Pathophysiology of GERD: Esophageal Epithelial Defense

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Gastroesophageal reflux disease, also known as GERD, is defined as damage to the esophageal epithelium from exposure to the gastric refluxate. Gastroesophageal reflux (GER) is a normal physiologic phenomenon that under special circumstances can lead to varying degrees of injury. Over the course of the day each episode of GER is quite brief, three to five minutes, yet at the end of the day, the esophageal mucosa has spent one to two hours in contact with the gastric refluxate. The exact mechanism by which GER leads to GERD is unknown but it is likely multifactorial and due most likely to defects in one or more of the three esophageal defenses, i.e. the antireflux barriers, luminal clearance mechanisms and/or resistance of the epithelium (1).

AGENTS OF INJURY

The refluxate is composed of many noxious substances including: acid (HCl), pepsin, bile salts, and pancreatic enzymes (trypsin and lipase). Acid and pepsin are produced in the stomach by parietal and chief cells, respectively, while bile salts and pancreatic enzymes are secreted into the duodenum and then are themselves refluxed into the stomach. While any of these agents could ultimately lead to epithelial damage, evidence suggests that the main culprits in humans are acid and pepsin (1). When conjugated bile salts induce cellular damage they leave behind pathologic “footprints”: intracellular bile salt deposition apparent on light microscopy and cell membrane microvesiculation apparent on electron microscopy (2,3). Neither of these footprints has been identified in patients with GERD. Pancreatic enzymes are quickly inactivated and unconjugated bile salts are insoluble in acidic environments, making it unlikely that either one contributes to epithelial injury. Pepsin, on the other hand, has been shown to accelerate epithelial damage in the presence of high acidity (pH < 3.0) (4). Since the duration of most episodes of reflux reside at luminal pHs of 3.0–4.0, however, the exact amount of damage that is attributable to pepsin is unclear. This is the case especially since available agents which inhibit acid, inactivate pepsin (e.g. H₂-antagonists, proton pump inhibitors), and agents which inhibit pepsin, neutralize acid (e.g. sucralfate). Consequently, determination of pepsin’s true role in reflux disease awaits the testing of a pepsin antagonist that does not also affect the level of luminal acidity. Nonetheless it is apparent that the agent responsible for the majority of cell damage is acid. Acid’s role in epithelial injury is supported by studies showing symptom (heartburn) control and lesion healing (erosions) in response to fundoplication and to anti-acid medications (1). Since acid and pepsin

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inflict most of the epithelial injury, and these secretions are secretive in relatively similar amounts between healthy subjects and those with GERD, the possibility that GERD develops from an increase in refluxate potency appears unlikely.

**MECHANISMS OF DEFENSE**

The esophagus has several mechanisms to prevent injury from gastric acid and pepsin (Table 1). These defenses fall into three categories: a) the antireflux mechanisms which reduce the frequency and volume of the refluxate, b) the luminal clearance mechanisms that reduce the time the target epithelium is in contact with the refluxate and c) epithelial resistance to the refluxate.

### REDUCE FREQUENCY AND VOLUME OF REFUXATE

The antireflux mechanisms prevent gastric contents from passing from the low pressure stomach into the low pressure esophagus by generation of a high pressure zone between the two organs. This high pressure zone is comprised principally of the lower esophageal sphincter (LES) but is also supported extrinsically by the right crus of the diaphragm. The LES, a thickened ring of circular smooth muscle in the distal 3 cm of the esophagus, is normally contracted. Relaxation occurs during swallowing to allow passage of food boluses into the stomach and transient LES relaxations (TLESRs) occur in response to gastric fundic distention to release air swallowed while eating and talking. Most episodes of GER occur during TLESRs (5). In general patients with GERD reflux acid more than healthy subjects so failure of the LES is an important factor in the development of GERD. Failure may occur through incompetence of the sphincter, increased intra-abdominal pressure, or increased acid escape during TLESRs. An example of sphincter incompetence is the reduction in LES pressure that accompanies loss of diaphragmatic support in those with non-reducing hiatal hernias (6,7).

