CARDIAC AND PULMONARY REPLACEMENT

MATURE PULMONARY LOBAR TRANSPLANTS GROW IN AN IMMATURE ENVIRONMENT

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Objective: Mature lobar transplantation will increase the pediatric donor organ pool, but it remains unknown whether such grafts will grow in a developing recipient and provide adequate long-term support. We hypothesized that a mature pulmonary lobar allograft implanted in an immature recipient would grow. **Methods:** We investigated our hypothesis in a porcine orthotopic left lung transplant model using animals matched by the major histocompatibility complex to minimize the effects of chronic rejection. Twenty-three immature animals (<12 weeks of age and <10 kg total body weight) received either sham left thoracotomy (SH control, $n = 4$), left upper lobectomy to study compensatory growth (UL control, $n = 4$), age-matched immature whole left lung transplants (IWL TXP, $n = 6$), mature (donor > 1 yr in age and > 40 kg in total body weight) left lower lobe transplants (MLL TXP, $n = 5$), or mature left upper lobe transplants (MUL TXP, $n = 4$). Twelve weeks after implantation, functional residual capacity of the left lung was measured and arterial blood gas samples were obtained after the native right lung had been excluded. The graft was excised and weighed, and samples for microscopy and wet/dry ratios were collected. **Results:** Initial and final graft weights were as follows: IWL TXP group (34.6 ± 1.5 and 107.8 ± 5.9 gm, $p < 0.0001$), MLL TXP group (72.4 ± 6.8 and 111.4 ± 8.7 , $p < 0.001$), and MUL TXP group (32.8 ± 1.3 and 92.8 ± 7.1 gm, respectively, $p < 0.004$). No significant differences between groups were demonstrated when functional residual capacity, wet/dry ratios, or oxygenation were compared. Immunohistochemical staining for the nuclear antigen Ki-67 demonstrated dividing pneumocytes. **Conclusions:** We conclude that a mature lobar graft implanted into an immature recipient grows by pneumocyte division in this model. Mature lobar transplants can be expected to grow and provide adequate long-term function in developing recipients. (J Thorac Cardiovasc Surg 1997;114:186-94)

Despite advances in all aspects of pulmonary transplantation, this successful therapeutic option remains limited by a critical shortage of suitable donors. Approximately 25% of patients on recipient lists will die of their underlying pulmonary disease

while awaiting identification of a suitable donor.¹ The pediatric population awaiting lung transplantation is further restricted as organ availability is limited by size discrepancies between the potential recipient and donor.² Reduced-size lung transplan-

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tation has been studied in experimental models and applied clinically.^{3,4} Such a strategy could provide a partial solution to the critical donor shortage and the difficulties related to size disparity in pediatric lung transplantation.

In addition to a paucity of lung donors, the other critical issue in pediatric lung transplantation pertains to lung growth. It remains unknown whether a transplanted graft will continue to develop in a growing individual, thus supporting the long-term respiratory requirements of the recipient.⁵ Growth potential is especially in question when pertaining to mature reduced-size grafts, and the long-term efficacy of these grafts has been questioned.⁶ It is possible that such grafts may serve only as a temporary bridge to retransplantation, at which time the recipient's thorax could accommodate an anatomically mature lung. Our laboratory has previously reported growth of a mature lobar transplant in a domestic swine model as noted by gross weights and fixed lung volumes.⁷ However, a possible limitation of this study may have been the effects of chronic rejection. In addition, the cell type responsible for this growth was not identified, nor was the growth potential of transplanted immature lung tissue investigated. In an effort to minimize the confounding effects of chronic rejection in these long-term studies and ensure transplantation of fully mature donor lung tissue, we developed a similar model in a strain of miniature swine matched according to major histocompatibility complex (MHC).⁸ In the present study we investigated the growth and function of immature whole lungs, mature lower lobes, and mature upper lobes transplanted into immature recipients using an orthotopic left lung transplant model. To identify the cell type responsible for lung growth, we used an immunohistochemical staining method that uses an antibody to the nuclear antigen Ki-67.

