DISRUPTION OF ESOPHAGEAL TISSUE HINDERS ORAL TOLERANCE INDUCTION TO OVALBUMIN

A THESIS

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Introduction

The immune system is constantly in contact with foreign but harmless antigen such as food proteins, and a mechanism must be in place to avoid needless immune responses against these antigens. Oral tolerance is the active suppression of an immune response to fed antigen, upon subsequent challenge with that same antigen [1]. Importantly this tolerance is induced throughout the gut-associated lymphoid tissue (GALT) and other associated mucosal tissues.

Mucosal membranes line the surface of the respiratory, gastrointestinal (GI), and urogenital tracts and contain unique structures and components, both of lymphoid and non-lymphoid origin, that provide protection for the body and comprise the mucosal immune system [2]. The main role of the GALT is the interaction and ingestion of harmless food proteins, and so it acts to provide a tolerogenic atmosphere through several inductive sites including Peyer's patches (PPs), and mesenteric lymph nodes (MLN)[1]. In mice it has been shown that antigen encountered in the periphery by antigen presenting cells (APCs) is identified and transported to the MLNs through CCR-7 dependent signaling and this process is necessary for tolerance induction [3]. PPs are located throughout the GI tract and contain specialized M-cells, which allow for constant sampling of antigen, and subsequent processing by immune cells contained within [2].

These immune cells include T regs [4], DCs [5-7], intestinal epithelial cells (IECs) [6] and intraepithelial lymphocytes (IELs) [8].

Once these immature cells arrive within the mucosal tissue they are uniquely conditioned to elicit a tolerogenic response by the presence of anti-inflammatory components such as IL-10, [9, 10] TGF-β, [11] and retinoic acid, [12] which are present within the mucosal environment. Under normal conditions, immature DCs process foreign but harmless antigen and act to suppress or delete autoreactive T-cells and stimulate Treg differentiation in order to create a tolerant atmosphere [7]. However, once inflammation has been induced through pathogen invasion, tissue damage, or various other mechanisms, the release of pro-inflammatory cytokines forces the maturation of immature DCs, putting them on high alert, and elevating the inflammatory response to antigen, regardless of its harmful or harmless nature.

The larynx is the gateway to the GI tract and therefore serves as one of the first sites of antigen introduction to the immune system. Recent examination of the epithelial cells of the human larynx, have revealed the presence of unique expression patterns of antigen presenting molecules within the laryngeal tissue [13]. A high amount of MHC Class I was observed in the deep layers of tissue, however a reduction of MHC class I and an increase in CD1d was observed on the superficial layers of laryngeal tissue. Secondly, the epithelial cells were found to contain MHC class II, which is normally present only on professional APCs. It is possible that the decreased expression of MHC class I molecules as opposed to other areas such as the spleen, as well as the presence of MHC class II in the absence of co-stimulatory molecules aids to establish a tolerant atmosphere to the barrage of external antigen it receives from the outside environment.

Although the esophagus lies proximal to the larynx, even less is known about its immunologic architecture or its role in initiating immune responses. However, given its location and the knowledge of interplay and trafficking of cells between mucosal tissues, it is possible that it too may have a role in establishing a tolerant response to fed antigens.

In the present study, we investigated whether physical damage of the esophageal mucosal tissue would abrogate oral tolerance induction to fed antigen. This hypothesis was tested by feeding mice the antigenic protein, ovalbumin (OVA) via feeding needle gavage over a 14-day period. The levels of OVA-specific IgG were measured via ELISA to assess tolerance induction, while changes in cell populations were measured via immunohistochemistry to assess the severity of damage from the needle-gavage treatment.

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 CO_2 asphyxiation and esophageal tissue was harvested for histological analysis.

5 days following the final feeding, 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 CO_2 asphyxiation, blood was collected using cardiac puncture, and serum was isolated. This treatment plan is outlined in Figure 1.

Enzyme-linked Immunosorbant Assay (ELISA)

Levels of OVA-specific IgG present in the serum were detected using an indirect ELISA. 96-well plates were coated with OVA (0.4mg/well) (Sigma-Aldrich, St. Louis, MO) in 100μL coating buffer (Bethyl Laboratories, Montgomery, TX) and placed at 4°C overnight. Plates were then washed three times in wash buffer (Bethyl Laboratories) between every step. Plates were then blocked for 30 minutes with blocking solution (Bethyl Laboratories). Serum from each mouse was diluted 1:100, 1:5000, 1:2500, and 1:12500, added to the plate, and incubated for 2 hours at room temperature. Following sample addition, plates were incubated with 100μL rat anti-mouse IgG-AP, human adsorbed (1:1000) (Southern Biotech, Birmingham, AL). To catalyze the enzymatic reaction *para*-Nitrophenylphosphate (0.4mg/well) (Sigma-Aldrich) in 100μL enzymatic substrate solution (Bethyl Laboratories) was used for detection. Samples were analyzed in duplicate using a microplate reader (Model 680, BIO-RAD).

