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TITLE: Actin Fence Therapy for Acute Lung Injury

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14. ABSTRACT

F-actin is known to be a major regulator of molecular trafficking between intracellular organelles (e.g. the Golgi) and the cell membrane. However, very little is known regarding f-actin regulation in the AE, the major site of the severe alveolar inflammation that causes lung injury. Our findings indicate that enrichment of the lung's epithelial lining with an f-actin stabilizing biologic blocked the surface display of a proinflammatory receptor, abrogating both the inflammatory response to LPS as well as the associated mortality. By contrast, in the absence of the biologic, receptor-medicated signaling leading to loss of the actin fence resulted in a major surge of proinflammatory receptor display. As proof-of-principle, we addressed these f-actin dependent responses in terms of TNFR1 expression. Strengthening of the fence by f-actin stabilization was we believe, a major factor in abating receptor expression, hence accounting for better mouse survival.

15. SUBJECT TERMS

Inflammation, f-actin, alveolar epithelium, mouse lung, lung compliance.

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1. **INTRODUCTION:**
The goal of this project is to develop a therapeutic strategy to inhibit the progression of alveolar inflammation and the associated lung injury resulting from persistent expression of proinflammatory receptors on the apical surface of the alveolar epithelium (AE). The hypothesis is that the surface receptor expression on the AE is regulated by the cortical layer of f-actin that forms a fence restricting receptor trafficking to the plasma membrane. A test of this hypothesis, therefore, requires the development of technology to reliably quantify f-actin in live alveoli in order to determine real-time mechanisms that might play a role in regulating, possibly enhancing the f-actin fence.

2. **KEYWORDS:**
Inflammation, f-actin, alveolar epithelium, mouse lung.

3. **ACCOMPLISHMENTS:**
Major Task 1. Determine real-time f-actin re-modeling in alveoli of live mouse lungs: projected end day July 2020 (to date 90% completion).

Major Task 2. Determine the role of the actin fence in regulating alveolar expression of proinflammatory receptors: projected end day July 2020 (to date 50% completion).

Major Task 3. Establish strategies enhancement of the alveolar f-actin fence as rapid therapy for ALI: projected end day July 2020 (to date 80% completion).

Update:
Our overall findings in this project show that TNFR1 ligation induced loss of the F-actin fence in the alveolar epithelium, causing receptor hyperexpression and alveolar injury. Rac1 delivery to

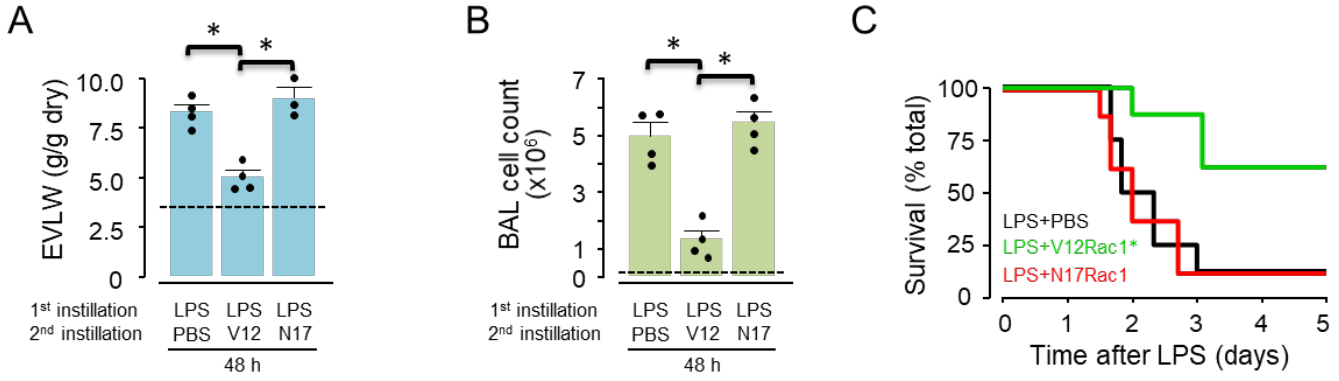

FIG. 1. (**A-C**) Mice received two intranasal instillations. The first instillation was a lethal dose of LPS, followed 24 hours later by the second instillation, which was PBS, or TAT-conjugated proteins. (**A, B**) Data were obtained 48 hours after LPS. Bars show blood-free extravascular lung water (EVLW) (**A**) and total leukocyte count in BAL (**B**). Mean±SE, *p<0.001 vs. as indicated using ANOVA with Bonferroni correction. n=4, except TAT-N17Rac1 in D (n=3). Each dot shows data for a single lung. Baseline values shown as dashed line (**C**) Kaplan-Meier plots for mouse survival after LPS. For each group, n=8. *p<0.01 vs LPS+PBS using log-rank test.

the alveolar epithelium enhanced the fence, blocking the receptor hyperexpression, injury and pre-treatment with i.n. TAT-V12Rac1 markedly reduced mortality due to LPS.