Materials and methods

Experimental protocol. Twenty-three immature swine comprised five study groups: two control groups and three transplant recipient groups. Nine mature and six immature swine (MHC-matched with recipients) served as lung donors. Immature recipients were less than 12 weeks of age and averaged 9.7 kg in total body weight. Mature donors were at least 1 year of age and averaged 42.2 ± 6.4 kg in total body weight. Immature donors were age- and size-matched with the recipients, averaging 7.9 ± 0.5 kg in total body weight. Group I (SH control) underwent sham left thoracotomy ($n = 4$). Group II (UL control) underwent left upper lobectomy ($n = 4$). Group III (IWL TXP) received age- and size-matched immature whole left lung

transplants ($n = 6$). Group IV (MLL TXP) received mature left lower lobe transplants ($n = 5$). Group V (MUL TXP) received mature left upper lobe transplants ($n = 4$). All lungs were flushed with cold Euro-Collins solution before preparation of the graft for implantation. After transplantation the animals were allowed to develop for 12 weeks before final study.

Harvest procedure. In accordance with the following experimental model, previously described by our laboratory,^{9,10} nine adult Hanford miniature swine served as mature lobar donors. Six immature age-, size-, and MHC-matched swine served as immature whole lung donors. Anesthesia was maintained with 2.0% inhalational halothane. A left posterolateral thoracotomy was performed. The hemiazygos vein was ligated and divided, facilitating exposure to the left hilum. The incomplete fissure between the left upper lobe and lower lobe was sharply divided in the mature lobar donors. Care was taken to carry out the dissection on the side of the unused lobe so as to avoid air leaks in the recipient. Each animal was then given intravenous heparin sodium (200 U/kg). The main pulmonary artery was clamped and a 16-gauge catheter was inserted into the distal vessel. A left atriotomy was then performed to vent the heart. One liter of Euro-Collins solution at 4° C was infused into the pulmonary artery from a height of 30 cm. Topical cooling was achieved with cold saline slush. Room air ventilation was continued during the Euro-Collins flush. The lung was then excised and prepared for implantation.

Mature lobar grafts were prepared for implantation *ex vivo*. Lower lobe grafts were prepared by performing an upper lobectomy. The donor lower lobe pulmonary artery and bronchus were then transected. The pulmonary artery was transected just distal to the lingular artery while still allowing enough length to perform the anastomosis without obstructing the artery to the superior segment. In animals in which the lingular artery branched off distally, it was ligated and the anastomosis was performed proximal to the takeoff. The bronchus was divided as close to the takeoff of the sixth segmental bronchus as possible. Upper lobe grafts were prepared by performing a left lower lobectomy. The upper lobe bronchus was prepared with a cuff of main-stem bronchus to allow for size mismatching. The venous anastomoses were accomplished with a left atrial cuff. The upper and lower lobe veins were identified as they entered the atrium and they were appropriately ligated. Before implantation all grafts were weighed.

Recipient pneumonectomy and implantation. Fifteen immature MHC-matched swine underwent a left pneumonectomy and served as left lung recipients. In addition, four immature animals underwent a sham left thoracotomy and four animals underwent a standard left upper lobectomy. All animals received cyclosporine (18 mg/kg) (Sandimmune, Sandoz Pharmaceuticals Corp., East Hanover, N.J.; INN: ciclosporin) and aspirin (325 mg) 1 day before transplantation. Azathioprine (1 mg/kg) and methylprednisolone (500 mg) were administered intravenously along with cefazolin (250 mg) before making the skin incision. Anesthesia was maintained with 1.5% inhalational isoflurane. Through a left posterolateral thoracotomy, the left lung was mobilized and the hilum dissected

Fig. 1. Chest roentgenogram demonstrating normal aeration of a mature lower lobe transplant after 12 weeks of development.

as in the donor pigs. Dissection around the recipient bronchus was minimized. The recipient was then given with intravenous heparin sodium (200 U/kg), the pulmonary artery was clamped, and the pulmonary veins and pulmonary artery were ligated and divided. The left main-stem bronchus was then lightly clamped, and the left lung was excised.