Timepoint Studies

Mice were either handled (to mimic handling that would occur via intragastic gavage) or fed water via intragastric gavage daily for a total of 7 days. Mice were selected at random from the group, sacrificed at Day 0, 1, 3, and 7 via CO_2 asphyxiation, and esophageal tissue was harvested for histological analysis.

Tissue Harvesting

For both the Oral Tolerance and Timepoint studies, esophageal tissue 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. Embedding, processing, and staining were performed at the Indiana University School of Medicine Immunohistochemistry Laboratory (Indianapolis, IN).

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. An anti-major basic protein (MBP) stain was also performed to assess eosinophils within the esophageal tissue. This stain was provided by

and performed by Dr. Marc Rothenberg's lab at the University of Cincinnati Children's Hospital. 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 μm^2.

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. All pathology scores were performed by a "blinded" investigator evaluating only the H&E stained tissues. The pathology scale is as follows: Grade 1: The tissue is considered normal and less than 2 cells/μm^2. No abscesses, mucosal shredding, or cell aggregates are present. Grade 2: The tissue is considered normal but more than 2 cells/μm^2 are present. No abscesses, mucosal shredding, or aggregates are present. Grade 3: Mucosal shredding is visible and less than 2 cells/μm^2. Grade 4: Mucosal shredding is visible and more than 2 cells/μm^2. Grade 5: 1-2 cell aggregates are present. Grade 6: Greater than 2 cellular aggregates or the presence of abscesses. Grade 7: Any combination of mucosal shredding, presence of aggregates of immune cells, and/or presence of abscesses in the tissue are present.

Statistical Analysis

All results from immunohistochemistry cell counts were analyzed using a Mann-Whitney rank sum test to determine statistical significance. All ELISA results were analyzed using a one-way ANOVA with Holm-Sidak post-hoc analysis. Pathology

grading scores were analyzed using a Kruskal-Wallis one-way ANOVA. A p-value < 0.05 was considered significant for all experiments. All statistical analysis was performed using SigmaStat 11 software.

Results

Extended treatment with intragastric gavage abrogates oral tolerance induction

Previous work in our lab suggested that extended use (14 days or more) with a feeding needle led to inconsistency in studies examining oral tolerance induction to OVA. To further examine the possibility that use of intragastric gavage for a period of 14 days was sufficient to cause a breakdown in oral tolerance induction, mice were fed water (sNT/nNT) or 3mg OVA (sOT/nOT) using either the syringe (sNT/sOT) or intragastric gavage (nNT/nOT) method daily for a period of 14 days (Figure 1). Following the feeding treatments, mice were challenged with OVA and levels of OVA-specific IgG were determined. A significant decrease in OVA-IgG levels was seen in the orally-tolerized syringe-fed mice compared to the non-tolerized, syringe-fed mice, demonstrating that tolerance induction to OVA was successful in the syringe-fed mice (Figure 2). However, orally-tolerized (nOT) mice fed via intragastric gavage failed to induce tolerance as evidenced by OVA-IgG levels comparable to non-tolerized (nNT) mice fed via intragastric gavage.

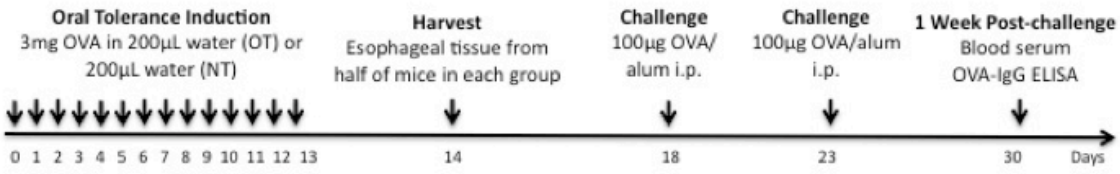

Figure 1. Experimental design.
Balb/c mice (8-12wks) were fed 200 µL water (NT) or OVA (3mg) (OT) every day for 14 days as shown above. Twenty-four hours following the final feeding, half of the mice from each group were sacrificed and esophageal tissue was harvested for immunohistochemical analysis. Five days following the final feeding treatment, the remaining mice were challenged with i.p. (100µg) OVA in a 50% alum solution, with a second challenge 1 week later. One week following the second challenge, mice were sacrificed, and blood serum was collected for analysis.

