

Radical induction theory of ulcerative colitis

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Abstract

To propose a new pathogenesis called Radical Induction to explain the genesis and progression of ulcerative colitis (UC). UC is an inflammatory bowel disease. Colonic inflammation in UC is mediated by a buildup of white blood cells (WBCs) within the colonic mucosal lining; however, to date there is no answer for why WBCs initially enter the colonic mucosa to begin with. A new pathogenesis termed "Radical Induction Theory" is proposed to explain this and states that excess un-neutralized hydrogen peroxide, produced within colonic epithelial cells as a result of aberrant cellular metabolism, diffuses through cell membranes to the extracellular space where it is converted to the highly damaging hydroxyl radical resulting in oxidative damage to structures comprising the colonic epithelial barrier. Once damaged, the barrier is unable to exclude highly immunogenic fecal bacterial antigens from invading the normally sterile submucosa. This antigenic exposure provokes an *initial* immune response of WBC infiltration into the colonic mucosa. Once present in the mucosa, WBCs are stimulated to secrete toxins by direct exposure to fecal bacteria leading to mucosal ulceration and bloody diarrhea characteristic of this disease.

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INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease characterized by infiltration of white blood cells (WBCs) into the colonic mucosa resulting in tissue destruction and recurrent bouts of bloody diarrhea. The initial inflammatory reaction begins in the rectal mucosa in over 95% of cases and may extend in a contiguous fashion to involve the whole colon^[1]. Often, young individuals in the prime of life are struck with this disease whose course can be severely debilitating, unpredictable and unrelenting. Treatment

modalities are few and unsatisfactory with total colectomy being the only option for individuals unresponsive to the limited medical therapy currently available.

Since the history of medically treated UC is characterized by lifelong repeated episodes of this disease, it appears that no currently available medical therapeutic modality is capable of addressing the fundamental disorder present and therefore unable to alter the natural history of this condition.

Several immunologically oriented hypotheses regarding the etiology and pathogenesis of UC have been advanced^[1]. All remain ill defined, fall short of promoting a clear understanding of this illness and lack predictive value for therapeutic development. These postulates provide no basis for individual risk factor profiling and are unable to explain the known histological, biochemical, immunological and epidemiological abnormalities associated with this disease.

WBCs found within the colonic mucosal lining mediate the tissue injury in active UC; however, to date there is no satisfactory answer for why these WBCs accumulate within the colonic mucosa to begin with. It is the purpose of this paper to propose a new evidenced based pathogenesis termed "Radical Induction Theory" to explain the genesis of this initial influx of WBCs which leads to UC.

The Radical Induction Theory of UC states that excess un-neutralized hydrogen peroxide, produced within colonic epithelial cells as a consequence of aberrant cellular metabolism, diffuses through cell membranes to the extracellular space where it is converted to the highly damaging hydroxyl radical, which is capable of causing extensive oxidative damage to structures responsible for maintaining the colonic epithelial barrier function.

Once damaged, the epithelial barrier is no longer able to exclude highly immunogenic fecal bacterial antigens from invading the normally sterile submucosal tissue. This antigenic exposure provokes an *initial* immune response consisting of WBC infiltration into the colonic mucosal surface in an attempt to "plug the hole" and prevent systemic bacterial invasion and fatal sepsis. Once present within the mucosa, WBCs are stimulated to secrete toxic substances by direct exposure to high concentrations of fecal bacteria leading to mucosal ulceration and bloody diarrhea characteristic of this disease.

BACKGROUND

It is perhaps among the greatest physiological wonders of evolution that the most highly evolved immune system ever engendered can remain unperturbed while surrounding the highest concentration of bacteria on the planet, separated only by a tenuous sheet of tissue one cell thick.

This unlikely truce describes the living conditions of the normal human colon where the luminal concentration

of potentially pathogenic bacteria is estimated to be 10^{12} (one trillion) colony-forming units (viable bacterial cells) per gram of colonic contents^[2].

Previous attempts at creating an animal model of UC have met with limited success. No current animal model is perfect^[3] and experimental attempts to create an animal model of human UC using rectal instillation of toxic chemicals are inherently limited in their ability to faithfully reproduce the disease due to complex psychological, physiological, genetic, environmental and immunological interactions that antecede and contribute to the pathogenesis of this condition in humans^[2].

