Inflammatory Mediators in Gastroesophageal Reflux Disease (GERD): Impact on Esophageal Motility, Fibrosis and Carcinogenesis

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Gastroesophageal reflux disease (GERD) is one of the most common problems in clinical practice today. It is widely believed that functional and structural abnormalities of the gastroesophageal junction as well as an abnormal exposure to gastroduodenal contents are the main contributors to its pathogenesis. Novel findings of the inflammatory process in GERD suggest a far more complex process involving multifaceted inflammatory mechanisms. This review summarizes knowledge about the expression of inflammatory mediators in GERD, their potential cellular sources and provides an integrated concept of disease pathogenesis. In addition we evaluate the contribution of inflammatory mediators to well-known complications of GERD, namely motility abnormalities, fibrosis and carcinogenesis. Novel findings regarding the pathophysiology of esophageal inflammation should enhance our understanding of GERD and its complications and provide new treatment insights.

KEYWORDS
GERD, cytokines, fibrosis, motility, carcinogenesis
**The Clinical Problem**

Gastroesophageal reflux disease (GERD) is one of the most common problems encountered in clinical practice today (31). The pathophysiology of GERD is complex, involving diverse factors such as gastric acid secretion, dysfunction of the anti-reflux barrier, gastric emptying disturbances and abnormalities in esophageal defense mechanisms. How these different factors cause GERD is incompletely understood but they all share one common initiating event: increased exposure of the esophageal squamous epithelium to gastric contents, namely acid, pepsin, trypsin and bile acids (22). Mucosal injury, characterized by a non-specific inflammatory infiltrate surrounding the acid damaged epithelial cells, may occur in the setting of pathologic reflux. This leads to the endoscopic findings of mucosal breaks, strictures, columnar metaplasia (Barrett’s esophagus) and adenocarcinoma (52, 80). Several publications have summarized our current understanding of the pathophysiology of GERD (89, 90), but work on mechanisms of disease has focused primarily on damage to the tight junctions and loss of epithelial integrity in response to acid (89, 90). Surprisingly little information is available about esophageal inflammation in GERD even though changes at the molecular level occur prior to macroscopic or even microscopic signs of inflammation. A subgroup of patients, most notably non-erosive reflux disease, responds less readily to conventional therapies with proton pump inhibitors (PPIs) (30) and GERD is a chronic relapsing condition with the potential for severe long-term complications.

The goal of this review is to summarize our current understanding of the origin and role of inflammatory mediators in GERD with an emphasis on their impact on three major GERD-related complications: motility disturbances, fibrosis and carcinogenesis.

**Inflammatory Mediators in the Inflamed Esophageal Mucosa**
Inflammation in any organ involves a highly complex environment which is rich in biological mediators. These include vasoactive amines and peptides, complement components, lipid mediators, proteolytic enzymes, cytokines, growth factors and chemokines, which act in an autocrine, paracrine or endocrine fashion (Tab. 1).

Cytokines and chemokines

Cytokines and chemokines are small peptide molecules synthesized and released by nearly all cell types present in the human body. They play a key role as communicators between cells modulating a wide variety of functions. Cytokines are known to induce, amplify, perpetuate and terminate inflammation (105). Extensive studies exist on cytokine profiles of various chronic inflammatory disorders, such as rheumatoid arthritis, psoriasis, multiple sclerosis or inflammatory bowel disease (76, 93, 105, 109). Surprisingly, in GERD the nature of the tissue response is still poorly defined and data on the expression profile of cytokines in the mucosa are limited.

Most studies in GERD have focused on a small group of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and IL-8. Even though their presence is not specific to the esophagus, the functional changes they induce might be distinct, as specific complications can be triggered through different mechanisms in different anatomic locations. Thus, even though the pro-inflammatory mediators present in the esophagus are not different from those associated with inflammation in other organs, the functional changes they produce in the esophagus may be characteristic for this organ.
IL-8 has been extensively studied in GERD and is expressed in high amounts in the affected mucosa of GERD patients (36, 55, 56, 83, 138). IL-8 is a powerful chemoattractant and activator of leukocytes and other non-immune cells. IL-8 levels in the esophagus correlate and increase with both endoscopic and histologic disease severity (54, 56, 138). In a Japanese study of patients with non-erosive reflux disease (NERD) with minimal mucosal involvement, as determined by endoscopy, IL-8 mRNA levels were increased when compared to NERD patients with no mucosal involvement and with controls (63). After successful treatment with PPIs or Nissen fundoplication, IL-8 levels declined (55, 83, 138). High mucosal IL-8 levels predicted an increased relapse rate for GERD within three years, indicating a potential prognostic role for this cytokine (53).