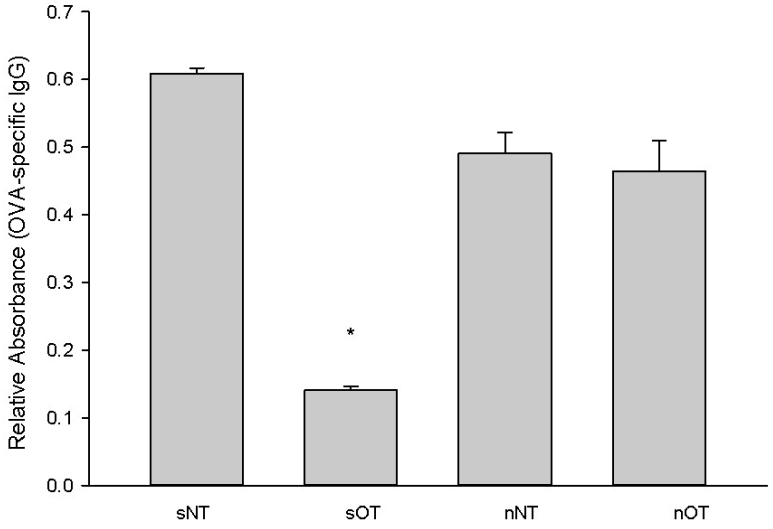

Figure 2. Oral tolerance induction to OVA is successful with syringe feeding, but not intragastric gavage.

Mice were divided into 4 treatment groups (n=3-4/group): syringe-fed, non-tolerized (sNT); syringe-fed, orally tolerized to OVA (sOT); needle-fed, non-tolerized (nNT); and needle-fed, orally tolerized to OVA (nOT). Mice were administered water (sNT/nNT) or 3mg OVA (sOT/nOT) every day for 14 days. At 5 and 12 days post-feeding, all mice were immunized with 0.1mg of OVA. Seven days after the second immunization, blood serum was isolated and serum levels of OVA-specific IgG were determined by indirect-ELISA. * = $p < 0.05$ sNT vs sOT.

Extended treatment with intragastric gavage alters total T-cell populations within the esophageal submucosa and epithelium

Given the importance of mucosal tissues in the induction and maintenance of oral tolerance, it was important to assess the effect of intragastric gavage on the integrity of the esophageal tissue. With the knowledge that there is constant interplay and trafficking of immune cells throughout the mucosal tissues, an influx of cells may indicate inflammation and the presence of this inflamed tissue during antigen introduction would be detrimental to establishing oral tolerance to that antigen. To assess the presence of inflammation within the esophagus (as demonstrated by an influx of cells into the tissue), mice were sacrificed 24 hours following the final feeding, and esophageal tissue was harvested for staining. Tissues were stained with H & E and cells within the submucosa were enumerated. No significant difference in total cells in the esophageal tissue was observed between syringe-fed mice and mice fed via intragastric gavage (Figure 3A). To identify changes in any specific cell populations, esophageal tissues were also processed and stained using an anti-CD3+DAB to identify T cells. In contrast to the anticipation that the esophageal tissue might be inflamed and thus have an increase in cells present, analysis of the stained tissues demonstrated a decrease in T cells in mice fed via intragastric gavage n compared to needle-fed mice (Figure 3B).