What is clear from animal studies is that the integrity of the colonic epithelial surface barrier is paramount in maintaining immune quiescence within the colonic tissues and preventing the colonic immune system from mounting an immune response to the high concentration of bacterial antigen that is poised to invade the normally sterile sub-epithelial environment.

Cellular mechanisms involved in maintaining the integrity of the colonic surface barrier function may therefore be compromised early on in the pathogenesis of UC. Dysfunction of a vital process required to maintain mucosal integrity must therefore be an early and necessary part of a sequential series of events ultimately leading to deterioration of epithelial barrier function with subsequent mucosal immune activation secondary to antigenic penetration into the normally sterile colonic sub-mucosal tissues.

In other words, the additive effects of abnormal cellular stressors focused on a common biochemical pathway are acting in concert to disrupt an intracellular biochemical process that contributes a required function necessary for maintaining colonic surface barrier integrity.

The high incidence (over 50%) of spontaneous improvement and relapse seen in UC^[4] suggests a reversible disruption and the possibility of a self-replenishing depletion syndrome affecting a crucial element required for mucosal integrity.

In 1951 Science published an article entitled "A New Concept of the Pathogenesis of Ulcerative Colitis"^[5]. In this seminal publication, the authors demonstrated that patients with UC have either completely absent or severely damaged colonic epithelial basement membranes (BMs). An important observation was that total destruction of BM was seen in the absence of any mucosal inflammation (no WBCs present) and in many areas the BM was noted to be "thinned out". The authors ascribed an important pathogenetic role to the BM destruction seen in colonic biopsy samples of their patients with UC.

The first real clue came from initial observations that BM was destroyed in areas uninvolved in active inflammation^[5,6]. It was already known that UC was an inflammatory condition with infiltration of WBCs (neutrophils) into the mucosal lining of the colon and that these WBCs were capable of causing inflammation and tissue damage. However, the presence of damaged BM in tissue areas without inflammation suggested that a prior process anteceded the WBC involvement.

The presence of "thinned out" BM suggested that a gradual, non-immune mediated, erosion had taken place.

The authors also noted sections of epithelium which had "sloughed" away from seemingly intact BM. This suggested that the epithelial cells themselves played a role in the process that led to the BM alterations and their own (epithelial cell) detachment from the BM. It also suggested that this process began in the interface between the BM and epithelium. Since BM regeneration was noted after successful treatment, it appeared that the process could be halted and reversed. What this process was and what effector molecules, if any, were involved could not be determined from histological studies alone, and in 1951 there was no animal model of UC to experiment with. However, there was a human model of this disease readily available for study which provides a second clue.

The second clue came from a series of case reports from dedicated clinicians over the span of several decades. For many years during the 20th century, hydrogen peroxide (H_2O_2) enemas were routinely employed and recommended by physicians for the evacuation of fecal impactions. However, in the 1930s reports began to surface regarding the development of rectal bleeding and colitis subsequent to the use of hydrogen peroxide enemas^[7]. A fatal case of UC subsequent to hydrogen peroxide enema was first recorded in 1948^[8]. In 1951, Pumphery reports severe ulcerative proctosigmoiditis following hydrogen peroxide enemas in two patients^[9].

In 1960 Sheenan and Brynjolfsson^[8] were able to reproduce acute and chronic UC by rectal injection of rats with a 3% solution of H_2O_2 . This was the first animal model of UC and it mirrored the effects of human UC. Microscopic examination of killed rats revealed colonic mucosal ulceration and WBC (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 wk 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.

Despite the demonstrated adverse effects of hydrogen peroxide it continued to be used as an enema and, in 1981, 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"^[10]. Even small amounts of hydrogen peroxide could cause human UC as was reported by Bilotta and Wayne in 1989^[11] after experiencing an epidemic of hydrogen peroxide-induced colitis in the GI endoscopy unit at their institution due to the inadvertent instillation of hydrogen peroxide during colonoscopy. These results indicate that, when in contact with the colonic mucosa, small amounts of hydrogen peroxide can, in predisposed individuals, produce a clinical and histological picture, which is indistinguishable from spontaneously occurring primary idiopathic human UC.

The data presented thus far reveals that epithelial cell "sloughing" (detachment) from BM and BM erosion (in non-inflamed areas only populated by colonic epithelial

cells) are fairly characteristic histological findings in UC suggesting that epithelial cells play a role both in their own detachment and in BM erosion. Additionally, clinical reports and experimental results reveal that UC is a “fairly predictable” occurrence when hydrogen peroxide comes in contact with rectal epithelium.

