Reduction of Interleukin 8 Gene Expression in Reflux Esophagitis and Barrett’s Esophagus With Antireflux Surgery

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Hypothesis: Chronic inflammation of esophageal mucosa secondary to refluxed gastric juice increases gene expression of interleukin 8 (IL-8). Antireflux surgery can reduce this overexpression.

Design: Prospective analysis of archival paraffin-embedded tissue.

Setting: Academic tertiary medical center.

Patients and Methods: One hundred eight patients with reflux symptoms were classified according to pH monitoring and endoscopic and histologic findings. Twenty patients did not have reflux or mucosal injury; 47 had reflux disease (16 esophagitis and 31 Barrett’s esophagus), 20 had dysplasia, and 21 had adenocarcinoma. Microdissection was performed to exclude inflammatory cells and stromal tissue. After RNA isolation and reverse transcription, IL-8 messenger RNA expression was measured using quantitative real-time polymerase chain reaction. All patients with reflux disease had Nissen fundoplication with biopsies at matched levels within the esophagus preoperation and postoperation.

Results: Expression of IL-8 was increased in patients with reflux compared with those without reflux. Patients with the highest IL-8 expression were those with Barrett’s dysplasia and adenocarcinoma (P<.001). In patients with reflux, Nissen fundoplication led to significantly decreased IL-8 expression compared with preoperative levels in esophagitis (P=.01) and Barrett’s esophagus (P=.03).

Conclusions: Interleukin 8 messenger RNA expression increases during the progression of reflux disease from normal squamous mucosa to esophageal adenocarcinoma. Elimination of reflux with Nissen fundoplication significantly reduces IL-8 expression in both squamous and Barrett’s mucosa. These results demonstrate that effective antireflux surgery can modulate the gene expression of esophageal mucosa and may impact the natural history of reflux disease.

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Increased exposure of the normal esophageal squamous mucosa to gastric juice in the setting of gastroesophageal reflux disease (GERD) results in mucosal damage and tissue inflammation. At the macroscopic level, this can be recognized endoscopically as esophagitis with ulceration and mucosal disruption or as a columnar-lined distal esophagus. At a sublier level, injuries can be identified microscopically as alterations in the histology of the mucosa with the presence of inflammatory cells or intestinal metaplasia. Recently, ultrastructural changes in the intercellular gaps of the mucosa seen on electron microscopy have also been correlated with tissue injury. These forms of mucosal injury can be associated with serious consequences, particularly, because it has been shown that chronic inflammation is associated with carcinogenesis. This is exemplified in GERD, where chronic inflammation of the squamous mucosa can lead to intestinal metaplasia, dysplasia, and ultimately esophageal adenocarcinoma.

Prior to macroscopic or even microscopic evidence of inflammation, there are molecular changes occurring within mucosal cells exposed to refluxed gastric juice. These molecular changes result in alterations in the expression of genes that affect cellular repair, proliferation, and migration. A more sensitive assessment of mucosal injury might therefore be the measurement of inducible genes known to be involved in these processes. Real-time polymerase chain reaction is a method that allows quantitative measurement of messenger RNA (mRNA) and, in combination with tissue microdissection, allows precise assessment of alterations in gene expression. These gene expression changes within mucosal cells exposed to reflux provide insight into the molecular correlates of mucosal injury and go beyond the subjective assessment of injury with the endoscope or microscope.
There is growing evidence that at the molecular level some of the pathways associated with inflammation and injury are similar to those involved in carcinogenesis. One such mechanism involves the nuclear factor-kB pathway, in which interleukin 8 (IL-8) is a major product activated immediately downstream. Nuclear factor-kB is a transcription factor that regulates many genes involved in the inflammatory response, and it is now thought to also play a role in activating genes involved in the progression of cancer, including that of the esophagus. Interleukin 8, a member of the CXC chemokine family, is a unique protein with dual roles in both inflammation and carcinogenesis and is known to be directly up-regulated by nuclear factor-kB activation. Interleukin-8 is both a neutrophil chemoattractant as well as a stimulant of cellular proliferation and angiogenesis, and it appears to be uniquely situated to mediate the transition from inflammation to carcinogenesis in GERD.

