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New Onset Ulcerative Colitis: Case Analysis and Correlations to Pathogenesis

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Abstract

New onset ulcerative colitis appearing with or soon after an associated comorbidity represents a unique opportunity to analyze and identify the events that may have triggered the colonic inflammation. The characteristic colonic mucosal inflammatory manifestations observed in ulcerative colitis lends itself to a common pathway analysis within the pathophysiology of associated comorbid triggering conditions. Under these unique circumstances the pathophysiology of one disease becomes the pathogenesis of another, in this case ulcerative colitis. Since the pathophysiology of the triggering comorbidity is usually known, all that remains is to identify a common pathophysiological event in each of the triggering comorbidities that can serve as a common pathway in the pathogenesis of ulcerative colitis (triggered condition). For this common pathway analysis six case reports have been chosen from the literature in which new onset ulcerative colitis is associated with a comorbid condition that is presumed to have triggered the inflammatory bowel disease, with the aim of identifying the common pathway leading to ulcerative colitis. The results suggest a pathogenesis in which an oxidative stress pathway culminates in the production of excess hydrogen peroxide within colonic epithelial cells. Hydrogen peroxide is a toxic by-product of normal metabolism that can initiate mucosal inflammation after diffusing out of colonic epithelial cells.

Keywords: Ulcerative colitis; Inflammatory bowel disease; Homeostasis; Biological pathway

Introduction

New onset Ulcerative colitis (UC) diagnosed after the appearance of other conditions has been reported [1]. This suggests that these associated co-morbidities are acting as a trigger for UC.

In contrast to environmental exacerbating factors, pre-existing comorbidities afford the unique opportunity to examine distinct diseases for a common biological pathway leading to UC. Although seemingly different, a shared aspect of these associated UC co-morbidities is a metabolic/bioenergetic response suggesting that UC is the result of a common effector molecule that is generated as a result cellular biochemical activity.

The absence of immune dysfunction associated with the variety of co-morbidities appearing to act as a trigger for UC suggests that the final common effector molecule is generated within the colonic epithelial cell itself. This is supported by inflammation that is consistently limited to the colonic mucosa.

This is not the first time a colonic epithelial cell origin has been considered in the pathogenesis of UC. A 1949 seminal study that examined colonic biopsies of 180 UC patients concluded that the "Presence of early lesions in a uniform location within the crypts suggests that the irritant is being released by the mucosa" [2]. Since then, compelling evidence has emerged to implicate excess colonic epithelial hydrogen peroxide (H_2O_2) as the "irritant" substance having a causal role in the pathogenesis of UC.

Knockout mice lacking glutathione peroxidase (the main H_2O_2 neutralizing enzyme) develop colonic inflammation analogous to human UC [3]. Two widely used animal models of ulcerative colitis (2,4,6-trinitrobenzenesulfonic acid and dextran sodium sulfate) deplete cellular glutathione (GSH) and upregulate glutathione peroxidase, both being indicators of elevated mucosal H_2O_2 levels in the colonic mucosa [4].

Replenishment of glutathione (needed for H_2O_2 neutralization) attenuates experimental colitis [5]. And critically, studies have documented significantly elevated levels of H_2O_2 in mucosal biopsy samples from quiescent (non-inflamed) regions of colonic mucosa taken from individuals with UC [6]. This is highly significant because cellular levels of H_2O_2 are normally close to zero.

This is supported by studies utilizing recital infusion of H_2O_2 in a murine model of UC, which produced a macroscopic and histological picture that is indistinguishable from human UC [7]. Case reports have shown that "ulcerative colitis appears to be fairly reproducible occurrence after H_2O_2 enemas" in humans [8]. In 2005 the author of this paper compiled evidence that is highly suggestive of a causal role for excess colonic epithelial H_2O_2 in the pathogenesis of UC [9].

The evidence strongly suggests that the colonic mucosa is incapable of neutralizing metabolically generated H_2O_2. Excess H_2O_2 diffuses out of the colonocyte and oxidizes (disintegrates) epithelial tight junctional proteins. The resulting increase in epithelial permeability to lumenal flora leads to mucosal inflammation (Figure 1). As a potent neutrophilic chemotactic agent, H_2O_2 also enhances inflammation by attracting neutrophils into colonic mucosa [10]. This strongly suggests that UC is the end result of a primary disturbance in colonic redox homeostasis.