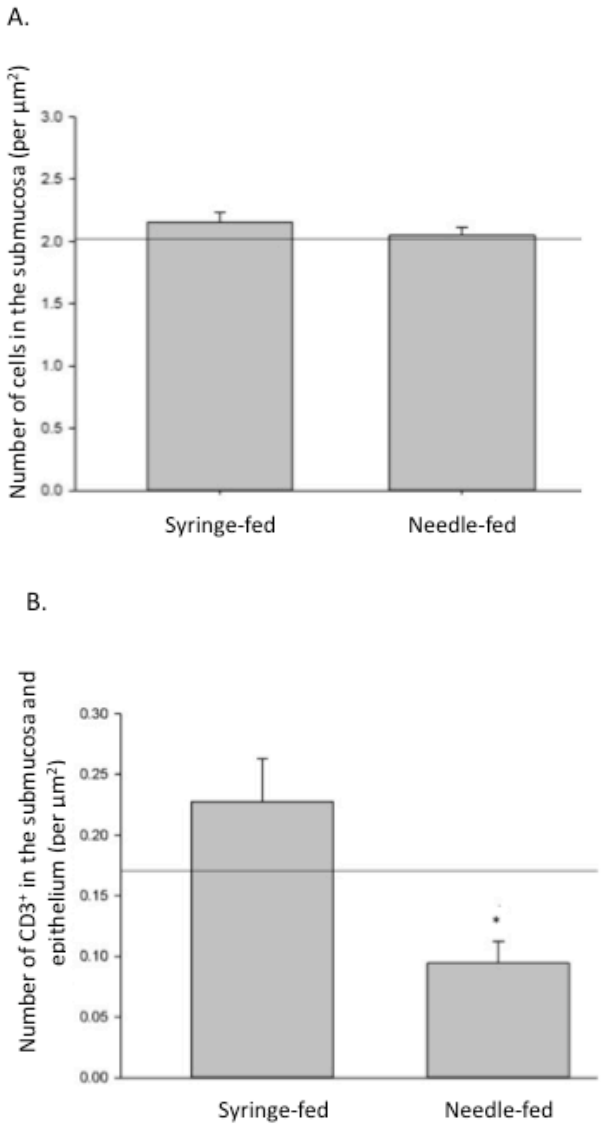

Figure 3. Intragastric gavage reduces levels of T cells in the submucosa and epithelium of the esophagus.

Mice were divided into 4 treatment groups (n=3-4/group): syringe-fed, non-tolerized (sNT); syringe-fed, orally tolerized to OVA (sOT); needle-fed, non-tolerized (nNT); and needle-fed, orally tolerized to OVA (nOT). Mice were administered water (sNT/nNT) or 3mg OVA (sOT/nOT) every day for 14 days. Twenty-four hours following the final feeding, esophageal tissue was harvested for immunohistochemical analysis of total cell infiltration via the H & E stain (A) and T cell infiltration via an anti-CD3 antibody + DAB stain (B). Horizontal lines indicate average cell counts from Day 0 mice. * = $p < 0.05$ syringe-fed vs needle-fed as determined by Mann Whitney Rank Sum Test

Total granulocytes but not eosinophils are altered after extended syringe feeding

In addition to T cells, an increased presence of granulocytes is an indicator of inflammation. Furthermore, eosinophils are not normal residents of the esophagus and, their presence can indicate disease such as eosinophilic esophagitis (EE) [14]. To determine if alterations in specific populations of granulocytes correlated with intragastric gavage treatments and an inability to induce tolerance, mice were sacrificed 24 hours following the final feeding, and esophageal tissue was harvested for staining. Tissues were stained using the Leder stain to identify and enumerate total granulocytes within the mucosa and submucosa. The results demonstrated a slight increase in granulocytes in the esophageal tissues of mice syringe-fed mice compared to control (Day 0) and mice fed via intragastric gavage (Figure 4A). To identify if eosinophils contributed to the slight increase in granulocytes seen in the syringe-fed mice,tissues were stained with anti-MBP antibody and examined (Figure 4B). No difference, however, was seen in total eosinophils in mice fed via intragastric gavage when compared to syringe-fed mice indicating this change in granulocytes is not driven by eosinophil migration to the esophagus.

Taken together these data fail to demonstrate an influx of cells in the mice fed via intragastric gavage, which would be indicative of inflamed tissue but suggest that other cellular alterations within the esophageal tissue may correlate with an inability to induce tolerance.

