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Acute Lung Injury: Making the Injured Lung Perform Better and Rebuilding Healthy Lungs

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14. ABSTRACT Acute lung injury (ALI) is a complex condition associated with diffuse injury to the distal alveolar epithelial gas exchange surface, resulting in marked impairment in the ability to oxygenate blood. Treatment is generally supportive. This condition commonly afflicts patients with cancer. In this regard, cancer patients are especially susceptible to developing acute lung injury (ALI) due to the immunosuppressive and toxic effects of chemotherapy and the debilitating effects of cancer. This condition also afflicts military personnel that experience severe trauma or receive multiple transfusions, and also commonly accompanies severe infections. The over-arching goal of our original application was to derive new approaches to treat ALI with an emphasis on developing new modes of mechanical ventilation and developing cell based strategies to directly repair the injured lung. In Project 1, we proposed to optimize so-called variable ventilation with the intent of minimizing the known injurious effects of conventional mechanical ventilation in patients with ALI. In this past year, where this grant has been in a limited no-cost extension, we have not been using DOD funds for Project 1. Based on data generated from this proposal, however we have been able to secure funds from the Coulter foundation to continue this work by performing a limited clinical study on the safety and efficacy of variable ventilation in patients with ALI. Using the funds that have been available in this no-cost extension has permitted us to continue work on Project 2, which is the cell therapy component. For Project 2, we have continued to develop protocols for the identification and derivation of lung stem cells that can be used for therapeutic purposes in ALI. We also have been identifying other cell populations that can be manipulated to hasten lung repair.					
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Introduction

The over-riding goal of this application has been to develop new treatments for patients with acute lung injury syndromes (ALI). As we have previously noted, ALI is the end-result of a variety of dramatic conditions that unfortunately are relatively common and afflict thousands of Americans. These conditions include: sepsis, exposure to toxins, multiple blood transfusions, and long bone fractures. Notably, this is a common problem amongst military personnel who experience severe trauma during battle. In addition, patients suffering from cancer are particularly susceptible to developing ALI as a result of the immunosuppressive effects of chemotherapy. This reflects that fact that cancer leads to severe infections and overall well-being. While poorly understood, it is also the case that certain chemotherapeutic agents themselves cause a syndrome with many features of ALI. The pathology of ALI is very complex and includes prominent acute inflammation and cellular injury to the lung. From a physiological stand point, the profound injury to the distal lung alveolar epithelial gas exchange region causes a marked impairment in the ability to oxygenate the blood. Thus, such patients require high flow supplemental oxygen and mechanical ventilation to ensure adequate oxygenation. Type I cells, which comprise the vast majority of the gas exchange surface are particularly susceptible to injury. To meet our goal, this grant has had 2 Projects. . In the original grant, we proposed in Project 1 to develop a better means of mechanical ventilation of patients with ALI. In this context, the way in which patients are ventilated, *by itself*, worsens lung injury. This is likely due to non-physiological stretching of the alveolar cells along with further stimulation of local inflammation. Using data generated from prior years of this grant, we have fortunately been able to continue this clinical study using funds secured from the Coulter foundation. In this no-cost extension year, the major emphasis year has been on Project 2 where we have continued to study, develop; and identify endogenous or derived cell populations that can be manipulated either in vivo or after injection to repair the injured gas exchange surface in ALI. These are pre-clinical studies that utilized mice, cell culture, and decellularized mouse lung preparations.

Body of Progress Report

Below is a summary of the progress and achievements for the 2 Projects that comprised the original parent proposal.

Project 1

As we discussed in earlier reports and letters, we had unforeseen delays in the initiation of the Clinical Study comparing variable ventilation to conventional ventilation in patients with acute lung injury. These delays included completing a legal agreement with the ventilator manufacturer (Covidien^R), overcoming several distinct software programming issues (eg, adding ability to titrate inspiratory time and inspiratory flow ratio), and then securing approval of an IDE from the FDA for this work. On 11/8/2012 we confirmed cessation of DoD funding for the clinical protocol of Variable Ventilation in Acute Lung Injury.

Since 11/8/2012 we have secured new funding from the Wallace Coulter Foundation to conduct a first-in-human clinical trial of variable ventilation in adult human patients. Our ability to obtain such funds and to design a safe rational trial directly relates to and builds upon prior work that was supported from this grant. Through this prior work, we were able to clearly develop this alternative means of ventilation in a way that is safe and can be easily monitored. To date, we have enrolled two patients of a planned 16 patient proof-of-concept study. Thus, variable ventilation has now been used with adult human patients with ALI. We will continue to enroll patients into our variable ventilation trial during the next year. We are hopeful that this will represent a major advance in the field, since new modes of ventilating patients with ALI are desperately needed. Our intent is to use data from this initial and limited clinical study to justify a more extensive study that might include other institutions.

Project 2: We will establish a pre-clinical program conducted in laboratory mice with the objective of developing cell-based treatments for ALI.

