Role of neutrophils in intestinal mucosal injury

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A single layer of epithelium, which provides a barrier between the gastrointestinal tract lumen and the subepithelium and circulation, lines the intestinal mucosa. Continuity of the mucosa is critical to prevent the absorption of resident bacteria and bacterial products such as endotoxin. Mucosal barrier function may be compromised by such insults as ischemia, subsequent reperfusion, ethanol, nonsteroidal anti-inflammatory drugs, and infective agents. Neutrophils play a central role in all of these mechanisms of mucosal injury, and inhibition or modulation of neutrophil function may provide innovative treatment strategies. For example, much attention has been focused on attenuating ischemic mucosal damage by modulating the reperfusion cascade; however, recent evidence suggests that therapeutic strategies may be better directed at modalities that enhance mucosal healing after the initial injury has occurred. Nevertheless, neutrophils continue to infiltrate the mucosa during the reparative phase of mucosal injury. Although neutrophil-induced injury has been attributed to the release of reactive oxygen metabolites, results of recent studies suggest that the physical presence of neutrophils within the intestinal mucosa compromises intestinal permeability. Depletion of circulating neutrophils by the administration of an antineutrophil serum improves microvascular perfusion, attenuates mucosal injury during reperfusion, and hastens repair. Modulation of neutrophil-induced potentiation of mucosal injury is an attractive prospect for hastening mucosal repair and, therefore, reducing the clinical severity of gastrointestinal tract inflammation.

Intestinal mucosal injury secondary to ischemia may be exacerbated during subsequent reperfusion through the generation of reactive oxygen metabolites (ROM), and the recruitment and activation of neutrophils (Fig 1). During ischemia, adenosine triphosphate (ATP) is broken down to hypoxanthine, which accumulates in the tissues. Concurrently, xanthine dehydrogenase is converted to xanthine oxidase (XO). During reperfusion, oxygenation of ischemic tissues results in the XO-catalyzed production of superoxide anion radicals (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$).

Results of a number of studies indicate a dramatic increase in neutrophil infiltration of the gastrointestinal tract mucosa and submucosa during ischemia and reperfusion. For example, during 3 hours of low-flow ischemia in the feline jejunum the amount of the neutrophil-associated enzyme myeloperoxidase (MPO) detected in the tissue increases 5- to 7-fold. During reperfusion, the amount of MPO increases 18 fold above control concentrations. Influx of neutrophils into the tissue after ischemia enhances injury by releasing ROM and proteases. These products react with tissue cells leading to necrosis and cellular dysfunction via the oxidation of sulfhydryl groups, inactivation of hemeproteins and cytochromes, and the degradation of amino acids and proteins. The intercellular tissue matrix is degraded via uncontrolled elastase release and by the inactivation of α1-antitrypsin by hypochlorous acid. Tissue-derived ROM also contribute to neutrophil-induced injury in part by regulating neutrophil influx into the tissue. Pretreatment of tissues with superoxide dismutase (SOD) or allopurinol prevents mucosal damage by decreasing neutrophil influx. Mucosal reperfusion injury can also be prevented experimentally by pretreating animals with the antioxidant catalase, deferoxamine, or dimethylthiourea (a hydroxyl radical scavenger). These agents were initially thought to attenuate damage by inhibiting xanthine oxidase catalyzed release of free radicals. However, they also dramatically decrease the amount of MPO within the intestinal mucosa after ischemia and reperfusion as compared with control tissues, suggesting that they decrease the influx of neutrophils. These findings are consistent with the hypothesis that ox-
The intestinal barrier may be compromised by the physical infiltration of neutrophils across epithelial tight junctions (Fig 2). The transmigration of neutrophils across the intestinal epithelium is a hallmark sign of acute inflammatory bowel disease in human patients. Migration of neutrophils across the epithelium causes a decrease in transepithelial resistance and an increase in permeability. This decrease in barrier function may be explained by the following 2 mechanisms: 1) the physical disruption of the epithelium and impairment of the epithelial intercellular tight junctions by transmigrating neutrophils, and 2) an increase in permeability caused by epithelial cell death. Study findings suggest that cell death in response to neutrophil infiltration is not an important mechanism affecting mucosal function. Results of these same studies indicate that an important contributor to the decreased barrier function is a neutrophil-associated disruption of tight junctions. For instance, pretreatment of cell monolayers with SOD, catalase, sodium azide (a MPO inhibitor), or protease inhibitors did not substantially decrease epithelial injury; compared with untreated monolayers, suggesting that the decrease in mucosal barrier function is not caused by the release of cytotoxic substances by neutrophils. Furthermore, results of in vitro studies have indicated that the transmigration of neutrophils across an epithelial monolayer causes erosions attributable to the physical lifting of the epithelial cells from the underlying matrix.

