic rejection and infection in OB development. A survey of 27 patients by the Pittsburgh group has correlated CMV post-operative infections with an increased risk of developing chronic rejection.\(^{100}\)

Infectious complications, without necessarily involving rejection are an important cause of morbidity and mortality.\(^{37}\) An incidence of infection as high as 86% was reported in two early series \(^{19,43}\) and 75% of all deaths have been related to infection in the overall Pittsburgh experience.\(^{37}\)

In addition to immunosuppression, the tendency to develop infections is probably associated with early post-operative ischaemic injury, impaired pulmonary drainage and interrupted lymphatic drainage.\(^{37}\) The pathogens in heart-lung and lung transplantation infection can be bacterial, viral, protozoal or fungal.\(^{68}\) The transplanted lung is particularly sensitive to bacterial infection and a prevalence of bacterial pneumonia of greater than 60% has been reported.\(^{37}\) CMV infection which frequently occurs during the first months after surgery, may lead to a high morbidity and mortality in lung allograft recipients.\(^{19,81}\) Patients with biopsy or cytology-proven CMV pneumonia have an increased prevalence of chronic allograft rejection, demonstrable by transbronchial biopsy, and a much worse overall survival.\(^{44}\) Other important herpes virus infections following pulmonary transplantation are herpes simplex virus (HSV) and Epstein-Barr virus (EBV).\(^{19,43,188}\)

**Bronchial artery ligation:** Evidence for the role of bronchial artery ligation in the pathogenesis of OB is less clear than that of infection. Following pulmonary transplantation, one of the major sources of early morbidity and mortality is the occurrence of ischaemic airway complications.\(^{140,166}\) Deprived of its physiologic arterial blood supply, the transplanted airway depends on the retrograde perfusion
from bronchopulmonary collaterals in the initial post-operative period. Closset and co-workers investigated the effect of bronchial artery severing on ischaemia of airways and subsequent development of OB. They saw no significant ischaemic lesions occurring during reperfusion of the bronchiolar vascular bed and concluded that even if ischemia does occur it is not a significant factor in OB. However, other studies have shown that reimplantation of the bronchial artery results in significant improvement of graft bronchial blood flow although restoration of perfusion to normal levels was not achieved. It was suggested that this may be due to a defect in the microcirculation as a result of endothelial damage on ischemia-reperfusion injury, as the patency of the bronchial artery was confirmed by angiography. Bronchial revascularisation by graft in pulmonary transplantation is being used in human patients and is associated with improved airway healing.

**Denervation:** Heart-lung and single and double lung (singly or en bloc) all result in total extrinsic denervation of the transplanted organs and the long term effects of this phenomenon are unclear. Denervation was not thought to be the cause of changes in growth in the immature allogeneic and isogeneic transplanted rat lung. In this study, these immature lungs grew with an increase in alveolar number, airway diameter and total lung volume compared to control lungs and was thought to have a humoral basis. Auto-transplantation of single porcine lobes has been shown to lead to small airway flow obstruction in the absence of immune rejection and this was thought to be due to denervation, rather than immunosupression or rejection in the immature pig lobe. However again, further work by the same investigators showed transplantation of the mature porcine lobe did not result in abnormally small airways. Thus, they concluded that abnormally small airways of the transplanted or reimplanted immature porcine lung were likely to be due to impaired airway development and not to bronchoconstriction caused by denervation.
This theory is supported by a study on post-natal development of the denervated rat and rabbit lung which concluded that innervation does not play an important role in the post-natal development of the lung.\textsuperscript{128}

There is evidence that the transplanted human heart does not reinnervate,\textsuperscript{15} however the situation in the lungs is less clear. Higenbottam and workers found a diminished cough response when ultra-sonically nebulised distilled water was distributed to the central airways and concluded that these structures do not become reinnervated after heart-lung transplantation.\textsuperscript{73} An immunocytochemical study has proved the persistence of intrinsic neurones in the human respiratory tract after heart-lung transplantation.\textsuperscript{177} Peptide-containing nerves were still detectable, in reduced numbers following transplantation. In addition there seemed to be a possible phenotypic change in the intrinsic cholinergic neurones. Thus, more work is needed to understand the effects of denervation on the structure and function of the transplanted lung and the possible role of denervation in the development of OB.

**Role of growth factors in obliterative bronchiolitis:** Recent findings have suggested a role for platelet-derived growth factor (PDGF) in the pathogenesis of OB.\textsuperscript{71} PDGF is a potent modulator of mesenchymal cell function and is closely associated with the presence of established disease. Alveolar macrophages are one source of PDGF. One study demonstrated that bronchial alveolar lavage (BAL) fluid from OB patients who had increased PDGF levels significantly stimulated fibroblast migration, whereas fluid from healthy lung transplantation patients did not. Prospective evaluation of sequential BAL samples from a patient who developed OB demonstrated markedly increased PDGF concentrations before the onset of irreversible airflow obstruction.\textsuperscript{71} They extended this work by showing that administration of PDGF and basic fibroblast growth factor (bFGF) to transplanted murine airway isografts into the subcutaneous tissue produced a fibroproliferative response.\textsuperscript{4} In addition, it has been demonstrated
that soluble IL-2 receptor levels in serum samples increase during acute rejection and that monitoring of the BAL IL-2 level may therefore be of predictive value.\textsuperscript{109}

G) The future of pulmonary transplantation.

In 1996, the Registry of the International Society for Heart and Lung Transplantation recorded actuarial survival rates of 60\% and 45\% for 1 and 3 years respectively following heart-lung transplantation. Lung transplant patients fared better. Single lung and bilateral/double lung transplantation both resulted in a 70\% and 55\% survival at 1 and 3 years respectively.\textsuperscript{78} Heart-lung transplantation in children with a mean age of 12.2 years at Great Ormond Street Hospital, London had a 63\% and 48\% survival rate at 1 and 3 years reported in 1994.\textsuperscript{203} A high incidence of OB was experienced similar to that found in adult pulmonary transplants. By contrast, lung transplantation in patients over the age of fifty is associated with a lower incidence of acute and chronic allograft rejection.\textsuperscript{176}

In the future, it seems that more single and double lung transplantations will be performed in patients with pulmonary disease, the donor heart being given to a cardiac patient. Lobar transplantation may also help to ease the demand for organs and may even prove to provide some immune protection if the lobes are donated by a living relative. However, at this stage the long-term prospects of this type of transplantation are unclear.
II. Airway structure.

In order to analyse airway structure in the transplanted lung, it is essential to study normal lung structure. In addition, when animal models are used it is necessary to know the similarities and differences in airway structure between the human airways and those of the animal in question so that changes can be interpreted appropriately. It is also essential to fully understand and compare experimental work carried out and reported in different animal models.

The rat has been used extensively to study heart-lung and lung transplantation. The dog has also been used, particularly for preservation and reperfusion studies. More recently the pig has also been used especially for lobar transplantation work. Both the rat and the pig have shown long term airway changes following transplantation and the pig has been shown to develop obliterative bronchiolitis following pulmonary transplantation. In the present study, the rat unilateral lung transplantation model has been used. In addition, cultured porcine intra-pulmonary airway cells have been used to examine cell to cell interactions within the airway wall.

A) Normal airway structure.

The airway structure of the rat is similar to the human, despite the size difference. The branching of human airways is dichotomous with several axial pathways in each lobe whilst in the rat it is monopodial with only a single axial airway in each lobe. An important difference between these two species is that there is cartilage present in the human airways until approximately halfway down the pathway. In the rat however, there is no cartilage beyond the extra-pulmonary bronchi. In the rat, the bronchial arterial supply reaches down as far as the terminal bronchioli and slightly further in the human lung.
B) Pulmonary epithelium.

There are at least eight lung epithelial cell types recognised which together make up the surface epithelium of the conducting airways: ciliated, basal, neuroendocrine, mucous goblet, Clara, serous, small mucous granule and brush cells. In this study we are primarily concerned with ciliated, Clara and basal cells (see Chapter 2). A summary of the morphologic features of airway surface epithelial cells and glands in more than 13 species, including human, rat and pig shows that these species have similar epithelial cell profiles.

A study by Jeffery and Reid examined the airway epithelium in the mature rat by comparing five airway levels from the upper trachea to the peripheral bronchioli in order to determine the distribution and frequency of each epithelial cell type as well as the epithelial thickness and the depth of the ciliary layer. In the smallest bronchiole studied (< 0.4mm), the dominant epithelial cell type was ciliated (152 +/- 2.0 per 1.8mm epithelium), Clara cells were the second most common epithelial cell type (45.6 +/-10.8 per 1.8mm epithelium) and basal cells were rare (1.2+/- 0.7 per 1.8mm epithelium).

A more recent report by Mercer and workers provides a detailed comparison of cell number and distribution in human and rat airways. This work included the study of smaller airways than those studied by Jeffery and Reid and utilised a different approach. Morphometric procedures were used to determine the number of cells, cell volume, cell diameter and the surface areas of the bronchi and bronchioli. The airway cell densities were given for the terminal bronchioli of the rat (diameter 0.23-0.15mm) as ciliated cells 9.1+/-1.2, basal cells 0 and other secretory cells (predominantly Clara cells) 6.8+/- 0.3 cells per mm² (x10³). The bronchioli of 1.0-0.4mm were the smallest airways of the human lung to be examined. The cell densities were ciliated 14.3 +/-2.9, basal 0 and other secretory cells (predominantly Clara cells) 10.6 +/-0.8, cells per mm²( x10³).
Some studies have concentrated on just one airway epithelial cell type. Studies on ciliated cells of the airway have concentrated on the effect of injurious agents. A study by Abdi and workers\(^1\) looked at the effect of cotton smoke exposure on ovine tracheal epithelium. They concluded that the loss of ciliated cells by sloughing was the first morphological indicator of injury after exposure to smoke. They suggested that the sloughing of intact ciliated cells was due to the effect of the smoke on the desmosomal attachments of these cells by an unknown mechanism. The function of ciliated cells in mucociliary clearance following transplantation is an important area of research. In canine left lung allografts, ciliated cell mucociliary clearance was examined using carbon particles placed at different locations on the mucosa. Clearance rates were decreased in the early post operative period. Histologic and electron microscopic examinations initially revealed focal denudation of ciliated cells. Twelve weeks post transplant there was regeneration of cilia and recovery of mucociliary function.\(^{45}\) Plopper in 1983 described and compared the morphologic features of bronchiolar Clara cells in 15 species, including human and rat. The Clara cell of the rat has agranular endoplasmic reticulum but this is not present in the Clara cell of the human airways.\(^{146}\) However Clara cells in both species have ovoid secretory granules, small mitochondria and lateral cytoplasmic extensions. This paper also emphasises the differences in Clara cell morphology and in the secretory products between perinatal and adult animals and therefore the need not only for the correct selection of species, but also the most appropriate age for study.

The importance of age in epithelial secretory cell morphology was also emphasised by Jeffery and co-workers.\(^{92}\) They examined the number and chemical composition of the human bronchiolar Clara cell at different ages. During development Clara cells are thought to appear during the second half of gestation from primitive glycogen-containing non-ciliated cells in the terminal airways. Maturation involves gradual loss of cytoplasmic glycogen, increasing ribosomal content and the appearance of the electron-dense secretory granules.
Structural analysis of airways has also been used to examine the role of the basal cells. Evans and co-workers in 1989 showed that the basal cells in the pseudostratified epithelium of the trachea are involved in the indirect attachment of columnar cells to the basal lamina. They determined the percentage of the columnar cell surface area associated with direct attachment to the basal lamina or indirectly attached via the basal cells in tracheal epithelium of different height. This varied from the 8.6μm tracheal epithelium of the hamster to the 21.4μm tracheal epithelium of the sheep. They found that the shorter the epithelium, the greater the percentage of columnar cell direct attachment to the basal lamina.

C) Methods used to study the structure of the transplanted lung.

Quantitative morphometric techniques have been applied to examine the effects of transplantation on the growth of the immature lung. Studies using light microscopy for point-counting, alveolar counts and airway diameter measurements have shown that following unilateral left lung transplantation in the rat, the immature lung continues to grow by both alveolar multiplication and increase in airway and alveolar size. Bullard and workers in 1991 used morphometric analysis of electron-micrographs to study changes associated with acute rejection in the gas exchange region of the lung in rats. The volume of endothelial and alveolar type I and II cells and of the interstitium was determined together with the total lung diffusion capacity, mean tissue thickness and tissue component of diffusion. Various Cyclosporine regimens were administered to rats undergoing allotransplantation. No significant changes were demonstrated between control (non-transplanted or hilus-stripped) and isografted or (immunosuppressed) allografted lungs for most parameters measured. They concluded that Cyclosporine can effectively block the development of injury in the gas exchange region, depending on the time of administration and dosage.
III. Airway epithelial cell/fibroblast interactions.

A) Proliferative response to lung injury.
McGauran and Brody examined the effect of chrysotile asbestos on the tritiated thymidine incorporation by epithelial cells. They showed that although the asbestos was deposited throughout all airway levels, increased thymidine incorporation was observed primarily in the peripheral airways. Earlier work showed that 48 hours after a one hour exposure, the number of epithelial cells is increased 10 fold and one month later interstitial fibrosis is observed at these sites.

Decampos and co-workers used functional and biochemical techniques to assess post preservation rat lung viability. They concluded that DNA synthesis is sensitive to ischaemic lung injury. Autoradiography of Euro-Collins preserved rat lungs revealed that alveolar macrophages and epithelial cells were the two major cell types synthesising DNA during ischemia at 4°C and 21°C. No other studies appear to have investigated the relationship between DNA synthesis and the conditions of ischaemic storage.

A study by Cagle and workers, examined cellular proliferation after pneumonectomy in rats. Autoradiography revealed that all cell types studied, both parenchymal and nonparenchymal, participated in lung growth. Pleural mesothelial cell tritiated thymidine labelling peaked on day two and declined thereafter. Tritiated thymidine labelling of all other cell types including bronchial epithelium occurred in parallel, peaking on day four. The different lobes had equal percentages of dividing cells, suggesting that a global stimulus such as an endocrine factor has a dominant role in postpneumonectomy lung growth.
Changes to the bronchiolar epithelium during chronic rejection and OB are well documented in clinical investigations and more severe proliferative epithelial changes in the transplanted lung have been reported by Benardi and workers. In a patient who died 6 months after heart-lung transplantation, histological studies demonstrated severe OB as well as diffuse hyperplasia, squamous metaplasia and dysplasia of the respiratory epithelium.

**B) Lung epithelial cell/fibroblast interaction.**

It is now recognised that the mesenchyme has important cell regulatory function in addition to providing mechanical support. Although the embryonic mesenchyme is known to have inductive capabilities, interactions between mesenchymal and epithelial cells in adult tissues are now recognised. The homeostasis of the airway cell population is influenced by both growth stimulatory and inhibitory substances and involves interactions through growth factors of epithelial cells and fibroblasts as well as other lung cell types. The study of the interactions between different cell types of the transplanted lung, whether *in vivo* or *in vitro*, may provide essential information concerning the mechanism of epithelial-mesenchymal disturbances. Current concepts suggest that re-epithelisation after airway injury and inflammation occurs as a result of migration, attachment and proliferation of epithelial cells at the site of injury. Extracellular matrix components have been shown to be attachment substrates for epithelial cells and fibronectin has also been shown to be chemotactic for epithelial cells. In sub-epithelial fibrosis conditions, the increase in extra-cellular matrix components is thought to be modulated by cytokines that activate fibroblasts following tissue injury. Airway epithelial cells are known to be capable of releasing a variety of active mediators and may well be important in regulating subepithelial connective tissue production.
C) Role of growth factors.

Transforming growth factors alpha and beta (TGF-α, TGF-β), insulin-like growth factor (IGF-1) and keratinocyte growth factor (KGF) released by fibroblasts and macrophages have all been reported to affect proliferation and differentiation of airway epithelial cells by autocrine and paracrine mechanisms.

Proliferation of epithelial cells can be controlled by members of the epidermal growth factor (EGF)-like family of molecules which include EGF and TGF-α. TGF-α in particular may play a major role during repair. EGF is a mitogen for both mesenchymal and epithelial cells but has not been shown to be synthesised by any type of lung cell. Numerous studies have shown that fibroblasts can stimulate epithelial cell proliferation and EGF-like molecules have been thought to play a role in this.

Treatment of cultured human or rabbit tracheobronchial epithelial cells with TGF-β1 induces irreversible growth arrest. TGF-β is known to influence fibronectin production in fibroblasts and is also thought to play a role in airway repair. The effect of TGF-β on bronchial epithelial cells is to increase the release of fibronectin into the media, increase cell associated fibronectin and increase de novo synthesis of fibronectin and fibronectin mRNA. It is known that fibronectin produced by bronchial epithelial cells acts as a chemoattractant for other airway epithelial cells and this activity may play an important role during tissue repair after airway injury by initiating prompt and efficient re-epithelisation of the bronchial wall. In addition, fibronectin production by bronchial epithelial cells can function as a chemotactic factor for lung fibroblasts. This production of fibronectin by bronchial epithelial cells may play an important role in regulating interaction between bronchial epithelial cells and the mesenchymal fibroblasts that underlie the epithelial basement membrane.

Kawamoto and workers have shown that bovine bronchial epithelial cells modulate collagen type I and fibronectin production by fibroblasts. The stimulating factor is
predominantly TGF-β and one of the inhibitory factors appears to be prostaglandin E2. Furthermore, TGF-β₁ is reported to increase the expression of cell surface receptors for fibronectin and collagen, as well as increasing the content of desmosomal plaque proteins I and II in cultured bronchial epithelial cells concurrent with an increase in the mRNAs encoding these proteins. The fact that TGF-β₁ has been localised at epithelial-mesenchymal interfaces during airway development suggests that this induction of desmosomal plaques may have significance in vivo.

The insulin-like growth factors are peptides able to promote proliferation and influence differentiation in a variety of eukaryotic cells. Lung fibroblasts have been shown to produce and release IGF-I and IGF-II in culture. The IGF-I released by lung fibroblasts is able to promote the proliferation of these cells in an autocrine manner. Airway epithelial cells can express the type 1 IGF-receptor and both IGF-1 and insulin are able to stimulate the growth of tracheobronchial epithelial cells in culture.

The synthesis and release of IGF-1 by mesenchymal cells in vivo may stimulate the growth of tracheobronchial epithelial cells in a paracrine manner. Such a mechanism may play a role in stimulating the growth of the airway epithelium during repair following injury.

Fibroblast-growth factor (FGF) stimulates growth and differentiation of fibroblasts and is likely to be important in repair. Keratinocyte-growth factor (KGF) is a newly identified growth factor produced by human embryonic lung fibroblasts. KGF belongs to the family of fibroblast growth factors. KGF is secreted by mesenchymal cells and is able to stimulate the growth of tracheobronchial epithelial cells, but has no effect on the proliferation of fibroblasts.

Platelet-derived growth factor (PDGF) was originally detected in blood platelets but is also produced by a variety of cells including alveolar macrophages. PDGF is
mitogenic and chemotactic for fibroblasts in a paracrine manner but in addition, interleukin-1 (IL-1) induces the expression of the alpha homodimer of PDGF in fibroblasts and thus PDGF may be involved in the IL-1 stimulated proliferation of these cells. IL-1, although produced principally by monocytes and macrophages, can also be synthesised by fibroblasts under appropriate conditions and may have autocrine activity. The peptide is mitogenic for fibroblasts and thus must be evaluated for its role in lung fibroblast proliferation. Tumour necrosis factor (TNF) also known as cachectin is synthesised principally by monocytes and macrophages. It induces monocytes and endothelial cells to release IL-1 which is a mitogen for fibroblasts. TNF is itself a mitogen for certain fibroblasts.

D) Cell culture of pulmonary cells.

The success of culturing airway epithelial cells and fibroblasts is influenced by the source of the tissue from which the cells are obtained, the isolation procedure, the cell culture substrate and the culture medium. In general, bronchial epithelial cells proliferate, differentiate and survive better in culture than epithelial cells isolated from tracheal or nasal tissue. Fibroblasts survive most mechanical and enzymatic treatments and can be cultured in many of the simplest media such as Eagle's basal medium.

Epithelial cell cultures can be started from either intact pieces of tissue that are explanted onto appropriate substrates or from disaggregated cells. Cells from an explant proliferate and spread onto the culture dish. Many of the cells in close proximity to the native tissue can be identified as ciliated and goblet cells by light microscopy. The number of well-differentiated cells decreases with increasing distance from the explant. The main problem with this type of culture is contamination with non-epithelial cell types, particularly fibroblasts, and the relatively long time required to establish these cultures.

In disaggregated epithelial cell populations, the specific cell types that attach, proliferate and give rise to the final cell culture have not been identified definitively.
Most of the cells are small, non-ciliated and non-granulated with a high nucleus/cytoplasm ratio, suggesting these cells may be basal cells, progenitor cells of the larger airways.\textsuperscript{16,125} No suitable molecular markers are currently available for identifying ciliated and goblet cells.

Most airway epithelial cell culture media are based on commercially available nutrient mixtures supplemented with specific hormones and growth factors.\textsuperscript{198,206} Serum may be added to the medium but can induce squamous differentiation.\textsuperscript{206} Serum-free medium is better defined and consequently gives more consistent results but there are problems with artefactual induction of growth and effects on differentiation. Insulin and endothelial cell growth supplement have both been shown to significantly increase proliferation of airway epithelial cells from several species.\textsuperscript{198,206} Insulin may be exerting an effect through the receptor for insulin-like growth factor (IGF).\textsuperscript{157} Transferrin, triiodothyronine, hydrocortisone, cholera toxin and epidermal growth factor can be added to medium and have minor effects on proliferation of airway epithelial cells, the effects being species-dependent.\textsuperscript{198,206} Triiodothyronine and hydrocortisone have been shown to improve morphological differentiation of canine tracheal epithelial cells resulting in increased numbers of ciliated cells.\textsuperscript{198} Epidermal growth factor induces multi-layering of squamous cells.\textsuperscript{198}

Co-culturing epithelial cells with fibroblasts or feeding with medium conditioned by fibroblasts increases the number of ciliated cells in cultures and increases ion transport activity of epithelial cells.\textsuperscript{206} Fibroblast conditioned medium also affects maintenance of goblet cell differentiation. Cells in serum-free medium exhibit few mucin granules whereas identical cultures maintained in fibroblast-conditioned medium contain goblet cells that can be identified by staining.\textsuperscript{198}

In order to identify and distinguish between epithelial cells and fibroblasts, fixed cells can be stained with antikeratin antibody which labels epithelial cells and antivimentin
which labels fibroblasts.\textsuperscript{174} It is also possible to use an Alcian-blue periodic acid Schiff stain which stains the granules of secretory cells to distinguish epithelial cells from fibroblasts and gives an indication of the degree of differentiation of the epithelial cells.

In order to assay cell replication rates in microwell culture, the incorporation of tritiated thymidine and visualisation by autoradiography is a well established technique,\textsuperscript{174} although direct innumeration and metaphase arrest can also be used.\textsuperscript{202} Olivier and co-workers have also devised a rapid assay for counting proliferating cells using a modification of the methylene blue assay for cytotoxicity.\textsuperscript{137}

\textbf{IV) Conclusion.}

In conclusion, the outlook for patients undergoing heart-lung and lung transplantation has improved over the last 15 years, with better immunosuppression and post-operative care. However, Obliterative Bronchiolitis (OB) remains the most important complication following transplantation and although the pathology of OB is well described, its pathogenesis is still unclear.

This study used rat unilateral left lung transplants to study the effect of preservation and reperfusion on the peripheral airways. The first aim was to determine whether reperfusion results in additional damage already sustained by the airways following preservation. The observations from the preservation/early reperfusion period in the transplanted lung were further investigated using cell culture, concentrating on epithelial cell/fibroblast interaction. The second aim was to relate the early damage incurred by the airways to the long term effects of transplantation in order to investigate whether early reperfusion might have any bearing on the development of OB following transplantation.
Chapter 2: Materials and Methods.
I. The Effect of Transplantation on Airway Structure.

A) Study Design.

Specific pathogen-free inbred strains of male Lewis and Brown Norway (BN) rats were used in all experiments. Five groups of rats were studied as shown in the flow chart overleaf (Figure 2.1). Table 2.1 shows the weight and number of the animals used in all groups. In addition for groups 4 and 5 lung weights and total lung/body weight ratios are shown in Tables 2.2 & 2.3, data were not available for Groups 1-3.

Study Groups.

**Group 1:** *Preservation study:* Lewis lungs were preserved for either 4 or 7 hours in Marshall's solution (see appendix A2.1).

**Group 2:** *Early reperfusion study:* transplants were performed between mature adult Lewis rats, the lungs having been preserved for either 4 or 7 hours in Marshall's solution. Transplanted left and native right lungs were studied 4, 12 and 48 hours post transplantation.

**Group 3:** *Allogeneic long-term adult study:* Donor lungs from young adult BN rats were transplanted into young adult Lewis recipient rats. Transplanted left and native right lungs were studied 5, 25 and 100 days post transplantation.

**Group 4:** *Isogeneic long term immature rat study:* transplants were performed between immature Lewis donor and recipient Lewis rats. Transplanted left and native right lungs were studied 14 days and 6 months post transplantation.

**Group 5:** *Allogeneic long-term immature rat study:* Donor lungs from immature BN rats were transplanted into immature Lewis recipient rats. Transplanted left and native right lung were studied 14 days and 6 months post transplantation.

Age and strain matched controls were examined for all groups. A minimum of 3 animals for each time interval within each group were examined.
Figure 2.1: Study Design.

AIM: To study the effect of ischaemic-reperfusion injury on the peripheral airways

Unilateral rat lung transplantation model

Group 1
Preservation study
Lewis lungs preserved for 4 or 7 hrs.

Light-microscopy study
Descriptive study
Epithelial cell counts on toluidine blue sections.

Electron-microscopy study:
Descriptive study: transmission and scanning EM.
Clara cell secretory granule counts on electron micrographs.

Group 2
Early isogeneic reperfusion study
Isogeneic mature adult Lewis transplants, lungs were preserved for 4 or 7 hrs followed by 4, 12 or 48 hrs reperfusion.

Group 3
Allogeneic young adult study
BN-Lewis young adult transplants. Lungs were examined 5, 25 and 100 days post-transplant.

Group 4
Isogeneic immature rat study.
Lewis-Lewis immature transplants. Lungs were examined 14 days and 6 months post transplant.

Group 5
Allogeneic immature rat study.
BN-Lewis immature transplants. Lungs were examined 14 days and 6 months post transplant.
Operative procedure, collection and fixation of tissue.

All rat transplantation operations and the collection and fixation of tissue were carried out by the members of the Department of Surgery, Mayo Clinic, Rochester, Minnesota USA and Newcastle University, UK under the supervision of Mr C.G.A. McGregor. The operative technique used was that described by Prop and Marck. This technique and care of animals are described in the appendix A2.1.

Both allogeneic transplant groups (3 & 5) were immunosuppressed post transplant to prevent acute rejection using 25mg/ml Cyclosporine in arachis oil. Group 3 rats received 25mg/kg intra-muscularly on days 2 and 3 followed by 2mg/kg subcutaneously daily from day 4 onwards. Group 5 received 25mg/kg intramuscularly only on days 2,3 and 4 post transplant.

For Groups 4 and 5, the immature rat lung transplants, the rats were weighed and then sacrificed by an intraperitoneal injection of pentobarbital. The pulmonary veins were occluded by a ligature passed around the base of the heart, through the transverse sinus. For light microscopic examination, the lungs were perfused with formol saline (10%) through both the trachea and pulmonary artery simultaneously at a constant pressure of 30cm H$_2$O until the lungs were fully distended. Lungs required for electron microscopy were perfused with 2.5% gluteraldehyde (BDH) in either 0.2M cacodyl ate buffer or Sorensen's Complete buffer (see appendix A2.2). The trachea and pulmonary artery were then tied off so that the lung was fixed in an inflated state. The lung and heart bloc was then removed from the body.

For light microscopy, the whole lung was immersed in formol saline (10%) until fixed. Tissue for transmission electron microscopy (TEM) was dissected immediately after lung perfusion into 1mm$^2$ blocks and fixed in a 2.5% glutaraldehyde solution. Once fixed, tissue was stored in Sorensen's buffer. Tissue for scanning electron microscopy was dissected into small blocks and also fixed in 2.5% glutaraldehyde, as for TEM.

For Group 3, the long-term young adult rat transplants, the animals were sacrificed and the heart-lung bloc was removed from the body before insufflating the lungs via the trachea using Histocon (Polysciences). The lungs were then separated and small 2mm$^2$ blocks were fixed in 2.5% gluteraldehyde and then stored in cacodylate buffer for TEM and the rest of the tissue was put in buffered formol saline for light microscopy studies.

Some tissue required for TEM was fixed in formol saline and so had to be post fixed. Tissue was first washed in Sorensen's Complete buffer and then placed in 2.5% glutaraldehyde solution which was exchanged for fresh solution 2 to 3 times, over a period of 48 hours.
Table 2.1.
Strain and Body Weight of Donor, Recipient and Control rats.

**Groups 1+2: Preservation and reperfusion study. (LEW-LEW)**

<table>
<thead>
<tr>
<th>n (of animals)</th>
<th>Average weight of animals (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4 hrs pres</td>
<td>3 269g</td>
</tr>
<tr>
<td>7 hrs pres</td>
<td>3 287g</td>
</tr>
<tr>
<td>4 hrs pres &amp; 4 hrs reper</td>
<td>3 415g</td>
</tr>
<tr>
<td>7 hrs pres &amp; 4 hrs reper</td>
<td>3 478g</td>
</tr>
<tr>
<td>4 hrs pres &amp; 12 hrs reper</td>
<td>3 267g</td>
</tr>
<tr>
<td>7 hrs pres &amp; 12 hrs reper</td>
<td>3 309g</td>
</tr>
<tr>
<td>7 hrs pres &amp; 24 hrs reper</td>
<td>3 431g</td>
</tr>
</tbody>
</table>

Maximum duration of study, 48 hours.

**Group 3: Young adult long-term allogeneic (BN-LEW)**

<table>
<thead>
<tr>
<th>n (of animals)</th>
<th>Average weight of animals (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN control</td>
<td>3 269g</td>
</tr>
<tr>
<td>LEW control</td>
<td>3 287g</td>
</tr>
<tr>
<td>BN control</td>
<td>3 415g</td>
</tr>
<tr>
<td>LEW control</td>
<td>3 478g</td>
</tr>
<tr>
<td>5d post T.x.</td>
<td>3 267g</td>
</tr>
<tr>
<td>25d post T.x.</td>
<td>3 309g</td>
</tr>
<tr>
<td>100d post T.x.</td>
<td>3 431g</td>
</tr>
</tbody>
</table>

**Group 4: Isogeneic long term immature rat study. (LEW-LEW)**

<table>
<thead>
<tr>
<th>n (of animals)</th>
<th>Average weight of animals (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control at d 0</td>
<td>3 92g</td>
</tr>
<tr>
<td>Control (for rats 1d post T.x)</td>
<td>3 215g</td>
</tr>
<tr>
<td>Control (for rats 6 mths post T.x)</td>
<td>3 467g</td>
</tr>
<tr>
<td>14 d post T.x.</td>
<td>3 198g</td>
</tr>
<tr>
<td>6 mths post T.x.</td>
<td>3 464g</td>
</tr>
</tbody>
</table>

**Group 5: Allogeneic long-term immature rat study (BN-LEW)**

<table>
<thead>
<tr>
<th>n (of animals)</th>
<th>Average weight of Animal (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN control at d 0</td>
<td>3 143g</td>
</tr>
<tr>
<td>LEW control at d 0</td>
<td>3 92g</td>
</tr>
<tr>
<td>BN control (for rats 1d post T.x)</td>
<td>3 215g</td>
</tr>
<tr>
<td>LEW control (for rats 1d post T.x)</td>
<td>3 215g</td>
</tr>
<tr>
<td>BN control (for rats 6 mths post T.x)</td>
<td>3 361g</td>
</tr>
<tr>
<td>LEW control (for rats 6 mths post T.x)</td>
<td>3 464g</td>
</tr>
<tr>
<td>14 d post T.x.</td>
<td>3 215g</td>
</tr>
<tr>
<td>6 mths post T.x.</td>
<td>3 500g</td>
</tr>
</tbody>
</table>

It is important to note that all post transplant animals are Lewis rats.

d = days
mths = months

36.
Table 2.2: Fixed lung weights in control Lewis rats and following isogeneic transplants between immature Lewis rats (Group 4).

<table>
<thead>
<tr>
<th></th>
<th>Left lung weight (g)</th>
<th>Right lung weight (g)</th>
<th>Body weight (g)</th>
<th>Total lung/body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lewis control</td>
<td>1.16 ±0.08</td>
<td>1.5 ±0.18</td>
<td>92 ±4.6</td>
<td>0.029 ±0.011</td>
</tr>
<tr>
<td>Young adult</td>
<td>1.85 ±0.17</td>
<td>2.7 ±0.18</td>
<td>215 ±4.8</td>
<td>0.021 ±0.001</td>
</tr>
<tr>
<td>Mature adult</td>
<td>2.84 ±0.39</td>
<td>4.2 ±0.6</td>
<td>467 ±8.3</td>
<td>0.015 ±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>1.7 ±0.13</td>
<td>3.97 ±0.31</td>
<td>198 ±10.9</td>
<td>0.029 ±0.02</td>
</tr>
<tr>
<td>6 months</td>
<td>4.76 ±0.57</td>
<td>7.9 ±0.74</td>
<td>464 ±48</td>
<td>0.028 ±0.001</td>
</tr>
</tbody>
</table>

▲ p=0.05, compared with immature Lewis control.  
* p=0.05, compared with age-matched controls.

