



# Experimental Laryngeal Granuloma in a Rat GERD Model

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## ABSTRACT

**Objective :** Acid reflux and mucosal injury of the larynx, caused by vocal abuse, chronic cough and intratracheal intubation, may be involved in the development of laryngeal granuloma<sup>1</sup>. However, the exact mechanism is unclear. We induced an experimental laryngeal granuloma by causing mechanical injury to the vocal cord mucosa in rats with chronic acid reflux esophagitis to clarify the pathological mechanism underlying laryngeal granuloma.

**Methods :** An experimental rat model of chronic acid reflux esophagitis was surgically created. A plastic bar was inserted into the trachea of the model rat, and was moved up and down three times in three seconds to mechanically injure the vocal cord mucosa. The pharynx, larynx and esophagus of these rats were observed histologically two weeks after the operation.

**Results :** A granuloma was observed in the vocal cord mucosa of the rat at two weeks after the surgery, and this presented the same pathological structure as human laryngeal granuloma. In contrast, only mucosal abrasions and blister formation of the vocal cord mucosa were observed in the control rats that did not undergo surgery.

**Conclusions :** The current results showed that both mechanical injury and acid reflux are involved in the development of a laryngeal granuloma.

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## INTRODUCTION

Globus pharyngeus and laryngeal granuloma are frequently observed in the clinical practice of otolaryngologists, and gastric acid reflux is one of the causes of these diseases. Direct acid damage and vagus -nerve- mediated reflex are thought to be the pathological mechanism of laryngopharyngeal reflux disease (LPRD)<sup>1</sup>, however, the association between the degree of gastric acid reflux and LPRD is unclear. To understand the mechanism and pathogenesis of LPRD, it is important to examine the pathological changes associated with gastric acid reflux in the mucosal epithelium of the pharynx and larynx. An experimental rat model of chronic gastro-esophageal reflux disease (GERD) was developed to study the histological changes of the pharynx and larynx with time progress<sup>2</sup>. Moreover, we induced an experimental laryngeal granuloma by causing mechanical injury to the vocal cord mucosa in the GERD rat model to clarify the pathological mechanism of laryngeal granuloma.

## METHODS AND MATERIALS

The GERD rat model was prepared by the methods described by Omura et al<sup>3</sup>. Male Wistar rats age 8 weeks, with a weight of 200-250 g fasted overnight and were anesthetized by inhalation anesthesia using ether. The abdomen was opened via a 3 cm long midline incision, and a piece of 18Fr Nelaton catheter was placed around the area of the pyloric sphincter in order to restrict gastric emptying. The transitional region between the forestomach and the glandular portion (limiting ridge) was ligated with 2-0 silk thread (Figure 1). The rats were then fasted for another 48 hr after the operation, but were allowed to drink water. Sham-operated rats, receiving only a midline incision, served as a control group.

Following surgery, the mouth of these rats was opened using forceps to observe the glottis, and a plastic bar was inserted into the trachea of a model rat, and moved up and down three times in three seconds, to mechanically injure the vocal cord mucosa (Figure 6).

The pharynx, larynx, trachea, lung and esophagus were excised under inhalation anesthesia using ether. Three rats were sacrificed every two weeks until 20 weeks after operation. However, in the rats received a mechanical injury to the vocal cord mucosa, five rats were sacrificed at two weeks after the operation.

## RESULTS

**Histological changes of the pharynx and larynx in the GERD rat model**  
Two weeks after the operation in the GERD rat model erosion was evident in the lower esophagus macroscopically (Figure 2A) and ulceration with loss of the esophageal epithelium was identified microscopically. Although there was acute inflammatory change with neutrophil infiltration in the ulcer (Figure 2B), at this point, no pathological changes had occurred in the mucosa of the pharynx and the larynx (Figure 2C & 2D).  
Eight weeks after the operation in the GERD rat model thickening of the pharyngeal mucosa associated with the proliferation of fibroblast and accumulation of collagen fibers was observed in the posterior wall of the hypopharynx (Figure 3A & 3B). Furthermore, in the GERD rat model 12 weeks after the operation, the thickening of the hypopharyngeal mucosa increased (Figure 4A), and inflammatory cell infiltration was apparent under the muscle coat (Figure 4B).  
Finally, in the hypopharyngeal mucosa of the GERD rat model at 18 weeks after the operation, extensive elongation of the papillae of the lamina propria into the epithelial surface was observed. Moreover, inflammatory cell infiltration with proliferation and dilatation of the capillaries was identified in the depth of the mucosa (Figure 5A). Surprisingly, no macroscopic changes were seen in the hypopharyngeal mucosa, despite the elongation of the papillae of the lamina propria into the epithelial surface and the inflammatory cell infiltration that was observed in microscopic study.

