



Cellular and Molecular Events Mediating Growth of Mature Pulmonary Lobar Transplants

Jeffrey T. Cope, James J. Gangemi, Aditya K. Kaza, Scott D. Ross, Stewart M. Long, Steven M. Fiser, Kimberly S. Shockey, John A. Kern, Curtis G. Tribble, Irving L. Kron, and Victor E. Laubach

Div. of Thoracic and Cardiovascular Surgery, Dept. of Surgery, University of Virginia Health System, Charlottesville, Virginia, USA



Background

- Lung transplantation is a successful therapeutic option for patients with end-stage lung disease.
- Widespread application of transplants, however, is limited by a shortage of donor organs, especially in the pediatric population.
- Donor lungs are even more scarce as a result of donor-recipient size discrepancies.
- Only 77 pediatric lung transplantations were performed worldwide in 1998.
- In some centers over 30% of candidates die while awaiting transplantation.

Reduced-size lung transplantation

- Reduced-size lung transplantation using a mature pulmonary lobar transplant (MPLT), in which an adult donor lobe is transplanted into a pediatric recipient, has been applied clinically as a partial solution to the organ shortage crisis.
- Although MPLTs function well, the nature and extent of the growth of such allografts remain uncertain.

Goals of this Study

In this study we sought to:

- 1) characterize the cellular and molecular features of MPLT growth, and
- 2) determine if MPLTs grow in a manner similar to classical compensatory growth.

Methods

Animal Model:

- Hanford miniature swine
- Mature animals: age > 5 mos., body wt. > 40 kg
- Immature animals: age < 12 wks., body wt. < 10 kg
- MHC-matched mature donor and immature recipient animals.

Animal Groups:

- **MPLT** (n = 5): Mature left lower lobe (LLL) transplanted into immature animals served as the experimental group.
- **LOB** (n = 5): Left upper lobectomy and study of left lower lobe growth in mature animal served as a compensatory growth control group.
- **NORM** (n = 3): Normal LLL tissue from mature animals served as the control group.

Methods

MPLT Surgery Protocol:

- 1-2% halothane inhalational anesthesia
- Left pneumonectomy in immature recipient
- LLL harvest from mature donor (cold Euro-Collins flush)
- Preparation of LLL allograft on back table
- Implantation:
 - Bronchial anastomosis (telescoped, 4-0 polypropylene)
 - PA-to-PA anastomosis (5-0 polypropylene)
 - Donor LA cuff-to-recipient LA appendage (5-0 polypropylene)

Immunosuppressive Regimen:

- Cyclosporine 18 mg/kg X 7 days
- Methylprednisolone 500 mg QD X 7 days, then prednisone 2 mg/kg QD X 11 wks.
- Azathioprine 1 mg/kg X 12 wks.

Data Collection:

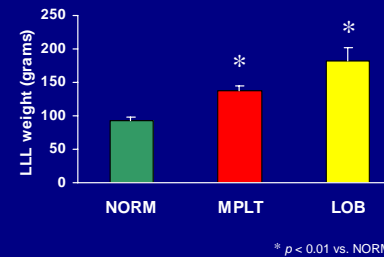
- LLL excised from MPLT and LOB 12 wks. postop. and weighed.
- Fresh tissue:
 - Wet/dry weight ratios
 - Western blot analysis for EGF receptor
- Fixed tissue:
 - Lungs fixed with 70% ethanol to 25 cm H₂O
 - H&E staining for morphometric analyses
 - BrdU immunostaining for cellular proliferation index (CPI)



Three MPLT recipients 24 hours after surgery

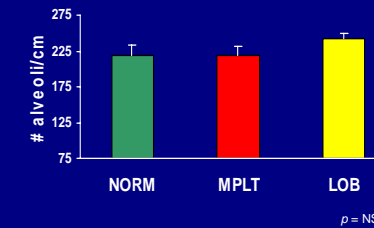
Results

LLL Graft Weights



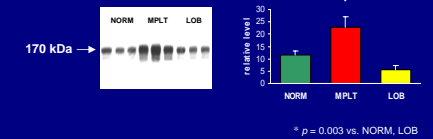
Results

Alveolar Surface Density



Results

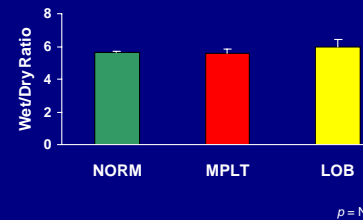
EGF Receptor Expression



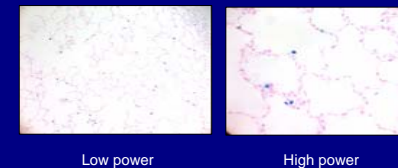
Conclusions

- MPLTs grow by alveolar cell division as indicated by increased CPI.
- MPLTs may grow by alveolar multiplication as indicated by increased volume of respiratory region while maintaining alveolar density.
- Increased graft weight of MPLT and LOB is not due to edema as indicated by wet/dry weight ratios.
- EGF-mediated processes may be involved in MPLT growth as indicated by EGF receptor upregulation.

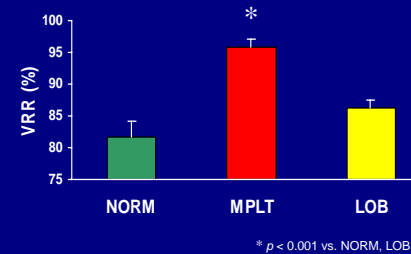
Wet/Dry Weight Ratios



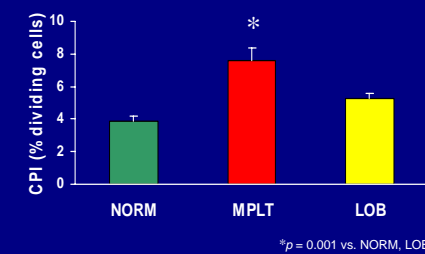
BrdU Immunostaining Example



Volume of Respiratory Region



Cellular Proliferation Index



Discussion

- Classical compensatory growth is triggered by increased stretch of the remaining lung, as seen in the LOB group.
- There is no stretch in MPLT since the grafts were at least as big as the recipient's excised lung.
- Taken together, these results indicate that MPLTs grow in a manner different from classical compensatory lung growth.