IL-1 and IL-6 are additional cytokines relevant to the pathophysiology of GERD, and both are key mediators in the control of inflammatory responses. The IL-1 family consists of different forms. IL-1α and IL-1β, both exert identical pro-inflammatory biological effects. A soluble IL-1 receptor antagonist (IL-1ra) acts as a negative regulator (21). IL-1 is a potent activator of many cell types and its expression is increased in inflammatory diseases of the bowel, skin, and other organs (62, 73, 117). IL-1β is increased in the esophageal mucosa during reflux induced inflammation, in animal models as well as in humans. It is produced upon contact of the esophageal mucosa with acid (11, 13, 36, 46, 103). IL-1β may be present in severe GERD cases only, and its expression is restricted to the lower third of the esophagus (103). Increased levels of IL-1β are found in the mucosa and muscle layers of the esophagus in animal models of esophageal inflammation as well as in GERD patients (9, 11, 103). IL-6 may precede and indirectly induce the formation of IL-1β (11).
Other cytokines have been investigated, namely tumor necrosis factor-α (TNF), IL-10 and IL-4. Hamaguchi et al. detected elevated TNF mRNA levels in a rat esophagitis model (46), a finding in contrast to data derived from a cat esophagitis model (9), as well as from human biopsies (103). IL-10 and IL-4 are considered anti-inflammatory or immunoregulatory cytokines, that exert an important function in the control of inflammation. They inhibit release of pro-inflammatory cytokines (105, 134). IL-10 and IL-4 mRNA were not increased in the mucosa of GERD patients versus controls (36).

**Platelet activating factor**
Platelet activating factor (PAF) is a potent pro-inflammatory phospholipid and chemoattractant, particularly for eosinophils (129). It enhances eosinophil adherence to vascular endothelial cells (65). PAF activates immune as well as non-immune cells and induces release of itself and other inflammatory mediators, including reactive oxygen species (ROS) (114, 140). PAF is produced by and released from the esophageal mucosa after acid exposure (11) and it is increased in the circular muscle layer of feline esophagitis model and in chronic esophagitis in humans (14, 16).

**Reactive oxygen species**
ROS are small molecules, including superoxide radical anions, singlet oxygen and hydrogen peroxide (H$_2$O$_2$). ROS are normal byproducts of oxygen metabolism. However, in inflammation, ROS levels can increase dramatically (66). ROS can induce their own production, cause lipid peroxidation of cellular molecules (58), release of intracellular calcium stores and may diffuse through cellular membranes to the nucleus, altering protein expression. The presence of increased levels of ROS results in a situation known as oxidative stress. The highly reactive oxygen species H$_2$O$_2$ is elevated in GERD (14). Antioxidants or radical scavengers that protect cells against
ROS, such as reduced glutathione, superoxide dismutase (SOD) and catalase are all depleted in esophagitis (70, 80, 84, 116, 130, 132). In addition lipid peroxidation is increased, indirectly indicating the increased presence of ROS. These responses and the mucosal damage are inhibited in experimental esophagitis by administration of antioxidants or free radical scavengers (70, 80, 84, 116, 130, 132).

In summary, studies examining the inflammatory mediator profile in GERD show an inflammatory response with increased levels of pro-inflammatory cytokines, such as IL-1β, IL-6 and IL-8, PAF and ROS in the esophageal mucosa. These findings are further supported by increased activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) in the mucosa of GERD patients (55, 81). NFκB is a ubiquitous transcription factor regulating pro-inflammatory responses. It can be activated by pro-inflammatory cytokines and NFκB can then mediate inflammatory mediator synthesis and secretion. To date the inflammatory infiltrate found in the esophageal mucosa of GERD patients appears to be non-specific, because the same pro-inflammatory cytokines can also be found in other inflammatory disorders of the esophagus, like eosinophilic esophagitis (104) or esophageal Candida infection (64). However it is possible that broader analytic approaches, such as microarray analysis, might reveal a distinct inflammatory profile for GERD.