The aim of this study was to quantitatively measure IL-8 mRNA expression in microdissected esophageal mucosal cells taken from patients with different stages of reflux-induced injury: esophagitis, Barrett’s esophagus, Barrett’s dysplasia, and esophageal adenocarcinoma. We also sought to assess the impact of the elimination of reflux with Nissen fundoplication on IL-8 gene expression. We hypothesized that IL-8 gene expression would increase with worsening reflux injury of the esophageal mucosa and that effective antireflux surgery would result in reduced expression of IL-8 mRNA.

**METHODS**

**STUDY POPULATION**

Approval for this study was obtained from the Institutional Review Board of the University of Southern California Keck School of Medicine. The study population consisted of 108 patients with reflux symptoms seen by members of the Section of Thoracic and Foregut Surgery at the University of Southern California between 1993 and 2006. Patients were selected at random and the final numbers in each study group were based on the availability of paraffin blocks, the adequacy of the tissue in the blocks, the quality of the ribonucleic acid isolated, and the availability of preoperative and postoperative biopsies at matched levels in those who had Nissen fundoplication. All patients were evaluated preoperatively with a symptom questionnaire; esophageal manometry with 24-hour pH monitoring (with the exception of patients with cancer); and upper endoscopy with biopsies taken according to a systematic protocol that included the gastroesophageal junction, squamous mucosa 3 cm above the squamocolumnar junction, and any columnar-lined segment of the esophagus or other mucosal abnormality. All patients stopped acid suppression medical therapy at the time of evaluation (2 weeks for proton pump inhibitors and 2 days for histamine receptor antagonists).

The patients were divided into 3 groups based on clinical, endoscopic, and pathologic findings:

1. No reflux (n=20). These patients were evaluated for foregut symptoms but had no evidence of GERD with normal acid exposure on 24-hour pH monitoring (median composite pH score, 2.5), normal endoscopic findings, and normal squamous histology seen on biopsies taken 3 cm above the squamocolumnar junction. The biopsies from these patients were used for baseline assessment of IL-8 gene expression.

2. Esophagitis (n=16). These patients had foregut symptoms and endoscopic or histologic evidence of reflux esophagitis (macroscopic ulcerations or cobblestone changes and/or microscopic evidence of intraepithelial eosinophils, basal cell hyperplasia, and papillary elongation). In addition, all patients had increased acid exposure (composite pH score >14.7) on 24-hour pH monitoring (median composite pH score, 36.5). Both macroscopic and microscopic esophagitis samples were considered together, as it has previously been shown that IL-8 levels increase to a similar degree in both stages of injury. All patients underwent Nissen fundoplication. Endoscopic biopsies of the squamous mucosa in these patients were obtained 3 cm above the squamocolumnar junction before and at least 6 months after Nissen fundoplication (median interval from surgery to follow-up biopsy, 29 months [range, 14.3-37.7 months]).

3. Barrett’s esophagus (n=31). These patients had foregut symptoms and a columnar-lined esophagus on endoscopy. All patients had increased acid exposure (composite pH score >14.7) on 24-hour pH monitoring (median composite pH score, 47.2). The diagnosis of Barrett’s esophagus was confirmed with biopsies showing cardiac mucosa with intestinal metaplasia. Patients with dysplasia or intestinal metaplasia at a normal gastroesophageal junction (cardia intestinal metaplasia) were excluded. All of these patients underwent Nissen fundoplication. The Barrett’s segment was short (<3 cm) in 24 patients and long (≥3 cm) in 7 patients, with a median length of 8 cm. Biopsies were obtained circumferentially every 2 cm along the columnar segment before and at least 6 months after Nissen fundoplication (median time interval from surgery to follow-up biopsy, 12 months [range, 8.1-22.1 months]).

4. Barrett’s dysplasia (n=20). These patients were found to have Barrett’s esophagus with dysplasia and all had esophagectomy. None of these patients had been previously treated for adenocarcinoma. The final pathologic diagnosis of the surgical specimen was high-grade dysplasia in 17 patients and low-grade dysplasia in 3 patients. Microdissection of the area with the greatest degree of dysplasia was performed in each specimen.