The third and final clue tying histological observations of Levine and Kirsner with the adverse clinical effects of hydrogen peroxide enemas came by way of biochemical studies undertaken by investigators in the early 1970s, which demonstrated that mammalian cells are constantly generating hydrogen peroxide as a byproduct of normal aerobic metabolism^[12].

WHAT IS HYDROGEN PEROXIDE?

Hydrogen peroxide is a colorless, highly damaging oxidizing agent, a powerful bleaching agent; used for wastewater treatment, and as an oxidant in rocket fuels. H_2O_2 has a ubiquitous presence in cells and is continuously being generated by the plasma membrane, cytosol and several different sub-cellular organelles including peroxisomes, endoplasmic reticulum, nucleus and by almost 100 enzyme systems^[12-15]. Under normal conditions, 90% of H_2O_2 is generated as a toxic by-product of mitochondrial electron transport chain (ETC) respiratory activity^[14,16]. The mitochondrial ETC is a series of proteins that channel the flow of electrons derived from ingested food into the synthesis of adenosine triphosphate (ATP), which is used as a chemical energy source for 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^[17-19]. These “leaked” electrons combine directly with molecular oxygen in the immediate vicinity, instead of the next carrier in the chain, to form the superoxide ($O_2^{\cdot-}$) radical^[20]. It is estimated that under normal conditions 2% of available oxygen is converted to superoxide by ETC “leakage”^[21].

Superoxide spontaneously dismutates to H_2O_2 or 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)^[12,14,17]. Complex I and III, of the ETC, are the source of electron leakage leading to the eventual intracellular generation of hydrogen peroxide^[22,23].

H_2O_2 is long lived and highly biomembrane permeable and must be immediately neutralized at the site of production to prevent diffusion throughout the cell or to the extracellular space^[12]. Sophisticated enzyme systems exist expressly for this purpose. These H_2O_2 neutralizing anti-oxidant enzymes are catalase (E.C. 1.11.1.6) and glutathione peroxidase (GPx, E.C. 1.11.1.9) with GPx responsible for 91% of H_2O_2 consumption^[24]. If allowed to accumulate H_2O_2 will diffuse from its site of production and generate hydroxyl radical ($\cdot OH$), which is the most damaging and chemically reactive radical formed in cellular metabolism. Hydroxyl will indiscriminately destroy everything it encounters^[17,25,26]. The hydroxyl radical is principally responsible for the cytotoxic effects of oxygen in animals^[25].

The iron catalyzed Haber-Weiss reaction ($O_2^{\cdot-} + Fe^{+3} \rightarrow O_2 + Fe^{+2}$), followed by ($Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + HO^{\cdot} + HO^{\cdot}$), is considered to be the major mechanism by which the highly reactive hydroxyl radical is generated^[27]. Molecules interacting with hydroxyl radicals sustain severe damage to the extent that the hydroxyl radical is able to crack polysaccharides; nucleic acids and proteins^[25]. H_2O_2 is also able to peroxidize and destroy lipids that make up cell biomembranes^[28]. Detoxification of hydrogen peroxide, the immediate precursor to hydroxyl radical, therefore is crucial to normal cellular function and survival.

MECHANISM OF DISEASE

The above data suggests a link between intracellular hydrogen peroxide production and UC. Since exogenously applied hydrogen peroxide can cause UC in humans, and colonic epithelial cells produce hydrogen peroxide, is it reasonable to speculate that excess hydrogen peroxide generated by colonic epithelial cells may be causing UC? How this may come about is suggested by the histological work of Levine and Kirsner (above) which hints of an extracellular process in the epithelial cell/BM interface causing epithelial cell detachment by erosion of subjacent anchoring BM and destruction of apical intercellular tight junctions (TJs). Together, these two bits of data suggest that colonic epithelial cells produce excess hydrogen peroxide, which exits the cell causing oxidative damage to BMs and TJs, which are structures responsible for physical epithelial integrity and barrier function. The resulting destruction of the epithelial barrier allows luminal bacterial antigens to enter the normally sterile submucosal layers of the colonic wall itself initiating an immune response leading to UC.