Possible Sources of Inflammatory Mediators Present in GERD

It is accepted that inflammatory responses are dominated by cells of the mucosal immune system. According to this view, activated immune cells, primarily represented by neutrophils, eosinophils, macrophages and T-cells play the role of aggressors, that attack and destroy nearby cells, either directly through physical contact or indirectly through the release of soluble
mediators (32). In this model the non-immune cells, such as epithelial, endothelial and mesenchymal cells, are passive bystanders, remaining quiescent until activated by the invading army of immune effector cells (32). However, this unidirectional concept of inflammation has been challenged by evidence showing that non-immune cells play an active role in inflammation. Epithelial, endothelial, mesenchymal and neural cells display a broad range of effector as well as regulatory functions, so that a multidirectional model of interactions between immune and non-immune cells more closely resembles the real situation in the setting of inflammation (Tab. 1). A multidirectional model may also serve as an alternative and more appropriate model to explain the symptoms and structural changes seen in patients with GERD such as pain, motility disturbances, fibrosis and carcinogenesis (Fig. 1A). This model has been applied to several organ systems – including the gut in inflammatory bowel disease, but has not been extensively studied in GERD. Therefore, some of the discussion below refers to data from publications outside the esophagus but has potential implications for ongoing inflammation in the esophagus.

**Epithelial cells**

The stratified squamous epithelium deserves special attention in the pathophysiological events leading to GERD. Twenty to thirty layers of keratinocytes provide a barrier between food, gastric contents and the underlying subepithelial region. The esophageal epithelium is embryologically, morphologically and functionally related to the skin epithelium, which is recognized as a major immunological organ (133). Esophageal keratinocytes most likely serve as the initiating cell type in esophageal inflammation (Fig. 1A). In addition to expressing activation and cell adhesion molecules, like HLA-DR and intercellular adhesion molecule-1 (ICAM-1) (51, 111), epithelial cells secrete a variety of pro-inflammatory cytokines affecting leukocyte recruitment and activity (59). Acid and bile salt activated keratinocytes cause significant increases in the chemotaxis of T
cells and neutrophils (118). Immunohistochemical studies demonstrate an increase of IL-1β, IL-8 and IL-10 in epithelial cells of GERD patients (36). Components of the gastric juice induce IL-1β, IL-6 and IL-8 production by esophageal epithelial cells, with acidification further enhancing the IL-8 release - an effect mediated via NFκB (103, 118, 137). IL-1β itself can induce IL-6 production (103). In addition acid induces PAF secretion by esophageal keratinocytes through vanilloid receptors (15).

Recently environmental factors, namely the esophageal microbiome, have been considered in the pathogenesis of GERD. The human body is colonized by a large number of microorganisms and their relationship with one another can range from mutualism to pathogenicity (50, 108). In a variety of diseases, the best example being inflammatory bowel diseases (IBD), the microbiota appears to play a key pathogenic role (60, 108). Although the exposure and response of the esophageal epithelium to gastric contents has been extensively investigated, little attention has been given to the microbiome, its effect on the esophageal epithelial layer and its potential changes in GERD (92). In a systematic analysis by Yang and coworkers the distal esophageal microbiome was analyzed in 34 subjects (136) and revealed a complex composition comparable to those in the mouth, stomach, vagina or skin (1, 6, 37, 39). Based on the bacterial genotypes two distinct microbial clusters were identified. A correlation analysis with host phenotypes, namely GERD, Barrett’s esophagus and controls, revealed that almost all controls (11/12) located to one cluster that was predominated by streptococci, whereas most of the abnormal samples (13/22) were associated with the other cluster, predominated by gram-negative bacteria (136). The microbiome did not differ between GERD and Barrett’s esophagus patients.
This finding is especially intriguing in lieu of the ability of bacteria and their products to activate epithelial cells and induce their secretion of proinflammatory cytokines (108). This therefore has the potential to contribute to the pathogenesis of GERD. The microbiome associated with GERD and Barrett’s esophagus is predominated by gram negative strains (136) and the effect of lipopolysaccharide, a major component of their cell wall, on epithelial cells is well established (108). The GERD associated esophageal microbiome had the strongest link with GERD related pathologic changes (odds ratio > 15) compared to all known environmental factors (136). It is however unclear if the esophageal microbiome is intrinsically stable in each individual or if the host response to GERD might influence the composition of the esophageal microflora, giving bacteria a secondary pathogenic role. In addition colonizing bacteria can have an important role in tissue homeostasis and therefore might present a counterregulatory anti-inflammatory mechanism (108). Proton pump inhibitors, a widely used therapy in GERD, have also been implicated in altering the bacterial flora, which can be a direct drug induced effect or indirectly mediated via the changes in gastric pH (4, 40, 72, 121, 125, 128).