Table 2.3: Fixed lung weights in control BN rats and following allogeneic transplants between immature BN and Lewis rats (Group 5).

<table>
<thead>
<tr>
<th></th>
<th>Left lung weight (g)</th>
<th>Right lung weight (g)</th>
<th>Body weight (g)</th>
<th>Total lung/body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BN control</td>
<td>1.43 ±0.12</td>
<td>1.99 ±0.18</td>
<td>143 ±5.2</td>
<td>0.023 ±0.003</td>
</tr>
<tr>
<td>Young adult</td>
<td>2.18 ±0.23</td>
<td>2.8 ±0.33</td>
<td>189 ±10.2</td>
<td>0.027 ±0.001</td>
</tr>
<tr>
<td>Mature adult</td>
<td>3.72 ±0.09</td>
<td>5.06 ±0.24</td>
<td>361 ±8.31</td>
<td>0.024 ±0.0007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>2.9 ±0.22</td>
<td>3.9 ±0.4</td>
<td>215 ±7.85</td>
<td>0.031 ±0.001</td>
</tr>
<tr>
<td>6 months</td>
<td>not available, total weight was recorded only 500 ±8.94</td>
<td>0.024 ±0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>post T.x.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

▲ p=0.05, compared with immature Lewis control.  
* p=0.05, compared with age-matched controls.
B) Preparation of tissue for light and electron-microscopy.

Selection of blocks.
Tissue was taken from each lobe of the lungs to be examined. Tissue for light microscopy was taken by cutting slices of each lobe to reveal the airways in transverse section. Tissue for electron microscopy was taken in the same way but the slice was cut into approximately 1mm² blocks.

Light microscopy (LM).

Processing and cutting of tissue for light microscopy.
Tissue was processed using a Shandon hypercentre 2. This involves progressive dehydration of the tissue and then infiltration with paraffin wax (see appendix A2.3 for detail). Tissue was then embedded in paraffin wax. 5µm sections were cut from the paraffin wax embedded specimens using a sledge microtome (Leica) and mounted onto slides (coated with glycerol) by floating sections onto a drop of water on the slide, on a hot box at 40°C and then heating for 15 minutes at 60°C. For stronger adhesion, slides were put in an oven at 60°C overnight. These sections were used for histochemical staining.

Staining Procedures.

*Haematoxylin and Eosin stain (H+E)*
Wax-embedded tissues were taken through xylene, alcohol and then water. (See appendices A2.4 for details). Sections were stained for 15 minutes with Ehrlich's Haematoxylin (BDH), differentiated in acid-alcohol and then stained with Eosin (BDH) for 3 minutes, before dehydration and mounting.  

*Elastic van-Gieson' (EVG)*
Wax-embedded sections were dewaxed, stained with Miller's elastic for 35 minutes, followed by 3% ferric chloride and 70% alcohol (10 minutes each) and then finally stained in van Gieson for 1 minute before dehydration and mounting (See appendix A2.5 for details). 
Transmission electron microscopy (TEM).

Processing and cutting of specimens for TEM.
Tissue was embedded as described in the appendix A2.6. Briefly, tissue was washed in Sorensen's Complete buffer and then placed in 1% osmium tetroxide for one hour. The tissue was then progressively dehydrated in increasing concentrations of alcohol, finishing in propylene oxide before embedding in araldite. Tissue in araldite was polymerised by incubation in either aluminium dishes or rubber coffin moulds at 60°C for 48 hours. Some tissue was processed by machine using a Lynx Microscopy Tissue Processor. Using an Ultracut E Reichert Jung Microtome, 1µm sections were cut with a glass knife, floated onto a drop of water on a slide and fixed by heating the slide on a hot-box (40-60°C). These sections were stained with toluidine blue (1% solution in a 1% Borax solution at pH 11.0), by placing a drop of the stain on the section whilst heating the slide and washing it off with distilled water after a dried ring appeared at the edge of the stain. These sections were used to identify airways of interest within the embedded specimen in which the cutting plane was transverse to the elastic lamina of the airway. Ultra-thin sections (70nm) of the selected area were then cut using a diamond knife and floated onto copper grids within the boat of the knife. The copper grids were pre-treated with acetic acid to ensure adhesion of the sections. These grids were blotted on non-fibrous filter paper and were left for at least an hour before staining.

Staining of grids for TEM.
Grids were stained in 35mm petri-dishes, first in uranyl acetate (BDH, 20% in 50% ethanol) for at least 8 minutes. The petri-dish was covered in aluminium foil as uranyl acetate is light sensitive. The grids were then picked up with forceps and rinsed with 0.02M NaOH and then double-distilled water. This was followed by staining in lead citrate (see appendix A2.7) for 4 minutes precisely. The grids were then rinsed in the same way as before, blotted on non-fibrous filter paper and stored in grid boxes.

Electron microscopy of grids.
The sections were observed and photographed on a JEOL 100X Transmission Electron Microscope using Kodak electron microscope film 4489, 8.3 x 10.2cm at magnifications between x2,000 and x16,000. Three airways at TB+1 level were examined for each lung. For each airway approximately 4 photographs were taken at a low magnification, around x2-3,000, and 5-10 photographs at higher magnifications, between x5,000 and x16,000.

Development of negatives
In the dark room, negatives were mounted in plastic holders and immersed in developer (Ilford phenisol) at 20°C for 3 minutes and then washed in running tap water for 1 minute. The negatives were then placed in fixative (Ilford Hipam) for 5
minutes and finally washed again in running tap water for at least 10 minutes. They were then air dried and stored in transparent envelopes until printing.

**Printing from negatives**

An enlarger was used with a 150mm lens to print images at x2.5 magnification from the negatives. AGFA photographic paper (grades 3 & 4) was used. The paper was exposed to the negative image and developed in AGFA rapidprint G18B and followed by fixation in AGFA rapidprint G380B. The print was then fixed in a 1:9 (aq) solution of fixer (Ilford Hipam) and finally washed in running tap water for 2-3 minutes before being dried.

**Scanning electron microscopy (SEM).**

**Preparation of lung tissue for SEM.**

Tissue was dehydrated in steps with increasing ethanol concentrations up to 100% ethanol. Specimens were then further dehydrated using a critical point dryer which replaces the alcohol with liquid CO₂, which is then removed by heating at 40°C. The specimens were then mounted on aluminium stubs using silver dag (Agar Scientific Ltd) and coated with gold for 2-4 minutes using a SEM coating unit (Polaron Equipment Ltd).

**SEM of specimens.**

Specimens were observed and photographed on a JEOL-35 scanning electron microscope using Ilford FP4 125 b/w 5x120 roll film at magnifications between x400 and x10,000. For each airway approximately 3 photographs were taken at low magnification, x400-1000 and 5 photographs at higher magnifications, x1-10,000.

**Development of SEM negatives**

The films were loaded onto reels in complete darkness and placed into a light-tight canister. 500ml of developer for each film were then poured in the canister. (Ilford Microphen 1:1 ratio with tap water at 20°C). The canister was inverted continuously for the first minute and then once each minute for the next 15 minutes. After this time, the developer was poured away. The films were then washed with tap water at 20°C and the fixer was poured on (Ilford Hipam 4:1 ratio with tap water, again 500mls per film). The canister was then left for 2-3 minutes and then the fixer was poured off but retained whilst checking the films for any cloudiness. If any was noticeable, the fixer was poured back for 1-2 minutes before proceeding and if not, the films were washed with cold running water for 10 minutes. After the water was poured away the films were removed from the canister and their reels and hung to dry in an oven.

**Printing of SEM images**

These were printed with the same apparatus as for the TEM images at a magnification of x2.5.
C) Definition of terms.

Airway generations.

Conducting airways.

*Trachea:* The trachea is the main conducting airway which runs from the larynx to the lungs, where it divides into two main bronchi.

*Bronchi:* The bronchi are conducting airways which lead from the trachea into the lungs. In humans these airways are cartilaginous (non-membranous) but in the rat there is no cartilage in the airways distal to the main extra-pulmonary bronchi.

*Bronchioli:* The bronchioli branch from the bronchi as progressively smaller generations of airways. In both the human and the rat lung these airways are non-cartilaginous (membranous).

*Terminal bronchioli (TB):* These are the smallest and last generation of bronchioli which lead into the respiratory unit and are lined entirely with cuboidal epithelium. TB+1 airways refers to airways one generation proximal to the terminal bronchioli.

Gas exchange region.

*Respiratory bronchioli:* These are the most proximal structures of the gas exchange region. They also act as conducting airways for the alveoli and are lined both with cuboidal epithelium and alveoli.

*Alveolar duct:* Alveolar ducts are airways lined with alveoli.

*Alveoli:* Alveoli are thin walled structures, surrounded by capillaries, providing the blood-gas barrier for gas exchange. Alveolar epithelium consists of two cell types:

*Type I (squamous pneumonocytes) cells* cover 95% of the alveolar surface.

*Type II (granular pneumonocytes) cells* are more numerous than type I cells but as they are cuboidal they cover only 5% of the alveolar surface. They have microvilli on their surface and lamellated bodies in their cytoplasm which contain surfactant.

*Acinus (terminal respiratory unit):* Acinus refers to all the structures distal to the end of a terminal bronchiolus. The branching pattern is species variable. In the human, there are usually two to five orders of respiratory bronchioli, the last of which leads into the first of two to five orders of alveolar ducts. In the rat, a terminal bronchiolus leads almost immediately into alveolar ducts.

Components of the airway wall

*Epithelium:* Airways from the trachea to the respiratory bronchioli are covered with an epithelial lining that rests on a thin basement membrane overlying an elastic lamina. This lining consists of a layer of cells (pseudostratified in the larger airways), which includes ciliated, secretory and basal cells.

*Basement membrane:* The basement membrane is a thin layer of collagen type IV, secreted by the epithelium.
Elastic lamina (lamina propria): A sub-layer, supporting the epithelium which contains elastic fibres and other connective tissue elements.

Submucosal region: The submucosa lies below the elastic lamina and contains the airway smooth muscle, collagen, bronchial arteries and veins, glands and nerves.

Bronchial smooth muscle: Bronchial smooth muscle is present in all conducting airways. In humans, muscle fibres have been identified as far down as the opening of the alveolar ducts. Rat airways contain muscle at least to the level of the terminal bronchioli.

Bronchial arteries: Bronchial arteries perfuse the airway wall with systemic arterial blood. In the rat they extend down to the terminal bronchioli.

Epithelial cell types.
The distal airways of the rat lung consist of the following cell types:

Ciliated cell: These are the most common cells of the epithelial layer. They line all the conducting airways, extending into the respiratory bronchioli and are terminally differentiated cells. Each cell has approximately 250 cilia, each approximately 6\(\mu\)m long and 0.3\(\mu\)m wide. Ciliary movement provides the force that impels the superficial layer of secretions upwards from the periphery of the lung to the pharynx.

Clara cell: These cells are present in the peripheral airways. Their exact structure and number varies between species. They are secretory cells which protrude into the lumen. In addition to their secretory granules, one of the most consistent features of the Clara cell is the abundance of smooth endoplasmic reticulum. They are thought to be one of the progenitor cells of the peripheral airways.

Basal cell: These cells are found on the basement membrane of the airway, making up the bottom layer of pseudostratified epithelium in the larger airways. They become less numerous in the more peripheral airways. They have numerous tonofilaments and a dense cytoplasm. Their function is a combination of structural stability and progenitor.

Intermediate cell: These cells are similar to basal cells, have fewer tonofilaments and lie just above the basal cells in pseudostratified epithelium. In the rat lung they reach the lumen where they exhibit a few microvilli. As they differentiate they become more electron-dense.

Unidentified cell: In the present study, these cells were epithelial cells which could not be positively identified under the light microscope.
D) Quantitative analysis of airway structure by light and electron-microscopy.

In addition to the descriptive studies carried out in all groups, light microscopy was used to measure the airway dimensions by length and the components by area in Groups 3, 4 and 5. Counts of the different types of epithelial cells were performed using light microscopy in all 5 groups. Electron microscopy was used to examine numbers of secretory granules in Clara cells in the TB+1 airways in all 5 groups. Within each of the 5 groups, animals were killed at different times and 3 animals were studied for each of the subgroups. At least 3 airways in each animal at each level (TB+1 and TB) were examined per lung, for both light and electron-microscopy. Thus at least 9 airways of each type were studied in the left lung and 9 in the right lung at each time point (Figure 2.2).

Analysis by light microscopy.

Selection of airways.

Airway dimensions and components were measured on 5μm wax-embedded sections stained with Millers elastic and van Gieson stain (EVG). Epithelial cell counts were performed on 1μm araldite-embedded sections stained with toluidine blue.

Methods of measuring and calculating airway dimensions

Selected airways from EVG stained sections at x10 or x20 magnification were selected by a colour camera (JVC video camera, Japan) from a light microscope (Leica Dialux 20) and transferred to a computer monitor (RasterOps) using the Image Grabber 2.1. These images were then transferred into "Optilab™ 24/2.1.1" image analysis package. This system was calibrated prior to the measurements being made in order to obtain data output in μm or μm². The total airway and lumen diameter of all airways was measured and a lumen/total airway diameter ratio was calculated (Figure 2.2). The total airway area was measured including the lumen. The lumen area was measured and subtracted from the total airway area to give the airway wall area. In the TB+1 airways, the epithelium, muscle and bronchial artery area were measured. These three component areas were subtracted from the total wall area to give connective tissue area. For the TB airways only the epithelial area was measured, this was subtracted from the total wall area to give the submucosal area. Submucosal area included the muscle and connective tissue in the TB airways as these components were too small to measure accurately. The mean and standard error were calculated for each feature, whether area or diameter. In addition the value of each airway component was expressed as a percentage of total airway area or wall area. Percentage values were normalised by arc-sin conversion to allow statistical analysis of data (see below). Data were analysed using 2 factor ANOVA tests to determine the effect of both transplantation and age on airway dimensions and areas.
Figure 2.2: Quantitative Analysis of Airways

**LIGHT MICROSCOPY**

**Airway Dimensions:**

The measurements below were made using Image grabber and Optilab packages. TB+1 and TB airways were examined.

- Total airway area/diameter.
- Lumen area/diameter.
- Wall area.
- Epithelial area/height.
- Connective tissue/submucosa area.
- *Bronchial muscle area.
- *Bronchial artery area.
- Not measured in TB airways.

**Epithelial cell counts:**

Cell counts were made using 1μm toluidene blue sections. Numbers of the different epithelial cell types were counted and expressed per unit length epithelium.

**ELECTRON MICROSCOPY**

**Secretory granule counts:**

Clara cell secretory granules per cell were counted on the electron micrographs.

**KEY.**

- connective tissue
- epithelial area
- muscle area
- bronchial artery area
Epithelial cell counts.

Methods of counting epithelial cells and calculating the number per unit-length. Airway epithelial cell counts were made on 1μm sections using light microscopy at x25 magnification. The cell types defined for this purpose were ciliated, Clara, basal and unidentified cells. Only cells with a sectioned nucleus visible were counted. The total length of epithelium per airway was measured for both the TB+1 and TB airways and the number of each cell type was counted and expressed per unit length (100μm). For each experimental time interval, a mean and standard error were calculated for each cell type for 3 airways in each of the 3 cases i.e a total of 9 airways.

Analysis by electron microscopy.
Selection of airways.
Araldite-embedded tissue was first cut as 1μm sections and stained with toluidene blue. These sections were then examined to identify airways of interest. As for the light microscopy studies, airway level was determined by first identifying terminal bronchioli. Once the airways were located on the toluidene blue sections the araldite block could be trimmed and ultra-thin (70nm) sections cut.

Counts of secretory granules in Clara cells.
The number of granules per Clara cell in the TB+1 airways were counted on electron-micrographs (magnifications between x3,000-x5,000) in all 5 groups. There were 3 animals per experimental group and at least 10 Clara cells per animal were counted, thus n= 30 cells per group.

E) Principles of statistical analysis.
The majority of the data complied to a normal distribution. Therefore after the mean and standard errors were calculated, analysis of variances (ANOVAs) were performed. One factor ANOVAs were calculated when transplantation was the only variable (Group 1 & 2 rats) and two factor ANOVAs were calculated when both transplantation and age were variables (Group 3, 4 & 5 rats). If the ANOVAs were significant, Student t-tests were used to calculate between individual data groups to determine which changes were significant. In some of the structural analyses, percentages were used to express results. Percentages and proportions are binominal variables and therefore direct statistical analysis is not possible. Instead, percentages were transformed with the object of making the variance independent of the mean. This angular transformation involved the conversion of percentages or proportions (r/n) to the angle q (expressed in degrees) where:

\[
\frac{r}{n} = \sin^2 q \quad \text{or} \quad q = \sin^{-1} \sqrt{\frac{r}{n}}.
\]

This results in the variance being independent of the mean of q and if n, the number of airways studied, is the same for all samples then the variance of q may be taken as
approximately constant so that one obstacle to the use of normal methods is removed. There is also the form of distribution of q to be considered. This distribution is not exactly normal but is sufficiently close to normality to ensure that reliable results are obtained by using normal methods. Such transformed data was then treated in an identical manner to the normal data. All data processing was carried out on a Apple Macintosh using Microsoft Excel and Statview software packages. Differences were considered to be statistically significant if p<0.05.

II. In vitro studies on airway epithelial cell/fibroblast interaction.

The effects of cooling (4°C) to mimic preservation, and rewarming (37°C) on porcine epithelial/fibroblast interactions were investigated by exposing epithelial cells and fibroblasts to fibroblast conditioned medium (FCM) harvested under different conditions. Growth stimulation was assessed using tritiated thymidine and autoradiography.

A) Preparation of materials.

Source and harvesting of cells.

Lungs were obtained from freshly killed 3-6 day old Large White pigs. Intra-pulmonary airways were dissected and placed in phosphate-buffered saline (PBS) which contained antibiotics, before use (See appendix A2.8). Each piece of bronchus was placed on a sterile petri-dish in a pool of PBS under a dissecting microscope. Any parenchymal tissue on the outside of the airway was first removed. The airway was then cut along its length, the epithelial sheet was revealed and strips of it were removed using fine forceps, cut up finely and placed in disaggregation medium (See appendix A2.9). Epithelial tissue was disaggregated for between one and one and a half hours. The cell suspension was then spun down at 4°C at 400rpm for 10 minutes in an IEC Centra-8R centrifuge. The supernatant was removed and replaced with medium 199 + EGF (epidermal growth factor) (See appendix A2.10). This suspension was then shaken to disperse the pellet of cells and any large pieces of tissue were then removed. The remaining suspension was recentrifuged, the supernatant removed and the cell pellet resuspended in M199+EGF.

The fibroblasts were isolated from the same airways. After the epithelial sheet had been removed, the remaining wall was minced and disaggregated for between 1.5-2 hours. The remaining cell suspension was treated as for the epithelial cells except that the fibroblasts were resuspended in RPMI medium (see appendix A2.11).
Plating of cells

Epithelial cells were plated on glass coverslips (19mm) in 12-well plates (Marathon). The coverslips were pre-treated by boiling in distilled water, allowing them to dry and then autoclaving them in glass petri-dishes to ensure sterility. By using a haemocytometer to count cells, cells were plated at a concentration of $4 \times 10^4$ per well in 0.5ml of M199+EGF and incubated in 5% CO$_2$ at 37°C (normal culture conditions). The cells were fed with the same medium on days 2-3 after plating and then as required.

Fibroblasts were plated in the same manner as epithelial cells on coverslips except that they were suspended in RPMI medium.

Fibroblasts were plated in 24-well plates pre-coated with 1% gelatin (Sigma). Cells were plated out at 3-4 $\times 10^4$ cells per well in 0.5 ml of RPMI medium. The plates were incubated in 5% CO$_2$ at 37°C as before. They were fed with RPMI medium as required.

Production of fibroblast-conditioned medium (FCM).

Preparation of fibroblasts on plastic.

Once fibroblasts were semi-confluent in the 24-well plates, the medium was removed and the cells were washed twice with serum free medium (SFM) (see appendix A2.12), before adding 1ml of SFM and incubating them as before. SFM contains no nutrients or growth factors for the cells and therefore the cells enter the steady state (G$_0$ part of cell cycle).

Cooling and rewarming of fibroblasts and collection of medium.

After 48 hours this medium was then removed and replaced with a fresh 1ml of SFM. The plates were then divided into two groups: FCM(normal) and FCM (from cooled then rewarmed fibroblasts). FCM (normal) plates were placed back in the incubator in 5% CO$_2$ at 37°C and medium was removed at 4 hours and then every 30 minutes for a further 4 hours and lastly, medium was taken at 28 hours. Once medium was removed from a well, the well was no longer used. The FCM (cooled and rewarmed) plates were treated in a similar way except that the first 4 hours of incubation was at 4°C, after which the first sample was taken. The remaining plates were placed in the incubator at 37°C and samples were taken every half hour for a further 4 hours, as before, and a final sample was taken at 28 hours. All the FCM collected was stored at -20°C until used.
B) Experimental design (Figure 2.3).

Group 1: The growth stimulatory effect of fibroblast-conditioned medium harvested from fibroblasts kept at 37°C, for each of the timed collections on a) epithelial cells maintained at 37°C and b) epithelial cells pre-cooled at 4°C. n= 4 experiments for both a) & b).

Group 2: The growth stimulatory effect of fibroblast-conditioned medium harvested from fibroblasts kept at 37°C for each timed collections, on a) on fibroblasts maintained at 37°C and b) fibroblasts pre-cooled at 4°C. n= 4 experiments for both a) & b).

Group 3: The growth stimulatory effect of fibroblast-conditioned medium harvested from fibroblasts which had been cooled at 4°C and rewarmed at 37°C, on a) epithelial cells maintained at 37°C and b) epithelial cells pre-cooled at 4°C. n= 4 experiments for both a) & b).

Group 4: The growth stimulatory effect of fibroblast-conditioned medium harvested from fibroblasts which were cooled at 4°C and rewarmed at 37°C on a) fibroblasts maintained at 37°C and b) fibroblasts pre-cooled at 4°C. n= 4 experiments for both a) & b).

C) Experimental procedure (Figure 2.4)

Step 1: Preparation of epithelial cells and fibroblasts.
Epithelial cells and fibroblasts were grown up to a near confluent state on the glass coverslips in 12-well plates. A measure of epithelial cell differentiation was determined using Alcian Blue-Periodic Acid Schiff (AB-PAS) stain which stains the granules of secretory cells; acid/sulphated mucopolysaccharides staining blue and neutral/non-sulphated mucopolysaccarides staining red (see appendix A2.13). The medium was removed and the cells were washed twice with SFM, put into 1ml of SFM and incubated for a further 48 hours prior to the experiment.

Step 2: Incubation of epithelial cells and fibroblasts in FCM/labelling with 3H thymidine
Both cell types were either pre-cooled at 4°C or maintained at 37°C for 4 hours. The SFM was removed from these cells and replaced with either 1ml of "normal" FCM or medium from cooled and rewarmed fibroblasts. Each well was also labelled with 1μl of tritiated (3H) thymidine (1μCi/μl) to give a final dilution 1μl/ml 3H thymidine. These plates were then incubated at 37°C in 5% CO2 for 24 hours.
Step 3: Measurement of $^3$H thymidine incorporation into cells by autoradiography.

After 24 hours of incubation with the $^3$H thymidine, the epithelial cells and fibroblasts were washed twice in SFM and then fixed in 100% ethanol for 15 minutes and air-dried.

Step 4: Coating of coverslips with photographic emulsion.

In a dark room, photographic emulsion (Ilford K5 emulsion) was warmed to ~ 40°C until it was the correct consistency to coat a blank coverslip smoothly. Coverslips were dipped into emulsion to coat them and were placed (cell side up) on a tissue-covered tray to dry. Once dry they were put back in the wells. Silica gel granules were then placed between wells to maintain a dry atmosphere and plates were then wrapped up in black plastic bags and were kept at 4°C for 3-4 days.

Step 5: Development, fixation of coverslips and counter-staining of cells.

Plates were unwrapped in the dark room and developer (Kodak D19) was pipetted into wells and was left for 10 minutes. It was removed and the cover-slips were placed in tap-water for 10 minutes and then this was replaced by fixer (Kodak Unifix) for a further 10 minutes. The coverslips were rinsed in tap-water and counter stained for 1 minute in Mayer's Haematoxylin, rinsed in water and then further stained in Eosin for 1 minute. Coverslips were rinsed in water and then dehydrated through alcohol to xylene before being mounted with XAM mountant, which was placed on the cell side of the coverslip. The coverslip was placed cell side down onto a microscope slide.

Step 6: Method of counting labelled cells.

For each time interval there were 2-3 slides per experiment. For each slide 10 areas were counted (each area containing 20-40 cells), using a grid eyepiece at x40 magnification. This gave an average of 300 cells counted per slide. The number of labelled and unlabelled nuclei were counted. The number of labelled cells was expressed as a percentage of the total cells counted.

D) Statistical analysis of cell counts.

For both cell types (epithelial and fibroblast) at both temperatures (37°C and 4°C) within each of the four groups the data expressed as percentages were first normalised by angular transformation (see page 45) before calculating the mean and standard error. Groups were compared using one or two factor ANOVA tests and Student t-tests were used to analyse the cell types studied at different temperatures in the same medium and at the same temperature in different media, using cells incubated in serum-free medium as controls. Differences were considered statistically significant if p<0.05.

49.
FIGURE 2.3: Experimental Design

**FCM 37°C:**
Fibroblast-conditioned medium from fibroblasts maintained at 37°C. Medium was collected after 4, 5, 6, 7, 8 & 28 hours incubation.

**FCM 4°C:**
Fibroblast-conditioned medium from fibroblasts precooled at 4°C for 4 hours and then rewarmed for 1, 2, 3, 4 & 24 hours at 37°C. Medium was collected at all time intervals.

**GROUP 1:**
- a) Epithelial cells maintained at 37°C.
- b) Epithelial cells precooled at 4°C for 4 hours.

**GROUP 2:**
- a) Fibroblasts maintained at 37°C.
- b) Fibroblasts precooled at 4°C for 4 hours.

**GROUP 3:**
- a) Epithelial cells maintained at 37°C.
- b) Epithelial cells precooled at 4°C for 4 hours.

**GROUP 4:**
- a) Fibroblasts maintained at 37°C.
- b) Fibroblasts precooled at 4°C for 4 hours.

**Controls:**
Both epithelial cells and fibroblasts at both temperatures were incubated in serum-free medium (SFM) for 24 hours at 37°C.

For each Group, the above procedure was performed 4 times.
Figure 2.4: Experimental procedure to investigate the effect of fibroblast-conditioned medium (FCM) on epithelial cells and fibroblasts.

Step 1.

Epithelial cells and fibroblasts on coverslips which have been in SFM for 48hrs are kept at 37°C or cooled at 4°C for 4hrs and washed 2 x SFM.

Step 2.

The cells are then incubated in 1 ml of FCM (from normal or cooled and rewarmed cells) and 1 ul of 3H thymidine for 24hrs at 37°C, 5% CO₂.

Step 3.

After 24 hours, medium is removed and the cells are washed 2x in SFM. Next the cells are fixed in absolute alcohol for 15 minutes and air-dried.

Step 4.

In a dark room the cover slips are coated in photographic emulsion which are first warmed to ~ 40°C to achieve the correct fluidity. The coverslips are laid out to dry on a paper towel before being stored back in their wells. These plates are stored, light and humidity-tight at 4°C for 3-4 days.

Step 5.

Coverslips were then developed and fixed in the dark room and washed thoroughly in tap water. Cells were then counterstained in haematoxylin and eosin and then mounted on glass microscope slides.

Step 6.

Cell nuclei counts:
For each coverslip, ten areas were counted using a x40 lens and grid square eye-piece. The number of labelled nuclei were counted and expressed as a percentage of total nuclei counted.
Chapter 3: Effect of preservation and early reperfusion on the peripheral airways of the isogeneic transplanted lung.
I. Qualitative Studies.

Control airways.
The healthy epithelium of the bronchioli of the rat consists mainly of ciliated and Clara cells. Clara cells protrude into the lumen in a characteristic manner and their apices contain secretory granules. Basal cells are rarely found in the bronchioli of the normal rat lung. The submucosa of these airways contain bronchial smooth muscle and sometimes nerves and bronchial arteries (Figure 3.1a, b & c).

A) Group 1: Preservation study.
Appearance of the peripheral airways.
Preservation of the Lewis lung for either 4 or 7 hours resulted in a similar degree of structural damage to the peripheral airways when observed by both light and electron microscopy. Light microscopy of the airways showed that the epithelium was intact. There appeared to be a decrease in the number of Clara cells which protruded into the lumen being replaced by a considerable number of unidentified cells with rounded apices. Electron microscopy showed a flattened epithelium with damaged epithelial cells exhibiting shrunken nuclei, dense cytoplasm, cytoplasmic clefts and swollen mitochondria and endoplasmic reticulum (Figure 3.2a & b). Some of these damaged cells could be recognised as Clara cells on the electron micrographs, because the cytoplasm contained secretory granules. Cell debris lay within the airway lumen, nearly always near the surface of the damaged cells. Like the overtly damaged epithelial cells, the comparatively healthy epithelial cells, predominantly ciliated cells, also appeared to have an increased number of cytoplasmic clefts and swollen mitochondria. Cilia appeared unaffected in number and structure under both light and electron microscopy.

Epithelial cell junctions were generally tight, with only a few examples of loss of cell to cell contact. The basement membrane was distinct and unbroken and below it the appearance of the elastic lamina was comparable to that seen in airways of control animals. Light microscopy revealed cellular infiltrate in the submucosa of some airways and electron microscopy showed an occasional lymphocyte within the epithelial layer. The endoplasmic reticulum and mitochondria of the airway smooth muscle cells were swollen.

Using scanning electron microscopy, control airways were seen to have healthy ciliated cells and protruding Clara cells (Figure 3.1c). After preservation, the surface of the epithelium showed that there was no evidence of loss of cilia (Figure 3.2c). The appearance of Clara cells varied from being slightly sunken to being completely flat and "blebby".

53.
Appearance of the alveolar region.
Using light-microscopy, after both 4 and 7 hours preservation, the structure of the alveolar region in the experimental animals was similar to that seen in the lungs of control animals, although there was an increase in the number of macrophages in the alveolar spaces (Figure 3.3a &b).

However, electron microscopic study of the alveolar Type II cells showed them to be slightly flattened compared with controls and their nuclei appeared to occupy an increased amount of the cell cytoplasm (Figure 3.3c &b). Within the cytoplasm, the mitochondria appeared swollen with distorted cristae and some mitochondria were fused. Lamellar bodies were clustered together and in some instances fused. In addition, some Type II cells contained large numbers of small empty vesicles. There was also a variety of individual mitochondrial abnormalities such as mitochondria within vesicles and distorted mitochondrial structures.

The preserved BN lung was also examined and the observations made were similar to those seen in the preserved Lewis lung.

B) Group 2. Early reperfusion study after isogeneic transplantation.
Appearance of the peripheral airways.
After 4 hours reperfusion.
After 4 hours reperfusion, there was an increase in the amount and severity of damage to the peripheral airways compared with the damage seen after preservation, which was similar whether the lungs had been preserved for 4 or 7 hours. Light microscopic study of the airways showed the epithelium to consist of cells of uniform height (Figure 3.4a). There were few identifiable Clara cells, although some cells still had a small number of secretory granules. There was a marked loss of cilia. Ciliary depletion was variable between airways, some exhibiting almost complete loss of cilia, others none at all and some with partial loss. By electron microscopy, it was still difficult to identify Clara cells due to the lack of secretory granules (Figure 3.4b). It was possible to see "ciliated cells" that had no cilia but had distorted basal bodies within the cytoplasm of the apical section of the cells. Some epithelial cells exhibited blebbing from their apices and had a greater number of inclusions than epithelial cells of control airways. This damage rendered it difficult to distinguish epithelial cell types even under the electron-microscope. However, epithelial cell nuclei were no longer shrunken as they had been after preservation nor were the mitochondria swollen.

Excessive loose interdigitation was present between some epithelial cells (Figure 3.4c). The basement membrane of the airway was not always distinct. However, there was no loss of integrity between epithelial cells and the basement membrane. The
elastic lamina had a normal appearance. There was no evidence of cellular debris in the lumen but ciliary debris was present in some airways. Infiltrate within the submucosa of the airways was present (mainly lymphocytes, with some macrophages, mast cells and basophils), although the amount varied between airways of the same level. The smooth muscle also appeared normal, with only a few areas of swollen endoplasmic reticulum.

Scanning electron micrographs supported the transmission electron microscope observations, showing large areas of flat cells (presumably both Clara and ciliated cells) which were not present in the control airways (Figure 3.4d).

**After 12 hours reperfusion.**
After 12 hours of reperfusion the epithelium of the peripheral airways still had a uniformly flattened look, although it was now possible to identify some Clara cells by light microscopy. Under the electron microscope however, there were still few or no secretory granules within the apical cytoplasm of the Clara cells (Figure 3.5a). Ciliary depletion was still evident but cells which did have cilia also had an increased undergrowth of microvilli and basal bodies in the apical region of the cell cytoplasm. The recovery of the Clara and ciliated cells varied between and within airways. Epithelial cell nuclei and mitochondria were normal in appearance, although some cells still exhibited blebbing. Epithelial cell to cell lateral junctions were still loose in some airways. The basement membranes and elastic laminae were distinct and unbroken.

