A Histological Grading System for the Analysis of Cellular Infiltration of Esophageal Mucosal Tissue

Senior Honors Thesis (Honors 499)

by

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Abstract

The body is constantly exposed to foreign molecules; some are harmful but most are harmless. Any molecule that interacts with the immune system (antigen) can activate it. To avoid activation of the immune system to harmless antigens, different immunologic mechanisms are employed. One such mechanism is oral tolerance, the generation of a suppressive (or tolerant) immune response to antigen initially administered to the body via the oral route. Subsequent exposure of this antigen to the body by any route will not generate an active immune response to that antigen since a tolerant response was generated initially. Mucosal tissues, particularly those of the upper and lower gastrointestinal tract, are critical for housing immune components necessary for initiating and maintaining tolerance. Recent work in our lab has demonstrated an inability to induce tolerance when a feeding needle is used to administer the treatments for 14 or more consecutive days. Preliminary data suggest that cellular alterations occur in the esophageal mucosa. The goal of this study was to examine all aspects of the esophageal mucosa that might be altered by repeated feeding needle treatments and correlate any changes with a hindrance in oral tolerance induction to fed ovalbumin. To do this, we designed a pathology scoring system to account not only for changes in cellular populations but aberrant cellular growth and physical alterations such as abscesses and mucosal shredding.

Introduction

The immune system is constantly in contact with foreign but harmless antigen such as commensal bacteria and food proteins, and a mechanism must be in place to avoid needless immune responses against these antigens. Antigen introduced orally into the body, induces a state of active suppression by the immune system known as oral tolerance (1). Tolerance requires immune cells that have specialized functions as part of the mucosal immune system. For
example, sampling of food particles and normal gut flora by dendritic cells results in their induction of T-regulatory cell differentiation instead of activating other T cell subsets (2).

Mucosal membranes line the surface of the respiratory, gastrointestinal and urogenital tracts (3). Mucosal tissues lining these tracts have unique structures and components, both of lymphoid and non-lymphoid origin, that provide protection for the body and comprise the mucosal immune system (3). Mucosal immunity is important for the induction of oral tolerance, which occurs following the first exposure of an antigen via the oral route, and is the active suppression of an immune response to a subsequent challenge with the antigen (2). The largest and most well studied mucosal tissue is the gastrointestinal (GI) tract.

Immune cells present in mucosal tissues are uniquely conditioned by their environment allowing them to stimulate suppressive immune responses and only generate active immune responses in the presence of pathogens or harmful antigens. Once dendritic cells have been activated, they travel in the lymph from the location of activation to the mesenteric lymph nodes, which contain dense populations of immune cells. It is here where the dendritic cells will prime naïve T-cells to initiate a proper immune response, always then traveling back to their resident mucosal tissues (4).

Since the larynx is the gateway to the GI tract it is one of the first sites of antigen introduction to the immune system. Recent examination of the epithelial cells of the larynx, have revealed the presence of unique expression patterns of antigen presenting molecules, such as MHC Class I and II and CD1 [2]. The decreased expression of these molecules as opposed to other areas such as the spleen, as well as the lack of co-stimulatory molecules aids to establish a tolerant atmosphere to the barrage of external antigen it receives from the outside environment.
Recent work in our lab has demonstrated an inability to induce tolerance when a feeding needle is used to administer the treatments for fourteen or more consecutive days. Preliminary data suggest that cellular alterations occur in the esophageal mucosa. The goal of this study was to examine all aspects of the esophageal mucosa that might be altered by repeated feeding needle treatments and correlate any changes with a hindrance in oral tolerance induction to fed ovalbumin. To do this, a pathology scoring system was created to account not only for changes in cellular populations but aberrant cellular growth and physical alterations such as abscesses and mucosal shredding.

**Materials and Methods:**

**Mice**

Balb/c mice (8-12 weeks) bred from mating pairs purchased from The Jackson Laboratory (Bar Harbor, ME), were used for each study. Mice were housed individually in cages and separated into four treatment groups, with no fewer than three mice per group and with equal numbers of each sex between groups. All methods involving mice were approved by the Ball State University Animal Care and Use Committee.

**Oral Tolerance Induction Studies**

Four treatment groups were used for each experiment sNT (syringe-fed non-tolerized), sOT (syringe-fed orally tolerized to OVA), nNT (needle-fed non-tolerized), and nOT (needle-fed orally tolerized to OVA). Mice fed via the syringe method received treatment, where the tip of the syringe (with no needle attached) was placed into the mouth of the mouse and the solution was administered drop-wise. Needle-fed mice were fed via intragastric gavage with a ball-tipped
18 gauge-feeding needle (SouthPoint Surgical Supply, Coral Springs, FL) for a period of up to 14 days.