### REDUCE TIME IN CONTACT WITH REFUXATE

To reduce the time of contact with the refluxate several mechanisms encourage quick removal of the refluxate from the esophagus. Luminal acid clearance is achieved by first removing the majority of the refluxate through bolus clearance and then neutralizing the remaining acidity through luminal alkalinization (8). Bolus clearance is achieved when peristalsis and gravity propel all but approximately 1mL of refluxate back through the LES into the stomach. Luminal alkalinization is achieved when bicarbonate-rich salivary and esophageal gland secretions neutralize the remaining refluxate (8,9). While there is no difference in either bolus clearance or salivary secretion between those with GERD and those who are healthy, several abnormalities that can delay acid clearance have been identified in those with GERD. These include: lower amplitude peristaltic contractions, more frequent aperistaltic contractions, and

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*Table 1*

**Esophageal Defense Mechanisms**

- **First Line: Reduce Frequency and Volume**
  - Anti-reflux barriers
    - LES
    - Diaphragm
- **Second Line: Reduce Contact Time**
  - Luminal clearance
    - Bolus clearance
    - Gravity
    - Peristalsis
  - Luminal alkalinization
  - Salivary gland secretions
  - Esophageal gland secretions
- **Third Line: Intrinsic Epithelial Resistance**
  - Pre-epithelial defenses
    - Mucous layer
    - Unstirred water layer
    - Surface bicarbonate
  - Epithelial defenses
    - Cell membranes
    - Intercellular junctions
    - Transport proteins
    - Intracellular and extracellular buffers
    - Cell replication
  - Post-epithelial defenses
    - Blood flow
    - Tissue acid base status

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more frequent failed contractions with swallowing (1,6). Luminal clearance mechanisms are just as important as the LES in promoting a healthy esophagus since the duration of esophageal acidification correlates more strongly with tissue injury than does the frequency of reflux events.

The failure of both antireflux and luminal clearance mechanisms can account for the increased frequency and prolonged exposure of the esophageal epithelium to an acidic refluxate as noted on pH monitoring in GERD patients; however, this observation alone is still inadequate to explain the development of GERD. Healthy esophageal epithelium, for instance, is able to resist acid contact without injury for extended periods of time, e.g. luminal pH 1.1 for up to 30 minutes (Bernstein test) (10), while many GERD patients have acid loads far less even over a 24 hr period. In addition, up to half of those with non-erosive reflux disease and one third of those with erosive esophagitis have normal acid contact times on pH monitoring (11), reflecting properly functioning antireflux and luminal clearance mechanisms. This indicates an important role in the pathogenesis of GERD for the third defense—namely, epithelial resistance—the major focus of this article.

**EPITHELIAL RESISTANCE**

Intrinsic epithelial resistance is provided by a number of processes which act in concert to enhance tissue protection against acid injury. For the sake of discussion these can be broken down into three compartments: pre-epithelial, epithelial, and post-epithelial (Table 2).

**Pre-epithelial Defense**
The pre-epithelial defense includes those factors that reside on the luminal side of the esophageal epithelium. In the esophagus, they are poorly developed when compared to those of the stomach (12). In the stomach the pre-epithelial defense is comprised of the mucous layer, unstirred water layer, and secreted bicarbonate by surface cells; these act in concert to create a barrier between the luminal content and gastric epithelial surface. The mucous layer which is comprised of high-molecular weight glycoproteins is a viscoelastic substance with gel-like properties that physically hinder pepsin diffusion from lumen to epithelial surface. Mucus, however, does not directly impede H+ diffusion, but contributes to its defense indirectly by expanding the unstirred water layer and increasing its capacity to trap bicarbonate. The bicarbonate-rich unstirred water layer then serves to neutralize the H+ as they diffuse toward the epithelial surface (13). While this defense is known to be effective in the stomach, it is weak at best in the esophagus even though the esophagus has bicarbonate secreting submucosal glands and is bathed by bicarbonate-rich swallowed salivary secretions. For instance, in vivo human studies show a buffering capacity of approximately one pH unit when luminal pH falls to 2.0 (i.e. surface pH is recorded at pH 3.0) (12). This observation in humans is also supported by animal studies showing that surface buffering can support a pH gradient only at levels of luminal pH above 3.0 (14). Although it is unclear why the pre-epithelial defense in esophagus is weak when compared to that of stomach; the possibilities include an absence of a surface mucous layer, an inability of stratified squamous epithelial cells to secrete bicarbonate, and/or a low rate of bicarbonate diffusion across the electrically-tight squamous epithelium. The absence of a mucous layer is surprising since

