High prevalence of abnormal gastrointestinal permeability in moderate-severe asthma

Abstract

Purpose: Abnormal gastrointestinal permeability (GIP) has been implicated in a number of diseases, including chronic intestinal inflammatory disorders such as Crohn's as well as non-intestinal immunologic diseases such as diabetes and multiple sclerosis. Although evidence in the literature demonstrates mucosal abnormalities of the digestive barrier in asthma, previous studies have assessed only colonic permeability, while ignoring the mucosal-associated lymphoid tissue (MALT) rich areas of the small intestine. Alterations in GIP may lead to increased entry of allergenic proteins from the gut lumen into the systemic circulation, thus priming and activating the adaptive immune system and leading to inappropriate allergen sensitization and/or deregulated extra-intestinal inflammation. This study examines GIP in adults with moderate to severe asthma.

Methods: Patients ingested a mixed-sugar solution and urine was collected. GIP was assayed using high-performance liquid chromatography. Demographics, atopy (assessed by allergen skin testing) and sputum cell counts were also assessed.

Results: Fourteen patients with moderate to severe asthma were studied, half of whom were found to have abnormal GIP. Abnormal GIP did not correlate with sputum cell counts and there was no apparent association between atopy and intestinal permeability.

Conclusion: This study demonstrated our ability to identify abnormal GIP in the MALT-rich, immunogenic small intestine of patients with asthma. The absence of a correlation between airway inflammation and increased GIP suggests that these two parameters are not causally linked, but rather define distinct entities that could separately or sequentially be involved with the development and propagation of asthma over time.

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Asthma is a multifactorial disease that involves genetic, environmental and immunologic components [1]. Asthma commonly presents with respiratory symptoms of wheeze, dyspnea, chest tightness and cough with increased sputum production, and is associated with airway inflammation, mainly reversible airflow limitation and airway hyper-responsiveness. Over time, asthma can lead to airway remodeling in a sub-group of individuals with the functional consequence of fixed airflow limitation, particularly in those with severe asthma (SA) [2].

The etiology and pathophysiology of asthma remains incompletely understood, and review of the literature reveals a growing recognition of asthma as a collection of heterogeneous disease phenotypes [3]. Patients with asthma may or may not identify specific allergenic and non-allergic “triggers” that provoke increased symptoms that can lead to an exacerbation [4]. Sensitization to sequential allergens, including occupational sensitizers, can occur over a lifetime [5].

Previously, a correlation was made between food allergy and asthma [6] and, in a recent study, two-thirds of the patients attending asthma clinics in Europe and Australia believed that certain foods induced their asthma-related respiratory symptoms [7]. The nature of these relationships is not well understood, and while gastro-esophageal reflux and sensitivity to cold foods may exacerbate asthma symptoms, the idea of gut-derived allergens as asthma triggers is intriguing. A recent double-blind, placebo-controlled study in children suggests food allergens may be inducers of asthma in approximately 7% of patients [8]. This number is relatively small, but becomes more significant when considered in context; dietary antigens would in fact represent a small minority of the antigenic proteins presented to the gut lumen when compared with the huge load of potential antigens represented by the resident bacterial microflora.

These observations lend support to the theory of a so-called common mucosal immune system, whereby a locally-evoked immune response could produce inflammation at a mucosal site distant from the initial contact site within the host. It is intriguing to speculate that antigens presented to the gastrointestinal mucosal immune system may play a pathogenic role in asthma. Specifically, an abnormal barrier to gastrointestinal antigens could confer a higher likelihood of inducing respiratory symptoms in a susceptible individual. The gastrointestinal mucosa represents both an innate and an active immunologic barrier, with constant sampling of the luminal antigens by a sophisticated process that remains incompletely elucidated. Tight-junction and adherens protein complexes provide a significant part of the intestinal epithelial barrier function. Abnormal intestinal permeability refers to a measurable increase in the passage of small water-soluble compounds across this paracellular (non-diffusive) pathway of the small intestine [9]. A defect in this barrier function has been associated with numerous diseases such as Crohn’s disease [10,11] and celiac disease [12], but interestingly, also with non-intestinal immunologic diseases such as insulin-dependent diabetes [13], eczema [14] and multiple sclerosis [15]. In the case of Crohn’s disease and type 1 diabetes, abnormal intestinal permeability may precede clinical disease, suggesting a potential etiologic role in disease development [16-18]. A 1996 study illustrated an increase in measured colonic permeability in adult asthmatics [19], but that study’s methodology precluded measurement of permeability in the small intestine. The small bowel is known to be rich in mucosal lymphoid tissue and is, arguably, a more immunologically relevant site than the colon. A subsequent pediatric study employed similar methodology to our own study [20], and reported that intestinal permeability is increased in children with asthma, but did not include an index of airway inflammation to correlate abnormal gastrointestinal permeability with active airway inflammation.