Since the last progress report which was submitted in May 2020, we further determined the therapeutic efficacy of TAT-V12Rac1 as a curative agent. We instilled the construct under conditions of established pathology, 24 hours after instilling a lethal LPS dose that caused major alveolar hyperpermeability and inflammation. In the group given TAT-V12Rac1 24 hours after LPS, evaluation of ALI after a further 24 hours indicated marked reduction of extravascular lung water (Figure 1A) and BAL leukocytes count (Figure 1B). Moreover, despite delayed curative intervention, V12Rac1 treatment resulted in marked reduction of LPS-induced mortality (Figure 1C). The non-active Rac1 mutant, TAT-N17Rac1 was not protective.

4. **Impact:** These studies indicate that (i) given intranasally, TAT-V12Rac1 protects against LPS-induced mortality due to ALI, (ii) delayed intervention with V12Rac1 is also protective in LPS-induced mortality, (iii) fence enhancement therapy might be more effective in early than late stages of ALI. These findings strengthen our conclusion that enrichment of the lung's epithelial lining with an F-actin abrogates the inflammatory response and protects against mortality. An important conclusion is that fence enhancement in the alveolar epithelium mitigates ALI well into the progressive phase of the disease and added to the translational significance of our study. Overall, our studies reveal the therapeutic efficacy of V12Rac1 in the setting of ongoing lung inflammation.

5. **Changes/Problems:**
   No major problem or changes are anticipated.
   - Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: NA
   - Significant changes in use or care of human subjects: NA
   - Significant changes in use or care of vertebrate animals: No
   - Significant changes in use of biohazards and/or select agents: No

6. **Products:**
   Publications, conference papers, and presentations Other publications, conference papers, and presentations. List presentations made during the last year (international, national, local societies, military meetings, etc.).
   a. Publications
      (1) Lay Press: None
      (2) Peer-Reviewed Scientific Journals: Journal of Clinical Investigation (In review)
      (3) Invited Articles: None
      (4) Abstracts: None

   b. Presentations made during the last year
      Invited Speaker: 2019 Stem Cell Therapies in Lung Biology and Diseases, University of Vermont
      Seminar Presentation Title: Live-Imaging Alveolar Defense Mechanisms

7. **Participants & Other Collaborating Organizations:** Nothing to report

8. **Special Reporting Requirements:** Nothing to report

9. **APPENDICES:** NA

COMPLIETED - STATEMENT OF WORK END DATE: July 2020

Columbia University Medical Center
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Specific Aim 1 (specified in proposal)	Completed	Animals used	Site 1
Major Task 1 Determine real-time F-actin re-modeling in alveoli of live mouse lungs	90%	125	
Subtask 1 Define the role of the alveolar cytosolic Ca^{2+}	90%	45	Dr. Gusarova
Subtask 2 Determine the role of calcineurin and cofilin	100%	45	Dr. Gusarova
Subtask 3 Determine the role of cytokines	50%	35	Drs. Gusarova and Quadri
Specific Aim 2			
Major Task 2 Determine the role of the actin fence in regulating alveolar expression of proinflammatory receptors (PIRs)	50	80	
Subtask 1 Determine dynamic expression of TNFR1 in live alveoli	100%	50	Dr. Gusarova
Subtask 2 Determine alveolar expression and IL-1β in real-time	0%	0	Dr. Gusarova
Subtask 3 Define global PIR expression in the lung in mouse models of ALI	45%	30	Dr. Gusarova
Specific Aim 3			
Major Task 3 Establish strategies to enhance alveolar F-actin fence as rapid therapy for ALI	80%	345	
Subtask 1 Establishment of acute lung injury (ALI) models	85%	90	Dr. Gusarova
Subtask 2 Expression, purification and conjugation of Rac1 mutant proteins (V12Rac1 and N17Rac1)	85%	0	Dr. Quadri
Subtask 3 Determination of alveolar Rac1 uptake in lungs with ALI	85%	55	Dr. Gusarova

Subtask 4 Assessment of protective effects of Rac1 constructs in ALI models	80%	155	Drs. Gusarova and Quadri
Subtask 5 Determination of off-target effects of Rac1 constructs	40%	45	Dr. Gusarova
Local IRB/IACUC Approval	We have IACUC approval for our projects		
Milestone Achieved: HRPO/ACURO Approval	NA		