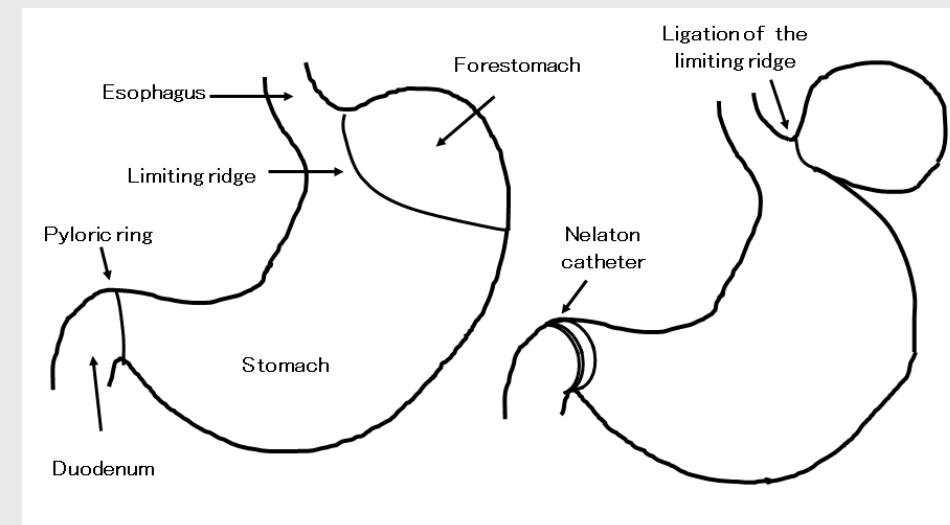


Figure 1. Preparation of the GERD rat model.

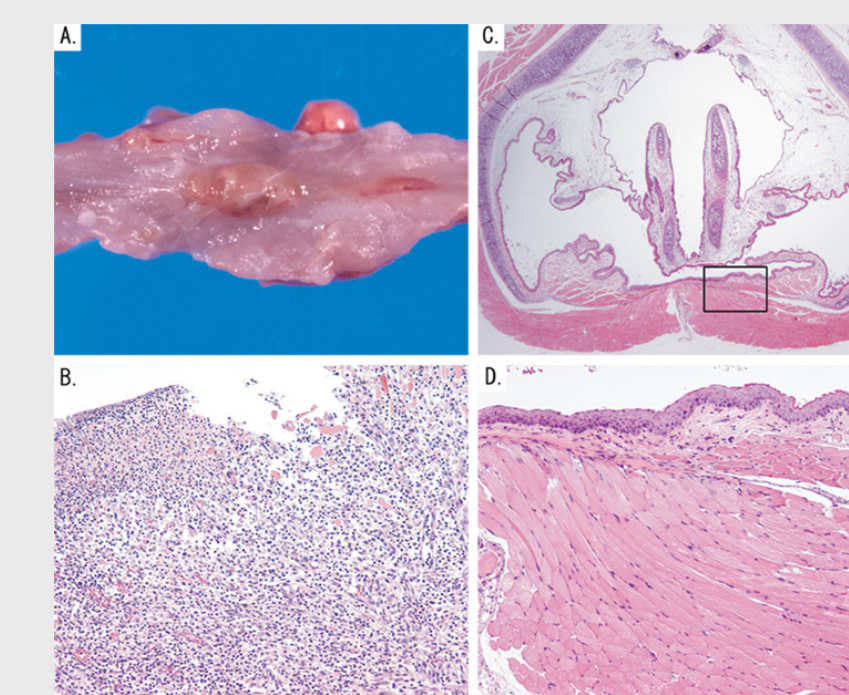


Figure 2. Histological changes of the GERD rat model at two weeks after the operation.

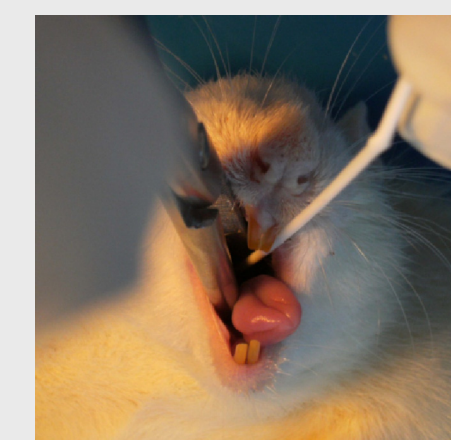


Figure 6. Insertion of a plastic bar into the trachea of a rat.