The amount of cellular infiltrate within the submucosa varied between airways, although it was always present to some extent. It consisted predominantly of lymphocytes but macrophages, mast cells and basophils were also present. A few macrophages were seen within the epithelial layer. There was no debris present in the lumen. Scanning electron-microscopy of these airways corroborated the findings on light and transmission electron microscopy, showing cells with ciliary depletion and growth of microvilli as well as newly protruding Clara cells (Figure 3.5b).

**After 48 hours reperfusion**
Peripheral airway structure appeared relatively normal at 48 hours post-transplantation (Figure 3.6a). Under the light microscope Clara and ciliated cells had a normal structure and distribution. However there were areas of epithelial hyperplasia due to the presence of basal cells, variable in extent within and between airways. Clara cells protruded into the lumen as in control airways (Figure 3.6b). Electron microscopic examination showed that the number of secretory granules within the apical cytoplasm of the Clara cells was similar to that seen in controls. The number and distribution of cilia on the ciliated cells was normal with only the occasional cell exhibiting loss of cilia and an increased covering of microvilli.
Lateral epithelial cell junctions were predominantly tight, with only a few examples of loose cell to cell connections. A few epithelial cells still exhibited blebbing on their apical surfaces. The presence of cellular (predominantly lymphocytic) infiltrate within the submucosa was similar to that seen after 12 hours reperfusion, variable in amount, but always present. The smooth muscle cells had a normal appearance. The scanning electron-micrographs showed protruding Clara cells and healthy ciliated cells with only the occasional flat or denuded apical surface of a epithelial cell (Figure 3.6c).

In this study, reperfusion is of the left transplanted lung only. The right lung of the recipient has also undergone an operation and is supplied by the same circulating blood. The airways of the native contra-lateral unpreserved right Lewis lung had a similar appearance to that of control airways in all cases of reperfusion studied.

**Appearance of the alveolar region.**

**After 4, 12 & 48 hours reperfusion.**

After 4 and 12 hours reperfusion, there were still a number of macrophages (with mast cells also present) in the alveolar spaces and some areas also showed cellular infiltrate within the alveolar walls. After 48 hours reperfusion, using light microscopy, alveolar structure appeared relatively normal. Both Type I and II cells appeared similar to the controls. Scavenging cells were still present in the alveolar spaces but there was little or no cellular infiltrate in the alveolar walls. Using electron-microscopy, the intra-cellular structure showed there were a few examples of fusion of lamellar bodies although the nuclei still appeared to be relatively large in comparison with controls. The cells no longer had a flattened appearance. Mitochondrial damage had resolved and the cytoplasm appeared normal (Figure 3.7a).
**Figure 3.1a:** Light photo-micrograph of a bronchiole in the control Lewis lung showing a single layered epithelium of ciliated and Clara cells (toluidene blue).

**Figure 3.1b:** Transmission electron-micrograph of the bronchiolar epithelium in the control Lewis lung showing protruding Clara cells with secretory granules and ciliated cells. Below the continuous elastic lamina in the submucosa there are smooth muscle cells surrounded by collagen.
Figure 3.1a:

--- 30 μm  
\[ E = \text{epithelium}, \; \text{LU} = \text{airway lumen}. \]

Figure 3.1b:

--- 1.5 μm  
\[ \text{Ci} = \text{ciliated cell}, \; \text{CL} = \text{Clara cell}, \; \text{EL} = \text{elastic lamina}, \; \text{SM} = \text{smooth muscle}. \]
**Figure 3.1c:** Scanning electron micrograph of the bronchiolar epithelial surface in the control Lewis lung showing apices of healthy Clara cells protruding between ciliated cells.

**Figure 3.2a:** Transmission electron-micrograph of a bronchiole in the preserved Lewis lung showing a flattened cell which is possibly a Clara cell, with intra-cellular damage and a shrunken nucleus. There is a lymphocyte within the epithelium. The ciliated cells have a relatively normal appearance with some intra-cellular damage.
Figure 3.1c:

--- 1.5μm

Figure 3.2a:

--- 1.5μm

Cl = ciliated cell, CL = Clara cell, SN = shrunken nucleus, L = lymphocyte

60.
Figure 3.2b: Transmission electron-micrograph of the intra-cellular bronchiolar epithelial damage in the preserved Lewis lung showing cytoplasmic clefts, swollen mitochondria, numerous small vesicles and a shrunken nucleus.

Figure 3.2c: Scanning electron-micrograph of the epithelial surface of a bronchiole in the preserved Lewis lung showing shrunken apices of Clara cells between normal cilia.
Figure 3.2b:

CC = cytoplasmic cleft, M = mitochondria, N = nucleus.

--- 0.5 μm

Figure 3.2c:

CL = Clara cell, Ci = ciliated cell.

--- 1.0 μm
Figure 3.3a: Light photo-micrograph of the alveolar region and vein of the control Lewis lung showing normal architecture of Type I and II cells.

Figure 3.3b: Light photo-micrograph of the alveolar region of the preserved Lewis lung showing a similar appearance to the control lung.
Figure 3.3a:

Figure 3.3b:

--- 40μm

AD = alveolar duct, V = vein.

64.
**Figure 3.3c:** Transmission electron-micrograph of the alveolar Type II cell in the control Lewis lung showing intra-cellular lamellar bodies.

**Figure 3.3d:** Transmission electron-micrograph of the alveolar Type II cell of the preserved Lewis lung showing a flattened cell with intra-cellular damage, swollen mitochondria, shrunken nuclei and many small vesicles.
Figure 3.3c:

--- 0.8µm

Figure 3.3d:

--- 0.8µm  LB= lamellar body, M= mitochondria, N= nucleus.

66.
**Figure 3.4a:** Light photo-micrograph of the bronchiolar wall after 4 hours reperfusion in the transplanted Lewis lung showing a flattened uniform epithelium with infiltrate present within the airway submucosa (toluidene blue).

**Figure 3.4b:** Transmission electron-micrograph of the damaged bronchiolar epithelium after 4 hours reperfusion in the transplanted Lewis lung showing a flattened epithelium consisting of unidentified cells and an intact elastic lamina.
Figure 3.4a:

12μm  E= epithelium, LU= airway lumen, SUB= submucosa, IF= infiltrate

Figure 3.4b:

2.0μm  EL= elastic lamina, UN= unidentified cell, BB= basal body.
Figure 3.4c: Transmission electron-micrograph of the damaged bronchiolar epithelium after 4 hours reperfusion in the transplanted Lewis lung showing intracellular damage and loose interdigitation between the epithelial cells.

Figure 3.4d: Scanning electron-micrograph of the bronchiolar epithelial surface after 4 hours reperfusion in the transplanted Lewis lung showing large areas of flattened unidentifiable cells.
Figure 3.4c:  

— 1.5μm  

Li= loose interdigitation, N= nucleus.

Figure 3.4d:  

— 2.5μm  

UN= unidentified cell.
Figure 3.5a: Transmission electron-micrograph of the damaged bronchiolar epithelium after 12 hours reperfusion in the transplanted Lewis lung showing an epithelial cell with no distinguishing features as well as a ciliated cell with few cilia but with basal bodies present in the apical section of the cell.

Figure 3.5b: Scanning electron-micrograph of the bronchiolar epithelium surface after 12 hours reperfusion in the transplanted Lewis lung showing recovery of previously flattened cells during early reperfusion.
Figure 3.5a: 

— 0.8\(\mu\)m

Figure 3.5b:

— 1.0\(\mu\)m

BB = basal body, Ci = ciliated cell, CL = Clara cell, UN = unidentified cell, MV = microvilli.
Figure 3.6a: Light photo-micrograph of a bronchiole after 48 hours reperfusion in the transplanted Lewis lung showing epithelial hyperplasia (toluidene blue).

Figure 3.6b: Transmission electron-micrograph of epithelial hyperplasia after 48 hours reperfusion in the transplanted Lewis lung.
Figure 3.6a:

- $30 \mu m$  $\text{EH}=\text{epithelial hyperplasia}$,  $\text{LU}=\text{airway lumen}$.

Figure 3.6b:

- $1.0 \mu m$  $\text{B}=\text{basal cell}$,  $\text{Cl}=\text{ciliated cell}$.
**Figure 3.6c:** Scanning electron-micrograph of the bronchiolar epithelial surface after 48 hours reperfusion in the transplanted Lewis lung showing healthy protruding Clara cells and a normal ciliated cell surface.

**Figure 3.7a:** Transmission electron-micrograph of the alveolar Type II cell after 48 hours reperfusion in the transplanted Lewis lung showing an appearance similar to controls.
Figure 3.6c:

--- 7.5μm

CL = Clara cell.

--- 0.8μm

LB = lamellar body, M = mitochondria, N = nucleus.

76.
II. Quantitative Studies.

A) Counts of different epithelial cell types using light microscopy.

At the end of the preservation period, the data obtained after 4 and 7 hours preservation for each of the cell types studied were found to be similar by one factor ANOVA tests, (and therefore the data were pooled). Moreover, after both 4 and 12 hours reperfusion, the findings between 4 and 7 hours preservation were also found to be similar by one factor ANOVA tests (and therefore these data were also pooled) (Table A3.1). Thus, 4 groups were formed: control, preservation, 4 and 12 hours reperfusion. A fifth group consisted of lungs reperfused for 48 hours, all the donor lungs in this group having been preserved for 7 hours. Within each of the 5 groups, both left and right lungs were examined. The total number of epithelial, ciliated, Clara, basal and unidentified cells were counted and expressed as the number of cells per 100μm epithelium. One factor ANOVA and Student t-tests were performed. The data are presented as figures in the text and the ANOVA values and data are tabulated in the appendices (Tables A3.2, A3.3 & A3.4).

Total epithelial cell number. In both the TB+1 and the TB airways, the mean total epithelial cell number per 100μm was similar to controls in the donor left lung immediately after preservation and following transplantation after 4, 12 and 48 hours reperfusion (Figures 3.8a &b). Findings were also similar to controls in the native right lung at the same time points.

Clara cells. The number of recognisable Clara cells in the TB+1 airways was significantly less than in controls following preservation and in both the transplanted and native Lewis lungs examined after 4, 12 and 48 hours reperfusion (Figure 3.9a). The reduction in mean Clara cell number was initially significantly greater in the transplanted than in the native right lung.

In the TB airways the number of recognisable Clara cells also decreased significantly in the Lewis lung after preservation and transplantation followed by 12 hours reperfusion. After 48 hours reperfusion, mean Clara cell counts were comparable with controls in both the transplanted left and native Lewis lung (Figure 3.9b).

Ciliated cells. In both the TB+1 and TB airways, mean ciliated cell number was similar to that in the normal lung immediately following preservation and after 4, 12 and 48 hours reperfusion. Findings were similar in the native right lung (Figures 3.10a&b).
Figure 3.8a: Total number of cells per unit length of epithelium in the TB+1 airways.

Number of cells per 100 μm epithelium

Control Preservation 4 hrs 12hrs 48hrs reperfusion

Lewis control
Tx left lung (Lewis)
native right lung (Lewis)

Figure 3.8b: Total number of cells per unit length of epithelium in the TB airways.

Number of cells per 100 μm epithelium

Control Preservation 12hrs 48hrs reperfusion

bars shown are standard errors
Figure 3.9a:
The number of Clara cells per unit length of epithelium in the TB+1 airways.

Figure 3.9b:
The number of Clara cells per unit length of epithelium in the TB airways.
Figure 3.10a:
The number of ciliated cells per unit length of epithelium in the TB+1 airways.

Figure 3.10b:
The number of ciliated cells per unit length of epithelium in the TB airways.
**Basal cells.** Preservation and up to 12 hours of reperfusion had no significant effect on the mean number of basal cells in the TB+1 airways of the transplanted left lung. After 48 hours of reperfusion, however, basal cell number had increased in the transplanted left lung in comparison with cell counts made after preservation and also after preservation followed by 4 and 12 hours reperfusion. There appeared to be more basal cells after 48 hours reperfusion than in controls, but the increase was not statistically significant. Basal cell number in the native right lung was unaffected by preservation followed by 48 hours reperfusion (**Figure 3.11 a**). In the TB airways of the transplanted lung, no basal cells were observed following preservation and after transplantation followed by 12 hours reperfusion which was comparable with control airways in which few or no basal cells were seen. Basal cells were present after 48 hours reperfusion in the transplanted lung but were not observed in the native Lewis lung (**Figure 3.11 b**).

**Unidentified cells.** Preservation of the left lung and preservation followed by reperfusion for up to 12 hours resulted in a statistically significant increase in the number of unidentified cells in the TB+1 airways of the transplanted left lung. After 48 hours reperfusion, the number of unidentified cells was less than after 12 hours but was still greater than controls but not significantly so. Few if any unidentified cells were present in the native right lung as in controls (**Figure 3.12a**). In the TB airways of the transplanted left lung, preservation and preservation followed by reperfusion resulted in an increase in the number of unidentified cells in comparison with controls. Cell number was still elevated after 48 hours reperfusion but not significantly so and was less than at 12 hours. In the native right lung there were no unidentified cells observed in the TB airways after 48 hours reperfusion (**Figure 3.12b**). Unidentified cells were most likely to be both damaged Clara and ciliated cells.

**B) Analysis of Clara cell secretory granules using electron-microscopy.**

The number of secretory granules in each Clara cell was similar after 4 and 7 hours preservation and therefore data were pooled (ANOVA p=0.14). Preservation resulted in a non-statistically significant decrease in the number of secretory granules per Clara cell in comparison with controls (counts: control 11.0 ±1.12, preservation 6.0 ±1.49, respectively). After 4 and 12 hours of reperfusion, Clara cells were severely damaged and therefore no counts were made. However, after 48 hours reperfusion, the number of secretory granules per Clara cell (17 ±3.5) was significantly greater than in the preserved lung but not significantly different from that seen in controls (ANOVA p=0.002) (**Figure 3.13**).
Figure 3.11a:
The number of basal cells per unit length of epithelium in the TB+1 airways.

![Graph showing the number of basal cells per 100μm epithelium across different conditions and time points.

The graph includes bars representing Lewis control, T.x left lung (Lewis), and native right lung (Lewis) conditions.

T-tests:
Compared with 48 hour data: +++ p<0.001

Figure 3.11b:
The number of basal cells per unit length of epithelium of TB airways.

![Graph showing the number of basal cells per 100μm epithelium across different conditions and time points.

The graph includes bars representing Control, Preservation, and reperfusion time points.

T-tests:
Compared with age-matched controls: ** p<0.01

No basal cells were observed after preservation and 12hrs reperfusion.

Bars shown are standard errors.
Figure 3.12a:
The number of unidentified cells per unit length of epithelium in the TB+1 airways.

Figure 3.12b:
The number of unidentified cells per unit length of epithelium in the TB airways.
Figure 3.13:
The number of secretory granules per Clara cell after preservation and reperfusion.

- Lewis control
- T.x left lung (Lewis).

\[ t\text{-}test: \quad ++ \quad p<0.01 \]

Bars shown are standard errors.
III) Summary of Chapter 3.

After preservation, the epithelium of the peripheral airways as shown by light, transmission and scanning electron microscopy was flattened, with particular damage to the Clara cells. These findings were confirmed by the epithelial cell counts: total epithelial, ciliated and basal cell number was unchanged whereas the number of identifiable Clara cells decreased with a corresponding increase in the number of unidentified cells. There was evidence of intra-cellular damage within the airway epithelial and smooth muscle cells. There was some cellular debris in the airway lumen and infiltrate within the submucosa. The alveolar region was less affected and its appearance was similar to that in control lungs, although the Type II cells had a flattened appearance and evidence of intra-cellular damage.

In the transplanted lung, following 4 hours of reperfusion, Clara cells remained damaged. In addition, the ciliated cells had become depleted of cilia, resulting in a flattened indistinguishable epithelial cell layer with loose lateral interdigitations. More cellular infiltrate was present within the airway submucosa and the alveolar walls than at the end of the preservation period. After 12 hours reperfusion, recovery of Clara and ciliated cells was apparent with evidence of ciliogenesis and Clara cells protruding into the lumen. However, Clara cell counts after 12 hours reperfusion were still lower than controls and the number of unidentified cells was still greater than in controls.

After 48 hours reperfusion, the appearance of the peripheral airways of the transplanted lung was similar to that of the controls except for areas of epithelial hyperplasia due to the presence of basal cells which was variable within and between airways. Clara cell number had increased between 12 and 48 hours, although it was still less than in control airways. The number of Clara cell secretory granules was also increased after 48 hours compared with 4 hours reperfusion. Within the airway submucosa, the cellular infiltrate present at 4 hours reperfusion was still evident after 48 hours reperfusion. The alveoli had a normal appearance, as did the Type II cells.

The appearance of the airways of the native right lung was similar to that of the control lungs at all times except for a significant decrease in Clara cell number after transplantation for up to 48 hours.

In conclusion, preservation of the lung resulted in damage to the epithelium of the peripheral airways which became worse after 4 hours of reperfusion. After 48 hours of reperfusion most of this structural damage had resolved but epithelial hyperplasia was apparent.
Chapter 4:
Long-term changes in peripheral airways after single lung transplantation (allogeneic) in adult rats.
I. Qualitative Studies.

Allogeneic single lung transplants were performed, the left lung of young adult BN rats (mean weight 265g) being transplanted into young adult Lewis rats (mean weight 295g). The Lewis rats were immunosuppressed with Cyclosporine continuously following transplantation and were killed at 5, 25 and 100 days post transplantation.

Control airways.
The appearance of the peripheral airways of the normal rat lung have already been described in Chapter 3. There were no differences observed in airway appearance between the control Lewis and BN rats.

Appearance, 5 days post-transplantation.
Five days post transplantation, light microscopic studies on the transplanted BN lung showed that there was lymphocytic infiltration around and within the airway submucosa in the majority of airways (Figure 4.1a). There was no oedema. There were areas of epithelial hyperplasia due to the presence of basal cells. The basement membrane of the airways was intact. The Clara cells of the epithelium seemed less prominent than in control airways. The ciliated cells appeared unaffected. Using electron microscopy, Clara cells looked slightly flattened but appeared to contain a normal number of secretory granules (Figure 4.1b). There also appeared to be an increase in basal cells, giving the epithelium a layered appearance. Epithelial cell junctions were tight and the basement membrane unbroken. Bronchial smooth muscle within the submucosa looked normal. Within the alveolar region, there were a large number of free macrophages in comparison with control BN lungs. If allogeneic transplants were not immunosuppressed peripheral airway structure was destroyed by acute rejection within 5 days following transplantation. Light microscopy showed massive infiltration and destruction of both airways and blood vessels. (Figure 4.1c).

Appearance, 25 days post-transplantation.
Using light microscopy the cellular infiltration of the peripheral airways was still evident 25 days post transplantation in the majority of airways, although it was less than at 5 days post transplantation and it varied in amount between airways. The epithelium was intact, although epithelial cell hyperplasia was still present. Using electron microscopy, the structure of the epithelial cells appeared normal despite an increase in number of basal cells. Cilia were unaffected and Clara cells now had a normal morphology and number of secretory granules. Epithelial cell junctions were tight and the basement membrane unbroken. The submucosa of the airways where lymphocytic infiltration was absent appeared normal. In the alveolar region there was still an increase in number of macrophages in comparison with control lungs, but less than at 5 days post transplantation.
**Figure 4.1a:** Light photo-micrograph of a bronchiole of the transplanted BN lung, 5 days post transplantation showing submucosal infiltrate (toluidene blue).

**Figure 4.1b:** Electron-micrograph of the peripheral airways of the transplanted BN lung, 5 days post transplantation showing a damaged layered epithelium consisting of Clara and ciliated cells reaching the lumen with a basal cell lying along an intact basement membrane.
Figure 4.1a:

![Image of Figure 4.1a]

75μm arrows showing infiltrating cells.

Figure 4.1b:

![Image of Figure 4.1b]

1.5μm B= basal cell, Ci= ciliated cell, CL= Clara cell.
Figure 4.1c: Light photo-micrograph of an acutely rejected lung showing massive infiltrate and destruction of airways and vessels 5 days after allogeneic transplantation with no immunosuppression (toluidene blue).

Figure 4.1d: Electron-micrograph of the peripheral airways of the transplanted BN lung, 100 days post transplantation showing the persistent epithelial layering consisting of Clara, ciliated and basal cells above an intact basement membrane.
Figure 4.1c:

--- 75μm  
Air = airway, V = vessel

Figure 4.1d:

--- 1.5μm  
B = basal cell, Ci = ciliated cell, CL = Clara cell.
Appearance, 100 days post-transplantation.
One hundred days post transplantation, light microscopy showed that the peripheral airways of the transplanted lung were structurally similar to controls, with only a few airways showing significant submucosal infiltrate. Epithelial hyperplasia was still evident in places (Figure 4.1d). The appearance of the alveolar region of the lung was similar to that of the controls.

The airways of the native, contra-lateral, non-transplanted Lewis lung had a normal structure except for epithelial hyperplasia similar to that seen in the transplanted lung, an appearance which was present at 5, 25 and 100 days post transplantation.

II. Quantitative Studies.

A) Analysis of airway structure.
The total airway and the lumen diameter of all airways was measured and a lumen/total airway diameter percentage calculated. In addition, the total airway, lumen and wall area were measured, as described in Chapter 2. Within the wall area of the TB+1 airways, area occupied by the epithelium, connective tissue, muscle and bronchial arteries was measured. The wall area of the TB airways was divided into epithelial and submucosal area only. Each of these airway components were then expressed as a percentage of a) total airway area and b) airway wall area.
Percentage values were normalised by arc-sin conversion to allow statistical analysis of the data (see Chapter 2). All data are summarised in Tables 4.1, 2, 3, 4, 5 & 6 at the end of the chapter. Data were analysed using 2 factor ANOVA tests to determine the effect of both transplantation and age on airway dimensions. ANOVA values from the statistical analysis are tabulated in the appendices (Tables A4.7-10).

Effect of age on airway dimensions and composition in normal rats.
All the young adult animals, control and experimental increased in weight during the study period (Table 2.1, Chapter 2). Therefore it was necessary to compare the morphology of the peripheral airways of control BN and Lewis lungs of the young adult (mean weight: 269g (BN) 287g (Lew)) and mature adult (mean weight 415g (BN) 478g (Lew)) rats.

Control BN Lung:
TB+1 airways: (Tables 4.1, 2 & 3) The mean airway diameter increased with age but the change was not statistically significant and there was no change in the lumen/total airway diameter%. Epithelial height was also unchanged with age. The mean total area
of the TB+1 airways increased with age and this appeared to be due to an increase in the wall area which was itself attributable to an increase in connective tissue area. However, none of these changes were statistically significant (Figure 4.2). Wall/total airway area% increased significantly with age and there was a corresponding decrease in the lumen/total airway area%. Both connective tissue/total airway area% and connective tissue/wall area% increased with age although only the former was statistically significant.

**TB airways:** (Tables 4.4, 5 & 6) There was a slight increase in mean total diameter and airway area but this was not significant (Figure 4.3). There was a significant reduction in the epithelial/airway wall area%. Epithelial height was also reduced although this change was not statistically significant.

**Control Lewis Lung:**

**TB+1 and TB airways:** (Tables 4.1-6) There was no significant change with age in the size of the TB+1 or TB airways or in the relative proportions of the components of the airway wall (Figures 4.4 & 5).

**Effect of transplantation on airway dimensions and composition.**

The effect of transplantation on the morphology of the peripheral airways was examined in the transplanted BN lung and native Lewis lung 5, 25 and 100 days post transplant and compared with controls matched for strain and age.

**Transplanted BN Lung.**

**TB+1 airways:** (Tables 4.1, 2 & 3) In the TB+1 airways of the transplanted BN lung the mean total diameter was normal 5 days post transplantation but was greater than in age-matched controls 25 and 100 days post transplantation, the increase only being statistically significant at 100 days. The mean lumen diameter and lumen/total diameter% were similar to those in control airways at all times post transplantation. The mean total area of the TB+1 airways of the transplanted BN lung was similar to that of age-matched control airways 5 and 25 days post transplantation but had increased significantly 100 days after transplantation (Figure 4.2). Mean lumen area was similar to controls at all times post transplantation and the increase in the total airway area appeared to be due to an increase in the wall area, although this increase was only statistically significant 25 days post transplantation due to wide variation (Figure 4.2). Within the airway wall, mean epithelial area and height were both significantly greater than in age-matched controls 100 days post transplantation and mean bronchial artery area was greater than in controls at 25 and 100 days post transplantation. Mean connective tissue area increased at all times following transplantation compared with controls but was not statistically significant. All other components and proportions of components of the TB+1 airways of the transplanted BN lung were within normal limits.
Thus, 100 days after transplantation, the transplanted lung showed a significant increase in total airway diameter and area, due predominantly to an increase in wall area. The epithelium also increased significantly in area and height.

**TB airways:** (Tables 4.4, 5 & 6) In the TB airways of the transplanted BN lung there was an increase in the mean total diameter at all times post transplantation compared with controls, statistically significant 25 days post transplantation. As in the TB+1 airways 100 days after transplantation, mean wall area increased although the change was not statistically significant but the mean submucosal area did increase significantly (Figure 4.3) and there was a small increase in epithelial area and height, statistically insignificant. All other components and proportions of components of the TB airways of the BN lung were within normal limits after transplantation.

**Native Lewis Lung.**

**TB+1 airways:** (Tables 4.1, 2 & 3) In the TB+1 airways of the native Lewis lung, the mean airway diameter and total airway area were greater than normal for age after the transplantation operation and in general the mean wall, epithelial and submucosal areas were also increased (Figure 4.4). None of these changes were statistically significant. However mean lumen/total airway diameter% was significantly reduced after 100 days. These findings suggest a disproportionate increase in airway wall thickness after transplantation of the contra-lateral lung. The mean epithelial area was significantly greater than normal 5 and 25 days after transplantation and the mean epithelial height was also greater than normal at these times but not significantly so. The epithelial/wall area% was significantly increased 5 and 25 days after transplantation while connective tissue/wall area% decreased.

**TB airways:** (Table 4.4, 5 & 6) In the TB airways of the native Lewis lung, the mean area of total airway, wall and submucosa were within normal limits following transplantation (Figure 4.5). There was a significant decrease in the mean lumen/total airway area% 5 and 100 days post transplantation compared with controls with a corresponding increase in the mean wall/total airway area%. The epithelial/total airway area% was greater following transplantation but the change was not statistically significant. Within the wall, the proportions of the different components did not change following the transplantation operation.
Figure 4.2: TB+1 airways of the control and transplanted BN lung

**Total Airway Area**

- Young adult control
- 5 days post Tx
- 25 days post Tx
- Mature adult control
- 100 days post Tx

**Airway Wall Area**

- Young adult control
- 5 days post Tx
- 25 days post Tx
- Mature adult control
- 100 days post Tx

**Airway Connective Tissue Area**

- Young adult control
- 5 days post Tx
- 25 days post Tx
- Mature adult control
- 100 days post Tx

* t-tests: Compared with age-matched controls:
  * p< 0.05

bars shown are standard errors

95.
Figure 4.3: TB airways of the control and transplanted BN lung

Total Airway Area

μm² x 10⁴

Young adult control 5 days post Tx 25 days post Tx Mature adult control 100 days post Tx

Airway Wall Area

μm² x 10⁴

Young adult control 5 days post Tx 25 days post Tx Mature adult control 100 days post Tx

Airway Submucosal Area

μm² x 10⁴

Young adult control 5 days post Tx 25 days post Tx Mature adult control 100 days post Tx

T-tests: Compared with age-matched controls: * p< 0.05 bars shown are standard errors

96.
Figure 4.4: TB+1 airways of the control and native Lewis lung

Total Airway Area

Airway Wall Area

Airway Connective Tissue Area

Bars shown are standard errors.
Figure 4.5: TB airways of the control and native Lewis lung

Total Airway Area

Airway Wall Area

Airway Submucosal Area

bars shown are standard errors

98.
B) Counts of different epithelial cell types using light microscopy.

Cell counts were made of the total number of epithelial, ciliated, Clara and basal cells per unit length of epithelium in both TB+1 and TB airways. Control airways were examined for the effect of age over the 100 days time scale of the present study. The data were subjected to the two factor ANOVA test, as it was possible that epithelial cell number could be affected by age as well as transplantation. Cell counts are presented as Figures 4.6-13 in the text and these data and the ANOVA values are in the appendices (Table A4.11-13).

**Total epithelial cell number.** In the normal animals, mean total epithelial cell number per unit length epithelium in the peripheral airways was unchanged with age in both the BN and Lewis control lungs. Comparing the two strains of rats, the total number of epithelial cells per unit length epithelium was greater in the BN than the Lewis TB+1 airways in the mature adult rats (Figure 4.6).

In the TB+1 airways of the transplanted BN left lung, the mean total number of epithelial cells was slightly but not significantly greater than in age-matched controls at all times post transplantation. In the TB+1 airways of the native Lewis lung, total epithelial cell number was similar to controls 5 and 25 days post transplantation but one hundred days post transplantation epithelial cell number was significantly greater than age-matched controls and also greater than in animals 5 days post transplantation (Figure 4.6).

In the TB airways of the transplanted BN left lung, the mean total epithelial cell number appeared to have increased at all times post transplantation but the increase was only statistically significant 100 days after transplantation. In the TB airways of the native Lewis lung, total epithelial cell number was similar to controls at all times (Figure 4.7).

**Clara cells.** In the normal animals, mean Clara cell number in the peripheral airways was unchanged with age in the BN and Lewis lung. Comparing BN to Lewis lungs the number of Clara cells was greater in the BN than the Lewis TB+1 airways in both the young and mature adult control rats but was similar in the TB airways (Figure 4.8).

In the TB+1 airways of the transplanted BN left lung, mean Clara cell number was significantly decreased 5 and 100 days post transplantation. In the TB+1 airways of the native Lewis lung, Clara cell number was unchanged by transplantation (Figure 4.8). There was no significant difference in Clara cell number in the TB airways of either the transplanted BN or native Lewis lung at any time after transplantation compared with the age-matched control airways (Figure 4.9).
Figure 4.6:
Total number of cells per unit length of epithelium in the TB+1 airways.

Figure 4.7:
Total number of cells per unit length of epithelium in the TB airways.
Figure 4.8: The number of Clara cells per unit length of epithelium in the TB+1 airways.

Figure 4.9: The number of Clara cells per unit length of epithelium in the TB airways.
Ciliated cells. In the normal animals, mean ciliated cell number per unit length epithelium did not change with age in the peripheral airways in either the BN or Lewis rats. There was also no difference between the two strains of rats. The number of ciliated cells of the TB+1 airways was within normal limits in both the transplanted BN and native Lewis lung at all times. However, the number of ciliated cells was significantly lower in the transplanted BN lung 5 days post transplant compared with 100 days post transplant and was also significantly lower in the native Lewis lung 5 and 25 days post transplant compared with 100 days post transplant (Figure 4.10). The mean number of ciliated cells in the TB airways of both the transplanted BN left and recipient Lewis lung was greater than in controls at all times post transplantation but none of these changes were statistically significant. (Figure 4.11).

Basal cells. In the normal animals, mean basal cell number was similar in both the TB+1 and TB airways in the young and mature adult control BN and Lewis lung. There was no difference in number between the two strains. In the TB+1 airways of the transplanted BN and native Lewis lung, there was a significant increase in basal cells at all times post transplantation in comparison with controls (Figure 4.12). There was an increase in basal cell number in the TB airways of the transplanted BN lung at all times compared with controls, statistically significant 25 and 100 days post transplantation. In the TB airways of the native Lewis lung, mean basal cell number was greater than in controls at all times post transplantation but the changes were not statistically significant (Figure 4.13).

C) Analysis of Clara cell secretory granules using electron-microscopy.

Clara cell secretory granule number was counted only in the TB+1 airways of the transplanted BN lung. There was no significant change in mean secretory granule number per Clara cell with age or following transplantation although in both cases the number decreased (Figure 4.14). The two factor ANOVA test showed that neither age nor transplantation independently was significant at a 95% confidence interval. However, there was a significant interaction between the two factors (ANOVA T.x p = 0.08, age p = 0.17, interaction p = 0.03). Thus the decrease in mean number of secretory granules per Clara cell after transplantation was due to both age and transplantation.
**Figure 4.10:**
The number of ciliated cells per unit length of epithelium in the TB+1 airways.

<table>
<thead>
<tr>
<th></th>
<th>Young adult controls</th>
<th>5 days post T.x.</th>
<th>25 days post T.x.</th>
<th>Mature adult controls</th>
<th>100 days post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ciliated cells per 100μm epithelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young adult controls</td>
<td>5 days post T.x.</td>
<td>25 days post T.x.</td>
<td>Mature adult controls</td>
<td>100 days post T.x.</td>
<td></td>
</tr>
<tr>
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<tr>
<td>t-tests:</td>
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<td></td>
</tr>
<tr>
<td>Comparison between 5 &amp; 100 days transplant data:</td>
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<td></td>
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</tr>
<tr>
<td>+</td>
<td>p&lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>p&lt;0.01</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Comparison between 25 and 100 days transplant data:</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>+++</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bars shown are standard errors.*

**Figure 4.11:**
The number of ciliated cells per unit length of epithelium in the TB airways.

<table>
<thead>
<tr>
<th></th>
<th>Young adult controls</th>
<th>5 days post T.x.</th>
<th>25 days post T.x.</th>
<th>Mature adult controls</th>
<th>100 days post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ciliated cells per 100μm epithelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young adult controls</td>
<td>5 days post T.x.</td>
<td>25 days post T.x.</td>
<td>Mature adult controls</td>
<td>100 days post T.x.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bars shown are standard errors.*
Figure 4.12:
The number of basal cells per unit length of epithelium in the TB+1 airways.

