A Murine Model of Airway Granulation and Subglottic Stenosis

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ABSTRACT

Objectives: The murine model, in which tracheas are transplanted into the subcutaneous tissue of recipient mice and subsequently studied for signs of granulation tissue, has been particularly successful in studying airway injury. Mouse laryngotracheal complexes (LTC’s) will undergo airway injury and transplantation into syngeneic recipient mice in order to develop a functional model of airway granulation tissue and subglottic stenosis. Study Design: IACUC (Institutional Animal Care and Use Committee) approved animal study Methods: The LTC’s of donor mice underwent direct airway injury through mucosal scraping using a wire brush or through application of hydrochloric acid (HCl) solution to the mucosa. A control group did not undergo any airway injury. LTC’s were harvested and transplanted heterotopically into the subcutaneous tissue of syngeneic recipient mice and harvested at 3 weeks post-transplantation. Harvested LTC’s underwent analysis by standard histochemistry using trichrome staining, specifically to highlight collagen formation and thus to examine degree of granulation tissue in the experimental groups compared to the control group. Results: At 3 weeks post-transplantation, trichrome staining showed that direct injury of the airway epithelium, both mechanically using a wire brush and chemically using HCl solution, results in the formation of granulation under the disrupted airway epithelium, with narrowing of the airway lumen and evidence of early fibrosis. Conclusions: The development of a murine model of airway granulation tissue is an efficient tool for characterizing the process of airway granulation and subglottic stenosis after mucosal injury. This will be instrumental in the development of preventative and treatment modalities.

METHODS AND MATERIALS

Three donor C57BL6 mice were euthanized using a compressed carbon dioxide (CO2) chamber. A vertical incision from the mentum to the sternum was made, and the laryngotracheal complex (LTC) was exposed through careful dissection and kept in situ. Two experimental groups underwent epithelial injury. Trauma: Direct mucosal scraping using a wire brush with diameter 0.007″ placed via a 3mm anterior pharyngotomy and passed 10 times through the LTC. Hydrochloric acid: Application of 0.5mL hydrochloric acid (HCl) solution, titrated to pH4, to the subglottic mucosa via a 1mL tuberculin syringe at the future inferior tracheal incision. After 5 minutes, the HCl solution was irrigated out of the airway using 0.5mL normal saline solution. Control: No airway injury

LTCs from the experimental groups and the control group were harvested and placed into normal saline solution for transplantation into syngeneic recipient mice. Recipient C57BL6 mice were anesthetized, and using sterile technique, 1cm incisions were made on the dorsum. Three separate subcutaneous pockets were created into which an LTC from each experimental group and from the control group were transplanted. The incisions were closed with absorbable suture.

RESULTS

Figure 1. Three subcutaneous pockets were created on the dorsum and closed with suture after transplantation.

Recipient mice were euthanized at 3 weeks post-transplantation using a compressed CO2 chamber. Transplanted LTCs were harvested via the previously placed incisions, carefully fixed in formalin, and blocked in paraffin.

Figure 2. Laryngotracheal complexes were harvested 3 weeks post-transplantation from their subcutaneous pockets.

Four slides of 5 micrometer thickness were made per millimeter of tissue and stained with trichrome, which stains collagen blue, cytoplasm light red or pink, and nuclei dark brown to black.

Figure 3. Control group at 4x and 40x magnifications harvested 3 weeks transplantation shows preservation of airway epithelium and no evidence of granulation. C=cartilage, L=lumen, E=epithelium.

Figure 4. Hydrochloric acid group at 4x and 40x magnifications harvested 3 weeks transplantation shows significant granulation, angiogenesis and an attenuated epithelium. C=cartilage, L=lumen, E=epithelium, A=angiogenesis.

Figure 5. Trauma group at 4x and 40x magnifications harvested 3 weeks transplantation shows significant granulation and a thickened epithelium. L=lumen, E=epithelium, G=granulation, C=cartilage.

REFERENCES