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**Table 2**

**Components of Esophageal Epithelial Resistance Against Acid Injury**

- **Pre-epithelial Defense:**
  - Mucous layer (minimal to non-existent)
  - Unstirred water layer
  - Surface bicarbonate ion concentration

- **Epithelial Defense:**
  - Cell membranes
  - Intercellular junctional complex
  - Tight junctions
  - Glycoprotein matrix
  - Buffers
  - Intracellular buffers
  - Membrane ion transporters

- **Post-epithelial Defense:**
  - Blood flow
  - Acid-base balance
both the salivary and esophageal submucosal glands secrete mucins (15). It may be explained by molecular differences in the mucins which prevent the cross-linking needed to form the viscoelastic layer or epithelial cell surface differences which prevent fixation of the mucins to the epithelium. Regardless of the mechanism, the inadequacy of this defense in the esophagus may explain why patients with GERD often require high levels of acid suppression (as those afforded by proton pump inhibitor therapy) for control of symptoms and lesion healing which contrasts significantly to patients with peptic ulcer disease that respond well to lower levels of acid inhibition afforded by histamine-2 receptor antagonists (16). Presumably peptic ulcer healing is enhanced at modest levels of acid inhibition because the presence of a pre-epithelial buffer zone in the form of a mucous cap enables the pH in the area of repair to approach neutrality.

**Epithelial Defense**

Epithelial defense becomes important when acid penetrates the pre-epithelial barrier and comes into contact with the epithelial cell surface. This defense is comprised of a combination of structural and functional elements within the epithelium proper and includes: cell membranes, intercellular junctional complex, transport proteins and intracellular and extracellular buffers (1) (Figure 1).

In humans the esophageal epithelium is a non-keratinized stratified squamous epithelium which is organized into three layers: the stratum corneum, stratum spinosum, and stratum germinativum. The stratum corneum consists of five to 10 layers of flat, pancake-shaped cells lining the luminal surface. These cells are in varying stages of desquamation and serve as a barrier layer for protection against both physical and chemical injury. Below the stratum corneum is the...
stratum spinosum. It consists of 10 to 20 layers of mature, somewhat less flattened cells which are metabolically active and principally responsible for the active absorption of sodium ions (Na⁺). In addition, these cells are transitional, migrating upward as they mature to replace the cells in the stratum corneum as they slough into the lumen. The stratum granulatum represents the lowest one to two cell layers. These cells are cuboidal to columnar in shape, are attached to the basement membrane by hemidesmosomes, and represent the only cells within the epithelium capable of mitosis and reproduction. Their main purpose is to maintain the integrity of the epithelial layer by generating new cells in response to cell death.