The following section analyzes six case studies from the literature to illustrate how preexisting disease can facilitate the production of excess H_2O_2 leading to the development of UC.

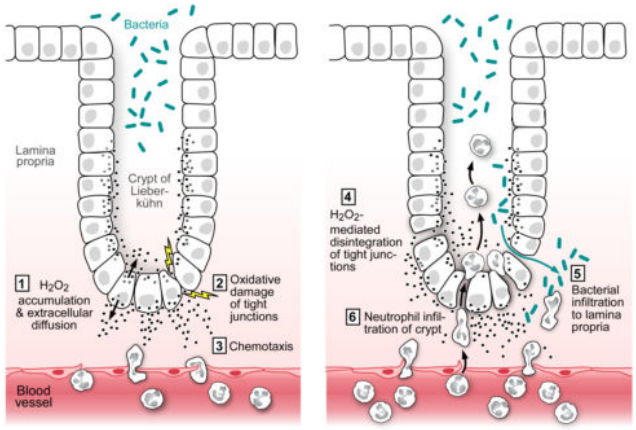

Figure 1: Metabolically generated H_2O_2 accumulates within colonic epithelial cells subsequent to oxidative stress exposure. H_2O_2 is cell membrane permeable and will diffuse through the cell membrane to the extracellular space (step 1). H_2O_2 is a potent oxidizing agent that will damage and dissolve tight junctional proteins (step 2). H_2O_2 is also a powerful chemotactic agent for neutrophils that extravasate from subjacent microvasculature via diapedesis and follow the H_2O_2 gradient back into the crypt of Lieberkühn (step 3). Continued H_2O_2 mediated destruction of epithelial tight junctional proteins (step 4) allows bacteria to infiltrate into the normally sterile lamina propria (step 5) while neutrophils are attracted into the crypts of Lieberkühn by lumenal bacteria (step 6). The end result is neutrophilic infiltration into the colonic mucosa and active mucosal inflammation with continued mucosal destruction when neutrophils are activated by contact with lumenal bacteria. This leads to the cryptitis and microscopic crypt abscesses accompanied by macroscopic surface mucosal ulcerations characteristic of ulcerative colitis.

Case 1: Respiratory Chain Mutations

This case history illustrates how oxidative stress from a genetic mutation in the electron transport chain can lead to the production of H_2O_2 and intractable ulcerative colitis.

Case

Two month old female develops two consecutive episodes of sepsis. At 3.5 months old she develops bloody diarrhoea. Biopsies reveal infiltration with neutrophils and crypt abscesses after which a diagnosis of ulcerative enterocolitis of infancy is made. At 6.5 months of age the patient becomes septic again and develops hypotension with positive blood cultures for *Staphylococcus epidermidis*. At 8 months of age serum lactate is recorded at 21 mmol/L (normal<1.5 mmol/L). At 10 months the patient dies of cardiac failure. Muscle biopsy biochemical analysis revealed severe mitochondrial complex I deficiency [11].

Analysis

Deficiency of electron transport chain complex I results in a significant increase in the production of superoxide [12,13]. This oxygen radical is promptly converted to H_2O_2 by superoxide dismutase.

The characteristic colonic biopsies revealing neutrophilic infiltration and crypt abscesses in this patient suggest locally produced H_2O_2-mediated colonic inflammation implying that the complex I deficiency that was found in muscle is likely to be present in colonic epithelial cells as well.

Although a differential white blood cell count is not reported, acquired H_2O_2 induced immunosuppression is suggested by the repeated episodes of sepsis. Lymphocytes undergo apoptosis at H_2O_2 exposure of only 1 μM H_2O_2 [14,15]. Normal blood H_2O_2 is close to zero and blood levels exceeding 550 μM have been reported in sepsis patients [16].

An elevated blood H_2O_2 would therefore be expected in this septic patient as well. Elevated blood H_2O_2 is further supported by the massive elevation of serum lactate of 20x normal. H_2O_2 can inhibit several enzymes of the Krebs cycle thereby decreasing the proton motive force required to transport pyruvate into the mitochondria [17-19]. The resulting increase of pyruvate in the cytosolic compartment drives the conversion to lactate via lactate dehydrogenase. Lactate is released to the extracellular space by the cell and subsequently into the bloodstream resulting in hyperlactatemia. Experimental sepsis studies in rats have documented hyperlactatemia resulting from ETC complex I inhibition [20]. H_2O_2 causes a dose dependent relaxation in arteriolar tone and studies have shown that intravenous infusion of H_2O_2 in rabbits results in hypotension [21-23]. This case history illustrates both the local (UC) and systemic (sepsis) toxicity of H_2O_2.