As stated previously, the long-term goal of this project has been to develop an autologous cell-based therapy to reconstitute and repair the injured distal lung epithelium in ALI. A key element of this work has been to identify and optimize exogenous progenitor cell populations with lung epithelial reparative properties. Such a cell population must be readily available and clinically scalable. As in previous years, we have been focused, at least in part on induced pluripotent stem cell, termed iPS cells along with the originally proposed studies utilizing embryonic stem (ES) cells. In the past year, we have identified a new endogenous lung stem cell population. By understanding the molecular signals that control this endogenous stem cell population, it may be possible to manipulate its behavior in vivo to facilitate repair of the injured lung in ALI.

With regard to the iPS cells, we have continued to derive and bank cells from humans and mice for further study. The general approach after derivation of iPS cells is to stimulate these cells through a series of steps that recapitulates embryonic development. The first important step is assumption of an endodermal fate, since the anterior endoderm gives rise to the lung epithelium. We continue to refine our methodology to induce directed

differentiation to definitive endoderm. Our efficiencies and purity continue to improve as demonstrated by the robust expression of key endodermal markers, Foxa2, Gata4/6, and Notably, the waves of marker genes (e.g. alpha fetal protein and albumin) expressed during further lineage specification of ES and iPS-derived endoderm appears to recapitulate what is found in the developing embryo. Of note, there is the possibility that endodermal derivatives may be an effective therapeutic for the injured lung. In other words, it may not be necessary to further differentiate these cells along a lung epithelial axis.

In order to optimally derive lung epithelium from endodermal derivatives, we have developed novel fluorescent based methods to track the differentiation of cells into specific precursor and lung lineages. Because expression of a discrete fluorescent protein marks cells that have assumed a lung-related lineage, we can employ cell sorting to collect cells for further testing as reparative cell populations. A major focus is on identifying conditions that optimally and efficiently induced differentiation into alveolar and airway epithelium. We have had modest success with deriving type II cells, type I cells albeit at low frequency, and more proximal airway epithelium, possibly including ciliated epithelium. The appropriate engraftment of these cells can be examined using the de-cellularized lung preparation that we employ. Several manuscripts are in preparation that details these findings.

Overall this set of studies and findings, as stated previously, have major implications for developing cell-based therapies to reconstitute diseased endodermal-derived tissues, such as the lung during ALI. As indicated flow cytometry-based sorting algorithms are being devised to both to reduce the presence of undifferentiated cells expressing and to expand the number of cells that have assumed the targeted cell fate. We hope that we are getting closer to a rational cell based therapy of ALI that uses iPS-derived lung cells.

Through related work, in the past year we have found that cells that reside in the overlying lung mesothelium (pleura) contain progenitor cells that give rise to vascular and bronchial cells. Notably, we have been able to show that sonic-hedgehog signaling in this cell population facilitates their movement into the underlying lung parenchyma. Our data also indicate that this cell population may be involved in lung regenerative events. Theoretically, therefore, strategies could be designed to stimulate these cells to facilitate lung repair. This is now being pursued.

Key Research Accomplishments

- Obtained supplemental funds to continue variable ventilation.
- Recruitment of patients with ALI to clinical trial
- Manuscript published on variable ventilation.
- Developed protocols with clinical and research staff to improve compliance with the control and research arms of the study
- Further optimized protocols to obtain alveolar epithelial cells from mouse iPS cells
- Further optimized in vitro engraftment for alveolar epithelial progenitors using de-cellularized rodent lung
- Further banking of iPS cells for future use
- Identified endogenous lung stem cell population that may be manipulated for lung regeneration and repair
- Several manuscripts have been published and are in preparation on lung stem cells and iPS cells
- Development of fluorescent reporters for lung epithelial differentiation tracking

Reportable Outcomes

- 1)** Manuscripts published lung stem cells and iPS cells
- 2)** Manuscript published on variable ventilation
- 3)** Human study in variable ventilation initiated.
- 4)** Coulter foundation grant secured to support variable ventilation study

Conclusion

We have made considerable progress in both Projects of the original parent grant. Building upon and extending data generated during early phases of this grant, we have been able to secure funds to perform a clinical study on variable ventilation in patients with ALI. This has allowed us to continue to meet the goals of Project 1 during this period of a limited no-cost-extension. This study is now active and we have already studied several patients

In Project 2, we have continued to focus on establishing and optimizing conditions that facilitate the differentiation of iPS cells to lung epithelium. As part of this, we have developed several unique tools that support this objective. This work continues to be a major advance for the field. Not only is this potentially critical for the field of cell based therapy, it also provides tools so that the signals that control lung lineage specification and epithelial growth can be identified. By identifying such signals, one may be able to develop strategies to facilitate endogenous stem cells to repair the injured lung. Indeed in related work, we have identified a new lung stem cell and one key signal that control its fate. This related but highly relevant work also has significant implications for developing strategies for lung regeneration and repair.