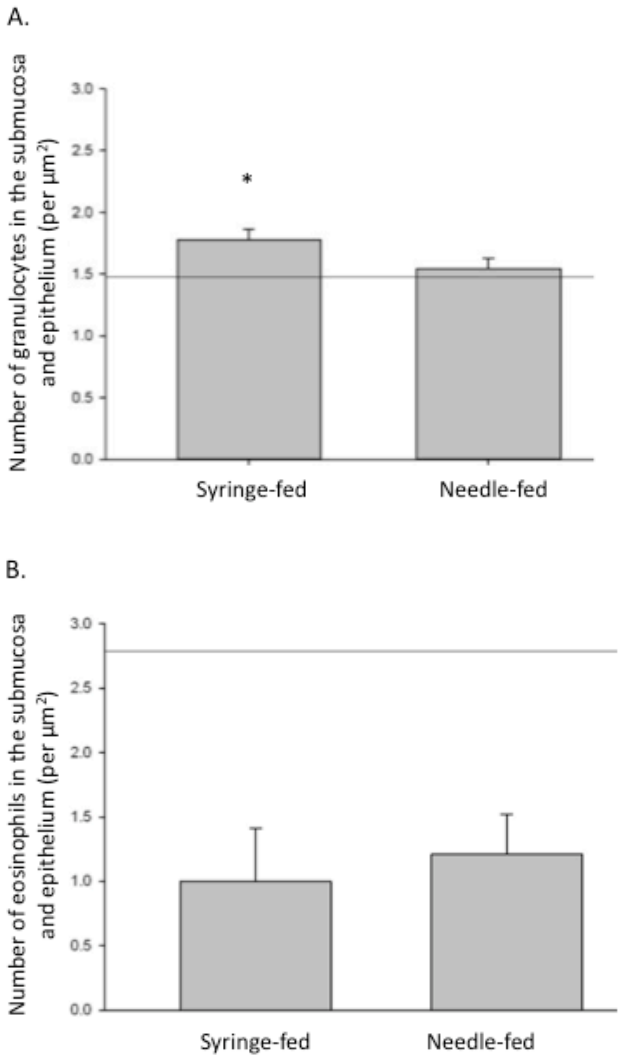

Figure 4. Levels of total granulocytes, but not eosinophils are increased in the submucosa and epithelium of the esophagus following syringe feeding

Mice were divided into 4 treatment groups (n=3-4/group): syringe-fed, non-tolerized (sNT); syringe-fed, orally tolerized to OVA (sOT); needle-fed, non-tolerized (nNT); and needle-fed, orally tolerized to OVA (nOT). Mice were administered water (sNT/nNT) or 3mg OVA (sOT/nOT) every day for 14 days. Twenty-four hours following the final feeding, esophageal tissue was harvested for immunohistochemical analysis of total granulocytes via the Leder stain (A) and total eosinophilic infiltration via an anti-MBP stain (B). Horizontal lines indicate average cell counts from Day 0 mice. * = $p < 0.05$ syringe-fed vs needle-fed as determined by Mann Whitney Rank Sum Test. Eosinophil data was also analyzed using a Mann Whitney Rank Sum Test, however no significant difference was found.

Granulocyte populations within the esophageal submucosa and epithelium fluctuate with feeding treatments.

To examine any changes in granulocyte populations that were occurring at earlier time points in the feeding regimen, tissues were harvested at day 0, 1, 3, and 7 and stained with the Leder stain. Although no difference was seen after the first day of feeding, a significant increase was observed at Day 3 in granulocytes in syringe-fed mice when compared to mice fed via intragastric gavage. This increase however was followed by a continual decrease in total granulocytes to normal levels by Day 7 (Figure 5).

Numbers of CD3+ cells in the esophageal submucosa and epithelium are significantly increased following 7 days of intragastric gavage feedings in mice

To examine any changes in the T-cell populations that were occurring at earlier points during the feeding regimen, tissues again were harvested at day 0, 1, 3, and 7 and stained with anti-CD3 antibody + DAB stain. No difference was seen in T-cell populations at day 0, 1, or 3 of the feeding schedule however, in contrast to the decreased numbers of T cells at day 14, levels of T-cells were increased at Day 7 in mice fed via intragastric gavage when compared to syringe-fed mice (Figure 6).