Case 2: Smoking Cessation

This case history relates the development of refractory ulcerative colitis after smoking cessation. As explained below, smoking has a significant impact upon the mitochondrial electron transport chain that can lead to excess H_2O_2 production.

Case

A 58 year-old man who develops ulcerative colitis 6 months after smoking cessation [24]. After a month of treatment with sulfasalazine (a 5-ASA pro drug) he felt well and did not receive any further treatment. Three years later the patient develops refractory ulcerative colitis and toxic megacolon, which prompted surgical intervention and colectomy.

Analysis

This patient smoked 3 packs of cigarettes daily for 35 years before discontinuing the habit 6 months prior to the onset of UC. Studies quantifying the effect of cigarette tar on mitochondrial electron transport activity report an 82% inhibition rate on whole chain respiration [25]. Under these circumstances, respiratory chain inhibition can lead to upstream accumulation of reducing equivalents (i.e. NADH, $FADH_2$) within colonocyte mitochondria [26]. Upon smoking cessation the inhibition is lifted and the accumulated electron transport chain (ETC) "fuel" is metabolized producing supraphysiological amounts of H_2O_2, which can overwhelm the colonocyte anti-oxidant (GSH) capacity and diffuse out of the colonocyte leading to colitis.

This patient also developed toxic megacolon, a dreaded complication of UC that presents with a non-functional dilated colon. Studies have shown that H_2O_2 is significantly elevated in UC colonic mucosa [8]. H_2O_2 is also neuro-toxic, which could disrupt colonic neural transmission resulting in colonic dysmotility and dilation [27]. Animal studies utilizing catalase (an enzyme that neutralizes H_2O_2) suggests that H_2O_2 is significantly involved in the colonic dysmotility leading to toxic megacolon [28,29]. Thus, H_2O_2 can account for the initial appearance of UC in the patient and the development of toxic megacolon. Conversely, conditions such as cigarette smoking that reduce ETC-generated H_2O_2 by ETC inhibition are associated with remission in UC patients [30].

Case 3: Prescription Drugs

The following case history demonstrates how systemic medications can impair the electron transport chain leading to excess H_2O_2 production and ulcerative colitis.

Case

A 63 year old male develops bloody stools 6 × daily, which began three days after finishing a 7 day course of *H. Pylori* eradication therapy [31]. The treatment consisted of lansoprazole, amoxicillin, and clarithromycin. Colonoscopy performed 40 days after termination of antibiotics showed proctosigmoiditis. Rectal biopsies revealed crypt abscesses, goblet cell depletion and inflammatory cell infiltration. A diagnosis of ulcerative colitis was made.

Analysis

Studies have demonstrated damage to electron transport chain (ETC) complexes with significant increases in the production of hydrogen peroxide and other reactive oxygen species with the use of bactericidal antibiotics including ampicillin and amoxicillin [32,33].

A damaged ETC will generate increased amounts of superoxide as a result of antibiotic induced impairment of electron transport through the chain. ETC impairment leads to an increased rate of electron leakage and H_2O_2 formation via the reduction of vicinal molecular oxygen to superoxide within mitochondria [34]. UC develops secondary to the diffusion of colonocyte H_2O_2 to the extracellular space as discussed above.

Case 4: Infections

This case report regarding new onset UC after infection illustrates how oxidative stress originating from local GI infections can initiate the development of new onset UC.

Case

A 27 year-old female, without history of ulcerative colitis, is admitted to hospital with an 8 day history of 3-4 liquid bloody stools per day [35]. A colonoscopy revealed continuous inflammation and diffuse hemorrhages of the rectal and descending colon. Histology revealed acutely inflamed mucosa with marked polymorphonuclear infiltrate of the crypts accompanied by numerous microabscesses. Enlarged cells containing typical cytomegalovirus (CMV) inclusions were noted and CMV viremia tests were positive. Appropriate antiviral therapy along with sulfasalazine and oral steroids was initiated. Physical exam was normal after one month and medications were progressively decreased and stopped. Two more episodes of CMV negative acute proctitis over the next 4 years were successfully treated with 5-ASA enemas.