Figure 4.13:
The number of basal cells per unit length of epithelium in the TB airways.

**t-tests:**
Comparison with age-matched controls:
* $p<0.01$
** $p<0.01$

**t-tests:**
Comparison with age-matched controls:
* $p<0.05$
Figure 4.14:
The number of secretory granules per Clara cell in the TB+1 airways of the transplanted BN lung.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>Controls</th>
<th>Post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult controls</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young adult controls</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature adult controls</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bars shown are standard errors

- Control BN lung
- T.x left lung (BN)
III) Summary of Chapter 4

Five days following transplantation, cellular infiltrate was present within and around the submucosa of the peripheral airways of the transplanted BN lung and there was an increase in macrophages within the alveolar region. These were still present at 25 days but to a lesser extent. One hundred days post transplantation, this infiltrate had resolved.

Airway structure was similar to control airways in both the transplanted BN and native Lewis lung following transplantation except for the presence of epithelial hyperplasia at all times following transplantation which was due to the presence of basal cells, variable within and between airways. The Clara cells were less prominent in the transplanted lung on the electron-micrographs than in controls and their number per unit length epithelium decreased following transplantation. Clara cell number in the native Lewis lung was similar to controls at all times post transplantation. Ciliated cells were unaffected following transplantation, although 100 days post transplant the number of ciliated cells was greater than controls in both the transplanted and native non-transplanted lung but not significantly so.

In the control BN lung, peripheral airway size increased with age between young and mature adult rats as expected, however following transplantation of the BN lung there was a further increase in airway size. In the control Lewis lung, peripheral airway size was similar between young and mature adult rats and although there was an increase in these airways of the non-transplanted Lewis lung following transplantation compared to controls, it was not statistically significant. This increase in airway size in both the transplanted BN and the native non-transplanted Lewis lung was due to a proportional increase in all airway components.
Table 4.1: Airway dimensions and component areas of the TB+1 airways in controls and after transplantation.

(Mean (X) +/- SE)

<table>
<thead>
<tr>
<th>Airway Dimensions</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 DAYS POST T.x.</th>
<th>25 DAYS POST T.x.</th>
<th>100 DAYS POST T.x.</th>
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</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>T.x BN Lung</td>
<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
</tr>
<tr>
<td>μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>6.0</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>T.x BN Lung</td>
<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
</tr>
<tr>
<td>μm</td>
<td></td>
<td></td>
<td></td>
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<td>8.5</td>
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<td></td>
<td></td>
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<td>T.x BN Lung</td>
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<td>T.x BN Lung</td>
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<td></td>
<td></td>
<td>T.x BN Lung</td>
<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
</tr>
<tr>
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<td>T.x BN Lung</td>
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<td>* 9.3</td>
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<td></td>
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<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
</tr>
<tr>
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<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
</tr>
<tr>
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<td>†12</td>
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<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
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<td>1.2</td>
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<td></td>
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<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
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<td></td>
<td></td>
<td>T.x BN Lung</td>
<td>T.x BN Native Lung</td>
<td>T.x BN Lung</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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</tr>
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<td>0.02</td>
<td>0.4</td>
<td>**0.8</td>
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<td>0.005</td>
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<td>0.00</td>
<td>0.001</td>
</tr>
</tbody>
</table>
| t-tests: Comparison with age matched controls of the same strain.  
* p<0.05  
** p<0.01  
*** p<0.001  
Comparison with 100 days post transplant data  
† p<0.05  

107.
Table 4.2: The airway components expressed as a percentage of the total airway area in the TB+1 airways in controls and after transplantation (Mean(X±SE)).

<table>
<thead>
<tr>
<th>% component of total airway area</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 DAYS POST T.x. T.x. BN Lung</th>
<th>Native Lewis Lung</th>
<th>T.x BN Lung</th>
<th>Native Lewis Lung</th>
<th>T.x BN Lung</th>
<th>Native Lewis Lung</th>
<th>100 DAYS POST T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area</td>
<td>X</td>
<td>37%</td>
<td>▲27%</td>
<td>39%</td>
<td>34%</td>
<td>33%</td>
<td>30%</td>
<td>31%</td>
<td>31%</td>
<td>38%</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1%</td>
<td>2%</td>
<td>1%</td>
<td>1.5%</td>
<td>0.5%</td>
<td>2.5%</td>
<td>3.5%</td>
<td>3.5%</td>
<td>0.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Wall area</td>
<td>X</td>
<td>63%</td>
<td>▲73%</td>
<td>61%</td>
<td>66%</td>
<td>67%</td>
<td>70%</td>
<td>69%</td>
<td>62%</td>
<td>77%</td>
<td>59%</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1%</td>
<td>2%</td>
<td>1.5%</td>
<td>1.5%</td>
<td>0.5%</td>
<td>2.5%</td>
<td>4%</td>
<td>0.5%</td>
<td>2.2%</td>
<td>2%</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>X</td>
<td>17%</td>
<td>11%</td>
<td>14%</td>
<td>19%</td>
<td>14%</td>
<td>26%</td>
<td>17%</td>
<td>18%</td>
<td>15%</td>
<td>19%</td>
</tr>
<tr>
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<td>2%</td>
<td>0.5%</td>
<td>1%</td>
<td>4%</td>
<td>1%</td>
<td>0.5%</td>
<td>2%</td>
<td>0.5%</td>
<td>2%</td>
<td>1%</td>
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<tr>
<td>Connective tissue area</td>
<td>X</td>
<td>37%</td>
<td>▲52%</td>
<td>46%</td>
<td>44%</td>
<td>42%</td>
<td>43%</td>
<td>36%</td>
<td>41%</td>
<td>49%</td>
<td>*35%</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>4%</td>
<td>2%</td>
<td>1%</td>
<td>0.5%</td>
<td>4%</td>
<td>1%</td>
<td>8%</td>
<td>5%</td>
<td>4%</td>
<td>1%</td>
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<tr>
<td>Muscle area</td>
<td>X</td>
<td>8%</td>
<td>9%</td>
<td>3%</td>
<td>2%</td>
<td>9%</td>
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<td>11%</td>
<td>3%</td>
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<td>0.5%</td>
<td>0.1%</td>
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<td>0.5%</td>
<td>0.5%</td>
<td>0.8%</td>
<td>0.15%</td>
<td>1%</td>
<td>0.2%</td>
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<tr>
<td>Bronchial artery area</td>
<td>X</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>2%</td>
<td>2%</td>
<td>0.5%</td>
<td>5%</td>
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<td>5%</td>
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<td>SE</td>
<td>0.7%</td>
<td>1.2%</td>
<td>0.15%</td>
<td>0.05%</td>
<td>0.2%</td>
<td>0.15%</td>
<td>0.6</td>
<td>0.15%</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

t-tests: Age comparison between controls of the same strain.
▲ p≤0.05
Comparison with age matched controls.
* p≤0.05

108.
Table 4.3:
The airway components expressed as a percentage of the airway wall area in the TB+1 airways in controls and after transplantation (Mean(X)+/-SE).

<table>
<thead>
<tr>
<th>Component of wall area</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 DAYS POST T.x. T.x BN Lung</th>
<th>25 DAYS POST T.x. Native Lewis Lung</th>
<th>100 DAYS POST T.x. T.x BN Lung</th>
<th>25 DAYS POST T.x. Native Lewis Lung</th>
<th>100 DAYS POST T.x. Native Lewis Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td>29% ± 5%</td>
<td>16% ± 0.6%</td>
<td>22% ± 0.25%</td>
<td>29% ± 3.3%</td>
<td>22% ± 2%</td>
<td>27% ± 1%</td>
<td>27% ± 6%</td>
<td>29% ± 0.5%</td>
<td>3% ± 35%</td>
</tr>
<tr>
<td>C'tive tissue area</td>
<td>57% ± 1%</td>
<td>71% ± 1.5%</td>
<td>74% ± 1%</td>
<td>74% ± 0.2%</td>
<td>67% ± 4%</td>
<td>61% ± 6%</td>
<td>48% ± 1%</td>
<td>67% ± 9%</td>
<td>64% ± 4%</td>
</tr>
<tr>
<td>Muscle area</td>
<td>13% ± 1%</td>
<td>12% ± 1%</td>
<td>3.5% ± 0.3%</td>
<td>3% ± 0.1%</td>
<td>14% ± 1%</td>
<td>3.3% ± 0.5%</td>
<td>18% ± 1.6%</td>
<td>4% ± 0.25%</td>
<td>10% ± 1.8%</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>2% ± 1.5%</td>
<td>1.5% ± 1%</td>
<td>2.7% ± 0.2%</td>
<td>3.2% ± 0.1%</td>
<td>3.2% ± 0.4%</td>
<td>0.7% ± 0.3%</td>
<td>8.5% ± 0.1%</td>
<td>3.7% ± 0.5%</td>
<td>4% ± 3%</td>
</tr>
</tbody>
</table>

T-tests: Comparison with age matched controls.
* p<0.05  
** p<0.01
Table 4.4: Airway dimensions and component areas of the TB airways in controls and after transplantation. (Mean(x)+/-SE).

<table>
<thead>
<tr>
<th>Component</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 DAYS POST T.x. T.x. BN Lung</th>
<th>Native Lewis Lung</th>
<th>25 DAYS POST T.x. T.x. BN Lung</th>
<th>Native Lewis Lung</th>
<th>100 DAYS POST T.x. T.x. BN Lung</th>
<th>Native Lewis Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total airway diameter</td>
<td>69</td>
<td>90</td>
<td>86</td>
<td>83</td>
<td>81</td>
<td>12.0</td>
<td>7.7</td>
<td>99 **120</td>
<td>100</td>
<td>118</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>47</td>
<td>56</td>
<td>60</td>
<td>64</td>
<td>46</td>
<td>6.2</td>
<td>68</td>
<td>63 **100</td>
<td>65</td>
<td>77</td>
</tr>
<tr>
<td>Lumen/Total diameter %</td>
<td>68</td>
<td>62</td>
<td>70</td>
<td>78</td>
<td>57</td>
<td>2.1</td>
<td>4.6</td>
<td>2.5</td>
<td>2.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Total airway area</td>
<td>3.3</td>
<td>5.4</td>
<td>4.2</td>
<td>4.4</td>
<td>3.4</td>
<td>0.5</td>
<td>0.3</td>
<td>0.7</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Lumen area</td>
<td>1.7</td>
<td>1.6</td>
<td>2.4</td>
<td>2.8</td>
<td>1.4</td>
<td>0.3</td>
<td>1.9</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Wall area</td>
<td>1.6</td>
<td>2.0</td>
<td>1.9</td>
<td>1.6</td>
<td>1.9</td>
<td>0.3</td>
<td>2.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>0.63</td>
<td>0.54</td>
<td>0.68</td>
<td>0.77</td>
<td>0.7</td>
<td>0.1</td>
<td>0.74</td>
<td>0.4</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>6.0</td>
<td>4.5</td>
<td>8.0</td>
<td>5.0</td>
<td>6.0</td>
<td>1.0</td>
<td>7.0</td>
<td>0.9</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>0.8</td>
<td>1.1</td>
<td>0.2</td>
<td>1.4</td>
<td>0.2</td>
<td>1.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

- t-tests: comparison with age-matched controls.
  - * p≤0.05
  - ** p≤0.01
**Table 4.5:**
The airway components expressed as a percentage of the total airway area of the TB airways in controls and after transplantation (Mean (X)±/SE).

<table>
<thead>
<tr>
<th></th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 DAYS POST T.x.</th>
<th>25 DAYS POST T.x.</th>
<th>100 DAYS POST T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>% component of total area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen area</td>
<td>49% ±0.6%</td>
<td>51% ±3%</td>
<td>55% ±0.5%</td>
<td>64% ±1.5%</td>
<td>46% ±2.8%</td>
<td>48% ±2.8%</td>
<td>55% ±0.5%</td>
</tr>
<tr>
<td>Wall area</td>
<td>51% ±0.6%</td>
<td>49% ±7.6%</td>
<td>45% ±0.5%</td>
<td>36% ±1.5%</td>
<td>58% ±2.8%</td>
<td>52% ±2.8%</td>
<td>45% ±3%</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>20% ±1%</td>
<td>19% ±2.7%</td>
<td>16% ±2.9%</td>
<td>18% ±0.2%</td>
<td>21% ±0.5%</td>
<td>19% ±0.1%</td>
<td>15% ±0.2%</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>31% ±0.3%</td>
<td>30% ±5%</td>
<td>28% ±4%</td>
<td>18% ±1.5%</td>
<td>31% ±1.8%</td>
<td>33% ±0.1%</td>
<td>33% ±1.3%</td>
</tr>
</tbody>
</table>

* t-tests: Comparison with age-matched controls.
  * p<0.05
  ** p<0.01

**Table 4.6:**
The airway components expressed as a percentage of the airway wall area in the TB airways in controls and after transplantation (Mean (X)±/SE).

<table>
<thead>
<tr>
<th></th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 DAYS POST T.x.</th>
<th>25 DAYS POST T.x.</th>
<th>100 DAYS POST T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>% component of wall area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial area</td>
<td>39% ±1%</td>
<td>▲27% ±2.7%</td>
<td>52% ±11%</td>
<td>37% ±1.6%</td>
<td>45% ±1.9%</td>
<td>37% ±1.6%</td>
<td>35% ±0.5%</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>61% ±1%</td>
<td>▲73% ±6%</td>
<td>48% ±11%</td>
<td>63% ±1.6%</td>
<td>55% ±2%</td>
<td>63% ±0.2%</td>
<td>65% ±0.5%</td>
</tr>
</tbody>
</table>

* t-tests: Age comparison between controls of the same strain.
  ▲ p≤0.05

111.
Chapter 5: 
The long-term changes in peripheral airways after single lung transplantation (isogeneic & allogeneic) in immature rats.
Isogeneic transplants: Lewis-Lewis.

I. Qualitative studies.

Single lung transplants were performed, the left lungs of immature Lewis rats (mean weight 168g) were transplanted into immature Lewis rats (mean weight 179g).

Appearance, 14 days post-transplantation.

Fourteen days after transplantation, the peripheral airways of the transplanted Lewis lung had a similar appearance to control airways as seen by light microscopy. The epithelium was intact with protruding Clara cells and healthy ciliated cells, although there were areas of cell hyperplasia within the airway epithelium. Electron microscopic examination of the airways revealed normal epithelial cell ultrastructure with an apparent increase in the number of basal cells (Figure 5.1a). The submucosa of the airways was normal with little or no evidence of cellular infiltrate. Muscle and connective tissue ultra-structure was normal.

The alveolar region was also normal in appearance when compared with control lungs.

Appearance, 6 months post-transplantation.

Six months after transplantation, the appearance of the peripheral airways of the transplanted lung by light microscopy was similar to the appearance after 14 days. There were still some areas of epithelial hyperplasia as a result of an increase in basal cells within the majority of airways. There was little or no evidence of infiltrate within the airway walls. Electron microscopic examination of the peripheral airways confirmed the light microscopic observations (Figure 5.1b).

The airways of the native right Lewis lung at both times were similar to that of the control Lewis lung except for epithelial hyperplasia due to the presence of basal cells which was present at all times post transplantation.
Figure 5.1a: Electron-micrograph of the peripheral airways of the transplanted immature Lewis lung, 14 days post transplantation, showing a layered epithelium consisting of ciliated and basal cells overlying an intact basement membrane, below which is the elastic lamina.

Figure 5.1b: Electron-micrograph of the peripheral airways of the transplanted immature Lewis lung, 6 months post transplantation, showing a layered epithelium consisting of Clara, ciliated and basal cells similar to that seen 14 days post transplantation. Below the epithelium the basement membrane and elastic lamina are intact and within the submucosa, muscle cells can be seen.
Figure 5.1a:
--- 1.0μm

Figure 5.1b:
--- 1μm

B= basal cell, Ci= ciliated cell, CL= Clara cell, BM= basement membrane, EL= elastic lamina, MC= muscle cell.
II. Quantitative Studies.

A) Analysis of airway structure.

Airway dimensions and component areas were measured and percentage values were calculated and normalised by arc-sin conversion to allow statistical analysis of the data (see Chapter 2). Firstly, one factor ANOVAs were performed between the control data to determine the effect of age and then two factor ANOVAs were performed between transplant and age-matched control data to determine the effect of both transplantation and age on airway dimensions and composition. P values for age comparisons calculated from two factor ANOVAs were only quoted if they provided additional information about the changes in airway dimensions.

The young and mature adult control rats for both the immature isogeneic and allogeneic transplant data are different animals from those used in the young adult transplants (Chapter 4) because of the method of tissue fixation (see Chapter 2) which may influence the results of quantitative analysis of airway structures.

All data are summarised in Tables 5.1, 2, 3, 4, 5 & 6 at the end of the chapter. Total airway, wall and lumen areas of both TB+1 and TB airways are presented in Figures 5.2-5. ANOVA values from the statistical analysis are tabulated in the appendices (Tables A5.7-10).

Effect of age on airway dimensions and composition in normal Lewis rats.

All immature animals, control and experimental increased in weight during the study period (Table 2.1, Chapter 2). Therefore it was necessary to compare the morphology of the peripheral airways of the control Lewis lungs of immature (mean weight 92g), young adult (mean weight 215g) and mature adult (mean weight 467g) Lewis rats. Young adult rats were used as controls for the rats killed at 14 days following transplantation and the mature adult rats were used as controls for the rats killed at 6 months following transplantation.

Control Lewis Lung.

TB+1 airways: (Tables 5.1, 2 & 3) In the TB+1 airways, there was an increase in both the mean total airway and lumen diameter between the immature and young adult control animals (t-test: p<0.001 for both). The mean total airway and lumen diameter of the TB+1 airways of the young and mature adult control animals were similar. There was also a significant increase in the mean total airway area between the immature and young adult rats and between the immature and mature adult rats. This increase in total airway area was due to an increase in lumen area, which
increased significantly between the immature and young adult rats. There was an increase in mean airway wall area with age but this increase was not statistically significant (Figure 5.2). However, there was a significant decrease in the mean bronchial artery/wall area% between the immature and mature control animals.

**TB airways:** (Tables 5.4, 5 & 6) In the TB airways, there was a significant increase in both the mean total airway and lumen diameter between the immature and young adult control airways. The mean total airway and lumen airway diameter of the TB airways of the young and mature adult control animals were similar. There were no other significant changes in composition or proportions of the TB airways of the control Lewis lung (Figure 5.3) except for a significant increase in mean epithelial area between the TB airways of the immature and mature adult control Lewis rats.

### Effect of Transplantation on airway dimensions and composition.

The effect of transplantation on the morphology of the peripheral airways was examined by looking at the transplanted and native Lewis lung 14 days and 6 months post transplantation.

**Transplanted Lewis Lung.**

**TB+1 airways:** (Tables 5.1, 2 & 3) In the TB+1 airways of the transplanted Lewis lung there was an increase in both the mean total airway and lumen diameter 14 days and 6 months post transplantation compared to control airways. However these increases were not statistically significant. There was a significant increase in mean total airway area 14 days and 6 months post transplantation probably due to an increase in lumen area 14 days and 6 months post transplantation as well as a significant increase in mean wall area 14 days post transplantation. The mean wall area was still greater than in controls 6 months post transplantation but not significantly so (Figure 5.2). Within the wall area, the mean epithelial, connective tissue and muscle area of the TB+1 airways had increased 14 days post transplantation but was not significantly different from control airways 6 months post transplantation. There was no other significant changes in the composition and component proportions of the TB+1 airways of the transplanted Lewis lung except for a decrease in the mean epithelial/total airway area% 6 months post transplantation compared with controls of the same age.

**TB airways.** (Tables 5.4, 5 & 6) In the TB airways of the transplanted Lewis lung there was an increase in both the mean total airway and lumen diameter 14 days and 6 months post transplantation compared to control airways although this increase was only statistically significant 6 months post transplantation. There was also an increase in the mean total airway area and wall area 14 days and 6 months post transplantation compared with control airways. This seemed to be to due to the submucosa in which
the mean area had increased significantly 14 days post transplantation. The mean lumen area was similar to control airways 14 days post transplantation but had increased 6 months post transplantation (Figure 5.3). Mean epithelial height had increased significantly compared with control airways 14 days post transplantation but at 6 months post transplantation was similar to controls. There were no other significant changes in the composition and component proportions of the TB airways of the transplanted Lewis lung.

Native Lewis Lung.

**TB+1 airways.** (Tables 5.1, 2 & 3) In the TB+1 airways of the native Lewis lung both mean total airway and lumen diameter increased compared with age-matched controls following transplantation but were not statistically significant. There was a significant increase in total airway area 14 days post transplantation compared with controls. This appeared to due to the significant increase in the mean lumen and wall area 14 days post transplantation (Figure 5.4). Within the airway wall, the mean connective tissue and muscle area had increased significantly 14 days post transplantation compared with control airways. The mean epithelial area had also increased 14 days post transplantation but this increase was not statistically significant. The mean epithelial/wall area% had significantly decreased 14 days post transplantation and the mean epithelial/total airway area% decreased 6 months post transplantation compared with control airways due to a greater increase in other wall area components. There were no other significant changes in the composition and component proportions in the TB+1 airways of the native Lewis lung following transplantation compared with controls.

**TB airways.** (Tables 5.4, 5 & 6) The TB airways of the native Lewis lung showed a transient and non-significant increase in the total airway and lumen diameter 14 days post transplantation compared with control airways. There was a decrease in the lumen/total airway diameter percentage both 14 days and 6 months post transplantation compared with control airways which appeared to be due to an increase in lumen and wall area following transplantation. However none of the above changes were statistically significant (Figure 5.5). Within the airway wall, there was a transient significant increase in epithelial area and submucosal area 14 days post transplantation compared with control airways. The epithelial/total airway area%, epithelial/wall area% decreased and the submucosa/wall area% increased 6 months post transplantation compared with control airways.
Figure 5.2: TB+1 airways of the control and transplanted Lewis lung. 

**Total Airway Area**

- Immature Lewis Control
- Young adult Control
- Mature adult Control
- 14 days post T.x.
- 6 months post T.x.

**Immature**

- 14 days

**Young**

- 6 months

**Mature**

- Lewis adult

- Adult control

### Airway Wall Area

- Immature Lewis Control
- Young adult Control
- Mature adult Control
- 14 days post T.x.
- 6 months post T.x.

### Lumen Area

- Immature Lewis Control
- Young adult Control
- Mature adult Control
- 14 days post T.x.
- 6 months post T.x.

**t-tests:**

- Compared with age-matched controls:
  - * p<0.05
  - ** p<0.01
  - *** p<0.001

- t-tests between controls not shown

Bars shown are standard errors.

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Figure 5.3: TB airways of the control and transplanted Lewis lung.

Total Airway Area

Airway Wall Area

Lumen Area

bars shown are standard errors

$t$-tests:
Compared with age-matched controls:
* $p<0.05$
*** $p<0.001$
Figure 5.4: TB+1 airways of controls and the native Lewis lung.

Total Airway Area

Airway Wall Area

Lumen Area

t-tests:
Compared with age-matched controls:
* p<0.05
** p<0.01
bars shown are standard errors
Figure 5.5: TB airways of the control and native Lewis lung.

**Total Airway Area**

![Bar chart showing total airway area for young adult and mature adult control, 14 days and 6 months post T.x.](image)

**Airway Wall Area**

![Bar chart showing airway wall area for young adult and mature adult control, 14 days and 6 months post T.x.](image)

**Lumen Area**

![Bar chart showing lumen area for young adult and mature adult control, 14 days and 6 months post T.x.](image)

Bars shown are standard errors.
B) Counts of different epithelial cell types using light microscopy.

Cell counts were of total epithelial, Clara, ciliated and basal cell number per unit length epithelium. The data were subjected to the two factor ANOVA test, as it was possible that epithelial cell number could be affected by age as well as transplantation. The data is presented as Figures 5.6-13 in the text and tabulated in the appendices (Tables A5.11 & 12). ANOVA values for these data are also tabulated in the appendices (Table A5.13).

**Total epithelial cell number.** Mean total epithelial cell number per unit length epithelium in both the TB+1 and TB airways was unchanged by age. Mean total number per unit length in the TB+1 airways of the transplanted Lewis lung 14 days and 6 months post transplantation was greater than controls but this increase was only statistically significant 6 months post transplantation (Figure 5.6). There was a statistically significant increase in the TB airways of the transplanted Lewis lung 14 days post transplant but by 6 months post transplantation the number was similar to that in control airways. In the TB and TB+1 airways of the native Lewis lung, there was no significant change in mean total epithelial cell number after transplantation (Figure 5.7).

**Clara cells.** Clara cell number did not change with age in either the TB+1 or TB airways in the normal Lewis rat. In the TB+1 and TB airways of the transplanted and native Lewis lung, there was no significant change in Clara cell number following transplantation. (Figures 5.8 &9).

**Ciliated cells.** Ciliated cell number was unchanged with age in the TB+1 airways of the control Lewis lung (Figure 5.10), but there was a significant increase in ciliated cell number with age in the TB airways (Figure 5.11). There was no significant change in ciliated cell number in the TB+1 airways of the transplanted and native Lewis lung compared with control airways (Figure 5.10). The TB airways of the transplanted Lewis lung, did show a significant increase in ciliated cell number 14 days post transplantation but by 6 months post transplant, numbers were similar to controls. In TB airways of the native Lewis lung, ciliated cell number was unchanged by transplantation (Figure 5.11).
Figure 5.6: Total epithelial cell number per unit length of epithelium in the TB+1 airways.

Total number of epithelial cells per 100µm epithelium.

Immature Lewis control 14 days post T.x Mature Lewis control

Immature Lewis control 14 days post T.x Mature Lewis control

Figure 5.7: Total number of epithelial cells per unit length of epithelium in the TB airways.

Total number of epithelial cells per 100µm epithelium

Immature Lewis control 14 days post T.x Mature Lewis control

Immature Lewis control 14 days post T.x Mature Lewis control

t-tests:
* p<0.05
*** p<0.001

bars shown are standard errors.

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Figure 5.8:
The number of Clara cells per unit length of epithelium in the TB+1 airways.

Figure 5.9:
The number of Clara cells per unit length of epithelium in the TB airways.

Bars shown are standard errors.
Figure 5.10:
The number of ciliated cells per unit length of epithelium in the TB+1 airways.

![Graph showing the number of ciliated cells per 100μm epithelium with bars for immature Lewis control, 14 days post T.x., mature Lewis control, and 6 months post T.x.]

Figure 5.11:
The number of ciliated cells per unit length of epithelium in the TB airways.

![Graph showing the number of ciliated cells per 100μm epithelium with bars for immature Lewis control, 14 days post T.x., mature Lewis control, and 6 months post T.x.]

t-tests:
* p<0.05
** p<0.01
*** p<0.001

bars shown are standard errors
**Basal cells.** Basal cell number was unchanged by age in both the TB+1 and TB airways (Figures 5.12 & 13). There was an increase in basal cell number in the TB+1 airways of both the transplanted Lewis and native Lewis lung compared with control airways at both 14 days and 6 months post transplantation but the increase 6 months post transplantation in the native Lewis lung was not statistically significant (Figure 5.12). In the TB airways of the transplanted Lewis lung, there was a significant increase in basal cell number at both 14 days and 6 months post transplantation compared with control airways. In the TB airways of the native Lewis lung, basal cell number was similar to controls 14 days and 6 months post transplantation (Figure 5.13).

**C) Analysis of Clara cell secretory granules using electron-microscopy**

Clara cell secretory granule number was counted in the TB+1 airways of the transplanted and control Lewis lung only. Data were subjected to the two factor ANOVA test to examine the effect of both transplantation and age on secretory granule number in Clara cells. Age had no effect on mean secretory granule number per Clara cell in the control Lewis TB+1 airways (ANOVA p= 0.77). There was a significant increase in mean secretory granule number in Clara cells after both 14 days and 6 months post transplantation compared to control cells (ANOVA p= 0.007) (Figure 5.14).
Figure 5.12:
The number of basal cells per unit length of epithelium in the TB+1 airways.

Figure 5.13:
The number of basal cells per unit length of epithelium in the TB airways.

**t-tests:**
* p < 0.05
** p < 0.01
*** p < 0.001

bars shown are standard errors
FIGURE 5.14:
The number of secretory granules per Clara cell in the TB+1 airways of the transplanted Lewis lung.

![Graph showing the number of secretory granules per Clara cell for young adult Lewis control, 14 days post T.x., mature adult Lewis control, and 6 months post T.x., with t-tests indicating ** p< 0.01. Bars shown are standard errors.]

bars shown are standard errors
Allogeneic transplants: BN-Lewis

I. Qualitative studies.

Single lung transplants were performed, the left lung of immature BN rats (mean weight 150g) being transplanted into immature Lewis rats (mean weight 162g).

Appearance, 14 days post-transplantation.

Using light microscopy, the appearance of the peripheral airways and alveolar region in the transplanted BN lung were similar to controls 14 days post-transplantation. There were areas of airway epithelial hyperplasia due to the presence of basal cells although this was variable within and between airways. Electron microscopy showed that cilia were normal in appearance and distribution on the ciliated cells. Clara cell morphology was normal and epithelial cells had tight junctions. The basement membrane was intact (Figure 5.15a). Within the submucosa there was lymphocytic infiltrate but this was below the muscle layer. The structure of the muscle and connective tissue was normal.

Appearance, 6 months post-transplantation

There were no distinguishing features between the light microscopic appearance of peripheral airways and alveoli 6 months after transplantation compared to control airways except for epithelial hyperplasia due to the presence of basal cells and cellular infiltrate within the submucosa of some airways. The electron microscopic observations verified the light microscopy observations (Figure 5.15b).

The airways of the native Lewis lung were similar to those of the control Lewis lung.
Figure 5.15a: Electron micrograph of the peripheral airways of the transplanted immature BN lung, 14 days post transplantation showing a layered epithelium consisting of Clara, ciliated and basal cells over an intact basement membrane.

Figure 5.15b: Electron micrograph of the peripheral airways of the transplanted immature BN lung, 6 months post transplantation showing a layered epithelium of Clara, ciliated and basal cells above an intact basement membrane and elastic lamina. The submucosa contains healthy looking muscle cells. The epithelial layering was less marked than that observed 14 days post transplantation.
Figure 5.15a: 2.0 μm

Figure 5.15b: 1.5 μm

B = basal cell, Cl = ciliated cell, CL = Clara cell.
II) Quantitative Studies.

A) Analysis of airway structure.

Airway dimensions and areas and calculations were performed as for the isogeneic immature lung transplants. All data are summarised in Tables 5.14, 15, 16, 17, 18 & 19 at the end of the chapter. Total airway, wall and lumen areas of both TB+1 and TB airways are presented in Figures 5.16-19. ANOVA values from the statistical analysis are tabulated in the appendices (Tables A5.20-23).

Effect of age on airway dimensions and composition in normal BN rats.

All immature animals, control and experimental increased in weight during the study period (Table 2.1, Chapter 2). Therefore it was thought necessary to compare the morphology of the peripheral airways of the control BN lungs of immature (mean weight 143g), young adult (mean weight 189g) and mature adult (mean weight 361g) rats. These comparisons for the control Lewis lung have already been given in the isogeneic transplant section of this chapter. t-tests between the controls are given in the text but not shown on the figures.

Control BN Lung (all p values are t-tests).

TB+1 airways: (Tables 5.14, 15 & 16) There was an increase in the mean total airway diameter, lumen diameter (p<0.05 for both) and total airway area (p<0.01) of the TB+1 airways of the BN lung between the immature and mature adult control rats. The increase in airway size was due to a statistically significant increase in both lumen (p<0.05) and wall area (p<0.001). There was also a significant increase in the mean wall area between the young and mature adult controls (p<0.001) (Figure 5.16). There was a decrease in epithelial/total airway area% between the immature and mature adult controls (p<0.05). Within the wall, mean epithelial area and connective tissue area of the TB+1 airways increased with age (p<0.05 for both). Mean bronchial artery/total airway area% (p<0.01) and bronchial artery/wall area% (p<0.001) of the TB+1 airways increased with age particularly between the immature and young adult controls.

TB airways: (Tables 5.17, 18 & 19) There was a significant increase in mean total airway (p<0.05) and lumen diameter (p<0.01) of the TB airways of the BN lung between the immature and young adult control rats with little further change with age. There were no other significant changes in the airway dimensions, component areas and proportions of the TB airways with age (Figure 5.17).
Effect of Transplantation on airway dimensions and composition.

**Transplanted BN Lung.**

*TB+1 airways: (Tables 5.14, 15 & 16)* In the TB+1 airways of the transplanted BN lung there was an increase in the mean total airway diameter, lumen diameter and total airway area 6 months post transplantation. This was due mainly to a significant increase in the lumen 6 months post transplantation (Figure 5.16). Wall area also increased but non-significantly 6 months post transplantation. Lumen/total airway area% increased significantly 14 days following transplantation. Mean epithelial/total airway area% decreased 14 days post transplantation in comparison with controls probably as a result of the increase in lumen area.

*TB airways: (Tables 5.17, 18 & 19)* In the TB airways of the transplanted BN lung, the airway size was similar to controls 14 days post transplantation. Six months post transplantation, the mean total airway area had increased significantly compared with controls. This was due to both a significant increase in lumen area and wall area (Figure 5.17). Within the wall, the mean epithelial height and area of the TB airways had increased 6 months post transplantation compared with controls. There were no other significant changes in the airway dimensions, component areas and proportions of the TB airways following transplantation.

