CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

Name(s)
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S0416

Project Title
Interaction of Integrin alpha-3/beta-1 and Laminin-5 Modulates Alveolar Epithelial Barrier Formation

Abstract

Objectives/Goals
This study investigated the interaction between integrin a3/b1and its ligand, laminin-5 in modulating alveolar epithelial barrier.

Methods/Materials
Cell Culture and preparation of slides- Primary type II cells were harvested from male Sprague Dawley rats. Procedures for cell extraction and animal sacrifice were pre-approved by review board. Culture media was changed every day. Slides that had an Ln-5 rich matrix were coated in Ln-5 for two hours and cells placed thereafter. Slides with extracellular matrix protein coating were also coated for two hours and cells placed thereafter.
Western Blot- Western blots were done on SDS-page using a 7.5% stacking gel. The protein concentration of samples was determined through Bio Rad DC protein assay kit. Standard Western Blot procedure was followed.
Immunofluorescence- Standard immunoflorescence procedure was followed.

Results
Cells plated on Ln-5 developed confluence at <24 h, those plated on plastic without Ln-5 developed confluence at 24-48 h, those plated on anti-integrin antibodies didn#t develop confluence. Uniform scratch made with needle on confluent monolayers of AECs. Wound closure measured serially +/- anti-integrin a3 mAb, or either anti-Ln-5 mAb with/without blocking activity. Closure faster on Ln-5-coated (at <48 h) to uncoated plastic (50% at 48 h). Presence of anti-integrin a3 mAb reduced rate of closure for cells plated on Ln-5, and prevented closure for cells on plastic

Conclusions/Discussion
. Interaction is likely important for alveolar epithelial cell migration and spreading during re-epithelialization of alveolar surface following lung injury in vivo. Cells coated on Laminin-5 had higher levels of Integrin a3-b1, which was probably attributed to the surface affinity of Laminin-5 or Laminin-5#s ability to up regulate levels of Integrin a3-b1. Interaction is likely to be important for epithelial barrier formation, which consists of cell adhesion, monolayer formation, migration and wound healing. Cell adhesion and spreading either induces Integrin a3-b1, is promoted by Integrin a3-b1 or both. The Laminin-5 coated filters bind cells at their a3b1 receptors, which accelerates attachment and spreading, and probably induces the expression of more a3b1, which further hastens the development of monolayers.

Summary Statement
Two proteins in the lungs are essential for the normal function and development of the lungs.

Help Received
Dr. Lubman and Dr. Hua Zheng gave me support and advice for this study. Parents gave me ride to and from lab.