Analysis

This patient had an infectious colitis due to CMV. A local immune response is mounted in response to the presence of the infectious agent. In addition to cytokines, infiltrating immune cells release massive amounts of H_2O_2 as a result of surface bound NADPH oxidase [36]. The H_2O_2 generated by just a single neutrophil can oxidize almost all the hemoglobin to methemoglobin in 5x as many erythrocytes after diffusing through the RBC cell membrane [37].

Normally, colonic mucosa is able to neutralize neutrophil derived extracellular H_2O_2 that diffuses into cells by utilizing enterocyte GSH to neutralize excess H_2O_2. However, after enterocyte glutathione is depleted local immunocyte production of H_2O_2 can no longer be neutralized. Excess local H_2O_2 will oxidize and dissolve epithelial tight junctional proteins allowing bacterial antigens to gain access to the normally sterile lamina propria. This attracts neutrophils from the subjacent vasculature which perpetuates the inflammation. H_2O_2 is also a membrane permeable neutrophilic chemotactic agent, which further acts to perpetuate the local inflammatory response (10).

Cells at the base of the crypts, such as stem cells, are much more sensitive to H_2O_2 toxicity than surface epithelium [38]. In response to H_2O_2 exposure, basal crypt cells can undergo a process of "ROS induced ROS release" (RIRR), which causes cells to continue secreting H_2O_2 after the original H_2O_2 exposure is no longer present [39]. Since cryptal stem cells provide progeny to populate the entire colonic epithelium, the progeny of stem cells that undergo RIRR can become perpetual H_2O_2 generators that can trigger lifelong episodes of UC relapse.

In summary, during colonic infection, H_2O_2 from neutrophils can diffuse into stem cells resulting in glutathione depletion along with oxidative damage to the ETC protein complexes and/or to mitochondrial DNA, all of which foster excess H_2O_2 production (RIRR). H_2O_2 induced oxidative damage to mitochondrial DNA introduces mutations into the mitochondrial genetic material (mitochondrial heteroplasmy) that subsequently miscodes during transcription of protein subunits destined for the Electron Transport Chain (ETC). The ultimate effect is the biosynthesis of faulty and mutated ETC subunits that lose electrons at a greater rate than normal (increased electron leakage). These electrons combine with vicinal molecular oxygen to form superoxide that is converted to H_2O_2 by superoxide dismutase. This auto-oxidation of ETC subunits to produce H_2O_2 is a normal occurrence in healthy cells but the introduction of ETC mutations increases the rate of enterocyte H_2O_2 generation [25,33]. The excess intracellular H_2O_2 production can overwhelm the enterocyte's antioxidant (GSH) system causing net extracellular H_2O_2 diffusion, which can initiate mucosal inflammation and UC relapse in the future.

Case 5: Hyperthyroidism

The following example explores how hyperthyroidism induced oxidative stress can lead to new onset ulcerative colitis.

Case

A 36 year-old female was diagnosed with hyperthyroidism (Graves' disease) and treated with Methimazole with a good response after which she gradually reduced the dosage until discontinuation approximately one year later [40]. Two months later she resumed Methimazole for 6 months with a good response.

Six months later she complained of 3-4 non-bloody bowel movements daily associated with lower abdominal colic pain and a 10 pound weight loss. Colonoscopy evaluation showed continuous inflammation from rectum to cecum, loss of vascular pattern, erythema, friability and mucosal erosions. Histological evaluation revealed acute and chronic inflammatory infiltrate, crypt abscesses, crypt architectural distortion and goblet cell mucin depletion. The patient was diagnosed with pan ulcerative colitis and achieved clinical remission with combined oral and topical 5-ASA.

Analysis

UC following hyperthyroidism has been reported previously since the 1970s [40-42]. Hyperthyroidism occurs significantly more in UC patients than controls and in more than half of the UC patients with a history of hyperthyroidism, the UC occurred after the onset of hyperthyroidism [38]. These reports suggests a cause and effect relationship. This is supported by the observation that hyperthyroidism tends to worsen the clinical features of UC and successful treatment of UC depends on normalization of thyroid function [43,44].