5. Esophageal adenocarcinoma (n=21). These patients were found to have esophageal adenocarcinoma and all had esophagectomy. None of these patients had received neoadjuvant therapy. The tumor invaded the muscularis propria in 4 patients, the adventia in 13 patients, and the adjacent adipose tissue in 4 patients. Lymph node metastases were present in all but 1 patient. Microdissection of the primary tumor was performed, avoiding areas of tissue necrosis.

Nissen fundoplication was performed by a member of the Section of Thoracic and Foregut Surgery at the University of Southern California, according to a standardized protocol. The procedure in all patients included esophageal mobilization, reduction of any associated hiatal hernia, crural closure with permanent suture, and a 360° Nissen fundoplication over a 60 French bougie. The operations were performed via a laparoscopic, open abdominal, or transthoracic approach, based on the severity of reflux and associated anatomic abnormalities, including the size and reducibility of the hiatal hernia. None of the patients had a previous antireflux operation. All postoperative endoscopies confirmed the presence of an intact fundoplication without recurrent herniation. Postoperative pH testing was performed on a selective basis after fundoplication.

In patients with short-segment Barrett’s esophagus who had endoscopic biopsies taken at 1 or 2 levels within the columnar segment, IL-8 gene expression represented the average of the values from all the biopsies. In those patients with long-segment Barrett’s esophagus where biopsies were taken at 3 or more levels, the biopsies were divided into lower, middle, and upper thirds based on the location within the columnar seg-
IL-8 mRNA expression was measured using a fluorescence-activated cell sorting (FACS) system, Foster City, Calif) relative to the expression of the housekeeping gene β-actin as previously described. All samples were run in triplicate. Interleukin 8 forward and reverse primers as well as the fluorescent probe were designed using published gene sequence data (National Center for Biotechnology Information GenBank) and Primer3 software to specifically amplify mRNA (sequences available on request from the corresponding author).

### STATISTICAL METHODS

Comparison of trend or analysis of variance was performed using the Kruskal-Wallis test. Interleukin 8 gene expression levels from matched pair biopsies before and after Nissen fundoplication were compared using the Wilcoxon rank sum test. The Mann-Whitney U test was used to compare continuous variables. Spearman correlation was performed to analyze the relationship between time from surgery and the pattern of IL-8 gene expression. Statistical significance was set at $P<.05$.

### RESULTS

Esophageal mucosal IL-8 mRNA expression was increased significantly in patients with abnormal esophageal acid exposure compared with symptomatic patients without gastroesophageal reflux (median, 0.23 vs 0.005, respectively; $P=.002$). The expression of IL-8 was increased further in patients with intestinal metaplasia compared with patients with esophagitis, though this did not reach statistical significance (median, 0.28 vs 0.05, respectively; $P=.41$). However, patients with Barrett’s dysplasia had a significant increase in IL-8 expression compared with patients with Barrett’s esophagus without dysplasia (Table 1). The highest IL-8 expression was found in patients with adenocarcinoma, in whom the median mRNA level was more than 700 times greater than that measured in the squamous mucosa of patients without reflux (Figure 1).

After Nissen fundoplication, IL-8 mRNA expression in the squamous mucosa of patients with reflux disease was significantly decreased compared with preoperative levels (median, 0.02 vs 0.12, respectively; $P=.01$) (Figure 2) and reached values similar to those found in the squamous mucosa of patients without GERD ($P=.90$) (Table 2). Interleukin 8 gene expression in Barrett’s esophagus before and after Nissen fundoplication is shown in Figure 3. Overall, postoperative IL-8 expression in Barrett’s esophagus was significantly lower.

### TISSUE PROCESSING AND IL-8 mRNA QUANTIFICATION

The formalin-fixed, paraffin-embedded biopsy and esophagectomy specimens were retrieved from the pathology archives. Hematoxylin and eosin slides were made from each block to confirm the histologic diagnosis and assess adequacy of tissue quantity for subsequent processing. Paraffin blocks were sectioned at a 10-µm thickness and the slides were then dried overnight and stained with nuclear fast red. Microdissection was performed to isolate the cells of interest using a combination of manual and laser capture techniques to minimize contamination by inflammatory cells or stromal tissue. Approximately 20 to 40 mm² of tissue was required to obtain a sufficient quantity for ribonucleic acid isolation, and the number of sections made from each block was adjusted accordingly. Ribonucleic acid was isolated and reverse transcribed to complementary DNA; IL-8 mRNA expression was measured using a fluorescence-based, quantitative, real-time polymerase chain reaction system (ABI Prism 7900 Sequence Detection System; Applied Biosystems, Foster City, Calif) relative to the expression of the housekeeping gene β-actin as previously described. All samples were run in triplicate. Interleukin 8 forward and reverse primers as well as the fluorescent probe were designed using published gene sequence data (National Center for Biotechnology Information GenBank) and Primer3 software to specifically amplify mRNA (sequences available on request from the corresponding author).