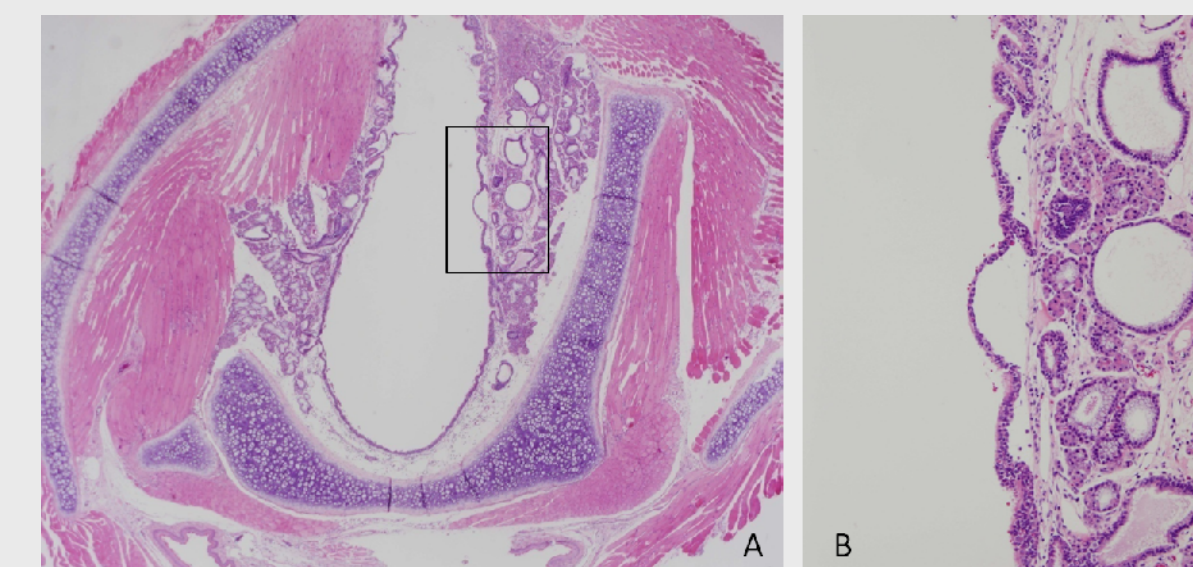


Figure 7. Histological changes of the larynx of the control rat model at two weeks after the operation.

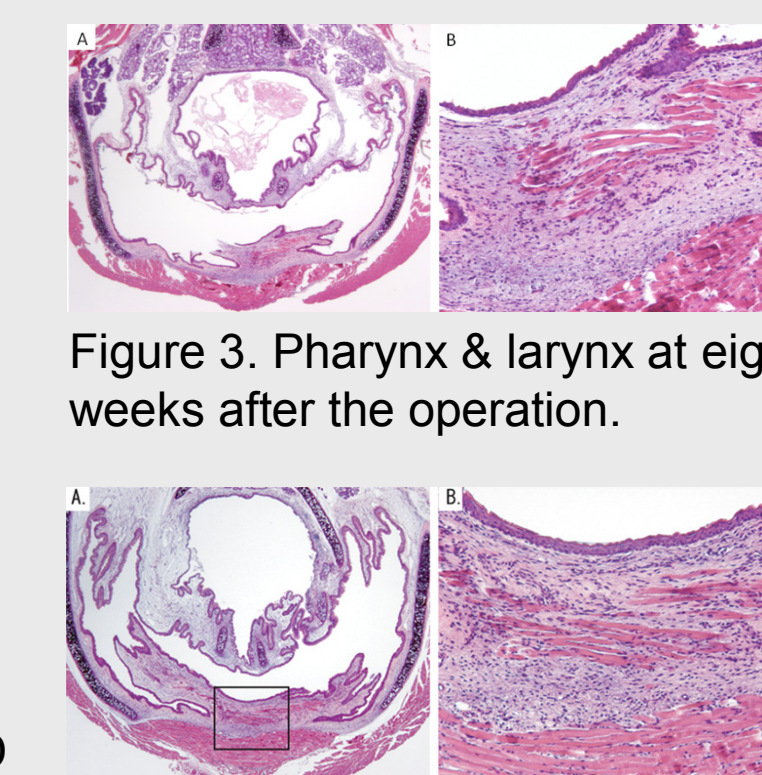


Figure 3. Pharynx & larynx at eight weeks after the operation.