**Native Lewis Lung.**

*TB+1 airways. (Tables 5.14, 15 & 16)* In the TB+1 airways of the native Lewis lung there was an increase in the mean total airway area 14 days post transplantation due to an increase in both the lumen and wall area (Figure 5.18). As in the transplanted lung, there was a decrease in the mean epithelial/total airway area% and epithelial/wall area% 14 days post transplantation compared to control airways. These values were similar to controls 6 months post transplantation.

*TB airways. (Tables 5.17, 18 & 19)* In the TB airways of the native Lewis lung, there was no change in airway dimensions and component areas compared with age-matched control airways (Figure 5.19). There was an increase in mean lumen/total airway area% and corresponding decrease in wall/total area% 14 days and 6 months post transplantation significant only after 6 months. This relative decrease in wall area was associated with a significant decrease in submucosa/total area% 6 months post transplantation. There were no other significant changes in the airway dimensions, component areas and proportions of the TB airways following transplantation.
Figure 5.16:
TB+1 airways of the control and transplanted BN lung.

Total Airway Area

Airway Wall Area

Lumen Area

Bars shown are standard errors.

T-tests:
Compared with age-matched controls:
* p<0.05
T-tests between controls not shown.
Figure 5.17: TB airways of the control and transplanted BN lung.

**Total Airway Area**

<table>
<thead>
<tr>
<th></th>
<th>Immature BN control</th>
<th>Young adult control</th>
<th>Mature adult control</th>
<th>14 days post T.x.</th>
<th>6 months post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values (µm² x 10⁴)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bars shown are standard errors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Airway Wall Area**

<table>
<thead>
<tr>
<th></th>
<th>Immature BN control</th>
<th>Young adult control</th>
<th>Mature adult control</th>
<th>14 days post T.x.</th>
<th>6 months post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values (µm² x 10⁴)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-tests: Compared with age-matched controls: * p&lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bars shown are standard errors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lumen Area**

<table>
<thead>
<tr>
<th></th>
<th>Immature BN control</th>
<th>Young adult control</th>
<th>Mature adult control</th>
<th>14 days post T.x.</th>
<th>6 months post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values (µm² x 10⁴)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bars shown are standard errors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

136.
Figure 5.18: TB+1 airways of control and native Lewis lung.

Total Airway Area

Airway Wall Area

Lumen Area

Bars shown are standard errors.

Comparison with age-matched controls:
- * p<0.05
- ** p<0.01
Figure 5.19: TB airways of the control and native Lewis lung.

Total Airway Area

Airway Wall Area

Lumen Area

bars shown are standard errors
B) Counts of different epithelial cell types using light microscopy

Cell counts made were total epithelial, Clara, ciliated and basal cell number per unit length of epithelium. The data were subjected to the two factor ANOVA test, as it was possible that epithelial cell number could be affected by age as well as transplantation. The basal cell data is presented as figures in the text (Figures 5.20 & 21) and all cell count data is tabulated in the appendices (Tables A5.24 & 5). ANOVA values for these data are also tabulated in the appendices (Table A5.26).

Total epithelial, Clara and ciliated cell number. There was no significant change in the mean total epithelial, Clara and ciliated cell number per unit length epithelium in either the TB+1 or TB airways of either the BN or Lewis lungs with age or following transplantation.

Basal cells. There was no change in mean basal cell number per unit length epithelium in either the TB+1 or TB airways of the normal BN or Lewis lungs with age. In the TB+1 airways of the transplanted BN lung there was a non-significant increase in mean basal cell number due to wide variation both 14 days and 6 months post transplantation compared to control airways. In TB+1 airways of the native Lewis lung, mean basal cell number was similar to controls at both 14 days and 6 months post transplantation (Figure 5.20). Mean basal cell number was increased significantly in the TB airways of the transplanted BN lung at both 14 days and 6 months post transplantation compared with control airways. In the TB airways of the native Lewis lung, mean basal cell number was similar to control airways at both 14 days and 6 months post transplant (Figure 5.21).

C) Analysis of Clara cell secretory granules using electron-microscopy

Clara cell secretory granule number was counted in the TB+1 airways of the transplanted BN lung and BN controls. Data were subjected to the two factor ANOVA test to examine the effect of both transplantation and age on secretory granule number in Clara cells.

There was a decrease in the number of secretory granules per Clara cell in the normal BN control TB+1 airways with age although this decrease was not statistically significant when t-tests were applied (ANOVA p= 0.011). Secretory granule number per Clara cell increased in airways both 14 days and 6 months post transplant but this increase was not statistically significantly in comparison with control cells (ANOVA p= 0.13) (Figure 5.22).
**Figure 5.20:**
The number of basal cells per unit length of epithelium in the TB+1 airways.

**Figure 5.21:**
The number of basal cells per unit length of epithelium in the TB airways.

- **Young adult controls**
- **14 day post T.x.**
- **Mature adult controls**
- **6 month post T.x.**

**t-tests:**
* p<0.05

Bars shown are standard errors

- control BN lung
- control Lewis lung
- left lung (BN)
- right lung (Lewis)
Figure 5.22:
The number of secretory granules per Clara cells in the TB+1 airways of the transplanted BN lung.

The number of secretory granules per Clara cell

<table>
<thead>
<tr>
<th></th>
<th>Young adult control</th>
<th>14 days post Tx</th>
<th>Mature adult control</th>
<th>6 months post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control BN lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.x left lung (BN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bars shown are standard errors.
III) Summary of Chapter 5.

The structure of the peripheral airways of the isogeneic and allogeneic transplanted lung was similar to that in control airways except for epithelial hyperplasia which was due to the presence of basal cells and varied within and between airways. This appearance was present both 14 days and 6 months following transplantation, although by 6 months the airways of the transplanted BN lung had a more normal appearance than those of the transplanted Lewis lung.

In the transplanted Lewis lung, mean basal cell number was greater than in controls at both times post transplantation in both the TB and TB+1 airways, although the number had decreased between 14 days and 6 months following transplantation. In the transplanted BN lung basal cell number increased after 14 days. 6 months post transplantation the number was less than after 14 days but was still greater than controls, although this increase was only significant in the TB airways.

Ciliated and Clara cell number was similar to controls after both isogeneic and allogeneic transplantation although the mean number of secretory granules per Clara cell was greater than in controls 14 days and 6 months post transplantation although only significantly so following isogeneic transplantation in Lewis rats.

In the normal Lewis and BN lung, airway size increased with age due to an increase in both lumen and wall area with all components of the wall increasing. Transplantation resulted in a greater than normal increase in airway size with age, although airway structure and composition were normal. However, following allogeneic transplantation, there was a disproportional increase in lumen area. This increase in airway size was statistically significant 14 days post transplantation in the isogeneic transplanted lung but by 6 months post transplantation the airway size of both the isogeneic and allogeneic transplants was significantly greater than controls, the greatest increase occurring in the allogeneic transplants.

In the native Lewis lung, epithelial hyperplasia was also present to a lesser extent following isogeneic transplantation, mean basal cell number was greater than controls 14 days and 6 months after transplantation. The airways of the native Lewis lung after both isogeneic and allogeneic transplants increased in size in a similar manner to those of the transplanted lungs after 14 days but values were similar to controls 6 months post transplantation. In all the measurements made of the immature rat transplants, it was the TB+1 airways which were more affected than the TB airways.
Thus, in both the control BN and Lewis lung the peripheral airway size increased with age due to an increase in the lumen and wall area. After transplantation, airway size was further increased due to an increase in lumen and wall area, the greatest increase occurring in the allogeneic transplants. In addition, both isogeneic and allogeneic transplantation resulted in epithelial hyperplasia in both the immature transplanted BN and Lewis lung and in the native Lewis lung of the isogeneic transplants which was due to a significant increase in basal cell number.
Table 5.1:  
**Airway dimensions and component areas** of the TB+1 airways in controls and after transplantation. (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th></th>
<th>Immature Lewis control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTHS POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AIRWAY DIMENSIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total airway diameter</td>
<td>148.7 ± 7.5</td>
<td>176.19 ± 16.7</td>
<td>215.30 ± 19.9</td>
<td>223.12 ± 16.1</td>
<td>190.14 ± 12.1</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal diameter</td>
<td>115.7 ± 7.5</td>
<td>143.15 ± 16.7</td>
<td>183.22 ± 19.9</td>
<td>189.18 ± 16.1</td>
<td>159.12 ± 12.1</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal/total diameter %</td>
<td>78.0 ± 1.7</td>
<td>81.0 ± 1.25</td>
<td>85.0 ± 2.7</td>
<td>85.0 ± 0.6</td>
<td>84.0 ± 1.73</td>
</tr>
<tr>
<td>Total airway area</td>
<td>5.60 ± 0.48</td>
<td><em>△</em> 6.6 ± 0.7</td>
<td><em>△</em> 8.0 ± 0.6</td>
<td><em>△</em> 11 ± 1.0</td>
<td><em>△</em> 11.6 ± 0.6</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen area</td>
<td>3.90 ± 0.31</td>
<td><em>△</em> 4.8 ± 0.5</td>
<td><em>△</em> 8.3 ± 0.5</td>
<td><em>△</em> 7.4 ± 0.9</td>
<td><em>△</em> 8.3 ± 0.5</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall area</td>
<td>1.80 ± 0.18</td>
<td>2.50 ± 0.38</td>
<td><em>△</em> 3.0 ± 0.16</td>
<td><em>△</em> 3.4 ± 0.26</td>
<td><em>△</em> 3.3 ± 0.39</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial area</td>
<td>0.78 ± 0.09</td>
<td>1.10 ± 0.15</td>
<td><em>△</em> 1.34 ± 0.06</td>
<td><em>△</em> 1.24 ± 0.13</td>
<td><em>△</em> 1.0 ± 0.1</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial height</td>
<td>6.01 ± 0.11</td>
<td>7.01 ± 0.23</td>
<td><em>△</em> 1.29 ± 0.23</td>
<td><em>△</em> 2.0 ± 0.14</td>
<td><em>△</em> 1.9 ± 0.3</td>
</tr>
<tr>
<td><em>μm</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connective tissue area</td>
<td>0.87 ± 0.11</td>
<td>0.90 ± 0.23</td>
<td><em>△</em> 1.49 ± 0.14</td>
<td><em>△</em> 2.0 ± 0.3</td>
<td><em>△</em> 1.3 ± 0.2</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle area</td>
<td>not measured</td>
<td>0.07 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td><em>△</em> 0.13 ± 0.01</td>
<td><em>△</em> 0.14 ± 0.02</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>0.017 ± 0.11</td>
<td>0.015 ± 0.03</td>
<td>0.015 ± 0.03</td>
<td>0.024 ± 0.01</td>
<td>0.025 ± 0.06</td>
</tr>
<tr>
<td><em>μm²</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Compared with immature controls
△ p<0.05
△△△ p<0.001
Compared with controls
* p<0.05
** p<0.01
*** p<0.001

144.
Table 5.2: The airway components expressed as a percentage of the total airway area in the TB+1 airways in controls and after transplantation (Mean (X) +/- SE).

<table>
<thead>
<tr>
<th>% component of total airway area</th>
<th>Immature Lewis control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS T.x Lew lung native Lew lung</th>
<th>6 MONTH T.x Lew lung native Lew lung</th>
<th>POST T.x Lew lung native Lew lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMEN AREA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>65% 2.5%</td>
<td>73% 0.15%</td>
<td>70% 1%</td>
<td>73% 0.1%</td>
<td>69% 1.2%</td>
<td>72% 2.4%</td>
</tr>
<tr>
<td>WALL AREA</td>
<td>35% 0.2%</td>
<td>27% 0.15%</td>
<td>30% 1.2%</td>
<td>27% 0.15%</td>
<td>31% 1%</td>
<td>28% 0.4%</td>
</tr>
<tr>
<td>EPIPHYRAL AREA</td>
<td>14% 0.1%</td>
<td>13% 0.3%</td>
<td>14% 0.25%</td>
<td>12% 0.1%</td>
<td>12% 0.15%</td>
<td>*11% 0.1%</td>
</tr>
<tr>
<td>CONNECTIVE TISSUE</td>
<td>15% 0.2%</td>
<td>13% 0.5%</td>
<td>15% 0.7%</td>
<td>13% 0.5%</td>
<td>19% 1%</td>
<td>15% 0.6%</td>
</tr>
<tr>
<td>MUSCLE AREA</td>
<td>Not Measured</td>
<td>1% 0.15%</td>
<td>1% 0.05%</td>
<td>1% 0.03%</td>
<td>1% 0.1%</td>
<td>1% 0.3%</td>
</tr>
<tr>
<td>BRONCHIAL ARTERY AREA</td>
<td>3% 0.3%</td>
<td>1.5% 0.05%</td>
<td>1% 0.1%</td>
<td>3% 0.1%</td>
<td>1% 0.2%</td>
<td>1% 0.1%</td>
</tr>
</tbody>
</table>
Table 5.3:  
The airway components expressed as a percentage of the airway wall area in the TB+1 airways in controls and after transplantation (Mean (X) +/-SE).

<table>
<thead>
<tr>
<th>% component of wall area</th>
<th>Immature Lewis control</th>
<th>Young Adult Lewis control</th>
<th>Mature Adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTH POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>43% 0.4%</td>
<td>48% 0.4%</td>
<td>45% 0.25%</td>
<td>47% 0.6%</td>
<td>40% 0.8%</td>
</tr>
<tr>
<td>Connective tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>48% 0.3%</td>
<td>47% 0.7%</td>
<td>48% 0.4%</td>
<td>50% 0.7%</td>
<td>59% 0.9%</td>
</tr>
<tr>
<td>Muscle area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>Not Measured</td>
<td>4% 0.4%</td>
<td>4% 0.11%</td>
<td>4% 0.15%</td>
<td>4% 0.11%</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>8% 0.1%</td>
<td>6% 0.15%</td>
<td>4% 0.5%</td>
<td>3% 0.4%</td>
<td>3% 0.4%</td>
</tr>
</tbody>
</table>

t-tests: comparison with immature controls
* p<0.05
Compared with controls
* p<0.05

146.
Table 5.4: Airway dimensions and component areas of the TB airways in controls and after transplantation. (Mean (X) +/-SE).

<table>
<thead>
<tr>
<th></th>
<th>Immature Lewis control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6MONTH POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AIRWAY DIMENSIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μm or μm² areas x 10⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total airway diameter</td>
<td>62.0 ± 1.5</td>
<td>101.0 ± 14.6</td>
<td>142.0 ± 18.7</td>
<td>144.0 ± 35.5</td>
<td>*172.0 ± 16.2</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>48.0 ± 0.7</td>
<td>84.0 ± 12.1</td>
<td>83.0 ± 16.0</td>
<td>115.0 ± 12.7</td>
<td>*147.0 ± 12.1</td>
</tr>
<tr>
<td>Total airway area</td>
<td>3.6 ± 0.2</td>
<td>3.7 ± 0.1</td>
<td>4.1 ± 0.3</td>
<td>*5.4 ± 0.6</td>
<td>***6.8 ± 0.3</td>
</tr>
<tr>
<td>Lumen area</td>
<td>2.5 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.3</td>
<td>3.6 ± 0.04</td>
<td>***5.0 ± 0.2</td>
</tr>
<tr>
<td>Wall area</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.04</td>
<td>1.3 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>*1.8 ± 0.1</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>0.52 ± 0.03</td>
<td>0.56 ± 0.03</td>
<td>0.66 ± 0.03</td>
<td>0.88 ± 0.08</td>
<td>*0.7 ± 0.05</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>3.0 ± 0.03</td>
<td>5.0 ± 0.06</td>
<td>6.0 ± 1.1</td>
<td>*8.0 ± 0.7</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>0.6 ± 0.08</td>
<td>0.5 ± 0.04</td>
<td>0.7 ± 0.08</td>
<td>*0.9 ± 0.12</td>
<td>*0.9 ± 0.08</td>
</tr>
</tbody>
</table>

Compared with immature controls.

* p≤0.05
*** p≤0.001

Compared with controls

* p≤0.05
** p≤0.01
*** p≤0.001
Table 5.5: The airway components expressed as a percentage of total airway area in the TB airways in controls and after transplantation (Mean (X) +/- SE).

<table>
<thead>
<tr>
<th>% component of total airway area</th>
<th>Immature Lewis control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTH POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area X SE</td>
<td>70% 0.25%</td>
<td>70% 0.2%</td>
<td>66% 1%</td>
<td>67% 1%</td>
<td>73% 0.2%</td>
</tr>
<tr>
<td>Wall area X SE</td>
<td>30% 0.25%</td>
<td>30% 0.2%</td>
<td>33% 1%</td>
<td>33% 1%</td>
<td>27% 0.2%</td>
</tr>
<tr>
<td>Epithelial area X SE</td>
<td>14% 0.3%</td>
<td>15% 0.2%</td>
<td>17% 0.5%</td>
<td>17% 0.5%</td>
<td>15% 2%</td>
</tr>
<tr>
<td>Submucosa area X SE</td>
<td>15% 0.5%</td>
<td>14% 0.2%</td>
<td>17% 0.5%</td>
<td>16% 0.5%</td>
<td>19% 4%</td>
</tr>
</tbody>
</table>

Table 5.6: The airway components expressed as a percentage of the airway wall area in the TB airways in controls and after transplantation (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>% component of wall area</th>
<th>Immature Lewis control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTH POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area X SE</td>
<td>49% 0.6%</td>
<td>52% 1%</td>
<td>50% 0.5%</td>
<td>52% 0.6%</td>
<td>45% 0.25%</td>
</tr>
<tr>
<td>Submucosa area X SE</td>
<td>51% 0.6%</td>
<td>48% 1%</td>
<td>50% 0.5%</td>
<td>48% 0.6%</td>
<td>55% 0.25%</td>
</tr>
</tbody>
</table>

Compared with controls.
* p<0.05
Table 5.14: **Airway dimensions and component areas** of the TB+1 airways in controls and after transplantation (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>Metric</th>
<th>Immature BN control</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTHS POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Airway Dimensions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total airway diameter μm</td>
<td>134 ± 5.0</td>
<td>193 ± 30</td>
<td>222 ± 20</td>
<td>180 ± 7.3</td>
<td>176 ± 19</td>
<td>219 ± 27</td>
<td>213 ± 16</td>
</tr>
<tr>
<td>Lumen diameter μm</td>
<td>107 ± 8.0</td>
<td>154 ± 27</td>
<td>196 ± 14</td>
<td>152 ± 8.0</td>
<td>143 ± 16</td>
<td>179 ± 24</td>
<td>185 ± 14</td>
</tr>
<tr>
<td>Lumen/total diameter %</td>
<td>69 ± 0.8</td>
<td>94 ± 1.0</td>
<td>13.2 ± 1.2</td>
<td>66 ± 0.3</td>
<td>80 ± 0.7</td>
<td>11.1 ± 0.1</td>
<td><strong>9.8 ± 0.7</strong></td>
</tr>
<tr>
<td><strong>Wall Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total wall area μm²</td>
<td>4.9 ± 0.5</td>
<td>6.1 ± 0.6</td>
<td>9.7 ± 0.7</td>
<td>4.8 ± 0.2</td>
<td>5.5 ± 0.5</td>
<td>8.1 ± 1.0</td>
<td><strong>7.4 ± 0.6</strong></td>
</tr>
<tr>
<td>Epihelial area μm²</td>
<td>1.9 ± 0.3</td>
<td>3.3 ± 0.4</td>
<td>3.5 ± 0.3</td>
<td>1.8 ± 0.1</td>
<td>2.5 ± 0.4</td>
<td>3.1 ± 0.6</td>
<td><strong>2.5 ± 0.1</strong></td>
</tr>
<tr>
<td>Connective tissue area μm²</td>
<td>0.8 ± 0.07</td>
<td>1.2 ± 1.5</td>
<td>1.4 ± 0.2</td>
<td>0.8 ± 0.04</td>
<td>1.1 ± 0.15</td>
<td>1.1 ± 0.12</td>
<td>0.94 ± 0.09</td>
</tr>
<tr>
<td>Muscle area μm²</td>
<td>7.0 ± 0.4</td>
<td>8.0 ± 0.8</td>
<td>7.0 ± 0.5</td>
<td>8.0 ± 0.9</td>
<td>7.4 ± 0.8</td>
<td>6.4 ± 0.1</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>Bronchial artery area μm²</td>
<td>1.0 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td>2.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.8 ± 0.4</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Compared with immature controls.</td>
<td>▲ p&lt;0.05</td>
<td>▲▲ p&lt;0.01</td>
<td>▲▲▲ p&lt;0.001</td>
<td>▲▲ p&lt;0.01</td>
<td>▲▲ p&lt;0.001</td>
<td>▲▲▲ p&lt;0.001</td>
<td>▲▲▲ p&lt;0.001</td>
</tr>
</tbody>
</table>

Compared with controls

* p<0.05
** p<0.01
Table 5.15: The airway components expressed as a percentage of total airway area in the TB+1 airways in controls and after transplantation (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>% component of total airway area</th>
<th>Immature BN control</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTHS POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMEN AREA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T.x BN lung</td>
<td>Native Lewis lung</td>
</tr>
<tr>
<td>X S E</td>
<td>73% 0.5% *65% 0.25%</td>
<td>73% 0.5% *65% 0.25%</td>
<td>73% 0.5% *65% 0.25%</td>
<td>70% 2% *73% 1.5%</td>
<td>74% 0.2% *73% 1.5%</td>
<td>78% 1.2%</td>
<td>70%</td>
</tr>
<tr>
<td>WALL AREA</td>
<td>27% 0.5% 35% 1%</td>
<td>27% 0.5% 35% 1%</td>
<td>27% 0.5% 35% 1%</td>
<td>30% 1.2% 27% 0.9%</td>
<td>27% 0.2% 27% 0.9%</td>
<td>26% 1%</td>
<td>22%</td>
</tr>
<tr>
<td>EPITHELIAL AREA</td>
<td>12% 0.1% 13% 0.05%</td>
<td>10% 0.15% 13% 0.05%</td>
<td>13% 0.3% 14% 0.25%</td>
<td>14% 0.3% 10% 0.1%</td>
<td>9.5% 0.1% 10% 0.1%</td>
<td>8% 0.2%</td>
<td>12%</td>
</tr>
<tr>
<td>CONNECTIVE TISSUE</td>
<td>15% 0.7% 20% 0.5%</td>
<td>15% 0.7% 20% 0.5%</td>
<td>15% 0.7% 20% 0.5%</td>
<td>16% 1.1% 15% 0.5%</td>
<td>15% 0.1% 16% 1.1%</td>
<td>13% 1.1%</td>
<td>16%</td>
</tr>
<tr>
<td>MUSCLE AREA</td>
<td>Not Measured 0.9%</td>
<td>1.4% 0.1%</td>
<td>1% 0.1%</td>
<td>1% 0.05% 1.5% 0.3%</td>
<td>0.7% 0.1% 1.5% 0.3%</td>
<td>1% 0.05%</td>
<td>1%</td>
</tr>
<tr>
<td>BRONCHIAL ARTERY AREA</td>
<td>0.11% 0.03% *3.5% 0.5%</td>
<td>0.78% 0.05% *3.5% 0.5%</td>
<td>1.5% 0.05% 1.5% 0.08%</td>
<td>1% 0.2% 1.5% 0.05%</td>
<td>1% 0.2% 1.5% 0.05%</td>
<td>1% 0.2%</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

* p<0.05
▲ p<0.01
▲▲ p<0.001

Comparison between 250g and 400g controls.
• p<0.05

Compared with controls:
* p<0.05

Compared with immature controls.

150.
Table 5.16: The airway components expressed as a percentage of the airway wall area in the TB+1 airways in controls and after transplantation (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>Component of wall area</th>
<th>Immature BN control</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 DAYS T.X BN lung</td>
<td>Native Lewis lung T.X BN lung</td>
<td>Native Lewis lung T.X BN lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial area</td>
<td>X S E</td>
<td>X S E</td>
<td>X S E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45% 1%</td>
<td>37% 0.4%</td>
<td>48% 0.4%</td>
<td>45% 0.25%</td>
<td>37% 2%</td>
</tr>
<tr>
<td></td>
<td>37% 0.4%</td>
<td>38% 0.7%</td>
<td>38% 0.7%</td>
<td>48% 0.4%</td>
<td>*38% 1.2%</td>
</tr>
<tr>
<td></td>
<td>0.4%</td>
<td>0.7%</td>
<td>0.4%</td>
<td>0.7%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>X S E</td>
<td>X S E</td>
<td>X S E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54% 1%</td>
<td>60% 1%</td>
<td>56% 0.7%</td>
<td>47% 0.7%</td>
<td>48% 0.4%</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>1%</td>
<td>0.7%</td>
<td>0.7%</td>
<td>4% 1.5%</td>
</tr>
<tr>
<td></td>
<td>0.4%</td>
<td>0.7%</td>
<td>0.4%</td>
<td>0.7%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Muscle area</td>
<td>X S E</td>
<td>X S E</td>
<td>X S E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not Measured</td>
<td>2% 1.5%</td>
<td>5% 1%</td>
<td>4% 0.4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>1.5%</td>
<td>5% 1%</td>
<td>4% 0.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>0.4%</td>
<td>4% 0.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>X S E</td>
<td>X S E</td>
<td>X S E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1% 0.1%</td>
<td>0.3% 0.03%</td>
<td>0.3% 0.03%</td>
<td>0.6% 0.15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3%</td>
<td>0.6%</td>
<td>0.7%</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03%</td>
<td>0.15%</td>
<td>0.1%</td>
<td>0.05%</td>
<td></td>
</tr>
</tbody>
</table>

Compared with immature controls.
▲ p≤0.05
▲▲ p≤0.01
▲▲▲ p≤0.001
Compared with controls:
* p≤0.05

151.
Table 5.17: Airway dimensions and component areas of the TB airways in controls and after transplantation (Mean (X) +/- SE).

<table>
<thead>
<tr>
<th></th>
<th>Immature BN control</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTHS POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIRWAY DIMENSIONS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total airway diameter</td>
<td>61.0 (1.8)</td>
<td>117 (30)</td>
<td>116 (31)</td>
<td>101 (15)</td>
<td>102 (19)</td>
<td>94.0 (32)</td>
<td>135 (24)</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>49 (1.7)</td>
<td>110 (32)</td>
<td>94 (22)</td>
<td>84 (12)</td>
<td>83 (16)</td>
<td>78 (29)</td>
<td>114 (21)</td>
</tr>
<tr>
<td>Lumen/Total diameter</td>
<td>80 (0.7)</td>
<td>94 (6.2)</td>
<td>81 (2.5)</td>
<td>83 (0.7)</td>
<td>80 (1.3)</td>
<td>82 (3.2)</td>
<td>84 (0.7)</td>
</tr>
<tr>
<td>Total airway area</td>
<td>3.6 (0.5)</td>
<td>4.5 (0.6)</td>
<td>4.4 (0.8)</td>
<td>3.7 (0.1)</td>
<td>4.1 (0.3)</td>
<td>3.9 (0.8)</td>
<td>4.8 (0.4)</td>
</tr>
<tr>
<td>Lumen area</td>
<td>2.4 (0.2)</td>
<td>3.3 (0.7)</td>
<td>3.1 (0.5)</td>
<td>2.6 (0.1)</td>
<td>2.7 (0.3)</td>
<td>2.8 (0.7)</td>
<td>3.5 (0.3)</td>
</tr>
<tr>
<td>Wall area</td>
<td>1.2 (0.3)</td>
<td>1.1 (0.06)</td>
<td>1.3 (0.3)</td>
<td>1.1 (0.04)</td>
<td>1.3 (0.1)</td>
<td>1.1 (0.2)</td>
<td>1.3 (0.04)</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>0.5 (0.1)</td>
<td>0.5 (0.02)</td>
<td>0.6 (0.01)</td>
<td>0.6 (0.03)</td>
<td>0.7 (0.03)</td>
<td>0.5 (0.07)</td>
<td>0.6 (0.05)</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>2.6 (0.5)</td>
<td>4.2 (1.1)</td>
<td>3.7 (1.3)</td>
<td>4.6 (0.6)</td>
<td>6.2 (1.1)</td>
<td>4.6 (1.7)</td>
<td>4.7 (0.7)</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>0.7 (0.1)</td>
<td>0.6 (0.06)</td>
<td>0.8 (0.2)</td>
<td>0.5 (0.04)</td>
<td>0.7 (0.08)</td>
<td>0.7 (0.1)</td>
<td>0.6 (0.08)</td>
</tr>
</tbody>
</table>

Between controls
▲ p<0.05
▲▲ p<0.01
Compared with controls
* p<0.05
Table 5.18:
The airway components expressed as a percentage of total airway area in the TB airways in controls and after transplantation (Mean(X)+/-SE).

<table>
<thead>
<tr>
<th>% airway component of total airway area</th>
<th>Immature BN control</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTHS POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area</td>
<td>X 1.2% 68%</td>
<td>72% 0.5%</td>
<td>70% 0.2%</td>
<td>67% 1%</td>
<td>70% 3%</td>
<td>75% 0.3%</td>
<td>68% 0.5%</td>
</tr>
<tr>
<td>Wall area</td>
<td>X 1.2% 32%</td>
<td>28% 0.5%</td>
<td>30% 0.2%</td>
<td>33% 1%</td>
<td>30% 3%</td>
<td>27% 0.3%</td>
<td>32% 1%</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>X 1.2% 14%</td>
<td>12% 3%</td>
<td>15% 0.2%</td>
<td>17% 0.3%</td>
<td>12% 1.2%</td>
<td>13% 0.15%</td>
<td>12% 0.25%</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>X 1.2% 18%</td>
<td>15% 0.5%</td>
<td>16% 0.5%</td>
<td>14% 0.2%</td>
<td>17% 0.5%</td>
<td>18% 1.5%</td>
<td>18% 1%</td>
</tr>
<tr>
<td>t-tests: comparison with controls * p&lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.19:
The airway components expressed as a percentage of the airway wall area in the TB airways in controls and after transplantation (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>% component of wall area</th>
<th>Immature BN control</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 DAYS POST T.x</th>
<th>6 MONTHS POST T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td>X 1% 43%</td>
<td>44% 1.2%</td>
<td>52% 1%</td>
<td>50% 0.3%</td>
<td>41% 2%</td>
<td>50% 0.3%</td>
<td>38% 2%</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>X 1% 57%</td>
<td>56% 1.2%</td>
<td>48% 1%</td>
<td>50% 0.5%</td>
<td>59% 2%</td>
<td>50% 0.3%</td>
<td>52% 2%</td>
</tr>
</tbody>
</table>

t-tests: comparison with controls * p<0.05
Chapter 6:
In vitro studies of bronchial epithelial cell/fibroblast interactions.
Introduction.
The growth stimulatory effect of fibroblast-conditioned medium (FCM) from fibroblasts either maintained at 37°C (FCM<sub>37°C</sub>) or cooled at 4°C and rewarmed (FCM<sub>4°C</sub>) on epithelial cells and fibroblasts was assessed using tritiated (³H) thymidine labelling. Before incubation with FCM the epithelial cells and fibroblasts had either been maintained at 37°C or cooled and then rewarmed. Alcian-blue periodic acid Schiff (AB-PAS) staining of the granules of the secretory cells was used to assess differentiation of the epithelial cells used in the experiments. This stain suggested that the epithelial cell cultures used 7 days after initial isolation still comprised a differentiated cell population (Figure 6.1a &b). The percentage of cells incorporating thymidine was determined by light microscopy (Figure 6.1c &d) and the percentages were normalised by angular transformation to permit conventional statistical tests to be carried out. Data are presented in Table 6.1 & 2 at the end of the chapter and in Figures 6.2-9.

I) The effect of cooling and rewarming on the response of bronchial epithelial cells to fibroblast-conditioned medium.

Epithelial cell thymidine labelling in serum free medium (SFM).
When incubated in SFM at 37°C the percentage of thymidine labelled epithelial cells was significantly reduced when the epithelial cells had previously been cooled at 4°C rather than being constantly maintained at 37°C (t-test p=0.013) (Figure 6.2).
It was necessary to determine whether it was the cooling of the epithelial cells that influenced their response to FCM or whether cooling of fibroblasts resulted in FCM which had a greater growth stimulatory effect on epithelial cells compared to normal FCM, or a combination of both effects.

The growth stimulatory effect of FCM<sub>37°C</sub> on epithelial cells.
FCM was harvested from fibroblasts incubated for either 4, 5, 6, 7, 8 or 28 hours at 37°C. Epithelial cells were incubated in each of these media for 24 hours at 37°C with the radio-labelled thymidine. When epithelial cells had been maintained at 37°C prior to incubation in FCM<sub>37°C</sub>, thymidine labelling of epithelial cells was significantly greater using FCM<sub>37°C</sub> collected after 6 and up to 24 hours incubation than using media taken after only 4 hours incubation (ANOVA p= 0.016) (Table 6.1). Using FCM<sub>37°C</sub> collected after incubations of 6-28 hours, thymidine-labelling was greater than seen in the controls incubated at 37°C in SFM, but this increase was not statistically significant. Thymidine labelling of epithelial cells that had been cooled and rewarmed was
significantly greater using FCM$_{37^0C}$ collected after incubations of 4-24 hours than epithelial cells in SFM (Table 6.1). The response at any point in time was generally greater in the epithelial cells which had been cooled at 4°C than in those maintained at 37°C. The latter increases were statistically significant except after incubation in 6 and 8 hours FCM (ANOVA p = 1.6 x 10^-6) (Figure 6.2).