Implantation commenced with the bronchial anastomosis, followed by the pulmonary arterial and left atrial anastomoses. All anastomoses were performed with absorbable suture to allow for growth. The bronchial anastomosis was done with a continuous 5-0 monofilament polydioxanone suture (PDS II, Ethicon, Inc., Somerville, N.J.). The donor bronchus was intussuscepted into the recipient bronchus approximately 4 to 6 mm to achieve a telescoping anastomosis. In animals receiving mature lower lobe grafts, the recipient bronchus was telescoped into the donor bronchus. The large diameter of the mature lower lobe donor bronchus as compared with the immature recipient's main-stem bronchus necessitated this difference in technique. The graft was ventilated with 100% oxygen until completion of the remaining anastomoses. The pulmonary arterial anastomosis was then completed with a continuous 6-0 polydioxanone suture and the left atrial cuff anastomosis was carried out with a continuous 5-0 polydioxanone suture. Intercostal nerve blocks were performed with 0.25% bupivacaine HCl (Sen-

soraine, Astra Pharmaceutical Products, Westborough, Mass.). The chest wall was closed in layers, with a chest tube connected to a waterseal chest drainage system (Atrium Medical Corp., Hudson, N.H.) being left in place. All animals were extubated within 1 hour after the operation.

Immunosuppression consisted of cyclosporine (18 mg/kg per day), azathioprine (1 mg/kg per day), and corticosteroids. Cyclosporine was continued for 7 days. Azathioprine was continued for the full 3-month growth period. Methylprednisolone (500 mg/day) was administered daily for 7 days and then switched to maintenance prednisone (2 mg/kg by mouth four times daily), which was continued for 3 months. Additionally, each animal received a daily 325 mg aspirin tablet and sulfamethoxazole-trimethoprim (Bactrim DS, Hoffmann-LaRoche) one tablet by mouth twice a day. Cefazolin (250 mg four times a day) was continued for 7 days after the operation. Chest roentgenograms were obtained 6 weeks after transplantation and at the time of the final study.

All animals received humane care in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No. 85-23, revised 1985.) The protocol was reviewed and approved by the Animal Review Committee of the University of Virginia.

Data collection. After 3 months of development, a second chest roentgenogram was obtained to document aeration of the transplanted lung. A tracheostomy was then performed, through which a 9F cuffed endotracheal tube was inserted. The right carotid artery and internal jugular vein were dissected, and a 16-gauge catheter was placed in the carotid artery for pressure measurements and blood gas analysis. A large-bore introducer was placed in the right internal jugular vein for intravenous access. Bronchoscopic examination was performed with a flexible pediatric bronchoscope (3.5 mm outer diameter). The bronchial anastomosis was examined for evidence of narrowing or technical complications.

Lung volumes. Functional residual capacity (FRC) data were recorded during double lung ventilation and again after exclusion of the native right lung. FRC was calculated by means of the helium dilution technique.¹¹ FRC of the transplanted lung was obtained by occluding the native right lung airways with a 6F Fogarty balloon catheter (model CV1048, Baxter Healthcare Corp., Santa Ana, Calif.). The catheter was positioned and inflated in the right main-stem bronchus under direct vision with the aid of a bronchoscope. The right eparterial bronchus, present in swine, was occluded by intubation of the proximal left main-stem bronchus. The endotracheal tube cuff was then inflated to occlude the airway to the right upper lobe.

Studies of gas exchange. Blood gas measurements were obtained under conditions of double lung ventilation and again during single lung ventilation 15 minutes after clamping of the right hilum. Arterial blood specimens were collected during room air ventilation and with an inspired oxygen fraction of 70%. Blood gas samples were run on a Corning blood gas analyzer (Corning 178 pH/Blood Gas Monitor; CIBA-Corning Diagnostics Corporation, Medfield, Mass.).

Fig. 2. Comparison between explanted lung weight (*Explant Wt*) and graft weight (*Graft Wt*) at the time of implantation. Mature lower lobe grafts (*MLL TXP*) were significantly larger than the recipient's explanted lung.