In this study, we hypothesized that intestinal permeability in patients with asthma would be increased. We further sought to determine whether increased intestinal permeability would correlate with airway inflammation through sputum cell counts [21]. We hypothesized that abnormal intestinal permeability could represent an underlying immunologic host defect, occurring independently from the presence or type of airway inflammation [22], and predisposing the host to an excessive pro-inflammatory response triggered by an overload of gut antigens with consequent effects in the airways.

We aimed to study gastro-intestinal permeability in adult patients with mild-moderate asthma followed longitudinally through a regional asthma center in Western Canada.

**Methods**

Gastrointestinal permeability testing is a clinical tool utilized routinely in Inflammatory Bowel Disease (IBD) clinics. It was incorporated into the evaluation of selected patients with asthma seen longitudinally within the Edmonton Regional Severe Asthma Clinic (with a catchment area encompassing the northern parts of the three most western provinces in Canada: Alberta, British Columbia and parts of Saskatchewan). Clinic patients with asthma were selected based on complaints of nonspecific gastrointestinal symptoms. Data were abstracted from the clinical records in accordance with institutional research ethics guidelines. A total of fourteen patients were included in this review. Data captured included demographics,
atopic status, sputum cell counts (within 3 months) and current pharmacologic therapies.

Of the fourteen patients included in the study, three were unable to produce a satisfactory induced sputum sample for quantitative cellular analysis on at least two occasions. Subjects were classified as atopic or non-atopic by allergen skin tests, using a standardized panel of allergen extracts.

**Measurement of gastrointestinal permeability**

Gastroduodenal and intestinal permeability was assessed by mixed sugar absorption assay as previously described [23]. Sucrose represents an ideal probe molecule to detect increased gastroduodenal permeability in a site-specific manner. In the presence of gastroduodenal damage, sucrose permeability is increased, but as sucrose is rapidly degraded within the small intestine, this probe does not detect abnormal small intestinal permeability, making it more specific for the gastric and duodenal mucosa [24]. In the healthy small bowel, the permeability for larger sugars such as lactulose is much lower than for smaller sugars such as mannitol. Under pathological conditions (such as mucosal inflammation) the permeability for the larger sugars increases, whereas the permeability of the smaller sugars remains the same or decreases. This results in an increased urinary excretion ratio of larger versus smaller sugars, and an increase in the lactulose to mannitol ratio. Collected urine was analyzed for these sugars using high performance liquid chromatography (HPLC). Specific methodology was followed as described by Hilsden et al. [25] to directly compare the results with established values for normal permeability. From their published series, standard upper reference limits (mean + 2 SD) were established at 0.0281 and 180 mg for the lactulose to mannitol ratio and excreted sucrose values, respectively.

**Induced sputum analysis**

Sputum was collected and processed as described by Pizzichini and colleagues [21], with non-squamous differential cell counts obtained in a standardized manner.

**Results**

Fourteen individuals with asthma underwent GIP testing, of whom seven were found to have increased GIP (Figure 1). While three of these seven patients showed an increase in gastroduodenal as well as small intestinal mucosal permeability, one patient showed an isolated increase in gastroduodenal permeability, and three patients demonstrated isolated increased small intestinal permeability. Table 1 compares the baseline demographics and characteristics between those patients with increased GIP versus those with normal GIP. Interestingly, of the seven asthma patients with normal GIP, five were male and only two were female. Corticosteroids are known to increase GIP and systemic corticosteroids were being used by three of the enrolled patients with abnormal GIP, in contrast to none of the group demonstrating normal permeability.