For hydrogen peroxide to be considered a primary etiologic agent in the pathogenesis of UC, a logical pathogenetic chain of events should be demonstrable starting from the generation of H_2O_2 within sub-cellular organelles to the eventual development of UC. H_2O_2 should possess distinct physicochemical attributes that render it uniquely qualified, to the exclusion of other agents, to induce UC.

In effect it must be demonstrated that H_2O_2 can be produced in excess in colonic epithelial cells and this leads to UC. H_2O_2 must also be capable of exiting colonic epithelial cells and be the source of damage to colonic barrier function structures (BMs and TJs), whose disintegration is important in the development of UC. Finally, in order to have clinical relevance it follows that conditions associated with UC must lead to excessive hydrogen peroxide within colonic epithelial cells. UC associated intracellular abnormalities such as impaired beta oxidation and neoplastic transformation should also be readily explainable. The following sections address these concerns.

1. Can H_2O_2 be produced in excess within colonic epithelial cells and does this cause UC? The answer came by way of genetic studies of knockout mice. These are mice that are genetically engineered with a deletion of a certain gene in order to isolate and study its effects. Knockout mice rendered genetically devoid of GPx (the main hydrogen peroxide neutralizing enzyme) spontaneously develop a crypt destructive colitis (mucosal inflammation -

similar to human UC) as early as 11 d of age with extension to the proximal colon by d 15^[29]. This indicates that, when the biological enzyme system needed to neutralize hydrogen peroxide is hindered, the resulting increase in un-neutralized colonic epithelial intracellular hydrogen peroxide can lead to UC.

2. Can hydrogen peroxide egress from the cell? This is important since H₂O₂ would need to exit the cell in order to cause the severe BM damage seen during histological examination of affected colonic tissue. It turns out that hydrogen peroxide is freely and highly permeable through biological membranes^[12] enabling its diffusion out of the cell from any site of excess production within the cell. H₂O₂ therefore is capable of reaching both the extracellular BMs and TJs from any intracellular location. H₂O₂'s proportionately variable production as a coupled consequence of fundamental cellular metabolic processes plus its ability to pass through biomembranes and produce damaging oxygen radicals far from its site of generation is a unique combination of properties not possessed by any other substance.

3. Can hydrogen peroxide damage BMs and TJs? It has been reported that extracellular hydrogen peroxide can severely damage BMs, TJs and colonic epithelial cell membranes by producing hydroxyl radical via a metal catalyzed Haber-Weiss reaction. Hydroxyl radical is able to damage proteins in BMs and TJs by cleavage of peptide bonds, formation of intra- and inter-molecular cross-linkages and oxidation of amino acids^[14,30,31]. The mechanism has been identified as a site-specific metal ion catalyzed oxidative damage and cleavage of amino acids and peptide bonds by hydroxyl radical. The *in vivo* source of all hydroxyl radical was identified as endogenously generated hydrogen peroxide^[32]. H₂O₂ can therefore disintegrate the micro-anatomical GI barrier structures that maintain epithelial integrity (BMs and TJs). Hydroxyl radical oxidizes and destroys everything it encounters resulting in microscopic alterations, which increase mucosal permeability allowing penetration of luminal proteins and antigens^[31,33-38].

Using a well-characterized model of BM, Riedle and Kerjaschki^[31] evaluated the *in vitro* effects of hydrogen peroxide induced changes on interstitial matrix proteins and the consequences for the integrity of the BM/matrix network.

The authors documented significant disintegration of matrix structure with 15% of matrix proteins being released into the incubation medium. This corresponded to seven times that was seen in control conditions without hydrogen peroxide. Importantly, extensive oxidative damage of individual amino acid residues (tryptophan) was noted without any morphological change to the BM/matrix network. The hydrogen peroxide-derived hydroxyl radical was found to be the main reactive oxygen species responsible for matrix protein disintegration. Laminin, a major BM structural protein, was also released from BM when incubated with low concentrations of hydrogen peroxide.

Hydrogen peroxide infused into rat renal artery produced local H₂O₂-derived oxygen radicals and subsequent marked glomerular protein leak suggesting an increased porosity of the glomerular BM secondary to oxidative damage of its constituent proteins^[35].