**Mesenchymal cells**

In inflammation, mesenchymal cells including fibroblasts, myofibroblasts and smooth muscle cells, are traditionally considered purely structural, filling the space around other cells that are more functionally important in the inflammatory process. Several lines of evidence suggest a broader range of activity. Mesenchymal cells can produce pro-inflammatory cytokines, express cytokine receptors (45, 120) and physically interact with immune cells, like T-cells and eosinophils that are also present in GERD (44, 126). In addition to secreting products of inflammation, mesenchymal cells are capable of modulating immune cell functions (110) and therefore directly influence the duration of the inflammatory process. In GERD, mesenchymal
cells are an active source of IL-6 (103) and IL-8 (unpublished observation). Esophageal circular smooth muscle cells may also contribute to the pool of H₂O₂ as well as PAF (16).

**Endothelial cells**

The human esophagus contains a rich microvascular bed within the submucosal stroma beneath the muscularis mucosae. In several organ systems including the intestine, endothelial cells actively contribute to inflammation by controlling the recruitment of leukocytes to the site of injury. Inflammation leads to an upregulation of adhesion molecules on endothelial cells and to increased binding of leukocytes (7). A key adhesion molecule expressed on endothelial cells is the mucosal addressin cell adhesion molecule (MAdCAM)-1 (29). Esophageal endothelial cells, upregulate MAdCAM-1, ICAM-1, vascular cell adhesion molecule (VCAM)-1 and E-selectin after activation. Cytokines secreted by endothelial cells influence surrounding immune and non-immune cells and vice versa. Esophageal endothelial cells are capable of secreting IL-8 (98).

**Immune cells**

Inflammatory effector cells may secrete all of the currently known inflammatory mediators. Data on the type of immune cells present in GERD are derived from animal models as well as from immunostaining of human biopsy specimens. The cell infiltrate is non-specific and classical pro-inflammatory in nature. Neutrophils, eosinophils, mast cells, Langerhans cells and macrophages can all be found, along with cells of adaptive immunity such as T- and B-cells (42, 48). Immunohistochemical detection of cytokines is difficult, but ample data on the ability of immune cells to secrete cytokines are available from other organs. In GERD immunohistochemical staining shows expression of IL-1β, IL-8, TNF and IL-10 (36, 46, 55). In addition, neutrophils, monocytes and macrophages are a major source of ROS and PAF in inflammation.
Integrating the Present Knowledge – New Insights into GERD

Inflammation in the esophagus is not driven by immune cells alone. Epithelial cells are most likely the initiators of inflammation, as they are exposed to and react to gastric contents, such as pepsin, trypsin, acid and gastric juice and potentially also to the esophageal microbiome. They actively secrete pro-inflammatory mediators, such as proinflammatory cytokines, ROS and PAF, which increase the epithelial response and epithelial damage itself but also activate mesenchymal and endothelial cells. Their activation leads to upregulation of molecules and mediators that allow communication with immune cells and amplify the immune cell response. The response includes upregulation of adhesion molecules and secretion of additional cytokines and chemoattractants with direct pro-inflammatory activity, which then culminates in perpetuation of inflammation in a self sustaining cycle (Fig. 1A&B). The esophageal non-immune cell compartment, activated by the esophageal epithelium, plays an important role, since under normal circumstances a minimal number of classical immune cells are present in the esophagus. Even in the initiating phases of GERD, this appears to be the case. This concept is supported by recent evidence showing that the inflammatory infiltrate in GERD starts in the submucosa and later progresses to the epithelium. Basal cell and papillary hyperplasia preceded the development of surface erosions (118). In fact the authors propose that injury to the epithelium is the result of the inflammatory immune cell response rather than its predecessor. Therefore the products secreted by immune cells are expected to contribute to later stages of esophageal inflammation. The recruitment and retention of classic immune cells (Fig. 1B) seems to be dependent on activation of endothelial cells and mesenchymal cells as well as the upregulation of adhesion molecules. Therefore, one can speculate that inhibition of endothelial- and mesenchymal activation may decrease the progression of esophageal inflammation.
In summary essentially all non-immune cell types of the esophagus – such as resident epithelial, mesenchymal and endothelial cells – actively contribute to the initiation and perpetuation of the inflammatory response. The inflammatory response in GERD is a result of non-immune/non-immune, non-immune/immune and immune/immune cell interactions. Inflammatory cytokines, PAF and ROS are all involved in the pathogenesis of GERD. Once the inflammatory cascade with its inflammatory infiltrate described above fully unfolds, complications induced by pro-inflammatory mediators can occur, such as motility disturbances, fibrogenesis and carcinogenesis.