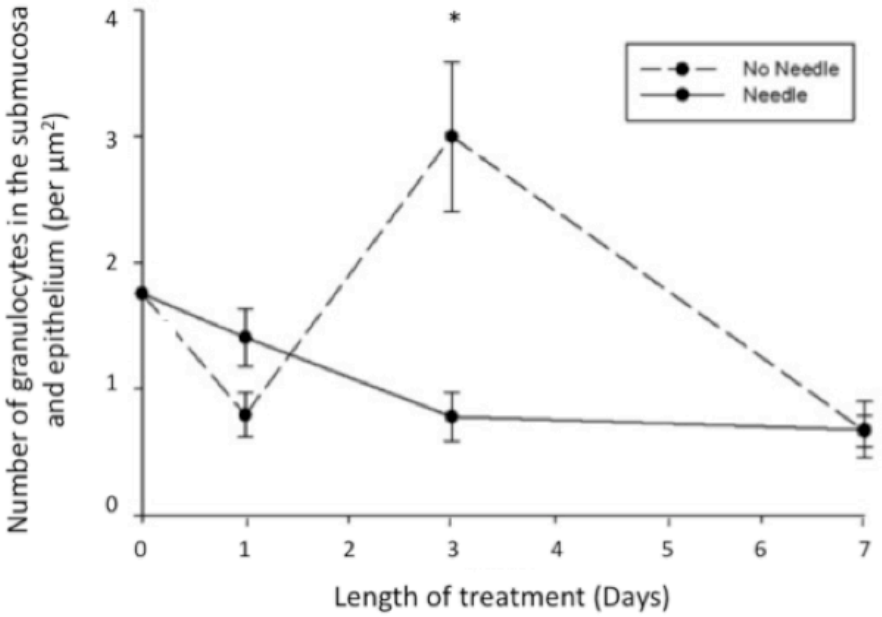

Figure 5. Granulocyte populations within the esophageal submucosa and epithelium fluctuate with feeding treatments.

Mice were divided into 2 treatment groups (n=12/group): Handled to mimic syringe-feedings (no needle) or fed water via intragastric gavage (needle) Mice were handled or fed water every day for 7 days. On day 0, 1, 3, and 7, 3 mice from each group were sacrificed and esophageal tissue was harvested for analysis of granulocyte infiltration via the Leder stain. Horizontal lines indicate average cell counts from Day 0 mice. * = $p < 0.05$ as determined by Mann Whitney Rank Sum test

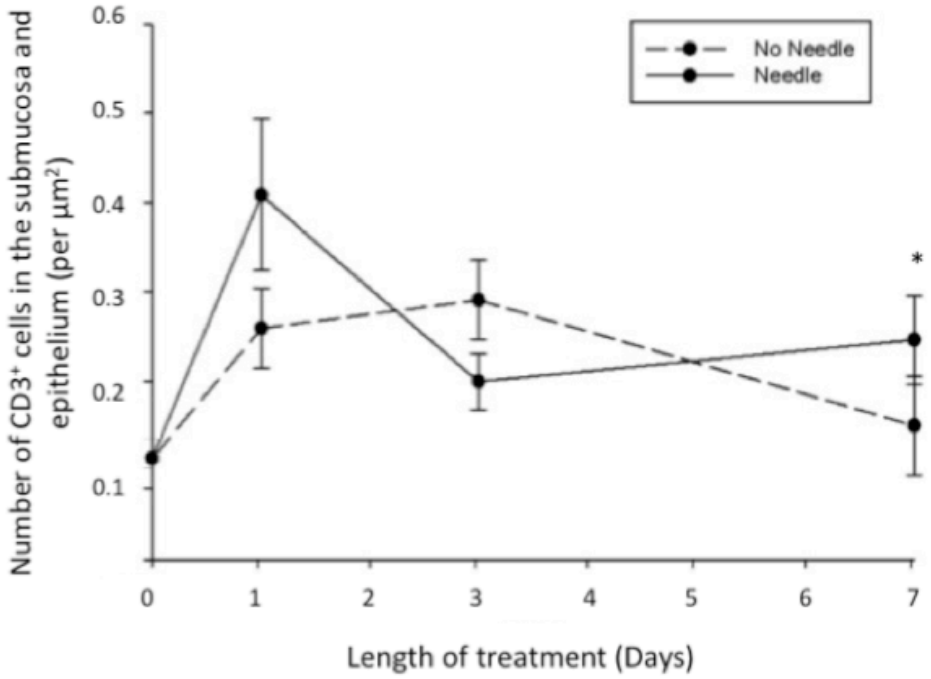

Figure 6. Numbers of CD3+ cells in the esophageal submucosa and epithelium are significantly increased following 7 days of intragastric gavage feedings in mice.

Mice were divided into 2 treatment groups (n=12/group): Handled to mimic syringe-feedings (no needle) or fed water via intragastric gavage (needle) Mice were handled or fed water every day for 7 days. On day 0, 1, 3, and 7, 3 mice from each group were sacrificed and esophageal tissue was harvested for analysis of T cell infiltration via the anti-CD3 antibody + DAB stain. Horizontal lines indicate average cell counts from Day 0 mice. * = $p < 0.05$ as determined by Mann Whitney Rank Sum test

Intragastric gavage feedings induce architectural modifications to esophageal tissue

In addition to differences in cell populations, physical alterations and damage to the esophageal tissue was observed. This included mucosal shredding as well as some aberrant cellular growth along the epithelial layer of some of the tissue. To account for this damage a pathology grading scale was used to assess differences in tissue condition. Although no significant difference was seen between intragastric gavage fed mice and syringe-fed mice, a significant increase was seen in the pathology score for the intragastric gavage fed mice when compared to tissue that was harvested at Day 0 of the feeding schedule (Figure 7). This data suggests that while use of the intragastric gavage may cause some physical alterations of the esophageal tissue, the stress induced through the prolonged handling of the mice while introducing liquid drop-wise via the syringe method induces some alterations as well.