### Table 1. IL-8 Gene Expression in Barrett’s Esophagus, Dysplasia, and Adenocarcinoma

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>Barrett’s Esophagus (n = 31)</th>
<th>Barrett’s Dysplasia (n = 20)</th>
<th>Esophageal Adenocarcinoma (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 mRNA expression</td>
<td>0.28 (0.01-0.56)</td>
<td>2.51 (1.03-5.11)†</td>
<td>7.14 (3.74-23.47)‡</td>
</tr>
</tbody>
</table>

Abbreviations: IL-8, interleukin 8; mRNA, messenger RNA.

*Values are median (interquartile range).
†$P<.001$ vs Barrett’s esophagus.
‡$P<.001$ vs Barrett’s esophagus and Barrett’s dysplasia.

**Figure 1.** Interleukin 8 (IL-8) messenger RNA (mRNA) expression in the spectrum of gastroesophageal reflux disease. There was a progressive increase in expression in esophageal mucosa with worsening stage of disease, with the highest expression observed in esophageal adenocarcinoma. Error bars represent interquartile range.
than in matched preoperative biopsies (median, 0.17 vs 0.26, respectively; \(P = .03\)) (Figure 4). However, the post-Nissen IL-8 mRNA level in patients with Barrett’s esophagus remained elevated compared with the values found in the squamous mucosa of patients with reflux disease (but no Barrett’s esophagus) who had Nissen fundoplication \((P < .03)\) (Table 2). There was no significant correlation between the time interval from operation and the pattern of IL-8 gene expression for the patients with reflux who underwent Nissen fundoplication (Spearman \(r = .15; P = .22\)).

**COMMENT**

By using quantitative gene expression measurements of microdissected mucosal tissue, we observed a progressive increase in IL-8 mRNA expression with worsening mucosal injury, beginning with very low expression in patients without reflux disease and peaking in esophageal adenocarcinoma. There was a 700-fold increase in median IL-8 gene expression during this sequence from normal squamous mucosa to adenocarcinoma. The most substantial increase occurred with the development of dysplasia within Barrett’s esophagus. These findings indicate that IL-8 is associated with the progression of mucosal injury in GERD. Furthermore, the significant increase in IL-8 gene expression with the development of dysplasia within Barrett’s esophagus indicates a potential role for IL-8 mRNA levels as a biomarker for disease progression in patients with intestinal metaplasia.

The findings of this study support the concept that inflammation and carcinogenesis are intimately linked processes. We demonstrated increased IL-8 expression both by injured squamous and metaplastic epithelium secondary to reflux and by high-grade dysplastic and malignant cells. This relationship between inflammation and cancer was first proposed by Dr Rudolf Virchow in 1863, based on the histologic findings of inflammatory cells in tumors. More than a century later, this relationship is slowly being elucidated at a molecular level. Current evidence indicates that, rather than being 2 distinct and separate processes, there are common biochemical pathways that link chronic inflammation and carcinogenesis. One protein that appears to function with this type of dual role is IL-8, a cytokine that was originally described as a chemoattractant for neutrophils. In this study we demonstrate that IL-8 gene expression is elevated in the squamous mucosa of patients with reflux disease. After antireflux surgery, the increased IL-8 expression returns to a level similar to that found in the squamous mucosa of patients without reflux disease. This suggests that the increased IL-8 gene expression in the squamous mucosa of patients with reflux disease is related to inflammation, as it is reversible with the elimination of reflux. In contrast, once intestinal metaplasia develops, IL-8 gene expression appears to take on a distinctly different characteristic. Whereas IL-8 expression decreased significantly after Nissen fundoplication in Barrett’s mucosa, the levels did not fall to the range of squamous epithelium in patients with reflux disease after Nissen fundoplication. This finding suggests that in Barrett’s mucosa, elevated IL-8 expression occurs secondary to both inflammation and perhaps a more sinister carcinogenic process. Elimination of reflux with Nissen fundoplication reverses the inflammatory component of increased IL-8 expression, but the expression of IL-8 remains elevated compared with squamous mucosa, secondary perhaps to a constitutive carcinogenic process in Barrett’s mucosa. Continued progression of Barrett’s esophagus toward cancer is associated with further increases in expression of IL-8.