The growth stimulatory effect of FCM$_{4^0C}$ on epithelial cells.
FCM was harvested from fibroblasts that had been cooled for 4 hours at 4°C and then rewarmed for 0, 1, 2, 3, 4 and 24 hours at 37°C. Medium was collected at each of these time points and epithelial cells were incubated in each medium with radio-labelled thymidine for 24 hours at 37°C. Comparing the response of epithelial cells maintained at 37°C and incubated in either FCM$_{4^0C}$ or SFM, the response of the epithelial cells at the end of the cooling period was similar but was significantly greater using FCM$_{4^0C}$ from fibroblasts which had been rewarmed for 2 and 3 hours (ANOVA p = 2.7 x 10^-3). Comparing the response of cooled epithelial cells to FCM$_{4^0C}$ and SFM at 4°C, the response was always greater using FCM$_{4^0C}$ (ANOVA p = 6.7 x 10^-7). (Table 6.1).

Thymidine labelling of epithelial cells maintained at 37°C and incubated in FCM$_{4^0C}$ was generally greater than in cooled and rewarmed epithelial cells, significantly so after incubation in FCM$_{4^0C}$ from fibroblasts rewarmed for 2 and 3 hours. (ANOVA p = 7.8 x 10^-6) (Figure 6.3).

Comparison of the growth stimulatory effect of FCM$_{37^0C}$ and FCM$_{4^0C}$ on epithelial cells maintained at 37°C.
Thymidine-labelling of epithelial cells maintained at 37°C was greater after incubation in FCM$_{4^0C}$ collected after 2 and 3 hours rewarmed compared with incubation in FCM$_{37^0C}$ for the same total incubation time suggesting that FCM$_{4^0C}$ had a greater stimulatory effect (ANOVA p = 5 x 10^-19) (Figure 6.4).

Comparison of the growth stimulatory effect of FCM$_{37^0C}$ and FCM$_{4^0C}$ on cooled epithelial cells.
Thymidine-labelling of epithelial cells cooled and rewarmed was generally greater following incubation in FCM$_{37^0C}$ rather than FCM$_{4^0C}$. This increase was statistically significant after incubation in 4, 5 and 6 hour FCM indicating a greater sensitivity of the cooled epithelial cells to FCM$_{37^0C}$ (ANOVA p = 2.6 x 10^-4) (Figure 6.5).
Figure 6.1a: Porcine bronchial epithelial cells 48 hours after initial isolation stained with AB-PAS (x25).

Figure 6.1b: Porcine bronchial epithelial cells 7 days after initial isolation stained with AB-PAS (x25).

Blue = acid/sulphated mucopolysaccarides.
Red = non-acid/non-sulphated mucopolysaccarides.
**Figure 6.1c:** Cultured porcine bronchial epithelial cells labelled with tritiated thymidine (x100).

**Figure 6.1d:** Cultured porcine bronchial fibroblasts labelled with tritiated thymidine (x50).

→ arrows indicate thymidine-labelled nuclei
Figure 6.2: The growth stimulatory effect of FCM$_{37^\circ C}$ on epithelial cells either maintained at 37°C or cooled and rewarmed.

![Graph showing the growth stimulatory effect of FCM$_{37^\circ C}$ on epithelial cells maintained at 37°C or cooled and rewarmed.](image1)

**t-tests:**
Comparisons between epithelial cells at 37°C and cooled and rewarmed:
* $p<0.05$
** $p<0.01$
*** $p<0.001$

Figure 6.3: The growth stimulatory effect of FCM$_{4^\circ C}$ on epithelial cells either maintained at 37°C or cooled and rewarmed.

![Graph showing the growth stimulatory effect of FCM$_{4^\circ C}$ on epithelial cells maintained at 37°C or cooled and rewarmed.](image2)
Figure 6.4: Comparison of the percentage of labelled epithelial cells maintained at $37^\circ$C incubated in FCM$_{37^\circ}$C and FCM$_{4^\circ}$C.

Figure 6.5: Comparison of the percentage of labelled cooled and rewarmed epithelial cells incubated in FCM$_{37^\circ}$C and FCM$_{4^\circ}$C.

**t-tests:**
Comparisons between FCMs:
*  
 ***  
 $p<0.05$
 ***  
 $p<0.001$
II) The effect of cooling and rewarming on the response of fibroblasts to fibroblast conditioned medium.

Fibroblast thymidine labelling in serum free medium (SFM).
It was found that cooling of fibroblasts prior to incubation in SFM at 37°C resulted in a reduced percentage of thymidine-labelled cells compared to fibroblasts maintained at 37°C. This decrease, however, was not statistically significant (t-test p = 0.076) (Figure 6.6).

As with the epithelial cells, it was necessary to determine whether it was the cooling of the fibroblasts that influenced their response to FCM or whether cooling of fibroblasts resulted in FCM which had a greater growth stimulatory effect on fibroblasts compared to normal FCM, or a combination of both effects.

The growth stimulatory effect of FCM\textsubscript{37°C} on fibroblasts.
FCM was harvested from fibroblasts after either 4, 5, 6, 7, 8 or 28 hours incubation at 37°C and fibroblasts were incubated in each of these FCM with radio-labelled thymidine for 24 hours at 37°C. Labelling of fibroblasts maintained at 37°C increased in response to FCM\textsubscript{37°C} from fibroblasts of increasing incubation time and this was statistically significant between fibroblasts in 28 hour FCM\textsubscript{37°C} compared with fibroblasts in all FCM\textsubscript{37°C} of lesser incubation time (ANOVA p = 0.04) (Table 6.2). There was also a significant increase in the number of thymidine-labelled cooled fibroblasts in response to all FCM\textsubscript{37°C} compared with those in SFM (ANOVA p = 0.003) (Table 6.2). The percentage of thymidine-labelled cooled fibroblasts was greater than that of fibroblasts maintained at 37°C after exposure to FCM\textsubscript{37°C} except when cells were exposed to FCM\textsubscript{4°C} which had been cooled for 4 hours followed by 4, 8 and 24 hours rewarming (ANOVA p = 2.6 \times 10^{-5}) (Figure 6.6).

The growth stimulatory effect of FCM\textsubscript{4°C} on fibroblasts.
FCM was harvested from fibroblasts that had been cooled for 4 hours at 4°C and then rewarmed for 0, 1, 2, 3, 4 and 24 hours at 37°C. Fibroblasts were incubated in each FCM\textsubscript{4°C} with radio-labelled thymidine for 24 hours at 37°C. There was no difference in the percentage of thymidine-labelled fibroblasts maintained at 37°C in response to all FCM\textsubscript{4°C} compared with fibroblasts maintained at 37°C in SFM (ANOVA p = 0.72) (Table 6.2). In contrast, there was a significant increase in the percentage of thymidine-labelled cooled and rewarmed fibroblasts in response to all FCM\textsubscript{4°C} compared with fibroblasts in SFM (ANOVA p = 3.9 \times 10^{-6}) (Table 6.2).
There was a significantly greater percentage of thymidine-labelled cooled fibroblasts compared to fibroblasts maintained at 37°C in response to FCM₄°C at nearly all times (ANOVA p = 7.6x10⁻¹⁴) (Figure 6.7).

**Comparison of the growth stimulatory effect of FCM₃₇°C and FCM₄°C on fibroblasts maintained at 37°C.**
There was no difference in the percentage of thymidine-labelled fibroblasts maintained at 37°C in response to the two different fibroblast-conditioned media (ANOVA p = 0.06) (Figure 6.8).

**Comparison of the growth stimulatory effect of FCM₃₇°C and FCM₄°C on cooled fibroblasts.**
The percentage of labelled cooled fibroblasts was similar in both FCM₃₇°C and FCM₄°C except for an increase of thymidine-labelled cooled fibroblasts after incubation in FCM₄°C of 1 and 2 hour rewarming, compared to FCM₃₇°C although only the former is statistically significant (ANOVA p = 7.5x10⁻⁵) (Figure 6.9).
These results suggest that it is the act of cooling fibroblasts that increases their response to FCM as opposed to the differential composition of the two FCMs.
Figure 6.6: The growth stimulatory effect of FCM₃⁷°C on fibroblasts either maintained at 37°C or cooled and rewarmed.

![Bar graph showing the growth stimulatory effect of FCM on fibroblasts maintained at 37°C or cooled and rewarmed.](image)

FCM (hours of conditioning)

- Fibroblasts maintained at 37°C
- Fibroblasts pre-cooled at 4°C

% of labelled cells

SFM 4hrs 5hrs 6hrs 7hrs 8hrs 28hrs

T-tests:
Comparisons between fibroblasts at 37°C and cooled and rewarmed:
* p < 0.05
** p < 0.01
*** p < 0.001

Figure 6.7: The growth stimulatory effect of FCM₄°C on fibroblasts either maintained at 37°C or cooled and rewarmed.

![Bar graph showing the growth stimulatory effect of FCM on fibroblasts maintained at 37°C or cooled and rewarmed.](image)

FCM (hours of conditioning)

% of labelled cells

SFM 4hrs 5hrs 6hrs 7hrs 8hrs 28hrs
Figure 6.8: Comparison of the percentage of labelled fibroblasts maintained at 37°C incubated in FCM$_{37°C}$ and FCM$_{4°C}$.

![Graph showing comparison of labelled fibroblasts incubated in different conditions.]

Figure 6.9: Comparison of the percentage of labelled cooled and rewarmed fibroblasts incubated in FCM$_{37°C}$ and FCM$_{4°C}$.

![Graph showing comparison of labelled cooled and rewarmed fibroblasts incubated in different conditions.]
III) Summary of Chapter 6:

Cooling and rewarming of both epithelial cells and fibroblasts enhanced their proliferative potential in response to components of FCM compared to cells maintained at 37°C.

The temperature regimen of the fibroblasts from which the FCM was harvested appeared to be less important suggesting that cooling and rewarming does not significantly increase the growth stimulatory effect of FCM especially for fibroblast culture.

Thus, cooling and rewarming of epithelial cells and fibroblasts appeared to increase their potential to enter the cell cycle and divide, irrespective of the conditions of the fibroblasts which had yielded the incubation medium. It is possible that cooling and rewarming may bring about a change in these cells, such as an increase in growth factor receptors.
Table 6.1: The effect of fibroblast conditioned medium from fibroblasts maintained at 37°C and from cooled and rewarmed fibroblasts on epithelial cells kept at either 37°C or 4°C and rewarmed. (Mean (X) +/-SE)

<table>
<thead>
<tr>
<th>Hours of medium conditioning by fibroblasts (serum-free medium)</th>
<th>Effect of fibroblast-conditioned medium from fibroblasts at 37°C</th>
<th>Effect of fibroblast-conditioned medium from fibroblasts cooled and rewarmed.</th>
<th>Hours of medium conditioning by fibroblasts</th>
<th>percentage of labelled cells</th>
<th>percentage of labelled cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hours</td>
<td>6.4% ± 0.03%</td>
<td>▲▲14.4% ± 0.07%</td>
<td>4 hours cooling at 4°C</td>
<td>6.4% ± 0.03%</td>
<td>▲8.3% ± 0.03%</td>
</tr>
<tr>
<td>5 hours</td>
<td>7.5% ± 0.04%</td>
<td>▲▲15.1% ± 0.06%</td>
<td>4 hours cooling +1 hour rewarmed</td>
<td>10.6% ± 0.02%</td>
<td>▲▲9.8% ± 0.06%</td>
</tr>
<tr>
<td>6 hours</td>
<td>▲▲10.5% ± 0.04%</td>
<td>▲▲13.1% ± 0.04%</td>
<td>4 hours cooling +2 hours rewarmed</td>
<td>▲▲20.6% ± 0.06%</td>
<td>▲7.6% ± 0.04%</td>
</tr>
<tr>
<td>7 hours</td>
<td>▲▲10.6% ± 0.02%</td>
<td>▲▲14.3% ± 0.04%</td>
<td>4 hours cooling +3 hours rewarmed</td>
<td>▲▲18.4% ± 0.05%</td>
<td>▲▲12.2% ± 0.04%</td>
</tr>
<tr>
<td>8 hours</td>
<td>▲▲11.1% ± 0.04%</td>
<td>▲▲11.8% ± 0.02%</td>
<td>4 hours cooling +4 hours rewarmed</td>
<td>11.1% ± 0.03%</td>
<td>▲▲10.4% ± 0.04%</td>
</tr>
<tr>
<td>28 hours</td>
<td>*9.4% ± 0.05%</td>
<td>▲15.3% ± 0.04%</td>
<td>4 hours cooling +24 hours rewarmed</td>
<td>9.3% ± 0.04%</td>
<td>▲▲13.2% ± 0.06%</td>
</tr>
</tbody>
</table>

Compared to 4 hr data
* p<0.05
** p<0.01
*** p<0.001

Compared to SFM data
▲ p<0.05
▲▲ p<0.01
▲▲▲ p<0.001

All other statistical differences are presented on the Figures 6.1-4.
Table 6.2: The effect of fibroblast-conditioned medium from fibroblasts maintained at 37°C and from cooled and rewarmed fibroblasts on fibroblasts kept at either 37°C or 4°C and rewarmed. (Mean (X)±/SE)

<table>
<thead>
<tr>
<th>Hours of medium conditioning by fibroblasts</th>
<th>Effect of fibroblast-conditioned medium from fibroblasts at 37°C</th>
<th>Effect of fibroblast-conditioned medium from fibroblasts cooled and rewarmed.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(serum-free medium)</td>
<td>Percentage of labelled cells</td>
<td>Percentage of labelled cells</td>
</tr>
<tr>
<td>4 hours</td>
<td>4.6% 0.22%</td>
<td>3% 0.01%</td>
</tr>
<tr>
<td>4 hours cooling at 4°C</td>
<td>**2.8% 0.02%</td>
<td>**5.4% 0.06%</td>
</tr>
<tr>
<td>5 hours</td>
<td>4 hours cooling at 4°C</td>
<td>4 hours cooling at 4°C</td>
</tr>
<tr>
<td>5 hours cooling at 4°C</td>
<td>**3.0% 0.02%</td>
<td>4.3% 0.02%</td>
</tr>
<tr>
<td>6 hours</td>
<td>4 hours cooling at 4°C</td>
<td>6 hours cooling at 4°C</td>
</tr>
<tr>
<td>6 hours cooling at 4°C</td>
<td>**3.5% 0.01%</td>
<td>**10% 0.1%</td>
</tr>
<tr>
<td>7 hours</td>
<td>4 hours cooling at 4°C</td>
<td>7 hours cooling at 4°C</td>
</tr>
<tr>
<td>7 hours cooling at 4°C</td>
<td>**3.9% 0.04%</td>
<td>**13.2% 0.1%</td>
</tr>
<tr>
<td>8 hours</td>
<td>4 hours cooling at 4°C</td>
<td>8 hours cooling at 4°C</td>
</tr>
<tr>
<td>8 hours cooling at 4°C</td>
<td>**3.8% 0.02%</td>
<td>**8.9% 0.07%</td>
</tr>
<tr>
<td>28 hours</td>
<td>4 hours cooling at 4°C</td>
<td>28 hours cooling at 4°C</td>
</tr>
<tr>
<td>28 hours cooling at 4°C</td>
<td>7.2% 0.02%</td>
<td>**7.2% 0.02%</td>
</tr>
</tbody>
</table>

Compared with 28h data
* p<0.05
** p<0.01

Compared with SFM data
▲ p<0.05
▲▲ p<0.01
▲▲▲ p<0.001

All other statistical differences are presented on the figures 6.5-8.
Chapter 7: Discussion.
I. Aims of study and principle findings.

The aims of this study were to describe the effects of preservation and reperfusion on the structure of the peripheral airways of the rat lung and to establish whether reperfusion produces additional damage to any sustained during preservation. A further aim was to determine whether any damaging effects at the time of transplantation might play a role in the long-term changes known to occur in the airways following transplantation. Long-term changes described in the airways of the transplanted rat lung may help us understand the mechanisms underlying the development of obliterative bronchiolitis (OB) in human lung transplantation.

Preservation of the lung resulted in significant damage to the peripheral airways, particularly to the epithelium, which became more evident during reperfusion. After 48 hours reperfusion, there were more basal cells in the epithelium of the peripheral airways of the transplanted lung than normal. This change persisted for up to 6 months after transplantation. The increase in basal cell number occurred in both the isogeneic and allogeneic immature and adult transplants, suggesting that the increase was a response to injury associated with transplantation, rather than the result of an immunological response.

Other investigators had shown that the external diameter of the peripheral airways increased compared with controls 14 days after transplantation of the immature rat lung and that allogeneic transplantation resulted in a greater increase in airway size than isogeneic transplantation. These observations were confirmed in the present study and in addition, an increase in external diameter of the peripheral airways was observed in the allogeneic transplanted adult rat lung after 25 days. In both the immature and adult rat lung transplants, this change persisted for the duration of the study and was due to a proportional increase in the area of all airway wall components, within the wall itself and the lumen.
Both the persistent basal cell hyperplasia and the increase in airway size were also observed in the native non-transplanted lung, but to a lesser extent. This suggests that transplantation of the left lung can also affect the non-transplanted right lung, possibly by circulating endocrine factors.

In order to further investigate these observations, cell culture studies were performed. Both pulmonary epithelial cells and fibroblasts exhibited an increase in proliferative potential in response to fibroblast-conditioned medium after they had been cooled and rewarmed. This occurred irrespective of the temperature (37°C or 4°C) at which the fibroblasts yielding the medium were maintained. This observation suggests that an alteration in epithelial cells and fibroblasts may occur during preservation and be responsible for the basal cell hyperplasia seen in vivo.

II. Methods used in transplantation studies.
A) The model of unilateral lung transplantation in the rat.

The present study used inbred, pathogen-free rats which underwent unilateral left lung transplantation using the modified Marck technique. This animal model has been used extensively for experimental studies concerning the immunological problems of lung transplantation as well as to study the effects of preservation and reperfusion on the blood-gas barrier. In the present study, the model was used to investigate the structure of the peripheral airways following preservation, reperfusion and transplantation. The study concentrated on a detailed description of the airways at terminal bronchiolar level and one generation proximal to this using both light and electron-microscopy. These are the airways in which OB develops in the human lung. The model proved to be suitable for these purposes. Structural abnormalities were seen in the peripheral airways after transplantation, as in the human situation. The animals survived for
up to 6 months despite the fact that these changes persisted. The nature of the abnormality, an increase in basal cell number, suggested that the change may be relevant to the development of OB.

In the present study, the allogeneic transplant of a Brown Norway (BN) rat lung into a Lewis rat was used. Prop found from his work on the RT₁ complex that Lewis recipient rats reject more readily than BN recipients, in this allogeneic combination. Thus, the BN lung to Lewis recipient combination is a more suitable model to study the long-term effects of transplantation on airway structure, given the vulnerability of the human lung in this respect.

Lewis lungs were transplanted into Lewis rats as an isogeneic model of lung transplantation. This allowed the effect of transplantation to be observed without the additional factor of an immunological barrier. This proved to be an essential part of the study, as following transplantation, basal cell hyperplasia and an increase in airway size were seen in both the isogeneic and allogeneic model, suggesting these changes occurred as a response to transplantation itself. However, the increase in airway size was more pronounced in the allogeneic model at the end of the study, implying that the immunological response may have enhanced this particular change following transplantation. The results of the studies on the epithelial cell counts suggested that the Lewis lung may respond differently to the BN lung following transplantation regardless of the presence or absence of the immunological barrier. Six months following transplantation the number of basal cells per unit length epithelium was no greater in the allogeneic than in isogeneic transplants, in fact it was rather smaller. In addition, basal cell number was greater in the native non-transplanted Lewis lung of the isogeneic transplants compared to the allogeneic transplants suggesting that greater basal cell number was due to a stronger stimulus from the transplanted Lewis lung. In retrospect, it therefore would have been helpful to have examined the BN lung
after isogeneic transplantation (BN-BN) in order to fully understand this observation.

Prop described the morphological phases of acute rejection of the rat lung following transplantation; latent, vascular, alveolar and destructive.\textsuperscript{149} In the present study, Cyclosporine was used as the immunosuppressive agent for the allogeneic transplants and no evidence of progressive rejection was observed except for mild persistent lymphocytic infiltrate around some of the airways examined. Immature Lewis rats received Cyclosporine on days 2, 3 and 4 after transplantation only, whilst it was necessary to give the adult Lewis rats Cyclosporine continuously following transplantation in order to control rejection. Transplantation of a BN lung into a Lewis recipient was equally successful in the immature and adult animals, indicating that both regimens of immunosuppression were sufficient to keep the rejection process under control for the duration of the study, allowing the long-term effects of transplantation to be studied. Allogeneic transplants which were not immunosuppressed showed acute rejection and destruction of peripheral airway structure within 5 days following transplantation.

The preservation fluid used for this study was Marshall's solution, which has been used successfully for kidney preservation\textsuperscript{163} in humans but not for lung preservation. It had however been used by other investigators to preserve the rat lung. In the experimental study by Hall and co-workers\textsuperscript{60} which looked at the blood-gas barrier, preservation injury was observed using Marshalls solution, but quickly resolved following reperfusion. In the present study, airways were damaged during preservation and this increased following reperfusion. However these acute structural changes had resolved by 48 hours following transplantation. Both this, and the fact the rats recovered quickly from the
operation, survived and appeared well for the duration of the study suggests
Marshall's solution is a suitable fluid for rat lung preservation.

Other investigators have performed lung perfusion scintigraphy and chest
roentgraphy. These tests were not carried out in the present study but the rats
survived and their weight was comparable to that of control animals at the end of
the study, suggesting that their lung function had not been severely
compromised. The structural studies confirmed this impression, showing the
lungs to be fully distended with no evidence of collapse and consolidation.

The rat is a good model for investigating the effects of transplantation on lung
structure. However it is also possible to use other animal models. Both the dog
and the pig have been used for transplantation studies and the pig in
particular has proved to be a good model for the study of OB and is probably
closest to the human system. However the transplanted rat lung is known
to reject in a similar way to the human lung and using the rat model is cheaper
than using a larger animal model, allowing a greater number of a defined strain of
animal to be used.

Thus the rat model appeared to be suitable not only to study the effects of
preservation and early reperfusion (up to 48 hours) but also to study the long-
term effects of transplantation and to determine whether there was any
relationship between the early damage associated with preservation and
reperfusion and the long-term structural changes. It also allowed a comparison to
be made between the long-term effects of transplantation in isogeneic and
allogeneic combinations, as well as studying the influence of age on peripheral
airway changes by using both immature and adult rats.
B) Methods of investigation of lung tissue.

Experimental and control animals were matched for age and strain. They also had to be matched according to source and technique of fixation. The adult rat lung transplants were performed in the Mayo Clinic, Rochester, USA, whereas the immature rat transplants were performed in Newcastle, UK. In the adult rats, the transplanted, native and control lungs were fixed in a partially distended state whereas the lungs of the immature rats were fully distended throughout the fixation procedure. Ensuring that each experimental group of animals had its own controls proved to be essential as quantitative analysis showed differences in airway size and in lumen to total diameter ratio between the two sets of normal control animals of the same strain.

In order to establish accurately the structure of both the transplanted left and native non-transplanted lung it was essential that the sampling of tissue was sufficient. Tissue sections were examined from all lobes of each lung and airways at terminal bronchiolar (TB) and one generation proximal to TB airways (TB+1), were analysed. All the airways of the same type within one lung exhibited similar histological features, suggesting that the sampling technique used was suitable for studying changes in airway structure following transplantation in that samples were representative of the whole lung.

Epithelial cell counts were made per unit length of epithelium. This method has been used previously by Jeffery and co-workers, and was a suitable method to quantify the damage seen in the transplanted lung. In the present study, cell counts per unit length of epithelium showed an increase in unidentifiable cells using light microscopy which was verified by electron-microscopy. If changes in epithelial area had been used to assess damage, it would not have provided such an accurate picture since although flattened Clara cells reduce epithelial area, ciliary depletion could not be accurately assessed in this way. Similarly, both
epithelial cell counts per unit length epithelium and electron-micrographs of the airways showed an increase in basal cell number, but this change was not evident when the airway composition was analysed with reference to epithelial area.

Airway dimensions and composition were measured using light microscopy and image analysis software. Hislop and co-workers had previously studied airway dimensions using light microscopy.\textsuperscript{74} In the present study, the light microscopic analysis of airway composition was extended using image-analysis. This approach allows structures of interest such as epithelium or bronchial smooth muscle to be studied in isolation. Accurate measurements of diameter, height and area of the components of the airway wall could be made, which is not possible in such an accurate way by using light microscopy alone. It was essential to consider the areas of each airway component as absolute measurements as well as to express each area as a percentage value of the total airway area and airway wall area. The absolute values showed which components of the airway had increased and the percentage calculations showed how changes in one part of the airway related to changes in other parts.

C) Methods of investigation using cell culture.

In the present study, porcine epithelial cells and fibroblasts from intra-pulmonary airways were cultured. Rat epithelial cells were not used because cells from the smaller intra-pulmonary airways of the rat proved to be difficult to isolate and culture, while the porcine cells were comparatively easy to grow in culture. Porcine cells were an appropriate choice as it is known that OB does develop in the pig following lung transplantation.\textsuperscript{3,84,85} Porcine intra-pulmonary airways are known to consist of the same cell types as those present in the rat airways, ciliated, secretory and basal.\textsuperscript{90} The cells were used in primary culture and the experiments were performed approximately 7 days after initial isolation. Alcian-Blue-Periodic acid Schiff (AB-PAS) staining of these cells throughout the culture
period showed that secretory cells remained differentiated and ciliary beating was observed for 3-4 days after isolation suggesting that the epithelial cells had not dedifferentiated significantly in culture.

The temperature and timing of the cooling procedure used was 4°C for 4 hours and thus was comparable to the clinical situation. In addition, in the present study rat lungs were preserved for either 4 or 7 hours and these times resulted in a similar amount of peripheral airway damage. It is important to note that the cells were cooled in culture medium, not preservation solution, as the aim of the study was to investigate the effect of cooling on pulmonary cell proliferative potential. However, if any further studies were carried out it would be essential to cool the cells in a preservation solution to give greater insight into the clinical situation.

In the present study, tritiated thymidine was used to measure the proliferative potential of the cultured epithelial cells. Cells were incubated for a 48 hour period in serum-free medium followed by incubation for 24 hours in conditioned medium containing tritiated thymidine. This time-span proved to give a measurable and reproducible number of labelled cells to allow quantitative analysis to be carried out.

Cells were grown on glass coverslips and after incubation in conditioned medium and thymidine labelling, were fixed prior to examination. For each coverslip, cells were counted and the percentage of labelled cells calculated. Ten grid areas per slide were counted using an eye-piece graticule. The grid areas were selected randomly by altering the position of the slide under a low magnification objective before changing to the higher magnification at which the cells were counted. This was done in order to ensure that a representative population of cells per slide was counted. The variation in cell counts within an experiment was low, suggesting that this method was appropriate.
III. Effect of preservation and early reperfusion on the peripheral airways.

A) Preservation injury.

After preservation, detailed studies on the Lewis lung showed evidence of intracellular damage within the airway epithelium, smooth muscle and alveolar Type II cells and some cellular debris in the airway lumen and infiltrate within the submucosa. The epithelium of the peripheral airways was flattened and showed particular damage to the Clara cells. The preserved BN lung was also examined and observations seen were similar to those in the preserved Lewis lung.

In the present study, the mitochondria of all epithelial cell types including alveolar Type II cells and bronchial smooth muscle cells appeared swollen with distorted cristae and some mitochondria had fused. Lehtola also described slight degeneration of cellular elements and a moderate degree of mitochondrial damage following preservation of the porcine lung using modified Euro-collins and flurocarbon preservation fluids. Mitochondrial damage during preservation suggests disturbance of cell metabolism, indicating that intra-cellular damage had occurred which in turn would lead to functional abnormalities. Other evidence of intra-cellular damage was seen on the electron-micrographs, all epithelial cells exhibiting shrunken nuclei, dense cytoplasm, cytoplasmic clefts and swollen endoplasmic reticulum.

In this study, it was the Clara cells which were most damaged during preservation, and secretory granules were lost. The susceptibility of the Clara cell to preservation injury may be due to the location of high concentrations of cytochrome P-450 and related monoxygenase enzymes in this sub-population of epithelial cells. Electron transfer processes, ongoing in the presence of dioxygen, are potential sites for free radical generation. Mitochondria of all cells can "leak" electrons via dioxygen reduction to produce superoxide via the

177.
The Clara cell, in addition has the cytochrome P450 system which also produces a basal level of superoxide radicals due to small electron leaks from its electron transfer systems. It is possible that these mechanisms are up-regulated as a result of preservation and thus cause particular damage to the Clara cells.

The number of secretory granules per Clara cell was calculated in the preserved and transplanted lung. Secretory granule number per Clara cell had increased 48 hours following transplantation compared to the number present at the end of the preservation period. The number of secretory granules per Clara cell was also greater than controls at both 14 days and 6 months following transplantation, in both the immature allogeneic and isogeneic transplanted lung, significantly so in the isogeneic transplanted lung. Secretory granule number per Clara cell was unchanged following allogeneic lung transplantation in the adult rats. The lung transplants between immature rats resulted in a greater number of secretory granules per Clara cell than in the adult lung transplants at the end point of study when the rats were all mature animals. This suggests that transplantation has a greater stimulating effect on secretory granule number in the Clara cell in the immature lung. Changes in secretory cell number and their secretory granules following an insult such as tobacco smoke have previously been reported. This observation suggests that the Clara cells of the transplanted immature lung are functioning at a higher metabolic rate than those in control lungs.

The condition of the airways of the lung is vital following preservation to ensure satisfactory lung function following transplantation. The present study suggests that preservation does result in damage to the airways but despite this, the epithelial layer remains intact and there is no significant oedema. In contrast, Hall and co-workers observed oedema in the alveolar walls at the blood-gas barrier and the thick side of the capillaries. Oedema may be more likely to form around
the blood vessels because of an increase in endothelial permeability following ischaemia. In other forms of lung injury, such as cotton smoke and cholera toxin exposure, more severe airway damage has been observed, such as sloughing of epithelial cells. However in the present study, preservation in Marshalls solution for up to 7 hours resulted in adequate preservation of airway structure.

In the present study, morphological changes did not correlate with the length of ischaemia. The appearance of the airways was similar following 4 and 7 hours preservation. This suggests that the damage sustained by the lungs following preservation occurs early, possibly during the cooling down period as opposed to the length of time the lung was actually kept at 4°C. This idea is supported by a study by Hidalgo and co-workers who showed that rat lungs stored for 24 and 48 hours are able to recover morphologically and present a nearly normal ultrastructure 4 weeks after transplantation. After 24 hours cold preservation, they observed some inflammation, small perivascular fibrotic areas and a mild degree of pleural thickening but no edema or hemorrhage.

The structure of the lungs preserved for 48 hours were considerably different from that of normal lungs. Four weeks after transplantation, the lungs showed fibrotic organisation with limited chronic inflammation of lymphocytes and plasma cells. Small foci of scarring with focal mild inflammation in the pleura were observed. Macrophages full of haemosiderin were occasionally seen in the unscarred parenchymal tissue. The general outcome was good for tissue stored for 24 hours, with thirteen out of fourteen lungs had more than 80% of the pulmonary tissue perfused like the native right lung after 24 hours preservation and less satisfactory (nine out of fourteen) after 48 hours of storage.

As the preservation time is less than 4 hours in clinical lung transplantation the findings in the present study suggest that airway damage would be similar or less in the human lung than that seen in the present study. Although there is not normally the opportunity to examine the pathology of the preserved human lung,
Fehrenbach and co-workers examined preserved human lungs which could not be matched with a potential recipient. Unfortunately only the alveolar epithelium was examined.\textsuperscript{49} The Type I pneumocytes were swollen and fragmented with some denudation. The extent of damage varied between lungs. Thus changes to the peripheral lung structures following transplantation have been observed in both the preserved human and rat lung.\textsuperscript{49,60}

B) Early reperfusion injury.

In the present study, the appearance of the airways deteriorated more during reperfusion than during preservation. Clara cells remained flattened and there was evidence of cilia loss from ciliated cells and all cells showed loose cell to cell connections. In addition, there was significant submucosal infiltrate.

The observation of additional damage to the airways following early reperfusion is in contrast to the absence of any structural evidence of additional injury to the pulmonary capillaries on reperfusion in the transplanted rat lung.\textsuperscript{60} The different response of the capillaries and the peripheral conducting airways may be due to the fact that the airways remain ischaemic immediately after transplantation due to bronchial arterial ligation. This may also explain why in the present study, generally it was the TB+1 airways which were more affected than the TB airways in terms of both epithelial cell number and overall airway size following transplantation. TB airways are nearer to the blood-gas barrier.

In previous experimental and clinical studies on the transplanted lung, cilia have only been studied in the more proximal airways in order to assess ciliary beat frequency (CBF) and mucociliary clearance (MCC).\textsuperscript{45,170} Both CBF and MCC are impaired around the anastomosis following transplantation. These studies cited several factors which could affect ciliary function following transplantation: immunological damage, repeated infections, denervation and loss of bronchial
artery supply, suggesting that these insults all damage the cells and disturb ciliary function. \(^{45,170}\) In the present study, immunological damage is unlikely as the transplants were isogeneic (Lewis-Lewis) and there was no evidence of infection. The influence of denervation and bronchial artery ligation on ciliary depletion is less clear. It cannot be ruled out that lack of innervation and a systemic arterial blood supply may influence ciliary structure directly or indirectly.