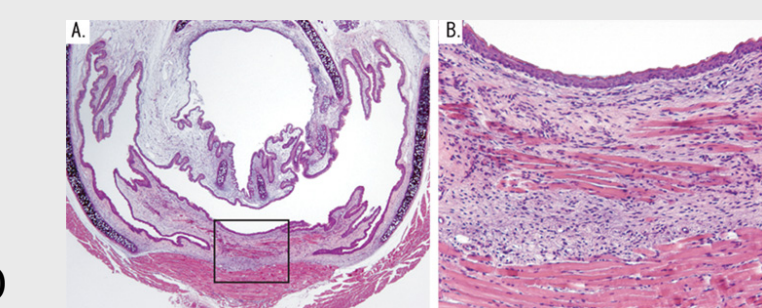


Figure 4. Pharynx & larynx at 12 weeks after the operation.

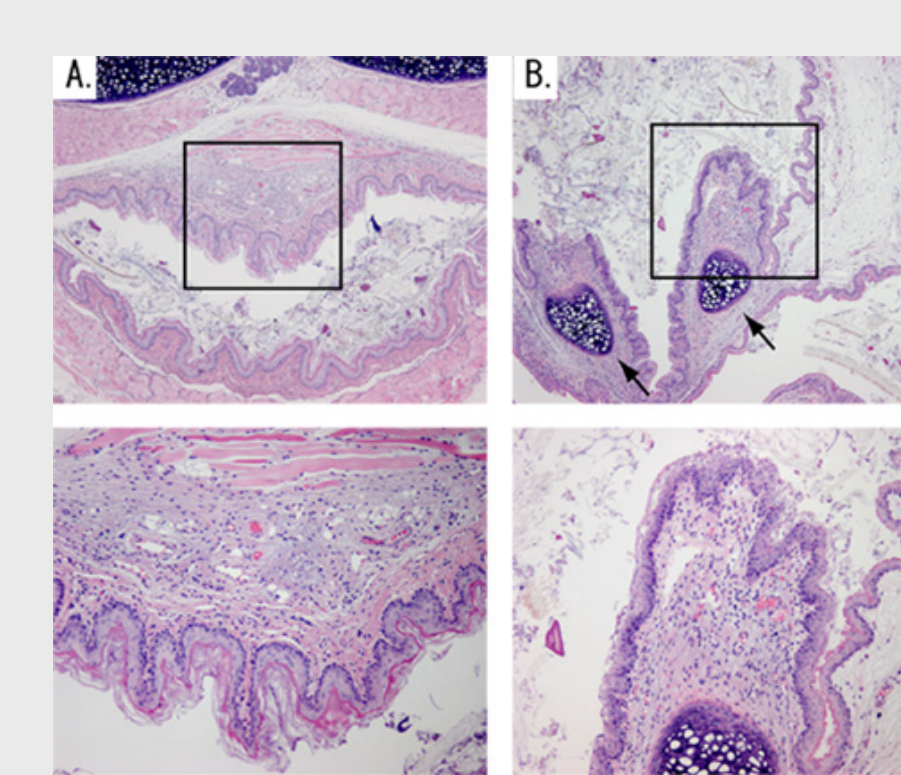


Figure 5. Pharynx & larynx at 18 weeks after the operation.