In addition to BM damage, hydrogen peroxide-derived

oxygen radicals are also able to disrupt colonic epithelial TJs. TJs are composed of thin bands of plasma-membrane proteins that completely encircle the apical (luminal) region of colonic epithelial cells and are in contact with similar thin bands on adjacent cells. These intercellular protein junctions fasten adjacent epithelial cells together forming a sealing gasket, which prevents the passage of most dissolved molecules and bacterial antigens from one side of the epithelial sheet to the other.

Since only a single layer of colonic epithelial cells separates the bacterial laden luminal contents from the subjacent lamina propria, the epithelial TJ constitutes the major primary barrier that prevents luminal bacterial antigens from gaining access to the effector immune cells and vasculature in the normally sterile lamina propria^[39]. Thus, the intestinal barrier function relies primarily on the tightness of the epithelial layer to maintain impermeability with sub-epithelial layers contributing a minor function^[37].

Hydrogen peroxide, at a low concentration of 0.2 mmol/L, was reported to increase *in vitro* epithelial monolayer permeability by disrupting paracellular junctional complexes^[37]. In experiments to assess the effect of hydrogen peroxide on intestinal permeability, Grisham *et al.* found a significant increase in mucosal permeability after *in vivo* perfusion of rat intestine with hydrogen peroxide^[38]. Altered epithelial permeability is also a consistent effect of hydrogen peroxide in other tissues including endothelial and renal cell lines^[33].

In studies to determine the *in vitro* effect of oxidative stress on TJ integrity, Parrish *et al.*^[36] studied the effect of chemically induced oxidative stress on the E-cadherin/catenin protein complex, which is the principal intercellular TJ (zonula adherens) anchoring protein. The authors bathed precision cut rat liver slices with non-lethal concentrations of oxidant chemicals (diamide and *t*-butylhydroperoxide), which penetrate the hepatocytes and oxidize both intracellular-reduced glutathione and NADPH. This depletes available glutathione stores and prevents the regeneration of reduced glutathione. This causes oxidation from both these added chemicals and any endogenously generated hydrogen peroxide. The authors found that this level of oxidative stress disrupted the E-cadherin/catenin cell-adhesion protein complex of the TJ.

4. Are BMs and TJs important in the pathogenesis of UC? BMs together with colonic epithelial cells and the TJs that bind them together are the micro-anatomical structures that comprise the gastrointestinal barrier, which prevents fecal bacteria from entering the sterile deeper layers of the colonic tissue and gaining entrance to the blood stream.

In an early study, BMs were found to be absent or severely damaged in UC^[5]. In a subsequent report of 29 patients with UC, Jacobson and Kirsner^[6] reported either completely destroyed or fragmented BM in all subjects. Of note was the observation of "thinned" out sections of BM consistent with a diffusible agent such as H₂O₂ causing membrane dissolution.

The authors also point out that destruction of BM was noted in the absence of leukocytic infiltration, which is also consistent with a diffusible agent of non-leukocytic (i.e. colonic epithelial cell) origin.

Colonic epithelial BM structure and function was also

found to be seriously disturbed in active cases of UC. In a study to determine the integrity of BM in active UC, Schmehl *et al.*, found no positive immunoreactivity for BM laminin in affected colonic tissue. The authors concluded that the three-dimensional network of colonic epithelial BM and its function is seriously disturbed in active UC^[40].

Using alternating current impedance analysis Schmitz *et al.* found that epithelial resistance in UC is strongly impaired and that this barrier defect was paralleled by a decrease in TJ strand count^[34].

Employing immunostaining with anti-human E-cadherin and catenin antibodies, Karayiannakis^[41] determined that E-cadherin expression was reduced in all cases of active UC but in none of the inactive cases. (The E-cadherin/catenin protein complex is the primary intercellular TJ (zonula adherens) anchoring protein.).

Altered a-catenin was also seen in all cases of active UC but was not altered in any case with inactive disease. Importantly, epithelial cells adjacent to mucosal ulcers showed loss of E-cadherin and a-catenin, while epithelial cells distant from the mucosal margin revealed normal E-cadherin and a-catenin expression. The authors found that altered E-cadherin always coexisted with abnormal a-catenin. This is consistent with an advancing margin of a diffusible agent which is disrupting the E-cadherin/catenin complex within the TJ.

TJ disruption was also found to correlate with the progression of UC. In studies employing immuno-cytochemistry, Western blotting and *in situ* hybridization, Jankowski found a strong correlation between E-cadherin disruption and the progression of UC^[42]. The authors showed that as disease activity progresses both cytoplasmic and membranous E-cadherin expression are lost. The authors propose that normal E-cadherin function is essential for the maintenance of normal colorectal epithelium.