Mice were fed water (sNT/nNT) or 3mg OVA (sOT/nOT) in a total of 200μL daily for a total of 14 days using either the syringe or intragastric gavage method, as mentioned above. Half of the mice from each group were sacrificed 24 hours following the final feeding via CO2 asphyxiation and esophageal tissue was harvested for histological analysis. The remaining mice were challenged via intraperitoneal immunization with OVA (0.1mg in 200μL of 50% alum solution) both 1 week and 2 weeks following the final feeding. One week following the second immunization, mice were sacrificed via CO2 asphyxiation, blood was collected using cardiac puncture, and serum was isolated.

Tissue Harvesting

Following treatments described above, esophageal tissue proximal to the larynx, was harvested from each mouse. Tissue was placed in a protective cassette and stored in 10% neutral-buffered-formalin (NBF) at room temperature for 6-8hrs. Following fixation in NBF, tissue cassettes were transferred and stored in 70% ethanol until embedding, processing, and staining could be performed.

Immunohistochemistry

Immunohistochemistry was performed on the esophageal cross-sections to assess the infiltration of immune cells within the tissue and identify any inflammation that may result from intragastric gavage. A hematoxylin and eosin (H & E) stain was performed to assess total immune cell infiltration in the submucosa. The chloroacetate esterase or Leder stain was used to
determine the presence of granulocytes in the submucosa and epithelial layer. T-cells in the submucosa and epithelial layer were identified using and anti-CD3 antibody + 3,3' diaminobenzidine (DAB) stain within the tissue. All stains were provided by and performed by the Indiana University School of Medicine Immunohistochemistry Laboratory (Indianapolis, IN). An anti-major basic protein (MBP) stain was also performed to assess eosinophils within the esophageal tissue. This stain was provided and performed by Dr. Marc Rothenberg’s lab at the University of Cincinnati Children’s Hospital (Cincinnati, OH).

Pathology Grading

A histological grading system was used to assess the damage to the tissue that could not be expressed by cell counts alone. Cell counts as well as tissue damage, abscesses, and aberrant epithelial cell growth was assessed using this grading scale. Cells were counted using a 10μm x 10μm grid at 5 different locations along the mucosal/epithelial layer of the esophageal tissue in order to get the best representation of the tissue condition. The average of these cells counts were taken to determine the amount of cells per μm². All cell counts were performed by a “blinded” investigator. All pathology scores were performed by a “blinded” investigator evaluating only the H&E stained tissues.

A histological grading scaled was development by examining twenty-nine slides of esophageal tissue in order to categorize and value anomalies found within the tissue that could not be designated by a simple cell count. The pathology scale descriptions are provided along with representative pictures exemplifying each grade.
Grade 1: The tissue is considered normal and less than 2 cells/μm². No abscesses, mucosal shredding, or cell aggregates are present.

Tissue from “syringe” treatment- normal feeding treatment
Grade 2: The tissue is considered normal but more than 2 cells/μm² are present. No abscesses, mucosal shredding, or aggregates are present.

Tissue from "syringe" treatment – normal feeding treatment
Grade 3: Mucosal shredding is visible and less than 2 cells/μm².

Tissue from “needle” treatment- experimental feeding treatment suspected of causing esophageal damage
Grade 4: Mucosal shredding is visible and more than 2 cells/μm².

Tissue from "needle" treatment—experimental feeding treatment suspected of causing esophageal damage
Grade 5: 1-2 cell aggregates are present. No samples scored a 5.

Grade 6: Greater than 2 cellular aggregates or the presence of abscesses.

Tissue from “needle” treatment - experimental feeding treatment suspected of causing esophageal damage

Grade 7: Any combination of mucosal shredding, presence of aggregates of immune cells, and/or presence of abscesses in the tissue are present. No tissue samples were scored a 7.
Results and Discussion:

Analysis of tissue samples using the pathology grading scale demonstrated that mice fed using a needle (intragastic gavage) had increased damage to the esophageal tissue compared to untreated “day 0” mice, but there was not a significant increase in damage compared to the syringe-fed mice (Figure 1). These data suggest that esophageal architectural changes may result simply due to the handling of mice without any needle/tube insertion into the esophagus. These data provide the basis for further examination into changes in the esophagus that result from feeding treatments so that investigators are aware of the comprehensive effects of treatments in mouse models.

Figure 1. Pathology Score

Figure 1: Intragastric gavage feedings induce architectural modifications to esophageal tissue.

Mice were fed drop wise via the syringe method, or by intragastric gavage daily, for a total of 14 days. 24 hours following the final feeding, esophageal tissue was harvested for analysis. H & E stained tissue were used for pathology grading. Cell counts and pathology grading were performed by a “blinded” investigator using a 10μm x 10μm grid and light microscope at 400X total magnification. * = p < 0.05 as determined by Kruskal-Wallis One Way Analysis of Variance on Ranks. Intragastric gavage results in some physical modifications in esophageal tissue compared to Day 0 control mice. It also appears that syringe-feeding results in some modifications as well, however these are not sufficient enough to disrupt tolerance induction to the fed ovalbumin.
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References:

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