No significant difference was found between abnormal GIP and atopy; three patients in the normal and four patients in the abnormal permeability groups had evidence for atopic asthma by prior skin prick testing (Table 1). Furthermore, no significant difference was found between abnormal GIP and measured airway inflammation through sputum cell counts (Figure 1).
TABLE 1. Patient demographics and characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sample size</th>
<th>M</th>
<th>F</th>
<th>Age (median, years)</th>
<th>Atopy</th>
<th>Systemic CS</th>
<th>ICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Permeability</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>44.6</td>
<td>3</td>
<td>0</td>
<td>7</td>
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<tr>
<td>Abnormal Permeability</td>
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<td>5</td>
<td>45.1</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
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CS, corticosteroid use; ICS, inhaled corticosteroid use

Discussion

This work expands upon previous literature that suggests that abnormal intestinal permeability exists in patients with asthma, and illustrates that increased intestinal permeability can be demonstrated in the absence of concurrent lower airway inflammation. This study is an investigator-initiated pilot project that was designed and conducted in part as a learner research project (Internal Medicine Residency Project), without external funding. As such, within this section our discussion touches upon not only our study results and their implications, but also includes a synopsis of the “lessons learned” with respect to study design, data collection and analysis.

This study found that 50% of individuals with asthma have demonstrable abnormalities in gastro-duodenal and small bowel permeability. This is consistent with the findings of previous reports [19,20] that abnormal GIP does not correlate with atopy. No correlation was demonstrated between active airway inflammation, as assessed by SCC, and abnormal GIP within our cohort. The absence of a correlation may be due to the small sample size or could alternatively suggest that abnormal GIP represents an underlying defect in innate mucosal immunity, as opposed to a systemic consequence of ongoing airway inflammation. Abnormal GIP has been demonstrated in numerous human diseases, most with a notable autoimmune/immunologic component. The theory of a common mucosal immune system, whereby a “trigger” recognized and responded to in the gut could provoke extra-intestinal inflammation, remains speculative but is increasingly supported by the literature. The intricate connection between the gut and the airways, compartments that share a similar embryologic origin, remains a fascinating area of study. No attempt to modulate antigen presentation in the gastrointestinal tract to modulate asthma has been reported, and would be an interesting and logical extension of the finding that GIP may be increased in asthma.

It is intriguing to note that abnormal GIP in this group of individuals with asthma was more common in females. Gender differences have been little explored to our knowledge, particularly with known gender differences in autoimmune diseases. In contrast, a recent study attempted to sub-classify Crohn’s disease patients with abnormal GIP, and found a predilection towards increased GIP in males [26].

Limitations of this work include our small sample size (unfunded study, learner-driven project), and as well the retrospective design of the study employed. It is possible that abnormal GIP may have an association with the development and propagation asthma; however, experimental numbers are too small at this juncture to exclude any definitive statements. Ideally, a larger prospective study should be designed with longitudinally-followed patients, which assesses GIP and asthma severity, along with sequential evaluations allergen sensitization, airway inflammation and different assays for those found to be positively screened with abnormal GIP (i.e., development of celiac disease, inflammatory bowel disease over the next 5-10 years). In addition, the possible impacts of diet modification should be considered. In the period following data collection and analysis, results of this study were presented at both local regional and international meetings with piqued interest, particularly in the area of gut and airway microbiome research. The response to our work from experts within the fields of respiratory medicine, gastroenterology and immunology has been invaluable, with very positive feedback at these venues. As a learner-led project, we have learned a lot from this early work and, with appropriate funding, wish to pursue next whether GIP in this patient group remains static or dynamic over the course of the individual’s airways disease in a prospective study. Based on work to date, we suggest that abnormal intestinal permeability may be an important determinant in the broader understanding of the pathophysiology of asthma – and possibly a factor in development of more severe disease within a proportion of these individuals.

Acknowledgments

JW, LD, JM and DV all contributed to the study design. DM and KP were responsible for data collection (including HPLC), and JW and DV analyzed the data and co-wrote the manuscript. Editorial input was received from all authors prior to submission of the manuscript, and JW and DV were responsible for verifying the accuracy of reported data and associated claims.
References