Ciliary depletion has been described in the peripheral airways following exposure to an insult such as cotton smoke\(^1\) and it is likely that the chemicals from this smoke caused intra-cellular damage leading to ciliary loss. Similarly, although the body of the ciliated cells appeared normal after preservation, significant intra-cellular damage may have occurred during preservation and caused cilia depletion in early reperfusion. Ciliary depletion in the transplanted lung leaves the airways in a vulnerable state due to inadequate mucociliary clearance and thus a lower ability to defend against infections and other foreign matter entering the lung. Although there was no evidence of infection in the present study, should such infection occur it would probably enhance any existing damage with further ciliary loss and cellular damage.

Epithelial hyperplasia of the peripheral airways of the transplanted lung due to the presence of basal cells was not present after 12 hours reperfusion but was observed after 48 hours. In other lung injury models, epithelial hyperplasia has been observed following an acute insult and during the subsequent repair process which re-established normal airway epithelial number and structure.\(^{120,151}\) In the present study, the observations suggest that epithelial cell proliferation was enhanced as a result of the damage sustained by the airway epithelium during preservation and reperfusion. The \textit{in vitro} studies showed that cooling and rewarming of pulmonary epithelial cells in culture increases their proliferative potential and this could explain the increase in basal cells observed in the
reperfused lung. The airways of the native, unpreserved, non-transplanted lung did not show basal cell hyperplasia immediately after transplantation but did later. Bishop and co-workers described how pulmonary arterial occlusion followed by reperfusion resulted in marked oedema and inflammatory infiltrates in the canine reperfused lung but also caused mild oedema and inflammation in the contralateral continuously perfused lung. Such changes were not seen in the native right lung of the transplanted animals in the present study but it is important to note that occlusion of the pulmonary arterial circulation to the lung in the Bishop study was maintained for 48 hours, considerably longer than the ischaemic time used in the present study.

Basal cell hyperplasia was found in the same peripheral airways as those in which OB develops in the transplanted human lung. It persisted for up to 6 months following transplantation and might be an important factor in the pathogenesis of OB.

IV. Persistent epithelial hyperplasia following transplantation.

A) Epithelial hyperplasia in the transplanted lung.

Epithelial hyperplasia due to the presence of basal cells was evident from 48 hours reperfusion until 6 months following transplantation. This observation is significant because epithelial cells have been implicated as the target cell in chronic rejection of the lung. Benardi and co-workers reported atypical changes in the bronchial respiratory epithelium in one combined heart-lung transplant patient 6 months following transplantation. They described diffuse hyperplasia, squamous metaplasia and dysplasia with foci of disorganised structure of the respiratory epithelium in both lungs. Although OB was present in some airways, these epithelial abnormalities were seen in all airways of each lobe, on almost all the sections examined. Benardi also suggested that it is
possible the damaged bronchial epithelium represents a target for rejection. This suggests that the epithelial hyperplasia seen in the rat peripheral airways in the present study is relevant to human lung transplantation.

One of the surprising features of the epithelial cell hyperplasia was that it was still present 6 months after transplantation. Previous studies examining the response of the airway epithelium to injury have concentrated on the acute phase of injury, not the long term effects. However, Forket using scanning electron-microscopy examined airway injury following a single exposure of trichloroethylene and observed Clara cell damage up to 60 days after chemical exposure, manifested as micronodules consisting of multiple Clara cells in the bronchiolar epithelium. This and the present study shows that a single damaging insult can result in persistent damage to the bronchiolar epithelium.

Late changes in the epithelium of the peripheral airways of the transplanted rat lung have not been reported previously. Uyama and co-workers have reported that in 4 out of 5 allografted lungs (BN-Lewis) 6 months after transplantation, the epithelium of the bronchi was interrupted or ulcerated with focal infiltration of lymphocytes in the underlying submucosa. In some parts of the bronchi, excessive granulation tissue grew into the bronchial lumen and caused airway narrowing. The bronchioli in the same study showed intact bronchial epithelium with mild lymphocytic infiltration. The rat lung transplants in the Uyama study were performed in a similar manner and with a similar immunosuppressive regime, although the age of the rats was not given.

These dramatic changes were not seen in the bronchi in the present study. In a later study, the same group conclude that alloreactivity could be demonstrated in rats with long-term surviving lung allografts and suggested that these late airway changes were immunologically mediated.
B) Epithelial hyperplasia in the native Lewis lung.

Epithelial hyperplasia due to the presence of basal cells in the native, unpreserved, non-transplanted lung was observed from 5 days following transplantation in the adult allogeneic model and from 14 days (first observation points) in the isogeneic and allogeneic immature rat model.

Cagle and co-workers studied the proliferation of various lung cell types in the remaining lung following single lung pneumonectomy in the rat using tritiated thymidine and autoradiography. Unilateral lung transplantation resembles single lung pneumonectomy in that although the native lung is replaced by the donor lung, denervation means the remaining native lung may not be "aware" of the transplanted lung. Cagle reported pleural mesothelial cell labelling peaking on day 2 and declining thereafter. However, labelling of the Type I and II pneumonocytes and bronchial epithelium occurred in parallel on day 4. Whether bronchiolar epithelial cell labelling occurred before the second post-operative day, when it would have coincided with the increase in basal cell number in the present study, was not commented upon. It is interesting that the pleural mesothelial cells were the first cells to start synthesising DNA. Cagle and co-workers did not comment on the connective tissue of the airways. It is possible that stimulation of fibroblasts in the airway submucosal could result in the production of growth factors which then stimulated the bronchial epithelial cells and other cell types 4 days after surgery. Fibroblasts are known to produce growth factors which affect epithelial cell proliferation and migration. In the present study, in vitro experiments showed that epithelial cells incubated in fibroblast conditioned medium had a greater proliferative potential than epithelial cells incubated in serum-free medium.

Cagle noted that the different lobes of the remaining lung had equal percentages of dividing cells and suggested that a general stimulus such an endocrine factor
was involved. McBride has shown that right-sided pneumonectomy in young ferrets results in an abnormally large left lung with airways of increased cross-sectional area.\textsuperscript{118} Hislop and co-workers,\textsuperscript{74,75} reported that single-lung transplantation of both isogeneic and allogeneic combinations in immature rats resulted in an increase in volume of the native recipient lung accompanied by an increase in alveolar number and peripheral airway size. It seems likely that a stimulus in the form of a growth factor(s) was influencing the transplanted lung and in turn affecting the remaining lung.

C) Role of basal cells.
In the normal rat lung, the basal cell is not a significant cell type in the peripheral airways in that it makes up less than 1\% of the normal bronchiolar epithelial cell population.\textsuperscript{91} Therefore other studies concerning the role of basal cells in the airway epithelium have tended to concentrate on the basal cells of the trachea, where it is one of the major cell types. It is generally accepted that both the basal and secretory cells have the capacity to divide and differentiate whereas ciliated cells are considered to be terminally differentiated and incapable of division.\textsuperscript{131} But it has been suggested that the basal cell may have limited progenitorial capacity and that their major role is structural.\textsuperscript{47} In the present study observations suggest that the basal cells are acting as progenitor cells, irrespective of any other structural function. The present study focused on the bronchiolar epithelium in which the Clara cell is thought to be the progenitor cell. But in both TB and TB+1 airways studied, Clara cell number was still less than in controls 48 hours post transplantation, whereas there was a significant increase in basal cell number at this time. It is known that bronchiolar Clara cells are capable of producing both Clara and ciliated cells.\textsuperscript{131} It may be that the basal cells of the bronchiolar epithelium in the post transplanted lung are progenitor cells, representing a repairing epithelium in which either basal cells divide to produce more basal cells as well as Clara and ciliated cells or Clara cell division gives rise to these poorly
differentiated cells which in turn differentiate into Clara and ciliated cells. Intermediate cells which were not in contact with the basement membrane were occasionally seen within the peripheral airway epithelium, but the number and frequency of these cells was so low as to make it impossible to perform any statistical analysis. In order to clarify the implications of these observations, it would be necessary to study epithelial cell proliferation following transplantation in vivo using tritiated thymidine and autoradiography to determine which cells were acting as the progenitor cells in the epithelium of the peripheral airways.

After 6 months, the total epithelial cell number per unit length epithelium had increased particularly following adult allogeneic and immature isogeneic lung transplants where Clara, ciliated and basal cells all increased in number. Given the presence of a persistent and disproportionate increase in basal cell number when the animals were sacrificed 6 months after transplantation, the primary cell to have been upregulated may have been the basal cell.

V. Peripheral airway size and composition following transplantation.

In the present study, an increase in peripheral airway size was observed in both the transplanted left and native right lung after isogeneic and allogeneic transplantation. Fixed lung weights were recorded from the isogeneic and allogeneic transplanted immature rat groups. The mean total lung weight was greater following transplantation than in control rats but not significantly so. Allogeneic transplantation resulted in the greatest increase in airway size and the effect was more pronounced in the transplanted lung than in the native lung after both types of transplant and was possibly influenced by age. These observations had been made previously by Hislop and co-workers. In the present study, the aim was also to find out which components of the airway contributed to the
increase in airway size, in order to determine whether such an increase could play a role in the development of OB.

**A) Peripheral airway size and composition in the transplanted lung.**

The increase in peripheral airway external diameter was mainly due to an increase in wall area in the adult rat transplants whereas there was an increase in both the lumen and wall area following transplantation in the immature rat. Although the lungs of the adult and immature animals were prepared using two different fixation techniques, each had its own controls prepared in the same way. The fact that the adult lungs were fixed in a partially inflated state does not therefore account for the observed differences with age. Analysis of the individual components of the airway wall showed that this increase in external airway diameter in the experimental adult animal was due to a proportional increase of all the wall constituents. Since normal adult animals show an increase in the area of all wall components with time it appears that transplantation exaggerates what should be happening in the peripheral airways of the normal lung with age.

Kern and co-workers suggested that mature porcine lobar transplants into growing piglets grew significantly due to an increase in connective tissue and cellular components of the lung. They reported an increase in fixed lobar volume and total lobar weight 12 weeks after transplantation despite a normal total number of alveoli, similar alveolar size and smaller parenchymal alveolar air space volume as a percentage of the transplanted lobe. This study by Kern did not measure airway size but in the present study, an increase in connective tissue and cellular components of the peripheral airways was observed, contributing to an increase in lung weight following transplantation.

Asthma is another condition which results in the remodelling of the airways. It involves activation of fibroblasts, collagen deposition and an increased synthesis
and release of extracellular matrix components. In cases of fatal and non-fatal asthma in humans, the area of the wall of the membranous airways (<2mm) has been reported to be significantly greater than in the normal lung. Unlike airways of the transplanted rat lung though, asthma is characterised by excessive airway narrowing during smooth muscle contraction as a result of the disproportionally increased thickness of the wall area. However, the increase in airway wall following transplantation in the present study and the hyperplasia of the connective tissue cells and fibres in the submucosa of asthmatic airways may both be associated with the release of cytokines and growth factors.

Dilated airways have been described following heart-lung transplantation in patients dying of OB accompanied by epithelial damage, frequently with superimposed infection. This is thought to be due to a lack of extrinsic innervation. The vagal nerve is thought to maintain a baseline bronchoconstriction and without this the airways may dilate in response to ventilation. However, the findings in this study do not suggest dilatation of the airways following transplantation.

B) Peripheral airway size and composition in the native Lewis lung.

The peripheral airways of the native non-transplanted Lewis lung became larger than normal after transplantation but in general the native lungs were less affected than the transplanted lungs. However, unlike the transplanted lung, peripheral airway external diameter in the native right lung 6 months post transplantation was similar following both allogeneic and isogeneic transplantation. This seems to suggest that the native non-transplanted Lewis lung responds to transplantation in a similar way in terms of airway size, whether it is in an immature or an adult rat, regardless of whether a BN or Lewis lung is transplanted and of whether or not the transplanted lung is from an immature or adult animal. Thus it appears to
be a response to transplantation per se. This conclusion confirms the work by Rinaldi and workers. They investigated the influence of the surgical factors without transplantation, right cardiac lobe resection, denervation and anastomosis stenosis individually on the increased right non-transplanted lung volume and increase in airway size in immature isogeneic Lewis rats. In none of these experimental groups was there an increase in airway size. Therefore they concluded that the changes in the non-transplanted right lung following unilateral left lung transplantation could only be explained in terms of transplantation itself.

In the native non-transplanted immature Lewis lung of both the allogeneic and isogeneic transplants, the increase in peripheral airway size was due to an increase in both lumen and wall area. McBride and workers reported an increase in central and more peripheral airway cross-sectional areas in the left lung following right lung pneumonectomy in the ferret. They suggested that increased mechanical stress following removal of one lung stimulates airway growth.

VI. Possible mechanisms for peripheral airway changes seen following transplantation.

It is possible that the epithelial abnormalities in early reperfusion described in this study resulted in the epithelium becoming vulnerable to further changes. Preservation and reperfusion of the rat lung resulted in early damage to the airway epithelium which persisted and was independant of the transplant being isogeneic or allogeneic, although the extent of the damage was influenced by age and rat strain. Similar abnormalities were also seen in the native non-transplanted lung. Transplantation also led to an increase in airway size. It is possible that an upregulation of pulmonary cell turnover was triggered in early reperfusion which may have resulted in abnormal epithelial/mesothelial interactions in the peripheral airways and that this in turn may have brought about an increase in airway size.
OB in the human transplanted lung is thought to start as a result of epithelial ulceration and denudation, which then allows fibroblasts from the submucosa to grow into the lumen. Persistent basal cell hyperplasia as seen in the present study is indicative of a damaged and thus vulnerable epithelium and the increase in airway wall thickness suggests fibroblast over-activity. Thus, changes in the epithelium and submucosa of the transplanted rat lung seen in the present study may be relevant to the initiation of OB in the transplanted human lung.

A) Epithelial cell/fibroblast interaction in the transplanted lung.

In the present study, the number of epithelial cells and fibroblasts entering the cell cycle following incubation in fibroblast-conditioned medium was increased when the cells had been cooled. Decampos and co-workers have shown that DNA synthesis is sensitive to ischaemia in the isolated rat lung. They used autoradiographic techniques to show that alveolar Type II cells and free macrophages were the major cell types synthesising DNA after lung ischaemia at both 4°C and 21°C. They did not comment on the cells of the peripheral conducting airways.

Adamson and co-workers examined the early proliferative response of mesothelial and subpleural cells of the mouse lung following asbestos fibre exposure and found that mesothelial cell proliferation can occur as a general response to lung injury and if the injury is more extensive prolonged cell proliferation occurs. Epithelial/mesothelial cell interaction is known to play an important role during repair and thus it may be that the damage to the peripheral airways following transplantation can influence the epithelial/mesothelial interactions in the airway wall. This may promote the persistent basal cell hyperplasia observed and up-regulate connective tissue synthesis in the airway wall following transplantation via autocrine and paracrine influences.
Both epithelial cells and fibroblasts produce growth factors which act in an autocrine and paracrine fashion. Macrophages are another source of growth factors in the lung and we found that these cells do increase in number in the alveolar region after transplantation and therefore may influence epithelial cell and fibroblast behaviour. In the present study, cooling and rewarming of fibroblasts did not seem to increase the production of autocrine/paracrine growth factors into the medium whereas cooling and rewarming of fibroblasts and epithelial cells increased their proliferative response to fibroblast-conditioned medium. This could have been due to an up-regulation of growth factor receptors on both cell types, increasing their response to any growth factors in the medium.

Madtes and co-workers have showed that bleomycin-induced injury in rat lung resulted in an increase in the expression of epidermal growth factor receptor (EGF-R) and transforming growth factor alpha (TGF-α) in airway epithelial cells, alveolar septal cells and macrophages. Using immunohistochemistry, the EGF-R staining remained intense up to 1 week post injury and was still present 2 weeks after the insult. Having also found an increase in TGF-α expression following bleomycin-induced injury, Madtes suggested that regulation of the EGF receptor may modulate the effects of TGF-α, which they considered an important mediator of lung injury in this experimental model. Since TGF-α is a potent mitogen and chemotactic factor, it might have facilitated epithelial cell and fibroblast proliferation in the transplanted rat lung. EGF receptors may have been up-regulated following cooling and rewarming in the present study.

Platelet-derived growth factor (PDGF) is produced by a variety of cells including alveolar macrophages. PDGF is mitogenic and chemotactic for fibroblasts in a paracrine manner. Bonner and co-workers have shown that chrysotile asbestos upregulates the gene expression and production of alpha receptors for PDGF-AA on rat lung fibroblasts and suggested that this alteration of the PDGF-A
population could be involved in the pathogenesis of pulmonary fibrogenesis.\textsuperscript{13} PDGF receptors may also have been up-regulated following cooling and rewarming and play a role in fibroblast proliferation and airway changes seen in the present \textit{in vivo} study.

Thus it is possible that preservation and reperfusion of the transplanted lung may result in an up-regulation of growth factor receptors in the peripheral airway cells and this in turn could lead to an increase in response to growth factors regardless of whether or not these were up-regulated. The increase in connective tissue within the airway walls may have been caused by the fibroblasts responding to several different growth factors via an increase in the number of receptors.

Both PGDF and basic fibroblast growth factor (bFGF) have been implicated in the development of OB in the transplanted human lung. Hertz and co-workers showed increased PDGF concentrations in bronchoalveolar lavage fluid obtained from human lung recipients with OB versus control recipients without OB.\textsuperscript{71} They extended this work by showing that administration of PDGF and bFGF to transplanted murine airway isografts in subcutaneous tissue produced a fibroproliferative response.\textsuperscript{4} These findings in the \textit{in vivo} model provide further evidence that PDGF and bFGF are relevant to the pathogenesis of OB after transplantation.

\textbf{B) Immunological influences in the transplanted and native lung.}

\textbf{Immunological influences and epithelial cell hyperplasia:} Both allogeneic and isogeneic transplantation resulted in an increase in the number of basal cells in the peripheral airways of both the transplanted and native right lung. The increase in basal cell number was no greater in the allogeneic than in isogeneic transplants, in fact it was rather smaller. Therefore epithelial
hyperplasia does not appear to have an immunological basis but occurs as a response to transplantation.

The fact that epithelial damage can persist until at least 6 months after transplantation and that epithelial cells have been cited as an immunological target in the chronic rejection of the allogeneic transplanted lung suggests that early epithelial damage could be of significance. The early damage sustained by the airways may be related to the expression of MHC II molecules on the epithelial cells. Rat airway epithelial cells do not express MHC II molecules in the normal lung but do express them following allogeneic single lung transplantation. It has been hypothesised that such an expression may contribute to the development of OB by rendering the epithelial cells open to attack by the immune system, initiating chronic rejection. This expression is seen 2 days following transplantation and corresponds to the time at which epithelial hyperplasia was seen in the present study. By contrast, another study suggested that the lung is a highly immunogenic organ and the lymphoreticulum tissue in the lung is normally held in a state of activation and thus expression of MHC II molecules on the airway cells following transplantation may not be such a useful indicator in rejection. In the human lung, Class II antigens are normally expressed on tracheal epithelium but expression on the bronchiolar epithelium is variable and sometimes absent.

**Immunological influences and peripheral airway size:** Peripheral airway size increased following transplantation and both the allogeneic immature and adult transplants resulted in a greater increase in airway size than in the immature transplanted Lewis lung at the end point of the study, 3 or 6 months. Thus it appears that allogeneic transplantation has a greater effect on peripheral airway size of the transplanted lung than isogeneic transplantation. However,
strain differences cannot be ruled out. This could be verified in future studies by carrying out isogeneic transplants using the BN rat.

C) Influence of rat strain and age.

Influence of rat strain and age on epithelial cell hyperplasia: The findings suggested that the epithelial cell hyperplasia of the peripheral airways might have been influenced by the strain of the transplanted rat lung and that the Lewis lung is more sensitive to the insult than the BN lung. It has been shown that Lewis recipients reject faster (BN-Lewis) than BN recipients (Lewis-BN) after single-lung allograft transplants. In the present study, it is possible that the transplantation of the immature Lewis lung caused greater epithelial damage which was then transmitted (possibly by a humoral mechanism) to the native Lewis lung. Although there was epithelial damage to the immature BN lung following transplantation it was apparently insufficient to trigger such a response in both lungs. However, epithelial hyperplasia was present in both the transplanted BN and native Lewis lungs of the adult rat transplants at all times after transplantation, unlike the allogeneic transplants of the immature rats. Thus the age of the transplanted lung may also influence the severity of epithelial hyperplasia. However the immunosupression regime of the 2 allogeneic transplant groups was different and this makes direct comparisons between these groups difficult.

Influence of rat strain and age on peripheral airway size: The maturity of the lung may also influence the increase in airway size following transplantation. When the transplanted lung was immature, the effects of the transplantation on peripheral airway size were more pronounced. Comparing the immature and adult BN lung, transplantation of the immature lung resulted in larger peripheral airways.
Hislop and co-workers described the effect of isogeneic and allogeneic single lung transplantation of an immature donor lung into a mature adult recipient rat.\textsuperscript{75} In this work, six months after transplantation the terminal bronchiolar diameter of the allogeneic transplanted lung had increased compared with controls and also appeared to increase in the native right lung, but the change in the right lung was not statistically significant. However, peripheral airway diameter was similar to controls after isogeneic transplantation in both the transplanted and native right lung. They suggested the growth of an immature transplanted lung is primarily under its own intrinsic control and that any circulating growth factors are likely to be less in a mature than an immature animal. It appears that the maturity of the lung may indeed influence post-transplant growth.

In summary the process of transplantation itself seemed to cause an increase in basal cell number rather than there being a determining immunological factor. The strain and age of the transplanted lung may also have had an effect. Both allogeneic and isogeneic transplantation resulted in an increase in peripheral airway size, but allogeneic transplantation had a greater effect than isogeneic transplantation, implying that there may have been an immunological influence. It is also possible that the increase in airway size is enhanced if the transplanted lung is immature.

D) Role of denervation, bronchial artery ligation, infection, immunosuppression.

Several experimental transplantation studies have suggested that denervation is not a cause of the abnormal increase in airway size seen after transplantation. Hislop and Rinaldi concluded that denervation of the transplanted rat lung did not seem to be the cause of abnormal increase in airway size.\textsuperscript{74,75,158} Kern and co-workers described abnormally small airways following porcine lobar transplantation which they thought was due to impaired development as opposed
to denervation.\textsuperscript{103,104} Also, although the lung is denervated, it does retain intrinsic nerves containing neuropeptides (personal observation) and it may be that they can act without extrinsic neural connections. Neuropeptides are thought to have trophic functions. Nilsson and co-workers have shown that substance P and K stimulated DNA synthesis in cultured human skin fibroblasts.\textsuperscript{132} It is possible that the lack of a functional vagal nerve, which is thought to maintain baseline bronchoconstriction in the normal innervated lung,\textsuperscript{188} allows increased growth of the airways to occur following transplantation.

There is no evidence that bronchial artery ligation plays a role in the increase in airway size following transplantation. In the adult BN lung transplants bronchial artery area increased after transplantation, significantly so, 25 and 100 days after transplantation. By contrast, no increase was seen in the native non-transplanted Lewis lung. Bronchial artery area also remained normal in both the transplanted and native non-transplanted lung of the isogeneic and allogeneic immature lung transplants. Whether this difference was due to age or to the different immunosuppressive regimes is unclear. An increase in bronchial artery area has been noticed in certain kinds of lung disease, especially chronic inflammation and neoplasms.\textsuperscript{129} Transplantation entails bronchial artery ligation and it is known that the bronchial arteries then fill in a retrograde manner from the pulmonary arterial system.\textsuperscript{129} The role, if any of denervation or bronchial artery ligation in basal cell hyperplasia has not been addressed. However bronchial artery ligation can lead to ischemia of the airways and thus must play some role in airway changes. In the present study, the TB+1 airways were generally more affected in terms of epithelial cell hyperplasia than the TB airways. It is possible that the TB airways are less ischemic following transplantation due to their proximity to the blood-gas barrier.

Infection is a possible factor in the post-transplant epithelial hyperplasia and increase in airway size. Castleman showed that a viral infection could induce
hyperplasia and hypertrophy of the developing rat lung. Five days after inoculation with Sendai virus weanling rats (22 day old), hyperplasia of non-ciliated bronchiolar epithelial cells was observed. Suckling rats infected during rapid post-natal lung growth at 5 days of age had a 48% greater mean TB cross sectional area compared with control rats at 22 days of age. However, the pathology of the lungs in the present study did not suggest any such infection.

Immunosuppression per se is unlikely to play a role in either basal cell hyperplasia or an increase in airway size following transplantation. Basal cell hyperplasia was seen in isogeneic transplants, which do not require immunosuppression. Cyclosporine can disturb growth but usually reduces it. In experimental animals high doses of cyclosporin suppresses total body growth.

VII) Relevance of findings to the development of OB in the transplanted human lung.

In the present study, unilateral lung transplantation in the rat resulted in basal cell hyperplasia and an overall increase in airway size in the terminal bronchioli and airways one generation proximal to them. These changes may provide an insight to the airways of the pre-OB transplanted human lung. OB in the human lung starts with ulceration and denudation of the epithelium resulting in necrotic debris in the lumen. This allows fibroblasts from the exposed submucosa to grow into the lumen. In the present study, although epithelial ulceration and denudation was not observed, basal cell hyperplasia was indicative of a damaged and thus vulnerable epithelium. In addition, the increase in area of the airway wall of the transplanted rat lung was partly due to an increase in connective tissue. This suggests an increase in fibroblast activity.

It is possible that the epithelium and fibroblasts of the peripheral airways of the transplanted human lung are also in a similar vulnerable state following
preservation and transplantation. The condition would then be aggravated by rejection and infection episodes, causing severe epithelial damage and further activation of fibroblasts, in turn leading to OB. Repeated, poorly controlled rejection and infection episodes are thought to be the main cause of OB in the transplanted human lung.\textsuperscript{169}

Winter and co-workers have reported that the typical features of human OB were present in the airways of the allogeneic transplanted rat lung when the rats were infected with Sendai virus 6 months following transplantation.\textsuperscript{204} They described epithelial necrosis and formulation of granulation tissue in both the large airways and the bronchioles and concluded that a respiratory viral infection induces severe damage to the airways of the rat transplanted lung but only in combination with chronic rejection.\textsuperscript{204}

Thus in the present study, the changes observed six months following transplantation suggest that the epithelium and fibroblasts of the peripheral airways may be vulnerable to further insult, whether rejection, infection or both. The airways of the transplanted human lung may also be compromised in a similar way.

\textbf{VIII) Future studies.}

The histological findings of the present study suggest that epithelial cell turnover increased in the peripheral airways following unilateral lung transplantation and to a lesser extent in the native non-transplanted lung. In future studies this could be verified by \textit{in vivo} labelling of proliferating lung cells following transplantation. Rats which have undergone unilateral lung transplantation should be injected with tritiated thymidine and sacrificed at regular intervals following transplantation, up to 6 months after transplantation. Lung sections would be cut and proliferating cells visualised using autoradiographic film. This would accurately determine when and in which components of the peripheral airways cell proliferation was upregulated.
This tissue could also be used to look at growth factors and their receptors in the post-transplanted lung, particularly platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) which have been implicated in the development of OB.\textsuperscript{71,4}

\textit{In vitro} studies of epithelial cell/fibroblast interaction should be extended. It would be important to look for the presence of specific growth factors in the fibroblast-conditioned medium from cooled and rewarmed fibroblasts, using Enzyme-Linked Immunosorbent Assays (ELISA). Also, receptor expression in airway epithelial cells and fibroblasts, using immunohistochemical staining could be studied. In addition, the effect of epithelial cell-conditioned medium (ECM) from cooled epithelial cells on both cooled epithelial cells and fibroblasts and the growth factors involved should be investigated.

**IX. Conclusion**

In conclusion, the results of this study suggest that injury to the peripheral airways of the lung caused by preservation and reperfusion may contribute to the long-term changes to these airways following transplantation. Damage to the peripheral airways following transplantation resulted in basal cell hyperplasia after 48 hours reperfusion. This appeared to be a physical response to injury, influenced by the strain and possibly age of the donor lung and persisted until at least 6 months post transplantation.

Airway size also increased following transplantation, an increase which was generally proportional and thus was an exaggeration of normal growth. Airway size increased after both isogeneic and allogeneic transplantation but was greatest after allogeneic transplantation and thus appeared to have some immunological basis and was also possibly influenced by the maturity of the lung.
The fact that these observations were also made in the native non-transplanted Lewis lung suggests a bi-lateral response to the influences inflicted onto the transplanted left lung, possibly by circulating endocrine factors. The effects on the contralateral lung however were less and in terms of airway size in both the allogeneic and isogeneic transplants, the size of the peripheral airways in the contralateral lung increased to a similar extent suggesting that the changes to the native lung are a response to the physical act of transplantation of the left lung.

This study suggests that the basal cell hyperplasia seen in the peripheral airways from early reperfusion may be linked to an increase in the production of submucosal collagen of the peripheral airways by a two-way interaction via growth factors. Cell culture studies showed that both epithelial cells and fibroblasts following cooling and rewarming are more responsive to fibroblast-conditioned medium and thus following the preservation/reperfusion period both cell types may respond to and influence each other in a different way to that in the normal lung.

If the epithelial cells and the fibroblasts of the submucosa of the peripheral airways of the transplanted rat lung are both in a sensitised state, an additional stimulus of a rejection and or infection episode could trigger the development of OB.

In summary, changes to the peripheral airways were evident following preservation and unilateral lung transplantation in the rat. Some of these changes were still present 6 months following transplantation. These observations may provide some insight to the development of OB in the transplanted human lung.

200.
Appendices.
Appendices for Chapter 2.
A2.1 Operative technique and care of animals.
Donor rats were anesthetised with intraperitoneal pentobarbital injection and their lungs ventilated with air. The thoracic organs were exposed through a long midline incision and the hilum of the left lung was dissected to mobilise the artery, vein and bronchus. The lungs were then flush perfused with Marshall's solution (Na 80, K 80, Mg 42, citrate 35, sulfate 35mEq/litre, mannitol 34g/litre, pH7.4 at 37°C and pH7.2 at 4°C, osmolarity 400mOsM Travenol, Newbury, England) at 4°C via the main pulmonary artery. Effluent flowed through the amputated left atrial appendage. Ventilation was continued during perfusion and the trachea was ligated during expiration with the lung inflated at a pressure of 2 to 3cm H2O. The heart and lungs were removed en bloc and stored in Marshall's solution at 4°C in the inflated state. Recipient rats were anaesthetised, their lungs were ventilated, and the temperature was maintained with a warming blanket. A left thoracotomy was performed and the left lung dissected and removed. The donor left lung was then implanted into the recipient by anastomosing the vein and artery. Interrupted sutures were used for part of the front wall of the vessels to allow for growth. The lung was reperfused, after which the bronchus was anastomosed. The chest wall was closed with a small drain in situ. This was removed during recovery from anesthesia. The animals were returned to their cages to recover. Animals received humane care in compliance with the British Home Office regulations and the "Principles of Laboratory Animal Care" formulated by the National Society of Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health (NIH publication No.18-23 1978).

A2.2 Sorensen's Complete.
Sorensen's A  9.5mls.  (Na$_2$HPO$_4$.H$_2$O  14.19g/L)
Sorensen's B  40.5mls.  (KH$_2$PO$_4$  13.6g/L)
Buffered to pH7.4.
A2.3 Embedding of tissue for light microscopy.
Tissue was processed in a Shandon Hypercentre 2 and was put through the following solutions.

1/ 10% formol saline 30 minutes.
2/ 50% absolute alcohol 2hrs.
3/ 70% absolute alcohol 2hrs.
4/ 90% absolute alcohol 1hr.
5/ 100% absolute alcohol 1hr
6/ 100% absolute alcohol 2hrs.
7/ 100% absolute alcohol 2hrs.
8/ Xylene 2hrs.
9/ Xylene 2hrs.
10/ Xylene 2hrs.
11/ Wax 2hrs.
12/ Wax 2hrs.

Tissue is then ready to embed in wax blocks.

A2.4 Haematoxylin and Eosin stain.
Paraffin wax embedded sections were stained as followed:
1/ Xylene for 5 minutes.
2/ Fresh xylene for 1 minute.
3/ Absolute alcohol for 30 seconds.
4/ Fresh absolute alcohol for 30 seconds.
5/ 70% alcohol for 30 seconds and rinse in tap water.
6/ Fresh 70% alcohol for 30 seconds.
7/ Ehrlich's Haematoxylin for 15 minutes.
8/ Rinced well in tap water.
9/ Differentiated in acid-alcohol for 15 seconds. (acid alcohol.1% HCL in 70% alcohol).
10/ Allowed to"blue" in tap water for 5 minutes.
11/ Aqueous eosin for 3 minutes.
12/ Quickly rinsed in tap water.
13/ Dehydrated swiftly through 70% alcohol, absolute alcohol and cleared in xylene.
14/ Sections were mounted in XAM (Raymond Lamb).
A2.5 Elastin Van Gieson's stain.
Paraffin wax embedded sections were stained as follows:
1/ Sections were rehydrated in water as before.
2/ Millers Elastic stain for 35 minutes (filtered before use).
3/ Rinsed well in tap water.
4/ 3% Ferric chloride for 10 minutes.
5/ Rinsed well in tap water.
6/ 70% alcohol for 10 minutes and then rinsed well in tap water.
7/ Van Geisons for 1 minute.
8/ Dehydrated swiftly through 70% alcohol, absolute alcohol and cleared in xylene
9/ Sections were mounted in XAM (Raymond Lamb).