Graft weight measurements. At the completion of the study, the animal was killed with a lethal dose of sodium pentobarbital, and the transplanted lung was excised. The transplanted graft was then weighed and samples for wet/dry analysis, microscopy, and immunohistochemical staining were obtained. Control lungs and lower lobes from the upper lobectomy control group were treated similarly.

Immunohistochemical staining. Ki-67 is a nuclear non-histone that begins to be expressed in the late G_1 phase, accumulates in the S phase, and reaches maximal levels in the M phase of the cell cycle. This nuclear protein is then rapidly lost from postmitotic cells.^{12, 13} For detection of Ki-67 antigen, the monoclonal antibody MIB-1 (AMAC, Westbrook, Mass.) was applied to paraffin-embedded tissue sections. Immunohistochemistry studies were performed by means of the avidin-biotin complex method modified by a microwave pretreatment of the paraffin sections for antigen retrieval.¹⁴ An automated immunostainer (Ventana ES Automated Slide Stainer, Ventana Medical Systems, Tucson Ariz.) was used to ensure uniform staining.

Statistical analysis. Measurements are reported as the mean $\pm$ the standard error of the mean. Analysis of variance was used to determine whether significant differences existed between groups. A p value of 0.05 or less was used to indicate a significant difference between measurements.

Results

Technical results. The mean ischemic times were as follows and demonstrate a trend toward increased ischemia for the mature lower lobe transplants: IWL TXP group (90.3 ± 3.9 minutes) and MUL TXP group (101.0 ± 6.1 minutes) versus MLL TXP group (110.2 ± 7.4 minutes), $p = 0.074$. No prolonged air leaks developed, and all chest tubes were removed

by postoperative day 2. All animals demonstrated normal aeration of the transplanted lung (Fig. 1). No airway complications of functional significance were identified. A granuloma developed at the anastomotic site in an immature whole lung recipient (IWL TXP group), and this granuloma resulted in a 20% narrowing of the airway. One mature lower lobe recipient (MLL TXP group) had an insignificant narrowing caused by a small cartilaginous shelf at the anastomosis.

Measurements of mass. Total body weight increased significantly in all animals. The mean recipient body weights at the time of transplantation were as follows: 9.1 ± 1.4 kg (SH control), 10.8 ± 1.4 kg (UL control), 9.1 ± 0.7 kg (IWL TXP), 9.8 ± 1.3 kg (MLL TXP), and 10.1 ± 1.4 kg (MUL TXP). After 12 weeks of development the total body weights were as follows: 26.0 ± 2.0 kg (SH control), 26.8 ± 1.6 kg (UL control), 26.0 ± 1.1 kg (IWL TXP), 28.3 ± 2.3 kg (MLL TXP), and 26.0 ± 1.5 kg (MUL TXP), demonstrating no differences in final body weights among the groups ($p = 0.81$). The mean graft weights at the time of implantation were 34.6 ± 1.5 gm (IWL TXP), 72.4 ± 6.8 gm (MLL TXP), and 32.8 ± 1.3 gm (MUL TXP), demonstrating significantly larger lower lobe grafts ($p < 0.001$) (Fig. 2). The recipients' mean explanted lung weights at the time of implantation were 41.5 ± 2.0 gm (IWL TXP), 42.2 ± 5.6 gm (MUL TXP), and 38.3 ± 4.5 gm (MLL TXP). The mean lung weights after 12 weeks of development were as follows: 75.5 ± 5.8 gm (SH control), 80.1 ± 7.3 gm (UL

Fig. 3. Initial graft weights at the time of implantation versus the final graft weights after 12 weeks of development. All groups demonstrated significant increases in lung mass, and no differences in final lung weight were demonstrated between groups.

control), 107.9 ± 5.9 gm (IWL TXP), 111.4 ± 8.7 gm (MLL TXP), and 92.8 ± 7.1 gm (MUL TXP), revealing significant increases in initial versus final graft weights (Fig. 3). Final/initial graft weight ratios for the transplant groups are as follows: 3.1 (IWL TXP), 1.5 (MLL TXP), and 2.8 (MUL TXP). The graft/body weight ratios after 12 weeks of development are as follows: 0.0029 ± 0.0001 (SH control), 0.0030 ± 0.0003 (UL control), 0.0041 ± 0.0001 (IWL TXP), 0.0040 ± 0.0003 (MLL TXP), and 0.0036 ± 0.0005 (MUL TXP), $p = 0.001$ (Fig. 4).