Neutrophils migrate via the extension of cytoplasmic pseudopodia through the tight junctions to unzip the cell-cell junction with subsequent movement of the cell through the epithelial layer. When low numbers of neutrophils are induced to migrate across the epithelium, the tight-junctions reseal rapidly and minimal barrier function is lost. However, when large numbers of migrating neutrophils penetrate the tight junctions, the healing process is overwhelmed, and mucosal ulcerations develop. Although healing of the mucosal lesions via epithelial restitution is generally complete within 18 hours of the insult, decreased barrier function of the epithelium can develop in patients in the absence of detectable mucosal ulceration. Collectively, these findings support the idea that the effect of transmigration of neutrophils and the associated increase in permeability is the result of physical disruption of the epithelium and not related to release of cytotoxins from the neutrophil.

These study findings have given rise to strategies to attenuate neutrophil-mediated mucosal damage by preventing neutrophil transendothelial migration into the intestinal mucosa and subsequent activation during inflammation. Transendothelial migration in response to injury or inflammation is a highly regulated, multistep process that requires a variety of adhesion molecules expressed on the leukocyte and the endothelial cell. Abundant experimental evidence indicates that adhesion molecules called leukocyte integrins are critical for neutrophil transendothelial migration and also for activation of a variety of effector functions within the tissues, including degranulation of protease containing granules and ROM production. Integrins are heterodimeric receptors composed of an α and a β chain that mediate cell-matrix and cell-cell adhesion in all cells of the body except RBC. Although neutrophils express several types of integrins, the leukocyte specific β2-integrin family (CD11/CD18 complex) is the most functionally important class of integrins in these cells. Calves, mice, and people who are genetically deficient of the β chain of β2-integrins have a profound immunodeficiency caused by an inability of neutrophils to migrate into tissues and to become activated, thus demonstrating the importance of the β2-integrins in neutrophil function. Inflammatory mediators activate endothelial cells to up regulate the expression of molecules called selectins (E- and P-selectin), which tether passing neutrophils in the circulation and cause them to roll along the vessel surface until they are activated. Beta2-integrins expressed on the neutrophil are activated to bind ligands called intracellular adhesion molecules (ICAM) on endothelial cells. To bind their ligands, β2-integrins must be activated to a high avidity state. Important mediators that activate integrin-mediated adhesion are produced in inflamed tissues and include the cytokines tumor necrosis factor-alpha (TNF-α) and IL-1, the chemokine IL-8, complement fragments, vasoactive lipids such as platelet activating factor.
(PAF) and leukotriene B4 (LTB4), and bacterial products. This binding of β2-integrins to ICAM causes the neutrophils to arrest and spread on the endothelial surface, which allows subsequent migration between endothelial cells, a process that also involves β2-integrins. Once in the submucosal tissues of the gastrointestinal tract, neutrophils are induced by soluble mediators that have yet to be identified to migrate through the epithelium, a process that contributes substantially to the leakage and damage to the mucosa (Fig 2). This complex regulation allows the cell to sense specific information about its environment, thus ensuring that they migrate into, and are activated in, appropriate tissues. However, overwhelming inflammation and inappropriate neutrophil responses are key elements of mucosal injury as described earlier.

Results of several studies have indicated that the functional inhibition of neutrophil β2-integrins decreases tissue injury during inflammatory diseases, including those of the gastrointestinal tract. For example, pretreatment of cats with a monoclonal antibody that blocks ligand binding by β2-integrins reduces the amount of MPO-positive granulocytes found within the tissues after ischemia and reperfusion by >80% as compared with control cats. This inhibition of neutrophil influx into ischemic and reperfusion-injured tissues resulted in a 60% reduction in mucosal injury as measured by blood to lumen clearance of 51Cr-EDTA. Interestingly, in this model, long-term pretreatment with the anti β2-integrin antibody was required for this effect, suggesting that resident neutrophils, which would not presumably be affected by short-term pretreatment with the antibody, have an important role in neutrophil-mediated mucosal injury during ischemia and reperfusion. Blockade of β2-integrin function also attenuates mucosal injury using models of gastrointestinal tract disease other than ischemia and reperfusion. Mucosal injury was markedly attenuated by pretreatment with anti-β2 antibodies in experimental models of acetic acid-induced inflammatory bowel disease and acute colitis in rats.

Neutrophils are critical elements of the cascade of events that culminates in mucosal damage in a host of inflammatory diseases of the gastrointestinal tract, including ischemia and reperfusion injury. It is clear that neutrophils mediate their detrimental actions by several mechanisms. However, the importance of the physical disruption of the epithelium is becoming increasingly recognized as a major mechanism of mucosal injury induced by neutrophils. Although anti-adhesion therapy is an attractive strategy to modulate neutrophil-mediated tissue injury, this treatment may be cost prohibitive in veterinary patients. Newer pharmacologic drugs that inhibit β2-integrin activation, and therefore β2-integrin function, may turn out to be clinically useful treatments to inhibit neutrophil-mediated eddy injury during inflammation. This area of research is active and may result in targeted strategies to inhibit not only neutrophil adhesion and activation in gastrointestinal tract diseases but in other inflammatory conditions.

References