In agreement with the above studies, results are obtained with genetically engineered chimeric mice expressing enterocytes with either normal or non-functional cadherin. Mice expressing non-functional cadherin developed an inflammatory bowel disease with histological similarities to human UC, including cryptitis, crypt abscesses and a neutrophilic infiltrate.

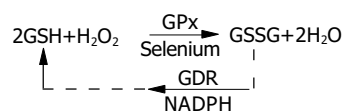
These inflammatory changes were confined to foci of epithelium expressing non-functional cadherin, confirming the absence of an autoimmune response^[43,44].

If extracellular hydrogen peroxide, originating from intracellular sources, is a causative factor in the breakdown of colonic barrier (TJ) function, then an increase in epithelial permeability should be an early manifestation in UC. Furthermore, these early permeability lesions should be macroscopically normal and occur in intact epithelium since the neutrophilic inflammatory process has not yet begun.

Gitter *et al.*^[45] found such early permeability increases in colonic tissue obtained from individuals with early (Truelove I) UC. The authors found that in seemingly intact epithelium there was a 35% increase in conductivity (ionic permeability) in early/mild (Truelove I) UC tissue samples with a 300% permeability increase in tissue samples showing a moderate to severe inflammation. These areas of early permeability increases correlated with foci of colonic epithelial apoptosis.

Both of these effects (i.e. increase in epithelial permeability and induction of apoptosis) are known consequences of tissue exposure to hydrogen peroxide^[33]. This is in agreement with permeability studies in animal models of UC, which demonstrated an increase in colonic mucosal permeability to tracer molecules prior to the appearance of a visible inflammatory process^[46].

5. Are conditions that enhance production of hydrogen peroxide also associated with UC? Elevated levels of intracellular hydrogen peroxide can result from increased production or decreased neutralization. H₂O₂ originates from two main intracellular sources. One is the mitochondrial ETC and the other is the total sum metabolic activity of nearly 100 oxidase enzymes distributed within most sub-cellular organelles and cytosol^[12-15,17,47,48]. H₂O₂ neutralization is mainly accomplished via the enzymatic action of GPx (E.C. 1.11.1.9) in conjunction with the anti-oxidant tri-peptide co-factor glutathione (GSH-reduced). Selenium is a required active-site co-factor for GPx enzymatic function. Oxidized glutathione (glutathione disulfide, GSSG) is converted back to the cytoprotective reduced state via the enzymatic activity of glutathione-disulfide reductase (GDR) (E.C. 1.8.1.7-formally E.C. 1.6.4.2.), with electrons supplied by NADPH which itself is generated by the pentose phosphate pathway (PPP). The chemical equation for H₂O₂ neutralization and glutathione regeneration is as follows:



Cytoprotective (reduced) intracellular glutathione is the main reducing agent available to colonic epithelial cells to neutralize hydrogen peroxide. Although other redox couples such as cysteine:cystine, NAD⁺:NADH, NADP⁺:NADPH and reduced:oxidized thioredoxin participate in the maintenance of the required intracellular reduction state critical to metabolic function, the glutathione redox couple (GSH:GSSG) is a two to four orders of magnitude more abundant than any other redox couple^[49].

Glutathione also serves as a common reducing agent for other redox couples^[49] and accounts for over 90% of intracellular H₂O₂ neutralization (catalase contributes a small amount to overall H₂O₂ neutralization). Glutathione is therefore the main supplier of reducing equivalents and a reliable indicator of the redox state of cells.

Intracellular H₂O₂ can thus be abnormally elevated secondary to increased production or impairment of any element or action required for its neutralization. The following sections illustrate this concept.

INCREASED H₂O₂ PRODUCTION

Increased oxidase enzyme activity

Oxidase enzymes utilize molecular oxygen as an electron accepting co-factor necessary for the enzymatic reaction to proceed. H₂O₂ is produced as an end by-product of these reactions. Thus, increased oxidase enzymatic activity can contribute to the generation of intracellular hydrogen peroxide. UC has been reported subsequent to the administration of certain xenobiotics (i.e. vitamin B-6)^[50].