Functional results. The FRC was determined with double lung ventilation and again after excluding the native right lung, thereby reflecting the volume of only the transplanted graft. The double lung FRC and single lung FRC are depicted in Table I. Oxygenation was equivalent between groups with an inspired oxygen fraction of 70%; however, worsening oxygenation in the IWL TXP group was demonstrated with the animals breathing room air (Table I). Wet/dry ratios were as follows and revealed no significant differences between groups: 4.88 ± 0.14 (SH control), 4.70 ± 0.10 (UL control), 4.84 ± 0.13 (IWL TXP), 4.72 ± 0.17 (MLL TXP), and 4.37 ± 0.33 (MUL TXP) (Table I).

Immunohistochemical staining. Immunohistochemical staining for Ki-67 demonstrated dividing

pneumocytes in all transplant groups and control groups (Fig. 5). Dividing inflammatory cells, including lymphocytes and polymorphonuclear leukocytes, were also stained. These cells were distinguished from pneumocytes by their morphologic characteristics.

Discussion

Lung transplantation has been increasingly applied in the pediatric population to treat a diverse group of primary pulmonary diseases, as well as secondary pulmonary hypertension as a result of congenital heart disease.¹⁵ Two critical issues are especially pertinent to pediatric lung transplantation. Most important, donor lung availability has limited the number of transplant procedures being performed. Second, it remains unknown whether transplanted lung tissue will continue to grow in a developing recipient to the extent that functional demands as an adult can be supported.

Mortality rates remain high on waiting lists for pediatric lung transplantation. A 32% mortality rate has been reported for pediatric patients awaiting identification of a suitable donor.² Appropriate size matching for small recipients places further restrictions on the number of available organs for pediatric transplantation, and only 44 pediatric lung or heart-lung transplantations were undertaken in 1995.¹⁶

Fig. 4. Graft/body weight ratios at the time of final study demonstrate significantly higher ratios when comparing the immature whole lung and mature lower lobe transplant groups with the control lobectomy and sham thoracotomy groups.

Table I. FRCs and wet/dry ratios demonstrate no significant differences between groups

Groups	FRC double lung (ml)	FRC transplant lung (ml)	Wet/dry ratio	PaO ₂ transplant lung room air (mm Hg)	PaO ₂ transplant lung FiO ₂ 70% (mm Hg)
SH control	504.5 ± 51.6	258.5 ± 24.2	4.9 ± 0.1*	85.2 ± 9.8	435.5 ± 68.9
UL control	527.5 ± 47.0	270.5 ± 32.8	4.7 ± 0.1	104.4 ± 11.6	382.2 ± 40.6
IWL TXP	457.2 ± 16.6*	200.5 ± 23.7†	4.8 ± 0.1	63.8 ± 4.2‡	318.2 ± 48.9§
MUL TXP	544.2 ± 21.6	292.1 ± 24.5	4.4 ± 0.5	74.0 ± 12.8	482.3 ± 19.2
MLL TXP	466.4 ± 37.2	244.0 ± 11.3	4.7 ± 0.2	77.3 ± 8.8	327.5 ± 37.4

A trend toward decreased FRC with the use of immature whole lungs is revealed. Immature whole lung transplants (IWL TXP) also demonstrated a significantly lower oxygenation on room air when compared with the upper lobectomy control group. (UL control). For description of groups, see text. PaO₂, Arterial oxygen tension; FiO₂, inspired oxygen fraction.

*p = Not significant.

†p = 0.12 versus MUL TXP.

‡p = 0.04 versus UL.

§p = 0.21 versus MUL TXP.