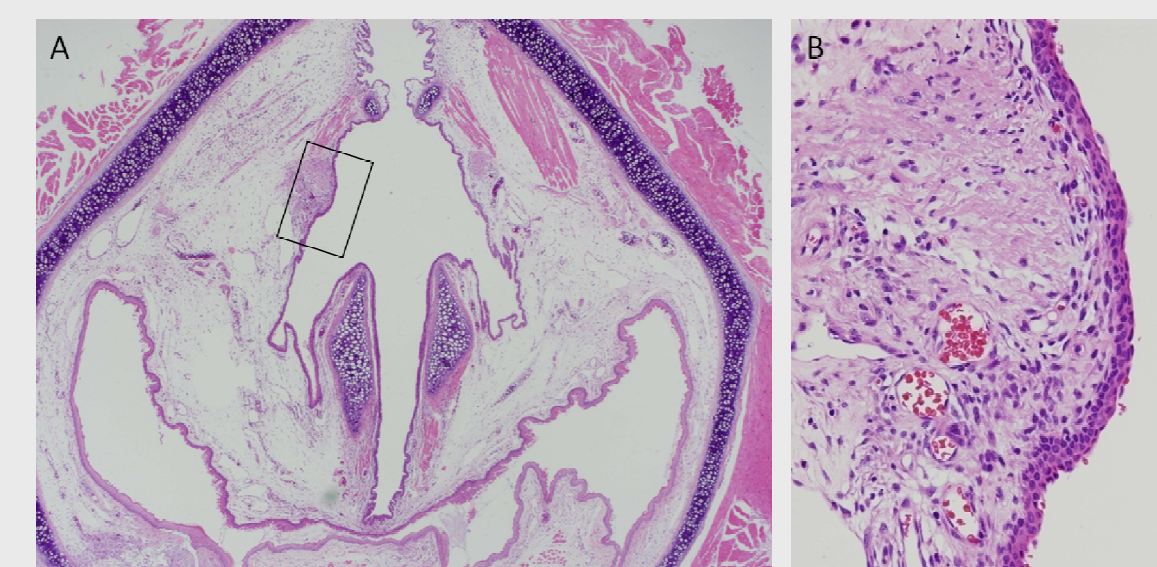


Figure 8. Experimental laryngeal granuloma in the GERD rat model.

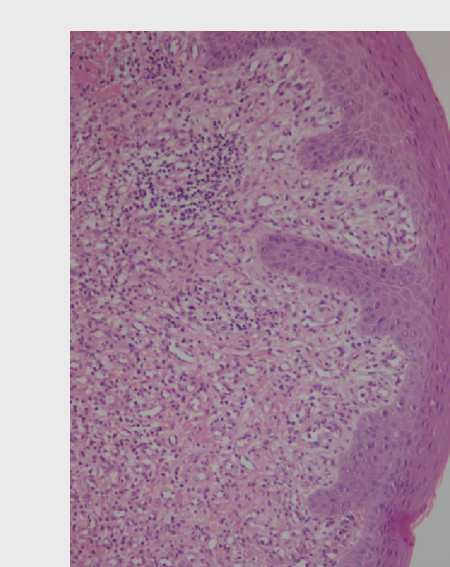


Figure 9. Pathological structure of a human laryngeal granuloma.

## RESULTS

The thickening of the mucosa and the proliferation and dilatation of the capillaries was noted in the mucosa around the arytenoid cartilage as in the hypopharyngeal mucosa, indicating that inflammation caused by gastric acid reflux had extended to the larynx. Moreover, the earliest change in the larynx was seen in the arytenoid which is a favorite site for a laryngeal granuloma (Figure 5B).

### Experimental laryngeal granuloma in the GERD rat model

In the larynx of the control model rats without acid reflux and that were only mechanically injured, only mucosal abrasions and blister formation on the vocal cord mucosa were observed (Figure 7). In contrast, in the vocal cord mucosa of the rat GERD model with mechanical injury, inflammatory cell infiltration was observed with the thickening of the mucosa and the proliferation and dilatation of the capillaries, and a granuloma was observed in the membranous portion of the vocal cord (Figure 8). The incidence of granuloma development was 60%, and no granuloma was observed in the control model rats. Moreover, this granuloma presented the same pathological structure as a human laryngeal granuloma (Figure 9). At this point, no histopathological changes, such as the inflammatory cell infiltration, were seen in the hypopharyngeal mucosa.

## DISCUSSION

In 1968, Delahunty, et al reported the experimental formation of a laryngeal granuloma by directly applying an acid solution in the canine vocal cord<sup>4</sup>. This was the first report that presented the association between gastric acid reflux and laryngeal granuloma using an animal model. The present study is the first to report the development of a laryngeal granuloma due to the chronic reflux of endogenous gastric acid in rats. Moreover, this is the first presentation using a rodent model. In this experimental rat model, the rats did not receive any surgical treatment along the esophago-gastric junction, and consequently, the defense mechanisms against regurgitation were preserved. In addition, the rats received a mechanical injury to the vocal cord mucosa for only short time. Therefore, the rat model used in this study was a model that closely resembled human laryngeal granuloma associated with gastric acid reflux and mechanical injury.

The development of the laryngeal granuloma was not observed in the vocal cord mucosa of the GERD rat model during the longitudinal observation of 18 weeks after surgery. Moreover, in the larynx of the control model rats without acid reflux and those that were only mechanically injured, only mucosal abrasions of the vocal cord mucosa were observed, and no laryngeal granuloma developed. In contrast, a laryngeal granuloma was successfully induced in the vocal cord mucosa of more than half of the GERD model rats by applying both gastric acid reflux and mechanical injury. As a result, our findings indicate that the laryngeal granulomas developed due to the synergic effects of mechanical injury, such as vocal abuse and chronic cough, and gastric acid reflux.

## CONCLUSIONS

This study developed a rat model of experimental laryngeal granuloma by mechanical injury to the vocal cord mucosa in rats with chronic acid reflux esophagitis. The developed laryngeal granuloma presented the same pathological structure as human laryngeal granuloma. The current results showed that both mechanical injury and acid reflux are involved in the development of laryngeal granulomas.

## REFERENCES

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