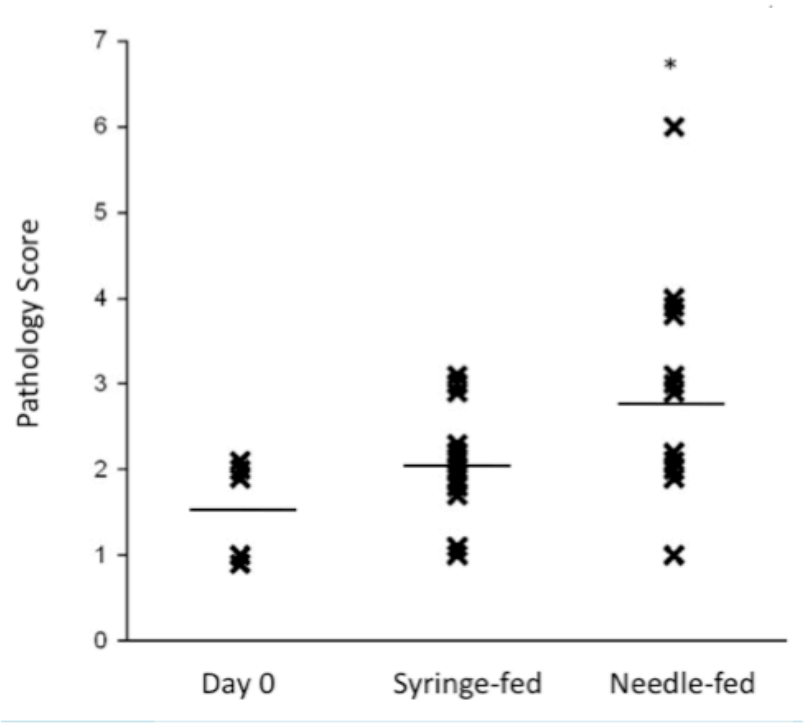

Figure 7: Intragastric gavage feedings induce architectural modifications to esophageal tissue.

Mice were divided into 4 treatment groups (n=3-4/group): syringe-fed, non-tolerized (sNT); syringe-fed, orally tolerized to OVA (sOT); needle-fed, non-tolerized (nNT); and needle-fed, orally tolerized to OVA (nOT). Mice were administered water (sNT/nNT) or 3mg OVA (sOT/nOT) every day for 14 days. 24 hours following the final feeding, esophageal tissue was harvested for analysis. H & E stained tissue 9were used for pathology grading. Cell counts were performed by a "blinded" investigator using a 10μm x 10μm grid. Horizontal lines indicate average cell counts from Day 0 mice. * = $p < 0.05$ as determined by Kruskal-Wallis One Way Analysis of Variance on Ranks.

Discussion

Problems with intragastric gavage have been previously documented [15-17]. This was a novel study to examine how extended use of intragastric gavage may alter mucosal tissues and subsequent immune mechanisms necessary for inducing tolerance to fed antigens. The data from this study demonstrate that oral introduction of OVA was sufficient to induce oral tolerance in syringe-fed mice. However, extended use of the intragastric gavage for a period of 14 days hindered oral tolerance induction to the fed OVA, such that levels of OVA-IgG in mice fed via intragastric gavage exceeded that of the non-tolerized control mice. Furthermore, these results demonstrated fluctuations of cellular populations within the esophageal mucosal that may provide insight into the tissue perturbations that may contribute to an inability to induce tolerance. Intragastric gavage is a commonly used method due to the ability to introduce a solution both quickly and accurately as compared to syringe-based or feed-based methods. It has been shown that handling of the mice alone for repeated and prolonged periods can induce stress responses as indicated by increased levels of plasma corticosterone and prolactin [18]. Previous studies however, have also found gavage feeding results in similar increased stress responses in mice [15] and rats [16] as indicated by increased levels of plasma corticosterone levels. Our study compliments these previous findings, demonstrating that although oral tolerance was induced in syringe-fed mice, both the syringe-feedings and

intragastric gavage resulted in increased alteration of esophageal architecture (identified as an increase in pathology score) as compared to unaltered mice (Figure 7).