Given this critical shortage of available organs, alternative reduced-size lung transplants have been proposed and used clinically.^{4, 17, 18} Although the intermediate results with reduced-size lobar transplants are encouraging, no long-term human data documenting continued growth of these grafts exists, nor is it known whether the functional capacity of these grafts can continue to support an immature recipient into adulthood. The growth potential of mature pulmonary tissue transplanted into an immature environment remains unknown. Dunnill¹⁹ reported that development of new alveolar units is virtually completed by 8 years of life and that

subsequent growth in lung volume occurs by an increase in linear dimensions. He further speculated that regeneration of the lung was unlikely after that age. Thurlbeck²⁰ later reported data supporting Dunnill's conclusion that the majority of alveoli are present by 2 years of life and that alveolar multiplication after that time is limited or does not occur at all. Can mature lung tissue regain its potential for further development? What effects does transplantation have on lung development? Transplantation departs from the normal developmental scenario as the graft is influenced by a new recipient environment, altered functional demands, host immuno-

Fig. 5. Immunohistochemical staining for Ki-67 demonstrating dividing pneumocytes.

logic responses, and immunosuppressive drugs. The long-term success of pediatric lung transplantation will require a transplanted graft, immature or mature, to continue to develop, both functionally and anatomically.

Several studies have demonstrated that immature lung tissue can continue to develop after transplantation. Haverich and coworkers⁵ reported that lung transplantation in growing piglets resulted in an increase in graft size in proportion to the somatic growth of the recipient organism; however, they also noted that somatic growth in transplant recipients was retarded as compared with growth in animals not receiving transplants. In a Lewis rat model, Hislop and coworkers²¹ concluded that the immature lung will continue to grow by formation of new alveolar units and hypertrophy of existing units after left lung transplantation. Our results corroborate these findings, as the immature whole lung transplants demonstrated a 212% increase in overall mass during the 12-week posttransplantation developmental period. These lungs also demonstrated a final FRC comparable with that of the mature lobar transplant recipients and control groups. We did not witness any aberrations in somatic growth as a result of the transplantation process or immunosuppressive therapy, although in this MHC-matched model cyclosporine was continued for only 7 days after the operation. Therefore immature lungs can continue to develop after transplantation, but can mature lung tissue?

To date, no clinical data and minimal experimental data exist to demonstrate that transplanted mature lung tissue can regain its previous developmental potential. Crombleholme and colleagues³ demonstrated the feasibility of lobar transplantation as they investigated the hemodynamic responses in an immature swine model, identifying the growth potential of a denervated mature lobar graft in a neonate as "another uncharted area." Although Backer and associates²² again succeeded with lobar transplantation in a survival model, their study was limited; because only two animals survived for an extended period, they were unable to demonstrate growth. Lillehei, Everts, and Shamberger²³ were able to transplant oversized mature grafts using staple pneumoreduction; however they too recognized that "the crucial unanswered issue is the long-term function and growth of these reduced-size allografts." Our group was the first to demonstrate the growth of mature lobar transplants in a porcine model.⁷ This growth was determined by gross weight and fixed lung volumes, but no increase in FRC was observed and the tissue component responsible for this increase in mass was not identified.

Our data support the finding of lung growth after mature lobar transplantation. All animals demonstrated normal somatic growth when compared with the sham thoracotomy control group (SH control), averaging an increase in body weight of 172% during the 12-week posttransplantation developmental period. During this time the transplanted

grafts increased 212% (IWL TXP group), 183% (MUL TXP group), and 54% (MLL TXP group) in mass. The diminished increase seen with the mature lower lobes is due to their greater initial weight at the time of implantation. When the graft/body weight ratios are compared, the transplant groups had higher ratios than both the sham thoracotomy (SH control) and upper lobectomy (UL control) control groups. The difference reached significance when control groups were compared with the immature (IWL TXP) and mature (MLL TXP) transplant groups. Extravascular water content could easily explain an apparent increase in mass. However the wet/dry ratios were similar among all groups, thereby confirming an increase in parenchymal elements. An appropriate increase in air space of the transplanted lung tissue was also seen, for the groups were equivalent when their FRCs were compared at the end of the developmental period.