Thyroid hormone is a critical regulator of basal metabolic rate in virtually every cell in the body. The energy required to sustain the hypermetabolic response as a result of a hyperthyroid state is mostly supplied by ATP. Increased oxidative phosphorylation supplies most of the required ATP during a hypermetabolic state. The increase in oxidative phosphorylation is powered by hyperactivity of the electron transport chain. The ETC is a series of mitochondrial protein complexes that channel the flow of electrons derived from ingested food into the synthesis of (ATP) that is used as a chemical energy source for almost all energy requiring cellular processes.

The transfer of electrons through the ETC, however, is not perfect. Up to 5% of electrons do not make it all the way through the chain and fail to combine with oxygen to produce water [45,46]. These "leaked" electrons combine directly with molecular oxygen in the immediate vicinity, instead of the next carrier in the chain, to form the superoxide ($O_2^{-\bullet}$) radical [47]. It is estimated that under normal conditions 2% of available oxygen is converted to superoxide by ETC "leakage" [48]. Superoxide undergoes enzymatic conversion to H_2O_2 at the site of production within mitochondria by the enzyme Superoxide Dismutase (SOD) (EC 1.15.1.1) [49]. Studies in isolated rat cardiac myocytes have shown that the production of H_2O_2 is proportionate to increases in metabolism [50]. Thus, a hyperthyroid induced hypermetabolic state will generate supraphysiological amounts of H_2O_2 in virtually all cells of the body.

Although most cellular H_2O_2 is generated within mitochondria GSH, the reducing (antioxidant) agent responsible for most of H_2O_2 neutralization, is not synthesized inside the mitochondrion where it is most needed. Instead mitochondria must import GSH from the cytoplasm where synthesis takes place [51]. Compounding this restriction in reductive (antioxidant) response, is the fact that mitochondria only maintain a relatively small reductive (antioxidant) reserve of 10-15% of cellular GSH content and recovery of mitochondrial GSH can take several hours after experimental GSH depletion [51- 55].

Therefore, the lack intramitochondrial GSH synthesis, the small mitochondrial GSH reserve and time delay for mitochondrial GSH importation all predispose to GSH depletion during a sustained hypermetabolic state such as hyperthyroidism. This is the Achilles heel of a sustained bioenergetic response, which can lead to unopposed H_2O_2 generation by the ETC that can diffuse out of colonic epithelial cells to initiate mucosal inflammation as described above.

Case 6: Stress

Stress is a known exacerbating factor for UC relapse. These two related cases report the appearance of new onset UC after individual and societal stress.

Case

A 16 year-old girl is reported to have developed new onset UC following a criminal rape. The author (Dr. Burrill Crohn) noting that that "the psycho-somatic aspect of this case was particularly significant" [56]. Case b: Bedouin Arabs are reported to have developed de-novo UC after being uprooted from their simple nomadic lifestyle and relocated to government provided housing. The author reports psychological stresses associated with relocation to a new and unfamiliar lifestyle as playing a significant role in the development of UC in this population [57].

Analysis

The importance of stress as an initiating factor can be seen in the cotton-top tamarin, a small monkey found only in northwest Columbia that spontaneously develops colitis while in captivity after having been deprived of its native habitat. Affected animals will enter remission when transferred to natural conditions indicating that the effects of stress can be reversed [58]. The impressive effect of emotional state on the appearance and functionality of the colon was reported in studies that documented a hyperactive, red, engorged and friable mucosa in ostomy patients during periods of anger and resentment [59].

Similar results were obtained in a study in which seven healthy medical students were outfitted with helmets containing 18 large screws that could be tightened against the head to produce a painful distressing headache during which time visual colonoscopic evaluation of the sigmoid colon was recorded. In each case the authors visualized severe colonic spasm, which was sufficient to occlude the entire lumen. Marked mucosal hyperemia and engorgement with intermittent blanching and flushing was also noted. During periods of maximum engorgement, gentle movement of the proctoscope caused a superficial injury with hemorrhage [60].