Interleukin 8 has been shown to have roles in cellular proliferation, angiogenesis, and metastases in numerous cancers, reflecting its unique role as a molecule involved in both inflammation and carcinogenesis. It is intriguing that the CXCR2 receptor for IL-8 is expressed by both inflammatory cells as well as by tumor cells, and that while IL-8 expression has been observed to be high in tumors, neutrophil infiltration is uncommon. In our study, all of the mucosal biopsies and esophagectomy specimens were microdissected to avoid inflammatory cells and surrounding stromal tissue in the samples. Therefore, the high levels of IL-8 mRNA expression in dysplasia and adenocarcinoma were seen in the mucosal cells themselves, which may indicate an autocrine role for IL-8 in the progression to cancer.

Despite the introduction of more potent antisecretory agents, the incidence of esophageal adenocarcinoma continues to rise at a rate unmatched by any other
malignancy in the United States, and use of proton pump inhibitors has been shown to increase the risk for this malignancy 3-fold compared with individuals not taking these medications. This may be due in part to alterations in the composition of the refluxate with these medications. An advantage of antireflux surgery is that the gastroesophageal barrier is mechanically restored and reflux of all types is prevented. We have previously shown that the expression of cyclooxygenase-2, another molecule with both inflammatory and carcinogenic roles, is reduced with effective antireflux surgery in the squamous mucosa of patients with reflux disease. To our knowledge, the present study is the first to show that gene expression within Barrett’s mucosa can be quantitatively altered by an antireflux operation. These results confirm that successful antireflux surgery can impact the esophageal mucosa at the molecular level, modulating the gene expression of IL-8 in both reflux-damaged squamous mucosa and in esophageal columnar mucosa. This genetic modulation likely occurs for other genes and may contribute to the quiescence observed in many patients with Barrett’s esophagus following antireflux surgery.

The debate over the efficacy of medical therapy vs antireflux surgery in the management of gastroesophageal reflux disease cannot be addressed in the present study, as a medical therapy arm was not included. There has been 1 published series reporting that proton pump inhibitor therapy can reduce IL-8 gene expression in squamous mucosa of the esophagus. However, because of differences in technique, data acquisition, and analysis, a direct comparison between our data and that of Isozumi et al cannot be made. Future studies are warranted to clarify this issue but must be designed carefully to take into account the heterogeneous response of individual patients to similar doses of proton pump inhibitors. The advantage of an antireflux operation, such as Nissen fundoplication, is that it is a standardized procedure that eradicates all reflux, regardless of its composition.

In conclusion, IL-8 mRNA expression is increased in both squamous and Barrett’s mucosa in patients with GERD and is maximal in esophageal adenocarcinoma. The most substantial increase in IL-8 expression was observed with the development of dysplasia within Bar-

Table 2. IL-8 Gene Expression in the Squamous Mucosa of Patients Without Reflux and in the Squamous Mucosa and Barrett’s Esophagus of Patients With Reflux Following Nissen Fundoplication

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>No Reflux/No Injury (n = 20)</th>
<th>Post-Nissen Squamous (n = 16)</th>
<th>Post-Nissen Barrett’s Esophagus (n = 31)</th>
<th>Kruskal-Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8 mRNA expression</td>
<td>0.01 (0.00-0.16)</td>
<td>0.02 (0.00-0.04)</td>
<td>0.18 (0.01-0.40)†</td>
<td>*P = .02</td>
</tr>
</tbody>
</table>

Abbreviations: IL-8, interleukin 8; mRNA, messenger RNA.
*Values are median (interquartile range).
†P < .03 vs no reflux squamous and post-Nissen squamous groups.
ret's esophagus, indicating that IL-8 gene expression may have a role as a biomarker for disease progression in patients with Barrett's esophagus. The increased IL-8 expression in the squamous mucosa of patients with reflux was reduced after Nissen fundoplication to the level found in patients without reflux disease. While IL-8 expression decreased in patients with Barrett's esophagus after antireflux surgery, the level of IL-8 expression remained increased compared with that in squamous mucosa. This suggests that IL-8 expression may become constitutive once intestinal metaplasia occurs, perhaps reflecting a carcinogenic rather than a purely inflammatory process in these patients.