Impact of Cytokines on Esophageal Motility Disturbances

One crucial component in the pathophysiology of GERD is the impairment of esophageal defense mechanisms due to defective motor function (99). Particularly after meals, reflux of gastric contents may occur as a result of transient relaxation of the lower esophageal sphincter (TLESR) unrelated to swallowing or to secondary peristalsis (18, 77) TLESR occurs in healthy individuals, but is more frequent in patients with gastroesophageal reflux disease (GERD) (22, 49, 77). In the early stages of GERD, TLESR accounts for the largest portion of reflux episodes (18, 77). However, with progressive severity of the disease the proportion of TLESR unrelated reflux increases and impairment of the lower esophageal sphincter (LES) tone and an altered contraction of the esophageal circular body musculature become more important (18, 61). As motor function becomes impaired the likelihood of further reflux episodes and of impaired acid clearance increases, aggravating the damage. The spiral of damage leading to further injury may contribute to permanent impairment of LES tone and of esophageal peristalsis. The underlying cause of this phenomenon has not been extensively explored.
To test the direct effect of acid exposure on esophageal motility Cheng and coworkers established an *in vitro* model of acute esophagitis (12, 13), addressing the sequential activation of inflammatory events in the mucosa. A tubular segment of cat esophageal mucosa was removed and tied at both ends to form a mucosal sac that was filled with hydrochloric acid (HCl). The medium surrounding the tied sac (supernatant) was collected and used directly in cat muscle contraction assays. In the presence of the sac supernatant neurally mediated contraction, induced by electrical field stimulation, was almost completely abolished. Muscle contraction induced by direct stimulation with acetylcholine (ACh) was not affected. This indicates muscle function is not affected. In contrast mediators released by the esophageal mucosa in response to HCl affect the neurons that mediate muscle contraction, inhibiting neurotransmitter release and depressing contraction of esophageal circular muscle. When human mucosa is used, the HCl filled mucosal sac releases PAF into the supernatant (11) and addition of a PAF receptor antagonist essentially abolished the supernatant-induced inhibition of neurally mediated circular muscle contraction. This indicates that PAF is the major mucosa-derived mediator that inhibits muscle contraction (11). But PAF can also activate the esophageal circular muscle to secrete IL-6 (Fig. 2) (11). The muscle derived IL-6 then leads to enhanced production of H₂O₂, which sequentially induces the secretion of IL-1β (11). H₂O₂ as well as IL-1β can themselves induce PAF production (Fig. 2). This indicates the existence of a self-perpetuating cycle of inflammation and motility abnormalities.

IL-1β, IL-6 and H₂O₂, can independently alter neurogenic esophageal muscle contraction (Fig. 2) (9, 10, 16). HCl applied directly to the muscle did not change contraction suggesting that inflammatory mediators are necessary for motility abnormalities.
In summary, mucosa and muscle derived pro-inflammatory mediators can induce their own secretion and influence muscle contraction in the esophagus. (Fig. 2).

Impact of Inflammatory Mediators on Esophageal Fibrosis

Peptic fibrosis, defined as an excessive accumulation of mesenchymal cells and extracellular matrix (ECM) in the esophageal wall, used to be a common and potentially severe complication of GERD. Up to 70% of all benign esophageal strictures in the US were caused by reflux disease (74, 96). With the emergence of PPIs in the 1990s, the incidence of esophageal strictures decreased dramatically (26, 107). However, chronic inflammation invariably induces fibrogenesis in all organ systems and most likely also in the esophageal wall. In other words, even though the incidence of the most severe form of fibrosis, stricture formation and stenosis, might be reduced, fibrosis on a subclinical level may still affect the esophagus. Fibrosis may therefore alter esophageal motility and contribute to the symptom of dysphagia. This phenomenon can also be observed in other intestinal diseases such as the colon in ulcerative colitis, a disease commonly thought of as being non-fibrotic, despite substantial ECM accumulation in the colonic mucosa (104) or in eosinophilic esophagitis, a disease in which subepithelial fibrosis is considered a major cause of dysphagia (2, 3).