The intercellular junctional complex is perhaps the most important structural defense within the epithelial layer—by virtue of being the most vulnerable to acid injury (see below). Morphologically, it is composed of tight junctions and an intercellular matrix of glycoproteins (17). The tight junctions are created by protein bridges that span the intercellular space between adjacent cell membranes where they act as a permeability barrier—allowing only ions and other small hydrophilic molecules to diffuse across them. Indeed, recent data suggests that this aqueous pathway excludes uncharged molecules whose size is ≥14 Angstroms while permitting uncharged molecules across whose diameter is approximately 7–8 Angstroms (18). Further the junctions are lined by negatively charged ions (carboxyl, phosphate and sulfate) so that the pathway is typically cation selective (19). While such selectivity may initially enable H⁺ diffusion, such diffusion can subsequently be inhibited as the anionic groups are titrated by H⁺, converting the junctions from cation to anion selective. Also, the esophageal epithelium is an electrically tight tissue, having an electrical resistance of 1000–3000 ohms cm². This resistance is created by both the layers of cells themselves and, specifically the series of membranes of the cells, in parallel with a series of intercellular junctional complexes between neighboring cells of the upper layers (19). Yet the electrical tightness of this tissue is somewhat surprising given that freeze fracture replicas of esophageal epithelium show the presence of a small number of protein strands within the tight junctions (17). Since this doesn’t account for the electrical tightness of the tissue, other resistors within the intercellular space have been sought—leading to the identification of an intercellular matrix of glycoprotein. What contribution this material ultimately makes to junctional resistance remains uncertain, though electron microscopy suggests that this material may contribute to the barrier for diffusion of electron dense material (lanthanum) across the paracellular pathway (17).

After acid and pepsin breach the structural defenses, the epithelium must rely for survival on its functional defenses, e.g. intracellular buffering, extracellular buffering and transport proteins. Proteins, phosphate, and bicarbonate are responsible for the buffering capacity both within the cell and intercellular space. Bicarbonate, the major buffer, is derived from two different sources: one is by diffusion from blood and the other is by its generation by the enzyme, carbonic anhydrase (20). The buffers work to prevent a fall in intracellular and intracellular pH (pHi) caused by the excess influx of H⁺ while, after pHi declines, the transport proteins on the basolateral membrane of the cell work to remove the excess intracellular H⁺. The two transport proteins are: a) a Na⁺/H⁺ exchanger and b) a Na⁺-dependent Cl⁻/HCO₃⁻ exchanger (21–23). Both exchangers are driven by the Na gradient which allows Na⁺ to enter the cell and either H⁺ to be removed or HCO₃⁻ to be absorbed into the cell. Either way the pHi increases towards neutrality. This process works effectively as long as the extracellular buffers are in sufficient supply to accept the increase in H⁺ load. If the extrusion of intracellular acid overwhelms the intracellular buffering capacity, then the intracellular environment becomes acidic and cell damage or death ensues. Notably, there is a third basolateral transport protein involved with regulation of pHi known as a Na⁺-independent Cl⁻/HCO₃⁻ exchanger. This permits Cl⁻ uptake into the cell in exchange for HCO₃⁻ (22). Under normal conditions this transporter—an acid absorber—is designed to maintain a neutral pHi under conditions in which the cell becomes too alkaline, e.g. during an overshoot in pHi created by the activity of the Na⁺/H⁺ exchanger.

Post-epithelial Defense

The post-epithelial defense consists of the blood supply to the epithelium. It is an integral part of the epithe-
ACID INJURY AND THE EPITHELUM

The pathophysiology of acid injury to the esophagus has been demonstrated using rabbit esophageal epithelium as a model (27–29). The rabbit has been studied because of its similarities to human esophageal epithelium including its capacity for Na⁺ absorption, high electrical resistance, tight junctions, acid-extruding Na⁺/H⁺ exchanger and pattern of change in potential difference during luminal acid exposure. More specifically, rabbits, like humans, have a biphasic change in the transmural electrical potential difference (PD) of the epithelium after exposure to acid (30) (Figure 2)—and this change in PD correlates with different phases of acid injury.

- The first stage is reflected in an increase in PD. This represents the diffusion of H⁺ from the lumen to the serosa.
- The second stage is reflected in the early decline in PD. This represents an increase in epithelial permeability.
- The third stage is reflected in the late decline in PD (effectively falling to zero). This represents both a further increase in epithelial permeability and inhibition of transepithelial ion transport.