The coordinated movement of food along the GI tract is dependent on 5-hydroxytryptamine (serotonin) mediated regulation of smooth muscle tone and peristalsis [61]. 95% of serotonin is stored in enterochromaffin cells (EC) that are present in the GI tract mucosa [62]. Serotonin is released from EC cells into the lamina propria to activate the submucosal sensory branch of the enteric nervous system and stimulate enteric nerve terminals to initiate a peristaltic wave [61,63]. The amount released is much more than needed and the excess serotonin is taken up by colonic epithelial cells via a surface bound serotonin reuptake transporter (SERT) and metabolized by

monoamine oxidase [61]. The neurotransmitter actions of serotonin are rapidly terminated by SERT, which plays a critical role in avoiding serotonin toxicity and hyper-stimulation of the bowel [61].

Mono-amine oxidase (EC#1.4.3.4), an enzyme present on the outer surface of mitochondria within colonic epithelial cells, catalyzes the oxidative deamination of both exogenous xenobiotic amines (i.e. medications) as well as endogenous catecholamine stress hormones (i.e. serotonin) and in the process reduces molecular oxygen to hydrogen peroxide [47]. The reaction catalyzed is $RCH_2+H_2O+O_2 \rightarrow RCHO +NH_3+H_2O_2$ [64].

Thus, stress can increase colonic epithelial H_2O_2 levels by providing additional metabolic substrate (i.e. serotonin) for colonic epithelial mono-amine oxidase. Thus, stress induced colonic hypermobility and spasm will release large amounts of serotonin into the colonic mucosa that will be metabolized to H_2O_2. Hence, severe acute stress (case 'a' above) or sustained stress (case 'b' above) are oxidative stressors that can increase colonic epithelial H_2O_2, which may overwhelm the enterocyte's antioxidant capacity resulting in H_2O_2 accumulation. Large amounts of colonocyte generated H_2O_2 can diffuse to the cell exterior and initiate new onset UC.

Discussion

In the absence of an infectious etiology and lacking evidence for immune dysfunction we are obliged to consider the third possibility in our quest to uncover the pathogenesis of ulcerative colitis, the colonic epithelium itself. This is not a new concept.

During his 1909 introductory address to the Royal College of Medicine on the subject of ulcerative colitis Dr. William Allchin stated "Are we not apt in the search for a specific causal organism to overlook somewhat the contributory part played by the individual's own tissues?". The concept of one's own tissues playing a causal role in this new and mysterious disease was revolutionary for its time (and our time as well) [65].

This idea was further refined 40 years later, in 1949, by Warren and Sommers after carefully examining colonic biopsies of 180 patients with UC and concluding that the characteristic and highly reproducible histologic inflammatory pattern observed in UC was best accounted for by the release of an "irritant" from the colonic epithelium into the crypts of Lieberkühn [2]. The nature of this irritant remained unidentified. However, in 1960 Sheenan and Brynjolfsson were able to reproduce acute and chronic UC by rectal injection of rats with a 3% solution of H_2O_2 [3]. Microscopic examination revealed colonic mucosal ulceration and neutrophilic infiltration, which was "sharply delineated from adjacent normal mucosa". The mucosal inflammation extended proximally over time. It was noted that, in surviving rats, most of the mucosal ulcerations were healed by 10 weeks with the exception of some ulcers which "were located almost always in the left colon a few centimeters above the anus". These three observations (sharp inflammatory tissue delineation from normal tissue, rectal inflammatory persistence and contiguous proximal extension) are also characteristic of human UC [9].

At the time, the H_2O_2 animal model of UC was simply a curiosity however, biochemical studies undertaken by investigators in the early 1970s demonstrated that mammalian cells are constantly generating hydrogen peroxide as a byproduct of normal aerobic metabolism [66]. This data served to place the "suspect" (H_2O_2) at the scene of the crime (colitis) but it was not until the early 1980s when Meyer reported three cases of acute UC after administration of hydrogen peroxide enema and stated that "acute ulcerative colitis appears to be a fairly predictable occurrence after hydrogen peroxide enemas" that H_2O_2 could be considered a theoretical effector molecule with a causal role in the pathogenesis of UC [67].

Thus, by the early to mid-1980s the cumulative data justified an examination of a possible role for colonocyte generated H_2O_2 in the pathogenesis of UC. H_2O_2 is a highly toxic oxidizing agent and by-product of normal aerobic cellular metabolism that is constantly being generated by almost all cells in the body, including colonic epithelial cells.