The pathophysiology of fibrosis in the esophagus is unclear (96, 101). It is believed, that fibrosis develops after epithelial injury, causing proliferation and activation of resident fibroblasts and deposition of ECM, as a response to tissue damage caused by acid peptic injury. Restoration of normal tissue architecture occurs in transient, mild inflammation. In chronic inflammation the extent of damage may exceed the intrinsic regenerative capacity resulting in scar tissue...
formation. This hypothesis is based on the association between severe, chronic esophageal inflammation with ulceration and the development of fibrosis (27). No data exist about the mechanisms of wound healing and fibrogenesis in GERD on a cellular or molecular level. The main effector cell in fibrotic processes is the mesenchymal cell, responsible for synthesis of several ECM molecules, such as collagens and fibronectins. Mesenchymal cells can differentiate and dedifferentiate among three interrelated cell types, the fibroblast, the myofibroblast and the smooth muscle cell (95). We will use the term “fibroblast“ interchangeably for the first two cell types. TGF-β is the prototypical fibrogenic molecule found in all organs (102). Although three isotypes of TGF-β exist (TGF-β1, 2 and 3), the β1 isoform is considered to be the main driver of fibrogenesis. Essentially all cell types can produce TGF-β1, the major ones being macrophages and fibroblasts. Information is missing with respect to the presence and levels of TGF-β1 in reflux esophagitis but TGF-β1 is elevated in a wide variety of inflammatory processes and also in eosinophilic esophagitis, another esophageal disorder associated with inflammation and fibrosis (3, 104). TGF-β1, and other proinflammatory mediators, such as IL-1β, IL-6 (43) or ROS (97), may activate fibroblasts to secrete enhanced amounts of ECM.

Local esophageal fibroblasts multiply in response to inflammatory signals, including IL-1β, IL-6 and PAF (43, 69, 106, 122, 124). Fibroblasts also proliferate in response to direct contact with inflammatory cells that are present in GERD, such as eosinophils, mast cells, and T-cells (135). In addition to expanding in number, fibroblasts migrate towards a chemotactic gradient generated by inflammation, but stop when inflammation subsides and the gradient disappears. Molecules inducing fibroblast migration can presumably also be found in the inflamed esophageal mucosa. Fibronectin is believed to be the most potent factor, but also platelet derived growth factor...
(PDGF)-A, PDGF-B, insulin growth factor-I and epithelial growth factor may contribute to fibroblast migration (71).

We conclude that chronic inflammation can drive fibrogenesis (Fig. 3A&B). All cell types can contribute to activation of local mesenchymal cells. Certain inflammatory mediators are key components in that process and can be found or are presumably present also in the chronically inflamed esophageal mucosa of GERD patients.

**Effect of Inflammation on Carcinogenesis**

The incidence of esophageal adenocarcinoma has increased almost 6-fold over the past decades in the US and elsewhere in the Western world (20, 94, 113). Unless diagnosed at an early stage the five-year survival rate with locally advanced or metastatic disease is below 20% (28, 68). Adenocarcinoma typically arises from Barrett’s esophagus and patients with Barrett’s esophagus have a 30-fold increased risk of progressing to esophageal adenocarcinoma (67, 82).

Inflammation appears to play an important role in carcinogenesis via at least two distinct mechanisms (Fig. 4) – first the release of inflammatory mediators from immune cells, mainly ROS, and second enhanced reparative mechanisms of the esophageal epithelium. As explained above, inflammation in GERD induces oxidative stress. Oxidative stress in animal models leads to the development of adenocarcinoma (112), that may be mediated through damage to DNA, RNA and lipids, specific gene alterations, genetic instability and aberrant DNA methylation (75, 127). These changes result in altered function of enzymes and proteins, such as activation of oncogene products (= carcinogenic) and/or inhibition of tumor suppressor proteins. Increased levels of ROS occur in the mucosa of GERD, Barrett’s esophagus and esophageal...
adenocarcinoma (85, 115). In metaplastic cells, ROS levels are elevated and antioxidant defenses are decreased (86, 131). Exposure of Barrett’s esophagus and esophageal adenocarcinoma cell lines to components of the gastric refluxate further enhances formation of intracellular ROS, that subsequently cause DNA damage through induction of double strand DNA breaks (17). Double strand breaks can promote genomic instability, which can then lead to carcinogenesis (57, 112).

Enhanced epithelial regeneration may also be a driver of carcinogenesis. Somatic genetic errors, such as chromosomal non-disjunction events and DNA base mismatch repairs, are normal occurrences during cellular proliferation, but rates of these anomalies are low and therefore manageable. As an increase in epithelial cell proliferation in response to inflammation is accompanied by a rise in frequency of replication errors, increased cellular turnover will also contribute to the fixation and expansion of these changes in the cell population (57). Acid and bile exposure is known to affect growth control, differentiation and apoptosis of esophageal epithelial cells (24, 34, 35, 119). Acid-induced H₂O₂ contributes to increased proliferation and decreased apoptosis in esophageal adenocarcinoma cells (38). In addition, other inflammatory mediators, such as IL-1, IL-6 or IL-8, are known to enhance epithelial turnover (47, 123, 139).