The mechanism by which this apparent lung growth proceeds continues to be speculative. Whether the increase in weight and air-space volume represents the formation of new alveoli or simply an increase in size of those alveoli present has been debated. From our results we cannot comment on alveolar size or numbers of functional units because morphometric techniques were not used. Many authors believe the theory and practice of counting alveoli are inaccurate and that morphometric evaluation of the lung is technically difficult.²⁴ We have, however, demonstrated definitively that dividing pneumocytes are present in transplanted mature lung tissue that has undergone an increase in mass and air-space volume. Using an immunohistochemical stain for the proliferating cell marker Ki-67, we have identified the presence of dividing pneumocytes in both immature and mature transplanted lung tissue. Such a finding in this MHC-matched model with minimal rejection leads us to conclude that the observed increase in mass is due to an increased number of pneumocytes. Thus lung growth occurs by hyperplasia as the transplanted lung tissue attempts to maintain an adequate gas exchange surface area. The role of these dividing pneumocytes, whether it be the formation of additional gas exchange units or the enlargement of existing units, will require further studies.

Similarly, the origin of these dividing pneumocytes remains unknown and is currently under investigation in our laboratory. Even during normal growth and development, the exact precursor cell of type 1 and type 2 pneumocytes is debated. Type 1

cells may be derived from undifferentiated epithelial cells or from differentiated type 2 pneumocytes. Terminal differentiation involves the loss of proliferative potential, and it is thought that type 1 cells cannot be induced to proliferate in mature lung, whereas type 2 pneumocytes may act as stem cells, providing new type 1 cells to line the gas exchange surfaces.^{25, 26} The effects of transplantation and the new host environment on mature lung tissue remain unknown. We have identified dividing pneumocytes in transplanted mature lung tissue. However, we have not determined at this time whether they represent (1) type 1 pneumocytes that have regained the potential for division or (2) cells derived from type 2 pneumocytes acting as a type of pleuripotent stem cell. The mechanisms responsible for pneumocyte differentiation and proliferation need to be determined, and transplantation models may provide unique insight into these issues.

Previous studies have implicated the volume of the thoracic cavity in the regulation of lung growth and regeneration. Early work by Cohn²⁷ in a rat model attempted to demonstrate that the increase in lung size is due "solely to the mechanical stimulus of the change in pull exerted by the alteration in size of the thoracic cage which the lung must fill." He concluded that removal of lung tissue is followed by restitution of lung weight in proportion to the body weight of the animal. Similarly, our data suggest that the host environment has an effect on final lung mass and volume after transplantation and after lobectomy. Although there was a trend toward larger final graft weights with MLL transplants (MLL TXP group), differences between groups were not significant. Regardless of initial graft weight, which ranged from 32.8 gm in the mature upper lobe transplant group (MUL TXP) to 72.4 gm in the mature lower lobe group (MLL TXP), the smaller mature upper lobe grafts reached a final weight and FRC that did not differ significantly from those of the controls or the initially larger mature lower lobe grafts. The upper lobectomy control group (UL control) had a final lower lobe weight comparable with that of the sham control left lungs, suggesting that regeneration occurred until the expected final lung weight was achieved. This similarity in final organ size, regardless of initial size, suggests that the recipient environment plays a regulatory role in lung growth and determination of final lung size. The mechanism for this regulation likely pertains to functional demands, but that remains to be elucidated.

In conclusion, we have demonstrated growth of mature pulmonary lobar allografts after implantation into an immature recipient using an MHC-matched porcine model. Extravascular water content is not responsible for the observed increase in allograft mass. This growth occurs by hyperplasia, inasmuch as dividing pneumocytes were identified with the use of an immunohistochemical stain for the nuclear antigen Ki-67. Similarly, compensatory lung growth after lobectomy occurs by hyperplasia. When comparing lung function between mature lobar transplants, immature whole lung transplants, and control thoracotomies, we identified no significant differences. Either mature lobar grafts or immature whole lung grafts can be expected to grow and provide excellent long-term function in developing recipients, provided rejection is adequately controlled.

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