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REFERENCES


DISCUSSION

Donald E. Low, MD, Seattle, Wash: This paper is another addition to the systematic assessment by the USC [University of Southern California] group of the relationship between GERD-related inflammation and injury and its potential association with Barrett's metaplasia and the increasing incidence of adenocarcinoma. Dr Oh has presented data demonstrating what appears to be a clear relationship of interleukin-8 levels in patients at various stages of the GERD injury spectrum. They have also demonstrated that IL-8 levels can be favorably impacted by successful antireflux surgery.

Their findings are of interest because of the potential of these measurements providing a more objective assessment of cellular injury. The unanswered question at this stage is whether IL-8 measurements will provide a clinically useful addition to genetic profiling and nuclear atypia assessment to identify which patients with Barrett's, and particularly with high-grade dysplasia, are at the greatest risk for developing cancer. In the short term, however, IL-8 levels would appear to be another option for those in a position to measure it, for measuring the effectiveness of antireflux surgeries.

I have several questions of Dr Oh, and I will ask them sequentially. Your study period spans 1995 to 2006, an 11-year period. I think I can reliably assume that during this period the Thoracic and Foregut Service at USC saw hundreds, if not thousands, of patients with reflux symptoms, esophagitis, Barrett's, and esophageal adenocarcinomas.

Your study cohort involves 108 patients. To minimize selection bias, most series study patients based on consecutive case series or according to specific criteria. Who selected this group of patients and what specific criteria were used to include these patients in particular from the large number of patients seen in your unit during the study period?

Dr Oh: The question of patient selection is critical, as you mention. There were more patients available, including those who underwent Nissen fundoplications and who had dysplasia and adenocarcinoma.
The biggest problem that we have when we randomly select these patients is the availability of tissue blocks. There is a lot of research going on in our institution using the same specimens that we are interested in, and most of the time this was the limiting factor.

The second issue is that of paraffin-embedded tissue and RNA [ribonucleic acid] degradation. Unfortunately, as tissue is archived for longer periods of time, intact RNA becomes more difficult to extract. To test this, we basically pull blocks and try them out and we screen to see if the RNA is of sufficient quality. A lot of times they are not, so we have to move on to the next patient. But those are the 2 main technical limiting factors in our patient selection.

Dr Low: The most pronounced changes in IL-8 levels before and after Nissen occur in your group with documented reflux. You state in your manuscript that histologic changes in this group could include macroscopic ulceration and/or microscopic evidence of inflammation. If you are assessing IL-8 levels as an expression of inflammation and tissue injury in a continuum, would it not be more pertinent to subdivide your reflux group into those with macroscopic ulcerations and those with only microscopic changes?

Dr Oh: Yes, that would be a great further analysis that we will consider doing. It is difficult, though, when patients have just esophagitis to obtain biopsies post Nissen, because usually they are doing very well and there is no need for surveillance endoscopies. To find esophagitis patients who come back for postoperative biopsies is uncommon. So in that sense we were limited by the number of patients available to us.

Dr Low: In your discussion, you hypothesized that IL-8 levels may be used as a biomarker for disease progression in Barrett's esophagus. Some of your group's previous work has demonstrated an IL-8 expression gradient at various levels of long-segment Barrett's. We know that even in patients with high-grade dysplasia, not all will progress to cancer. Have you studied IL-8 levels in the Barrett's mucosa of patients with established adenocarcinoma to see if levels are substantially higher than in patients with high-grade dysplasia who have not progressed to cancer?

Dr Oh: In other words, is there a field effect of IL-8 expression from adjacent dysplasia and carcinoma? That again would be the next step for us to investigate. For the purposes of this study, because of the issue of field effect, we purposely avoided patients who had cancer in the adjacent specimen. That is why we restricted our dysplasia group to esophagectomy patients, to ensure that there was in fact no cancer present elsewhere.