Except for a tiny amount used as an intracellular messenger, the vast majority of generated H_2O_2 must be neutralized in order for the cell to survive. H_2O_2 is converted to hydroxyl radical, the most reactive oxygen radical in biological systems. Hydroxyl radical will indiscriminately destroy any molecule it comes in contact with and will dissolve proteins, crack DNA molecules and peroxidize lipids [68,69]. The critical reducing agent needed by cells to neutralize almost all H_2O_2 is glutathione (GSH), a tripeptide cofactor used by glutathione peroxidase (GPx) that reduces H_2O_2 to water [52,70].

For each molecule of H_2O_2 that is neutralized the cell expends two molecules of GSH, as per the following reaction.

$$H_2O_2 \xrightarrow[2GSH]{GPX} GSSG + 2H_2O$$

Several observations single out H_2O_2 as a potential causal agent in the pathogenesis of UC:

1. H_2O_2 is produced as a by-product of aerobic metabolism in the colonic epithelium, which is the site of inflammation in UC [47].

2. H_2O_2 is cell membrane permeable allowing it to exit the colonocyte into the extracellular crypt of Lieberkühn where it is poised to initiate inflammation [71]. Extracellular H_2O_2 can oxidize (disintegrate) protein tight junctions leading to increased paracellular mucosal permeability to lumenal bacterial antigens and subsequent mucosal inflammation [9].

3. Glutathione peroxidase (GPx) is responsible for over 90% of H_2O_2 detoxification and GPx knock-out mice (unable to neutralize H_2O_2) develop a crypt destructive colitis analogous to human UC [72,73].

4. Animal models employing rectal infusion of H_2O_2 produced colitis with a histologic and macroscopic appearance analogous to human UC [3].

5. Human use of H_2O_2 enemas can result in acute ulcerative colitis that is histologically identical to the naturally occurring diseases [67].

6. Studies have documented significantly elevated levels of mucosal H_2O_2 in normal appearing colonic biopsies in human UC compared to normal controls ($P<.001$). This highly statistically significant difference strongly suggests a specific abnormality associated with UC colonic epithelium leading to excess H_2O_2 production [8].

Additional evidence for a causal role of H_2O_2 in the pathogenesis of UC is the use of 5-aminosalicilic acid (5-ASA). This agent has been the mainstay therapeutic agent for the treatment of UC since the 1950s. 5-ASA is an extracellular tetravalent reducing agent capable of donating four electrons per molecule for H_2O_2 neutralization [74]. The electrons are derived from the phenoxyl group of 5-ASA [75]. The therapeutic action of 5-ASA is topical (on the surface of the colonic mucosa) where

it serves to neutralize neutrophil derived H_2O_2 during acute inflammation. In a subset of patients this reductive (anti-oxidant) effect can resolve inflammation and induce remission.

Once acute inflammation is resolved 5-ASA's role switches to the neutralization of colonocyte derived H_2O_2 that would otherwise reinitiate mucosal inflammation. In other words, 5-ASA serves as a topical reductive sink for H_2O_2. The action of 5-ASA as a topical H_2O_2 reducing agent argues strongly in favor of a pivotal role for colonocyte H_2O_2 in the pathogenesis of UC. This is supported by a recent analysis regarding the therapeutic effect of 5-ASA, which concluded that 5-ASA has a specific effect on the inflammation occurring in UC and does not have a general anti-inflammatory effect on the colonic mucosa [76].

Conclusion

The cumulative evidence suggests that ulcerative colitis is the end result of the interaction between environmental oxidative stressors and individual reductive (anti-oxidant-GSH) capacity. Based on experimental and clinical data, a mechanism of disease emerges whereby an individual with compromised or inadequate reductive capacity (genetic predisposition-low GSH) is exposed to environmental oxidative stressors (environmental exacerbating factors) which upregulate biochemical reactions that generate H_2O_2 within the colonocyte. Excess un-neutralized H_2O_2 diffuses to the cell exterior and initiates mucosal inflammation by acting as a neutrophilic chemotactic agent and initiating oxidative damage to epithelial tight junctional proteins, both of which lead to neutrophilic mucosal infiltration and colitis. The rectum, a unique tissue having the least reductive capacity of the entire GI tract and the highest bacterial antigenic exposure of any other body surface [77], is particularly prone to oxidative (H_2O_2 induced) tissue damage and inflammation upon oxidative stress exposure.

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