A2.6 Embedding specimens in araldite for TEM.
1/ Specimens were washed in Sorensen's Complete for 2 x 10 minutes.
2/ 1 hour in 1% OsO4 (Agar Scientific Ltd) in the fume cupboard.
3/ 30% ethanol for 5 minutes.
4/ 50% ethanol for 10 minutes.
5/ 70% ethanol for 2 x 10 minutes.
6/ 90% ethanol for 10 minutes.
7/ 95% ethanol for 5 minutes.
8/ 100% ethanol for 3 x 10 minutes.
9/ Propylene oxide (BDH) for 15 minutes.
10/ Propylene oxide/araldite (Agar Scientific Ltd) 50/50 for 15 minutes.
11/ Put in araldite and placed in automatic stirrer for 30 minutes.
12/ Put in fresh araldite and then put into fridge overnight.
13/ Taken from fridge and put onto stirrer for 2 hours.
14/ Embedded in fresh araldite at 60°C for 48 hours either in aluminium dishes or rubber "coffin"moulds.

A2.7 Lead citrate stain.
Double-distilled water was first boiled to remove gases and then allowed to cool. 1.33g of Pb nitrate and 1.76g of Na citrate was then added to 30mls of the water with vigorous shaking and then shaken intermitently for 30 minutes. 8mls of NaOH was then added and the volume made up to 50mls with the same water.

A2.8 PBS (phosphate buffered saline) 6 x antibiotics solution.
160mls PBS (+Mg & Ca) (1 tablet in 200mls of distilled water) (Sigma).
20mls Penicillin and Streptomycin (Flow).
1.2mls Gentamycin (Roussel).
A2.9 Disaggregation medium.

50mls Hepes 199 (Flow)
50mg Collagenase (Sigma)
2.5u Elastase (Calbiochem)
0.075g Bovine serum albumin (Sigma)
0.05g Soyabean trypsin inhibitor (Sigma)
1% Vitamins in Modified Eagle's medium (Flow)
1% Amino acid solution (Flow)

A2.10 M199 + Epidermal growth factor (EGF).

440ml M199 medium (Flow)
50mls Fetal calf serum (FCS) (GlobePharm)
10ml Penicillin and streptomycin (Flow)
1.7ml Glutamine. (Flow)
25ng/ml EGF (Sigma)

A2.11 RPMI medium.

440ml RPMI (Flow)
50ml Fetal calf serum (FCS) (GlobePharm)
10ml Penicillin and streptomycin. (Flow)
1.7ml Glutamine (Flow)

A2.12 Serum Free medium (SFM).

490ml M199 medium (Flow)
10ml Penicillin and Streptomycin (Flow)
1µg/ml Hydrocortisone (Sigma)
5 µg/ml Transferrin (Sigma)

A2.13 Alcian-blue periodic acid Schiff stain.

Paraffin wax embedded sections were stained as follows:

1/ Sections were rehydrated in water as before.
2/ Stained in Alcian-Blue solution for 20 minutes.
3/ Rinsed in distilled water and then washed in running tap water for 5 minutes.
4/ Oxidised for 5 minutes in 1% (aq) periodic acid.
5/ Washed in running tap water for 5 minutes and rinsed in distilled water.
6/ Placed in Schiffs solution for 15 minutes.
7/ Washed for 30 minutes in water.
8/ Differentiated in acid alcohol for seconds and "blued" in running water.
9/ Sections were dehydrated in alcohol and xylene before mounting in XAM.
Appendices for Chapter 3.
Table A3.1:
ANOVA values of the epithelial counts of peripheral airways following preservation and reperfusion in Lewis rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TB+1 T.x Lewis lung</th>
<th>TB+1 native Lewis lung</th>
<th>TB T.x Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=18 airways</td>
<td>n=18 airways</td>
<td>n=18 airways</td>
</tr>
</tbody>
</table>

Comparing 4 and 7hrs preservation

<table>
<thead>
<tr>
<th></th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>total no. of cells</td>
<td>0.10</td>
<td>0.74</td>
<td>0.68</td>
</tr>
<tr>
<td>Clara cells</td>
<td>0.94</td>
<td>0.87</td>
<td>0.96</td>
</tr>
<tr>
<td>ciliated cells</td>
<td>0.72</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td>basal cells</td>
<td>0.93</td>
<td>0.73</td>
<td>0.93</td>
</tr>
<tr>
<td>unidentified cells</td>
<td>0.95</td>
<td>0.89</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Comparing 4 and 7 hrs preservation followed by 4hrs reperfusion

<table>
<thead>
<tr>
<th></th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>total no. of cells</td>
<td>0.74</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Clara cells</td>
<td>0.87</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>ciliated cells</td>
<td>0.67</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>basal cells</td>
<td>0.73</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>unidentified cells</td>
<td>0.89</td>
<td>0.77</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Comparing 4 and 7hrs preservation followed by 12hrs reperfusion

<table>
<thead>
<tr>
<th></th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>total no. of cells</td>
<td>0.68</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Clara cells</td>
<td>0.96</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>ciliated cells</td>
<td>0.68</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>basal cells</td>
<td>0.93</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>unidentified cells</td>
<td>0.77</td>
<td>0.53</td>
<td>0.53</td>
</tr>
</tbody>
</table>

NOTES:
No TB airways were examined in the native right lung.

207.
**TABLE A3.2:**
ANOVA values for the effect of preservation and reperfusion on epithelial cell counts in the TB+1 and TB airways.

<table>
<thead>
<tr>
<th>TB+1 airways</th>
<th>Transplanted Lewis lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell type</td>
<td>ANOVA values</td>
<td>ANOVA values</td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P = 0.16</td>
<td>P = 0.32</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P = 0.0001</td>
<td>P = 0.0025</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P = 0.78</td>
<td>P = 0.99</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P = 0.0001</td>
<td>P = 0.26</td>
</tr>
<tr>
<td>Unidentified cells</td>
<td>P = 0.025</td>
<td>P = 0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TB airways</th>
<th>Transplanted Lewis lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell type</td>
<td>ANOVA values</td>
<td>ANOVA values</td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P = 0.5</td>
<td>P = 0.14</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P = 0.004</td>
<td>P = 0.1</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P = 0.24</td>
<td>P = 0.83</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P = 0.0001</td>
<td>not calculated</td>
</tr>
<tr>
<td>Unidentified cells</td>
<td>P = 0.04</td>
<td>not calculated</td>
</tr>
</tbody>
</table>
TABLE A3.3:
Epithelial cell counts per 100µm epithelium in the TB+1 airways of controls and following preservation and early reperfusion. (mean (X) +/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>controls</th>
<th>preservation</th>
<th>4 hours reperfusion</th>
<th>12 hours reperfusion</th>
<th>48 hours reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.x Lewis lung</td>
<td>native Lewis lung</td>
<td>T.x Lewis lung</td>
<td>native Lewis lung</td>
<td>T.x Lewis lung</td>
</tr>
<tr>
<td>Total cell No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>15.7</td>
<td>12.9</td>
<td>11.9</td>
<td>12.1</td>
<td>11.7</td>
</tr>
<tr>
<td>SE</td>
<td>1.9</td>
<td>0.9</td>
<td>0.4</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Clara cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>5.8</td>
<td>***2.0</td>
<td>***1.8</td>
<td>**3.3</td>
<td>***1.6</td>
</tr>
<tr>
<td>SE</td>
<td>0.8</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>9.0</td>
<td>7.9</td>
<td>7.2</td>
<td>8.7</td>
<td>7.1</td>
</tr>
<tr>
<td>SE</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Basal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>0.8</td>
<td>0.4</td>
<td>0.7</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>SE</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Unidentifiable cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>0.1</td>
<td>*2.6</td>
<td>*2.3</td>
<td>0.0</td>
<td>*2.5</td>
</tr>
<tr>
<td>SE</td>
<td>0.1</td>
<td>0.2</td>
<td>0.6</td>
<td>0.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* p=0.05
** p=0.01 compared with controls.
*** p=0.001
++++ p=0.001 compared with preservation and reperfusion data
Table A3.4:
Epithelial cell counts per 100 μm epithelium in the TB airways of controls and following preservation and early reperfusion (mean (X) +/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>controls</th>
<th>preservation</th>
<th>12 hours reperfusion T.x Lewis lung</th>
<th>12 hours reperfusion T.x Lewis lung native Lewis lung</th>
<th>48 hours reperfusion native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell No.</td>
<td>X 12.8 0.7</td>
<td>10.6 0.8</td>
<td>11.1 1.5</td>
<td>12.5 0.6</td>
<td>11.1 0.4</td>
</tr>
<tr>
<td>Clara cells</td>
<td>X 4.1 0.5</td>
<td>*2.8 0.4</td>
<td>**1.8 0.4</td>
<td>3.7 0.1</td>
<td>3.1 0.4</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>X 8.1 0.5</td>
<td>7.0 0.6</td>
<td>8.2 0.8</td>
<td>6.8 0.8</td>
<td>7.9 0.5</td>
</tr>
<tr>
<td>Basal cells</td>
<td>X 0.5 0.1</td>
<td>0.0 0.0</td>
<td>0.0 0.0</td>
<td>**1.8 0.2</td>
<td>0.0 0.0</td>
</tr>
<tr>
<td>Unidentifiable cells</td>
<td>X 0.05 0.05</td>
<td>**1.1 0.3</td>
<td>**1.1 0.2</td>
<td>0.3 0.3</td>
<td>0.0 0.0</td>
</tr>
</tbody>
</table>

* p=0.05 compared with control airways.

** p=0.01
Appendices for Chapter 4.
TABLE A4.7:
ANOVA values for the effect of age and allogeneic transplantation on the airway dimensions and component areas of TB+1 airways.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Airway diameter</td>
<td>P=0.13</td>
<td>P=0.034</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>P=0.69</td>
<td>P=0.92</td>
</tr>
<tr>
<td>Lumen/total diameter</td>
<td>P=0.18</td>
<td>P=0.095</td>
</tr>
<tr>
<td>Total airway area</td>
<td>P=0.017</td>
<td>P=0.015</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.6</td>
<td>P=0.42</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.009</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.45</td>
<td>P=0.04</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>P=0.013</td>
<td>P=0.002</td>
</tr>
<tr>
<td>C. tissue area</td>
<td>P=0.012</td>
<td>P=0.88</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P=0.62</td>
<td>P=0.13</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P=0.6</td>
<td>P=0.007</td>
</tr>
</tbody>
</table>
TABLE A4.8: ANOVA values for the effect of age and allogeneic transplantation on airway component percentages of TB+1 airways.

<table>
<thead>
<tr>
<th>Component percentage of total airway area</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.013</td>
<td>P=0.19</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.01</td>
<td>P=0.18</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.35</td>
<td>P=0.59</td>
</tr>
<tr>
<td>C tissue area</td>
<td>P=0.034</td>
<td>P=0.95</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P=0.58</td>
<td>P=0.65</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P=0.56</td>
<td>P=0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component percentage of wall area</th>
<th>Age ANOVA</th>
<th>T.x ANOVA</th>
<th>Age ANOVA</th>
<th>T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td>P=0.14</td>
<td>P=0.89</td>
<td>P=0.13</td>
<td>P=0.016</td>
</tr>
<tr>
<td>C.tissue area</td>
<td>P=0.09</td>
<td>P=0.45</td>
<td>P=0.13</td>
<td>P=0.016</td>
</tr>
<tr>
<td>muscle area</td>
<td>P=0.18</td>
<td>P=0.82</td>
<td>P=0.93</td>
<td>P=0.96</td>
</tr>
<tr>
<td>bronchial artery area</td>
<td>P=0.25</td>
<td>P=0.08</td>
<td>P=0.34</td>
<td>P=0.9</td>
</tr>
</tbody>
</table>
### TABLE A4.9:
ANOVA values for the effect of age and allogeneic transplantation on the airway dimensions and component areas of the TB airways.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Airway diameter</td>
<td>P=0.09</td>
<td>P=0.04</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>P=0.85</td>
<td>P=0.3</td>
</tr>
<tr>
<td>Lumen/total diameter</td>
<td>P=0.78</td>
<td>P=0.48</td>
</tr>
<tr>
<td>Total airway area</td>
<td>P=0.4</td>
<td>P=0.63</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.96</td>
<td>P=0.09</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.14</td>
<td>P=0.2</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.35</td>
<td>P=0.19</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>P=0.48</td>
<td>P=0.18</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.13</td>
<td>P=0.024</td>
</tr>
</tbody>
</table>
### TABLE A4.10:
ANOVA values for the effect of age and allogeneic transplantation on airway component percentages of TB airways.

<table>
<thead>
<tr>
<th>Component percentage of total airway area</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.31</td>
<td>P=0.08</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.23</td>
<td>P=0.55</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.18</td>
<td>P=0.45</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.79</td>
<td>P=0.89</td>
</tr>
<tr>
<td>Component percentage of wall area</td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.023</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.65</td>
<td>P=0.07</td>
</tr>
</tbody>
</table>
Table A4.11:
Epithelial cell counts per 100μm epithelium in the TB+1 airways of controls and 5, 25 and 100 days post transplant (Mean (X)+/−SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 days post T.x.</th>
<th>25 days post T.x.</th>
<th>100 days post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell No.</td>
<td>14</td>
<td>16.7</td>
<td>12.6</td>
<td>▲13.5</td>
<td>16</td>
<td>13.4</td>
<td>15.6</td>
</tr>
<tr>
<td>SE</td>
<td>1.1</td>
<td>0.4</td>
<td>1.2</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Clara cells</td>
<td>5.8</td>
<td>4.9</td>
<td>▲▲▲3.0</td>
<td>▲▲3.6</td>
<td>**3.6</td>
<td>3.1</td>
<td>5.0</td>
</tr>
<tr>
<td>SE</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>9.3</td>
<td>11.4</td>
<td>9.1</td>
<td>9.5</td>
<td>8.0</td>
<td>6.3</td>
<td>9.2</td>
</tr>
<tr>
<td>SE</td>
<td>0.6</td>
<td>0.5</td>
<td>1.3</td>
<td>0.8</td>
<td>0.8</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Basal cells</td>
<td>0.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.2</td>
<td>**2.2</td>
<td>**1.7</td>
<td>**2.2</td>
</tr>
<tr>
<td>SE</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.05</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

compared to age-matched controls.

*  p= 0.05
** p= 0.01
*** p=0.001

comparison between 5 and 100 days post transplantation.

+  p=0.05
++ p=0.01
+++ p=0.001

comparison between 25 and 100 days post transplantation.

^  p=0.05

comparison between age-matched controls of different strains.

▲  p=0.05
▲▲▲ p=0.001
Table A4.12:
Epithelial cell counts per 100 μm epithelium in the TB airways of controls and after 5, 25 and 100 days post transplant (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>5 days post T.x.</th>
<th>25 days post T.x.</th>
<th>100 days post T.x.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T.x BN lung</td>
<td>native Lewis lung</td>
<td>T.x BN lung</td>
<td>native Lewis lung</td>
<td>T.x BN lung</td>
<td>native Lewis lung</td>
</tr>
<tr>
<td>Total cell No.</td>
<td>13.6 ± 0.6</td>
<td>13.8 ± 0.8</td>
<td>11.2 ± 0.8</td>
<td>11.6 ± 1.2</td>
<td>17.3 ± 1.6</td>
<td>13.6 ± 1.5</td>
<td>17.2 ± 1.8</td>
</tr>
<tr>
<td>Clara cells</td>
<td>3.8 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.6 ± 0.6</td>
<td>4.4 ± 0.6</td>
<td>4.8 ± 1.6</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>9.5 ± 0.3</td>
<td>10.3 ± 0.7</td>
<td>7.6 ± 0.6</td>
<td>7.8 ± 1.1</td>
<td>11.8 ± 0.8</td>
<td>10.3 ± 2.3</td>
<td>12.0 ± 1.3</td>
</tr>
<tr>
<td>Basal cells</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>1.8 ± 0.9</td>
<td>*1.8 ± 0.1</td>
</tr>
</tbody>
</table>

compared to age-matched controls.
*p = 0.05
TABLE A4.13:
ANOVA values for the effect of age and allogeneic transplantation on epithelial cell counts in the TB+1 and TB airways.

<table>
<thead>
<tr>
<th>TB+1 airways</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x. ANOVA</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P=0.08</td>
<td>P=0.23</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P=0.07</td>
<td>P=0.003</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P=0.052</td>
<td>P=0.94</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P=0.65</td>
<td>P=0.0003</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TB airways</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x. ANOVA</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P=0.91</td>
<td>P=0.005</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P=0.07</td>
<td>P=0.54</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P=0.99</td>
<td>P=0.06</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P=0.41</td>
<td>P=0.001</td>
</tr>
</tbody>
</table>
Appendices for Chapter 5.
Table A5.7: ANOVA values for the effect of age and isogeneic transplantation on the airway dimensions and component areas of the TB+1 airways.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control Lewis lung Age ANOVA</th>
<th>Transplanted Lewis lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway diameter</td>
<td>P=0.001</td>
<td>P=0.01</td>
<td>P=0.37</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>P=0.001</td>
<td>P=0.005</td>
<td>P=0.34</td>
</tr>
<tr>
<td>Lumen/total diameter</td>
<td>P=0.12</td>
<td>P=0.08</td>
<td>P=0.97</td>
</tr>
<tr>
<td>Total airway area</td>
<td>P=0.017</td>
<td>P=9.6 x10^-6</td>
<td>P=0.004</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.027</td>
<td>P=2.53 x10^-6</td>
<td>P=0.017</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.74</td>
<td>P=0.026</td>
<td>P=0.009</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.12</td>
<td>P=0.01</td>
<td>P=0.12</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>P=0.94</td>
<td>P=0.56</td>
<td>P=0.98</td>
</tr>
<tr>
<td>C. tissue area</td>
<td>P=0.41</td>
<td>P=0.046</td>
<td>P=0.026</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P=0.21</td>
<td>P=0.007</td>
<td>P=0.036</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P=0.54</td>
<td>P=0.19</td>
<td>P=0.28</td>
</tr>
</tbody>
</table>
Table A5.8:
ANOVA values for the effect of age and isogeneic transplantation on the proportions of the TB+1 airways.

<table>
<thead>
<tr>
<th>Component percentage of total airway area</th>
<th>Control Lewis lung Age ANOVA</th>
<th>Transplanted Lewis lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area</td>
<td>P=0.25</td>
<td>P=0.49</td>
<td>P=0.72</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.35</td>
<td>P=0.34</td>
<td>P=0.86</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.6</td>
<td>P=0.016</td>
<td>P=0.32</td>
</tr>
<tr>
<td>C tissue area</td>
<td>P=0.65</td>
<td>P=0.39</td>
<td>P=0.35</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P=0.34</td>
<td>P=0.73</td>
<td>P=0.58</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P=0.33</td>
<td>P=0.64</td>
<td>P=0.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component percentage of wall area</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td>P=0.81</td>
<td>P=0.69</td>
</tr>
<tr>
<td>C.tissue area</td>
<td>P=0.27</td>
<td>P=0.38</td>
</tr>
<tr>
<td>muscle area</td>
<td>P=0.58</td>
<td>P=0.25</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P=0.03</td>
<td>P=0.36</td>
</tr>
</tbody>
</table>
Table A5.9:
ANOVA values for the effect of age and isogenic transplantation on the airway dimensions and component areas of TB airways.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control Lewis lung Age ANOVA</th>
<th>Transplanted Lewis lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway diameter</td>
<td>P=0.001</td>
<td>P=0.002</td>
<td>P=0.14</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>P=0.001</td>
<td>P=0.002</td>
<td>P=0.34</td>
</tr>
<tr>
<td>Lumen/total diameter</td>
<td>P=0.15</td>
<td>P=0.39</td>
<td>P=0.2</td>
</tr>
<tr>
<td>Total airway area</td>
<td>P=0.27</td>
<td>P=4.8x10⁻⁶</td>
<td>P=0.14</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.68</td>
<td>P=7.19x10⁻⁶</td>
<td>P=0.5</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.07</td>
<td>P=0.001</td>
<td>P=0.051</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.04</td>
<td>P=0.053</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>P=0.28</td>
<td>P=0.004</td>
<td>P=0.21</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P= 0.11</td>
<td>P=0.008</td>
<td>P=0.033</td>
</tr>
</tbody>
</table>

222.
Table A5.10:
ANOVA values for the effect of age and isogeneic transplantation on the airway component percentages of the TB airways.

<table>
<thead>
<tr>
<th>Component percentage of total airway area</th>
<th>Control Lewis lung Age ANOVA</th>
<th>Transplanted Lewis lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area</td>
<td>P=0.35</td>
<td>P=0.41</td>
<td>P=0.35</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.35</td>
<td>P=0.41</td>
<td>P=0.35</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.42</td>
<td>P=0.12</td>
<td>P=0.012</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.42</td>
<td>P=0.49</td>
<td>P=0.44</td>
</tr>
<tr>
<td>Component percentage of wall area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.85</td>
<td>P=0.23</td>
<td>P=0.005</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.88</td>
<td>P=0.24</td>
<td>P=0.005</td>
</tr>
</tbody>
</table>
Table A5.11:
Epithelial cell counts per 100 μm epithelium in the TB+1 airways of controls and 14 days and 6 months post transplant (Mean (X) +/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 days post T.x</th>
<th>6 months post T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.x left lung native right lung</td>
<td>T.x left lung native right lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cell No.</td>
<td>X 15.2 12.7</td>
<td>X 18.4 16.3</td>
<td>X* 19.0 14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE 1.8 0.7</td>
<td>SE 1.8 1.5</td>
<td>SE 1.9 1.5</td>
<td></td>
</tr>
<tr>
<td>Clara cells</td>
<td>X 4.4 3.5</td>
<td>X 4.8 4.4</td>
<td>X 5.2 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE 0.6 0.3</td>
<td>SE 0.7 0.6</td>
<td>SE 0.8 0.5</td>
<td></td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>X 10.4 8.7</td>
<td>X 10.3 10.3</td>
<td>X 11.1 8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE 1.7 0.5</td>
<td>SE 1.1 1.3</td>
<td>SE 1.5 1.2</td>
<td></td>
</tr>
<tr>
<td>Basal cells</td>
<td>X 0.3 0.4</td>
<td>X*** 3.3 1.9</td>
<td>X** 2.3 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE 0.2 0.1</td>
<td>SE 0.6 0.5</td>
<td>SE 0.4 0.7</td>
<td></td>
</tr>
</tbody>
</table>

t-tests: compared to age-matched controls.
* p=0.05
** p=0.01
*** p=0.001
Table A5.12:
Epithelial cell counts per 100μm epithelium in the TB airways of controls and 14 days and 6 months post transplant (Mean (X) +/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 days post T.x</th>
<th>6 months post T.x</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.x left lung.</td>
<td>native right lung</td>
<td>T.x left lung</td>
<td>native right lung</td>
</tr>
<tr>
<td>Total cells</td>
<td>X 12.8</td>
<td>X 15.6</td>
<td><strong>17.1</strong></td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Clara cells</td>
<td>X 4.1</td>
<td>X 3.5</td>
<td>4.6</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.4</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>X 8.1</td>
<td>X 11.8</td>
<td>* 10.4</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Basal cells</td>
<td>X 0.5</td>
<td>X 0.3</td>
<td><strong>2.1</strong></td>
<td>*1.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Tests:
Comparison between controls
▲ p= 0.05
Compared to age-matched controls.
* p= 0.05
** p= 0.01
*** p= 0.001
TABLE A5.13:
ANOVA values for the effect of age and isogeneic transplantation on epithelial cell types in the TB+1 and TB airways.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>TB+1 airways</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transplanted Lewis lung</td>
<td>Transplanted Lewis lung</td>
</tr>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P=0.58</td>
<td>P=0.009</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P=0.9</td>
<td>P=0.25</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P=0.71</td>
<td>P=0.36</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P=0.35</td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell type</th>
<th>TB airways</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transplanted Lewis lung</td>
<td>Transplanted Lewis lung</td>
</tr>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P=0.12</td>
<td>P=0.0004</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P=0.08</td>
<td>P=0.55</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P=0.0001</td>
<td>P=0.05</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P=0.09</td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

226.
**Table A5.20:**
ANOVA values for the effect of age and allogeneic transplantation on the airway dimensions and component areas of the TB+1 airways.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control BN lung</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
<td>T.x ANOVA</td>
</tr>
<tr>
<td>Airway diameter</td>
<td>P=0.022</td>
<td>P=0.027</td>
<td>P=0.096</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>P=0.012</td>
<td>P=0.02</td>
<td>P=0.08</td>
</tr>
<tr>
<td>Lumen/total diameter</td>
<td>P=0.21</td>
<td>P=0.86</td>
<td>P=0.69</td>
</tr>
<tr>
<td>Total airway area</td>
<td>P=0.003</td>
<td>P=0.005</td>
<td>P=0.003</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.003</td>
<td>P=0.004</td>
<td>P=0.006</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.01</td>
<td>P=0.47</td>
<td>P=0.046</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.032</td>
<td>P=0.22</td>
<td>P=0.081</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>P=0.24</td>
<td>P=0.33</td>
<td>P=0.7</td>
</tr>
<tr>
<td>C.tissue area</td>
<td>P=0.02</td>
<td>P=0.73</td>
<td>P=0.065</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P=0.14</td>
<td>P=0.55</td>
<td>P=0.16</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P=0.41</td>
<td>P=0.27</td>
<td>P=0.12</td>
</tr>
</tbody>
</table>
Table A5.21:
ANOVA values for the effect of age and allogeneic transplantation on the airway component percentages of the TB+1 airways.

<table>
<thead>
<tr>
<th>Component percentage of total airway area</th>
<th>Control BN lung Age ANOVA</th>
<th>Transplanted BN lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area</td>
<td>P = 0.04</td>
<td>P = 0.024</td>
<td>P = 0.75 (age p = 0.02)</td>
</tr>
<tr>
<td>Wall area</td>
<td>P = 0.034</td>
<td>P = 0.23</td>
<td>P = 0.71</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P = 0.04</td>
<td>P = 0.003</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>C.tissue area</td>
<td>P = 0.43</td>
<td>P = 0.15</td>
<td>P = 0.59</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P = 0.99</td>
<td>P = 0.75</td>
<td>P = 0.75</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P = 0.003</td>
<td>P = 0.28</td>
<td>P = 0.28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component percentage of wall area</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td>P = 0.2</td>
<td>P = 0.22</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>C.tissue area</td>
<td>P = 0.14</td>
<td>P = 0.89</td>
<td>P = 0.08</td>
</tr>
<tr>
<td>Muscle area</td>
<td>P = 0.34</td>
<td>P = 0.24</td>
<td>P = 0.24</td>
</tr>
<tr>
<td>Bronchial artery area</td>
<td>P = 0.013</td>
<td>P = 0.81</td>
<td>P = 0.81</td>
</tr>
</tbody>
</table>
Table A5.22: ANOVA values for the effect of age and allogeneic transplantation on the airway dimensions and component areas of TB airways.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control BN lung Age ANOVA</th>
<th>Transplanted BN lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway diameter</td>
<td>P=0.35</td>
<td>P=0.54</td>
<td>P=0.18</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td>P=0.29</td>
<td>P=0.84</td>
<td>P=0.16</td>
</tr>
<tr>
<td>Lumen/total diameter</td>
<td>P=0.46</td>
<td>P=0.39</td>
<td>P=0.41</td>
</tr>
<tr>
<td>Total airway area</td>
<td>P=0.45</td>
<td>P=0.002</td>
<td>P=0.1</td>
</tr>
<tr>
<td>Lumen area</td>
<td>P=0.4</td>
<td>P=0.012</td>
<td>P=0.064</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.72</td>
<td>P=0.0035</td>
<td>P=0.27</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.85</td>
<td>P=0.0023</td>
<td>P=0.34</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>P=0.93</td>
<td>P=0.1</td>
<td>P=0.77</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.67</td>
<td>P=0.023</td>
<td>P=0.29</td>
</tr>
</tbody>
</table>
Table A5.23:
ANOVA values for the effect of age and allogeneic transplantation on airway component percentages of the TB airways.

<table>
<thead>
<tr>
<th>Component percentage of total airway area</th>
<th>Control Lewis lung Age ANOVA</th>
<th>Transplanted Lewis lung T.x ANOVA</th>
<th>Native Lewis lung T.x ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area</td>
<td>P=0.72</td>
<td>P=0.38</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Wall area</td>
<td>P=0.72</td>
<td>P=0.5</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Epithelial area</td>
<td>P=0.74</td>
<td>P=0.87</td>
<td>P=0.05</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.76</td>
<td>P=0.43</td>
<td>P=0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component percentage of wall area</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial area</td>
<td>P=0.96</td>
<td>P=0.39</td>
<td>P=0.37</td>
</tr>
<tr>
<td>Submucosa area</td>
<td>P=0.47</td>
<td>P=0.39</td>
<td>P=0.73</td>
</tr>
</tbody>
</table>
Table A5.24:
Epithelial cell counts per 100 μm epithelium in the TB+1 airways of controls and 14 days and 6 months post transplant (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 days post T.x. T.x BN lung native Lewis lung</th>
<th>6 months post T.x. T.x BN lung native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell No.</td>
<td><strong>X</strong> 12.0 0.6</td>
<td><strong>X</strong> 15.4 1.7</td>
<td><strong>X</strong> 15.2 1.8</td>
<td><strong>X</strong> 12.7 0.7</td>
<td><strong>X</strong> 16.4 4.6</td>
<td><strong>X</strong> 12.7 1.0</td>
</tr>
<tr>
<td>Clara cells</td>
<td><strong>X</strong> 3.7 0.5</td>
<td><strong>X</strong> 4.6 0.4</td>
<td><strong>X</strong> 4.0 0.6</td>
<td><strong>X</strong> 3.5 0.3</td>
<td><strong>X</strong> 4.0 1.7</td>
<td><strong>X</strong> 4.5 0.3</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td><strong>X</strong> 7.7 0.4</td>
<td><strong>X</strong> 9.3 1.7</td>
<td><strong>X</strong> 10.4 1.7</td>
<td><strong>X</strong> 8.7 0.5</td>
<td><strong>X</strong> 10.6 2.5</td>
<td><strong>X</strong> 7.8 0.7</td>
</tr>
<tr>
<td>Basal cells</td>
<td><strong>X</strong> 0.5 0.2</td>
<td><strong>X</strong> 0.5 0.3</td>
<td><strong>X</strong> 0.2 0.1</td>
<td><strong>X</strong> 0.4 0.1</td>
<td><strong>X</strong> 1.6 0.3</td>
<td><strong>X</strong> 0.2 0.1</td>
</tr>
</tbody>
</table>
Table A5.25:
Epithelial cell counts per 100μm epithelium in the TB airways in controls and 14 days and 6 months post transplant (Mean (X)+/-SE).

<table>
<thead>
<tr>
<th>CELL TYPES</th>
<th>Young adult BN control</th>
<th>Mature adult BN control</th>
<th>Young adult Lewis control</th>
<th>Mature adult Lewis control</th>
<th>14 days post T.x. T.x BN lung</th>
<th>native Lewis lung</th>
<th>6 months post T.x. T.x BN lung</th>
<th>native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell No.</td>
<td>15.9±0.8</td>
<td>15.2±0.6</td>
<td>12.8±0.7</td>
<td>15.6±0.6</td>
<td>14.9±0.6</td>
<td>13.2±0.9</td>
<td>16.3±0.6</td>
<td>11.1±0.7</td>
</tr>
<tr>
<td>Clara cells</td>
<td>3.2±0.6</td>
<td>3.2±0.4</td>
<td>4.1±0.5</td>
<td>3.5±0.4</td>
<td>2.8±0.6</td>
<td>4.4±0.5</td>
<td>2.6±0.5</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>11.9±0.9</td>
<td>11.4±0.4</td>
<td>8.1±0.5</td>
<td>11.8±0.7</td>
<td>10.4±1.1</td>
<td>8.1±0.6</td>
<td>13.1±0.6</td>
<td>8.1±0.5</td>
</tr>
<tr>
<td>Basal cells</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.5±0.1</td>
<td>0.32±0.19</td>
<td>*2.1±0.6</td>
<td>0.8±0.2</td>
<td>*0.8±0.2</td>
<td>0.4±0.2</td>
</tr>
</tbody>
</table>

* p= 0.05 compared to age-matched controls.
Table A5.26: ANOVA values for the effect of age and allogeneic transplantation on epithelial cell counts in the TB+1 and TB airways.

<table>
<thead>
<tr>
<th>cell type</th>
<th>TB+1 airways</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
<td>Age ANOVA</td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P=0.44</td>
<td>P=0.23</td>
<td>P=0.95</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P=0.78</td>
<td>P=0.61</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P=0.5</td>
<td>P=0.17</td>
<td>P=0.52</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P=0.44</td>
<td>P=0.15</td>
<td>P=0.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cell type</th>
<th>TB airways</th>
<th>Transplanted BN lung</th>
<th>Native Lewis lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age ANOVA</td>
<td>T.x ANOVA</td>
<td>Age ANOVA</td>
</tr>
<tr>
<td>Total epithelial cells</td>
<td>P=0.93</td>
<td>P=0.62</td>
<td>P=0.36</td>
</tr>
<tr>
<td>Clara cells</td>
<td>P=0.75</td>
<td>P=0.33</td>
<td>P=0.2</td>
</tr>
<tr>
<td>Ciliated cells</td>
<td>P=0.18</td>
<td>P=0.94</td>
<td>P=0.2</td>
</tr>
<tr>
<td>Basal cells</td>
<td>P=0.06</td>
<td>P=0.01</td>
<td>P=0.16</td>
</tr>
</tbody>
</table>

233.
Bibliography.


235.


237.


241.


246.


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