Dr Low: Your manuscript indicates that 3 patients with low-grade dysplasia had esophageal resections. Were preoperative biopsies more concerning, or did some other issues figure in the decision for resection in these 3 patients?

Dr Oh: In the low-grade dysplasia patients, they had other factors in their clinical picture, such as end-stage reflux disease and very bad motility, and it was felt that their esophagus was not salvageable. For that reason they underwent esophagectomy.

Dr Low: IL-8 expression is affected by inflammation and cellular injury as well as being a potential marker for carcinogenesis. It would seem a natural expectation that IL-8 levels might decrease following successful antireflux surgery. Where do you envision the greatest potential clinical application of your current study is in the long run?

Dr Oh: As far as the clinical significance, I think there are 2 aspects of that question. The first is measuring the clinical significance of an antireflux operation. Ultimately, the goal of antireflux surgery in the setting of Barrett's in particular is to interrupt the natural history of the disease. The goal is to prevent adenocarcinoma from occurring in these patients. And this has been a difficult question to answer by other clinical endpoints. For example, to show a decrease in the incidence of adenocarcinoma effectively would require a very large study that probably is not practical.

The second aspect of the clinical relevance is on a day-to-day basis. I think there is emerging data of many potential genetic biomarkers in disease progression, and I don't think it would be practical to use 1 gene as a test to indicate whether a patient is progressing, especially with Barrett's and dysplasia. I think the ultimate goal is to have a panel of genes that we could run on a day-to-day basis in these patients to show whether or not the operation is working and whether or not they need to go on and have an operation to begin with or something else, such as an esophagectomy, if they have indications of a malignancy imminent.

Jeffrey H. Peters, MD, Rochester, NY: Dan, as you just said, it is unlikely that this can be used as a marker of disease progression, more likely as a marker of how good our therapy is doing. This begs the question, where is the nonsurgical proton pump inhibitor (PPI) control group in your data?

Dr Oh: That is a great question, and it is something we are very interested in comparing. The only study that is out there that has actually looked at PPI therapy and IL-8 expression comes from Japan, where they treated patients with 8 weeks of PPI therapy. Again, their sample size is very small. And unfortunately, we can't compare their expression with ours because of laboratory-to-laboratory variability and differences in technique. Further, they didn't microdissect the tissue. But that would be a great question to answer with further studies.

Stephen G. Jolley, MD, Anchorage, Alaska: I have 3 questions, hopefully very short questions, that are procedural. The first is that you led the audience to assume that the postoperative pH studies in your patients who had Nissen were all well controlled and normal. Is that true? Were the postoperative acid exposures normal in those patient groups? Because I think that is important in the IL-8 determination.

The second is, why did you not disclose the pH studies performed preoperatively? Or were they performed preoperatively in the dysplasia group and the adenocarcinoma group? I think that is important in terms of holding to the thesis that GERD is responsible for all of this.

The third thing is, did you exclude patients or look at patients who may have food allergies? Because that is an inflammatory process that could potentially affect some of your results.

Dr Oh: As far as the first question regarding postoperative pH monitoring, we do not routinely monitor patients with pH monitoring after a Nissen fundoplication, only when there are clinical signs to suggest there may be something wrong. In those patients who actually did have a postoperative pH study, they were all normal.

As far as the specific values of pH data, I didn't present this because of time constraints. In the no reflux group the median pH or 24-hour composite score was 2.5. In the esophagitis group the median pH composite score was 3.5. In the Barrett's group it was 4.5.

In patients with food allergies, we did not specifically look at that question as far as whether or not they had allergies to food. I think with the combination of clinical symptomatology, increased esophageal acid exposure, and a defective LES [lower esophageal sphincter] on manometry with endoscopic indications of injury, these patients were classified as having reflux.

Dr Jolley: With respect to your preoperative pH studies, I think median values are really useless. I think you have a normal range and I think you need to list specifically the percentage that are abnormal and the percentage that are normal.

Dr Oh: Of all our reflux patients, the 16 with esophagitis and the 31 with Barrett's, had abnormal composite scoring. In our laboratory, that is defined as a score greater than 14.7.

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