Interestingly, polymorphisms of the IL-1ra, a genotype that is linked to high levels of IL-1β in vivo, could be found three times more frequently in patients with Barrett’s esophagus or esophageal adenocarcinoma compared to patients with erosive GERD only (81) emphasizing a potential role of IL-1β in more severe inflammation progressing to neoplastic or dysplastic complications through enhanced ROS and epithelial regeneration. However, this polymorphism exhibits a low overall frequency being present in only 7.2% of Barrett’s or esophageal adenocarcinoma patients vs. 2% of erosive GERD patients.
Taken together, inflammation, oxidative stress, and increased cellular turnover could work together to produce the events responsible for cellular transformation in the inflamed esophagus (Fig. 4). However, despite the emergence of PPIs and therefore enhanced control of GERD symptoms and inflammation, the incidence of esophageal adenocarcinoma is increasing. This is the case even though long-term inhibition of esophageal acid exposure by administration of PPIs in patients with Barrett’s esophagus decreases proliferation of metaplastic cells (91) and reduced the incidence of dysplasia in Barrett’s esophagus patients (25). In addition, only a small fraction of GERD patients develop Barrett’s esophagus or esophageal adenocarcinoma whereas the majority remain unaffected. This indicates that the specific host responses towards esophageal reflux or inflammation may be an important determinant if an individual will move along the neoplasia pathway or not.

Fitzgerald et al. found a qualitatively and quantitatively different expression profile of cytokines in GERD versus Barrett’s esophagus (36). In GERD a pro-inflammatory cytokine profile was present, whereas in the Barrett’s esophageal mucosa a relative increase in IL-10 and a highly significant increase in IL-4 was noted. All patients with Barrett’s esophagus had a low expression of pro-inflammatory cytokines irrespective of PPI therapy (36). In addition in Barrett’s esophagus versus GERD an increased proportion of Th2 effector cells and the formation of isolated lymph follicles can be found (79). This led to the hypothesis, that these two disease entities reflect distinct host responses to reflux disease, a notion that gained further support by the finding, that a polymorphism associated with increased IL-10 levels in vivo was approximately twice as common in Barrett’s esophagus and esophageal adenocarcinoma patients versus esophagitis patients (81). In further studies, an inflammatory gradient within Barrett’s segment was found,
with the maximal extent of inflammation at the neosquamocolumnar junction, with an associated increase in IL-1β and IL-8 (33). Pro-inflammatory cytokines were induced by exposure of the Barrett’s esophagus mucosa to acid and bile. In contrast to this, the distal part of the Barrett’s esophagus segment was characterized by low-grade inflammation and high levels of IL-10, despite being maximally exposed to gastric contents. It seems likely that the specific immune microenvironment within the Barrett's metaplasia may be an important driver towards dysplasia and carcinoma (33).

TGF-β signaling is known to demonstrate tumor suppressive activity by regulating differentiation and proliferation of epithelial cells (19). Gastrointestinal malignancies, such as gastric or colon cancer frequently display inactivating mutations of the TGF-β cascade (8, 23, 78). This also holds true for a subgroup of esophageal adenocarcinoma cases (41). Responsiveness to TGF-β is reduced in the esophageal epithelium during all stages of the metaplasia-dysplasia-carcinoma sequence in Barrett’s esophagus, due to abnormalities at several levels of the TGF-β pathway, in particular alterations in the major TGF-β signaling molecule Smad 4 (87). Barrett’s esophagus cell lines fail to mount an anti-proliferative response when exposed to TGF-β (88). Although mutations directly in the Smad 4 gene are an infrequent event in esophageal adenocarcinoma (5) it was suggested that promoter methylation, which leads to inhibited transcription of Smad 4, could be a cause of decreased Smad 4 mRNA and subsequent protein expression (88).

In summary, inflammatory mediators appear to be drivers of dysplasia and carcinogenesis in esophageal inflammation through genetic alterations and enhanced cell turnover. In addition the individual host response seems to be critical in rendering patient populations at risk for the development of esophageal adenocarcinoma. TGF-β exhibits distinct roles in cell proliferation...
and transformation. Knowledge about the specific inflammatory mechanisms present is crucial for developing not only anti-inflammatory therapies but also for controlling the rising incidence of Barrett’s esophagus and esophageal adenocarcinoma.