The stages above from a clinical perspective can be described as follows. During the first stage, acid enters the lumen and comes into contact with the epithelium. If the pH is sufficiently low to break the esophageal epithelial barrier—the junctions—then luminal acid diffuses across the epithelium via the paracellular route resulting in an increase in PD reflecting H⁺ absorption. The second stage reflects an acid-induced breakdown of the junctional complex sufficient to allow uninhibited diffusion of acid across the epithelium but insufficient to produce cell necrosis. This stage is characterized by an increase in paracellular permeability—not only to acid—but to larger molecular weight water soluble molecules and this apparent morphologically by the presence of dilated intercellular spaces on electron microscopy (18). The squamous cells themselves during this stage appear morphologically normal and the dilated intercellular spaces appear as a response to an increase in salt and water flowing into the intercellular space along an osmotic gradient. Notably, dilated intercellular spaces have been shown to be an early marker of reflux dis-

Figure 2. The percent change in rabbit esophageal transmural potential difference (DPD) is shown plotted against the time of exposure to 80 mmol HCl-80 mmol NaCl. A transient increase in PD occurs during the first ten minutes. This is followed by a progressive decline in PD until it reaches zero at 1hr. Values are means ± standard error, n = 11. PD = −30 mV ± 2 mV. (Reprinted with permission from Orlando RC, Powell DW, Carney CN. Pathophysiology of acute acid injury in rabbit esophageal epithelium. J Clin Invest, 1981;68: 286-293).
Table 3
Esophageal Epithelial Defense Mechanisms Following Acid Injury

- Inflammation
- Basal (squamous) cell replication
- Fibrosis
- Specialized columnar epithelium (Barrett’s Esophagus)

ease in humans as evident by their being present in patients with non-erosive reflux disease (NERD) as well as erosive esophagitis (Figure 3) (31). In effect, as a marker of NERD, the presence of dilated intercellular spaces offers an explanation for the generation of heartburn—this due to the diffusion of luminal acid into the intercellular space in sufficient quantities to stimulate the esophageal sensory neurons (nociceptors) residing within the epithelium (32). The third stage occurs when the paracellular diffusion of acid exceeds the buffering capacity of the intercellular space, resulting in intercellular acidification. Intercellular acidification in turn leads to intracellular acidification because of acid absorption across the basolateral membrane by the Na+-independent Cl−/HCO3− exchanger (26). This occurs because the cell mistakenly believes it must match its pHi with the pH of the extracellular space—normally having a neutral pH as a reflection of the blood supply. The ultimate outcome of the lowering of pHi is cell edema—a consequence of the inability of the cell to regulate its volume—and necrosis (33). Notably, as acid increases the rate of cell injury and death, other defenses available to the squamous epithelium to preserve itself are called into play—and these include tissue inflammation, squamous cell replication and aberrant repair (Table 3). Tissue inflammation results in activation of a variety of inflammatory cell types and release of chemical products with both good and bad features. On the one hand they aid the removal of cellular debris and clear the way for repair but on the other such products as oxygen-derived free radicals worsen the propensity for squamous cell injury and death in the area (34). Simultaneously as noted in patients with NERD, cell repair is ongoing in an attempt to preserve an intact epithelium (35,36)—the latter evident by the presence of a histopathologic lesion known as basal cell hyperplasia (37). Basal cell hyperplasia is a reflection of an increase in number and density of basal cells resulting from a stimulus for increased cell turnover to repair the damaged tissue. Ultimately, when repair is insufficient to meet the increased rate of cell destruction, the epithelium changes from being grossly normal in appearance to one that has clearcut visible erosions—which when visible endoscopically is referred to clinically as ‘erosive’ esophagitis.

Finally, it is of interest to note that two other reparative defenses are available to acid-injured squamous epithelium, but both of them are two-edged swords. One is the development of a fibroblastic reaction that can repair the damaged tissue by collagen deposition—a

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process that when aberrant can lead to dysphagia due to esophageal stricture formation. The other is by the outgrowth of a pluripotential cell from either the squamous epithelium or the duct cells lining the esophageal submucosal glands producing a lining of specialized columnar epithelium—a condition known as Barrett’s esophagus. While specialized columnar epithelium is more acid resistant and so a protective adaptation against further reflux injury, it is also known to be a pre-malignant lesion in that it increases the risk of esophageal adenocarcinoma (1).

References