This work was done to examine the hypothesis that use of a feeding needle to perform intragastric gavage for an extended period of time induces inflammation in the esophageal tissue, stimulating an immune response that is not tolerant to fed antigen. In contrast to our anticipated results, both granulocyte and CD3+T cell populations were reduced following intragastric gavage treatment (Figure 3). One explanation of the significant reduction in CD3+ T cells is that it is possibly a result of a reduction in γδ T cells. γδ T cells have been shown to have an important regulatory role in oral tolerance through their maintenance of the barrier function of the epithelium, control of intestinal epithelial cell (IEC) turnover, as well as having a protective role in DSS-induced colitis in mice [19] . This cross-talk between IECs and γδ T cells has recently been explored and has been shown to be crucial for routine maintenance and repair of the intestinal epithelium, which is important in establishing tolerance to fed antigen [8]. It is possible that the damage caused by the intragastric gavage is disrupting the balance between the IEC and γδ T cell population, which is disrupting their normal cross-talk and homeostasis. This likely would be a complex explanation however, as γδ T cells have also been shown to have inflammatory properties as well [19]. Alternatively, complete removal of the surface epithelial cells in both the small and large intestine of humans has been shown to allow for the migration of large amounts of T cells out of their normal residence in the lamina propria [20]. It is possible that prolonged use of the feeding needle results in similar damage to the esophagus allowing for migration of T cells out of

the tissue and causing the disparity in T cells between syringe and needle-fed mice. Despite these possibilities, it is most likely a complex mechanism that is responsible for the fluctuations seen.

It has been shown that handling and restraint of mice alone causes increased stress responses as indicated by increased plasma corticosterone levels [18]. It has also been shown that physical stress caused by strenuous exercise in humans, results in an increase in granulocytes as well as fluctuations in lymphocyte makeup within the blood, and these changes correspond to an increase in plasma catecholamine levels [21]. It is possible that the prolonged restraint of the mice during syringe treatments caused a similar response and account for the significantly increased granulocytes within the tissue at day 3 (Figure 5) and 14 (Figure 3B). Importantly, the ability to induce tolerance to fed OVA in these syringe-fed mice show that these brief granulocyte increases are not detrimental to the tolerance induction process. Conversely, granulocyte populations in gavage-fed mice do not significantly deviate from day 0 levels, indicating that although some physical damage may occur during gavage-feeding, this as well as the brief restraint during treatment is not enough to elicit the same response seen in syringe-fed mice.

In contrast to the granulocytes, levels of T-cells were significantly increased at Day 7 of treatment in intragastric gavage-fed mice. This is in opposition to the decreased numbers of CD3+ T cells in intragastric gavage-fed mice seen at day 14. This fluctuation may demonstrate an evolution of the immune response in this tissue such that minor damage to the esophageal epithelial cells initially results in decreased barrier function and subsequent antigen exposure to the lower, more pro-inflammatory layers of the mucosal

tissue, resulting in an inflammatory response and account for the increase in T cells at Day 7 of treatment. Repeated needle exposure could then result in compounding damage to the surface epithelial layer and eventually lead to the migration of T cells out of the tissue as mentioned above inducing the significant decrease of T cells seen at Day 14.

Taken together, these data demonstrate an inability to tolerize mice to OVA fed via intragastric gavage for 14 days or more and highlight alterations in the esophageal architecture that occur as a result of intragastric gavage treatments. Although this study did not reveal a specific subset of cells responsible for the loss of tolerance induction, it is clear that intragastric gavage causes major fluctuations in the T cell population as well as some significant structural alterations within the esophageal mucosal tissue. Interestingly, the data demonstrates that syringe-feeding also results in changes in the esophageal architecture, specifically a subtle increase in granulocyte populations as well as some minor alterations in esophageal tissue, however these changes are not enough to disrupt tolerance induction. This study provides initial evidence of the importance of esophageal integrity and cellular populations in oral tolerance induction and serves as a basis for future studies investigating the role of T cell subsets in the esophagus that contribute to tolerance induction and maintenance and the destruction of the barrier function of the mucosa by the physical trauma of intragastric gavage that may allow for systemic introduction of fed antigen.

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