Summary

It may be appropriate to consider a new conceptual paradigm for the pathogenesis of GERD and its complications. In addition to considering functional and structural abnormalities of the gastroesophageal junction as well as abnormal exposure to gastroduodenal contents, it may now be time to also focus on bacteria as epithelial cell activators, the cellular inflammatory processes involved in the pathogenesis of GERD and its complications. This approach may help in understanding why some patients develop GERD and its complications whereas others do not, despite similar exposure to acid and gastric contents. For instance it has been suggested that reflux esophagitis may develop as an immune mediated injury rather than a caustic chemical injury (118). In addition, further work in this area may enhance our understanding of the reasons why only certain subgroups respond to therapy. Current therapy continues to focus on acid secretion, modulation of TLESR and surgical approaches. The recent findings of rebound hypersecretion after PPI therapy (100) along with the well-recognized problems with antireflux surgery should further stimulate this line of investigation. Finally, enhanced control of inflammation could play a key role in the esophageal neoplasia pathway.
ABBREVIATIONS

ECM Extracellular matrix
GERD Gastroesophageal reflux disease
H₂O₂ Hydrogen peroxide
IL Interleukin
LES Lower esophageal sphincter
PAF Platelet activating factor
ROS Reactive oxygen species
TLESR Transient lower esophageal sphincter relaxation
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**FIGURE LEGENDS**

**Figure 1A:** Multidirectional model of interactions between immune and non-immune cells in GERD induced inflammation of the esophagus. This also explains the development of complications in patients with GERD such as motility disturbances, fibrosis and carcinogenesis.

**Figure 1B:** Hematoxylin & Eosin staining of GERD affected versus control mucosa. In GERD a non-specific infiltrate of inflammatory immune cells can be seen. Magnification 10x and 40x.

**Figure 2:** Working model for the effect of inflammatory mediators on muscle contraction. Acid reflux in the esophagus causes formation of PAF by the esophageal mucosa. PAF is then released from the mucosa to activate the circular muscle causing the sequential production of IL-6, H$_2$O$_2$ and IL-1$\beta$. H$_2$O$_2$ and IL-1$\beta$ induce production of PAF and IL-6, all of which are known to depress neurogenic muscle contraction by inhibiting release of ACh. PAF: platelet activating factor; IL: Interleukin, H$_2$O$_2$: hydrogen peroxide.

**Figure 3A:** Working model for esophageal fibrosis. Chronic inflammation can drive fibrogenesis. This process involves essentially all cell types that can contribute to the activation of local mesenchymal cells.

**Figure 3B:** Masson Trichrome staining of a peptic esophageal stricture. Collagen fibers are depicted in blue. Massive subepithelial and submucosal collagen accumulation (*) with neo-angiogenesis and an inflammatory infiltrate (arrows) can be noted. Magnification 10x.
Figure 4: Mechanisms of inflammation induced carcinogenesis in GERD. Genetic alterations and increased cellular regeneration link an inflamed and damaged epithelium to metaplasia, dysplasia and carcinoma.
### Table 1

#### Inflammatory mediators present in GERD

<table>
<thead>
<tr>
<th>Cytokines and Chemokines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proinflammatory</strong></td>
<td></td>
</tr>
<tr>
<td><em>IL-1</em></td>
<td>(9, 11, 13, 36, 46, 103)</td>
</tr>
<tr>
<td><em>IL-6</em></td>
<td>(9, 11, 12, 103)</td>
</tr>
<tr>
<td><em>IL-8</em></td>
<td>(36, 53, 54, 55, 56, 63, 83, 138)</td>
</tr>
<tr>
<td><strong>Immunoregulatory</strong></td>
<td></td>
</tr>
<tr>
<td><em>IL-4</em></td>
<td>(33, 36)</td>
</tr>
<tr>
<td><em>IL-10</em></td>
<td>(33, 36)</td>
</tr>
<tr>
<td><strong>PAF</strong></td>
<td>(11, 14, 16)</td>
</tr>
<tr>
<td><strong>Reactive Oxygen Species</strong></td>
<td>(14, 70, 80, 84, 116, 130, 132)</td>
</tr>
</tbody>
</table>

#### Cellular sources of inflammatory mediators in GERD

<table>
<thead>
<tr>
<th>Non-immune cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cells</td>
<td>(15, 36, 103, 118, 137)</td>
</tr>
<tr>
<td>Mesenchymal cells</td>
<td>(16, 103)</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>(98)</td>
</tr>
<tr>
<td><strong>Immune cells</strong></td>
<td>(36, 42, 46, 48, 55)</td>
</tr>
</tbody>
</table>
Figure 2

- Muscle
- Mucosa
- Reduced neurally mediated muscle contraction
- PAF
- IL-6
- H₂O₂
- IL-1β
- NADPH Oxidase