Vitamin B-6 is metabolized by pyridoxine 4-oxidase (EC 1.1.3.12), which generates H_2O_2 as a by-product.

Increased electron transport activity

UC can develop subsequent to hyperthyroidism^[51-55]. Hyperthyroidism is known to enhance ETC activity, which increases hydrogen peroxide generation. On the other hand, cigarette smoking, which inhibits ETC activity, is protective. Studies quantifying the effect of cigarette tar on mitochondrial electron transport activity report an 82% inhibition rate on whole chain respiration^[56], whereas cessation of cigarette smoking (which releases ETC inhibition) is a powerful risk factor for the development of UC^[2,57-59].

Colonocyte ETC activity can become a source of excess H_2O_2 if subjected to hypoxia and sudden re-oxygenation^[60]. This process of hypoxia and re-oxygenation increases the activity of the ETC due to the interim accumulation of reducing substrate resulting in increased production of hydrogen peroxide. Local colonic hypoxia/reoxygenation can be caused by stress. The following section reviews mechanisms of stress-related increases in colonocyte H_2O_2 .

Psychological stress

Psychological stress has long been recognized as an exacerbating factor for UC. Dr. Burrill Crohn was well aware of the psychological effects of stress on UC when, in the first issue of *Gastroenterology* in January 1943, he reported the appearance of acute UC in a 16-year-old girl following a criminal rape, noting that "the psycho-somatic aspect of this case was particularly significant"^[61].

During the 1950s, practitioners noticed the onset and/or exacerbation of UC commonly occurring subsequent to emotional disturbances^[62-64]. Early observations of severely emotionally disturbed individuals with UC reported resolution of the latter when the former was treated^[65,66].

More recently an association has been reported between stress and UC disease activity^[2,67]. Up to 40% of patients with UC report psychological stress as an exacerbating factor^[68]. Life stress has been reported to be associated with both objective and subjective aspects of activity in UC^[67] and high long-term stress was found to triple the risk of disease exacerbation^[69]. 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 when deprived of its native habitat while in captivity. Affected animals will enter remission when transferred to natural conditions indicating that the effects of stress can be reversed^[70].

The molecular basis of stress-induced exacerbation of UC can be correlated to both increased H_2O_2 production and decrease H_2O_2 neutralization secondary to the effect of stress on electron transport activity and cellular enzyme systems. These mechanisms may find expression either through systemic or local effects of stress on the colon as discussed below.

1. Acute systemic psychological stress increases the amount of systemic circulating biogenic amines (catecholamines), such as serotonin, epinephrine, nor-epinephrine and dopamine^[71]. 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 and in the process reduces molecular oxygen to hydrogen peroxide^[20].

The reaction catalyzed is $RCH_2 + H_2O + O_2 \rightarrow RCHO + NH_3 + H_2O_2$.

Stress therefore may increase H_2O_2 levels by providing additional metabolic substrate (endogenous catecholamines) for mono-amine oxidase. Thus, individuals with genetically diminished anti-oxidant (reductive, H_2O_2 neutralizing) capacity are at greater risk of developing UC when exposed to acute stressful events.

2. Chronic systemic psychological stress, such as depression, has been associated with circulating increased nor-epinephrine levels^[71]. Depression has been reported to precede the onset of UC significantly more often than expected^[72]. Depressive stress and anxiety, however, were found to be significantly more common after the appearance of Crohn's disease^[73]. This suggests that physiological alterations present in depression contribute to the appearance of UC in contrast to Crohn's disease where depression may be a psychological reaction to the appearance of the disease.

Chronic depression, therefore, may result in significant long-term increases in circulating endogenous catecholamine levels, which may elevate intracellular colonocyte H_2O_2 when metabolized via mono-amine oxidase. Chronically depressed individuals with marginal anti-oxidant capacity needed to neutralize this excess H_2O_2 are at increased risk for development of UC.

3. Local colonic perfusion/reperfusion (hypoxia/reoxygenation) can result from the effects of psychological stress on the colon. Stress-induced colonic spasm may result in local hypoxia and re-oxygenation, which can lead to oxygen deprivation of dozens of oxidase enzymes such as xanthine oxidase (XO)^[74-76]. In a seminal study, Almy and Tulin^[77] directly observed the effects of stress on the colonic mucosa of seven healthy volunteer medical students.

The students were fitted with a metal helmet containing 18 large screws that could be tightened against the head to produce a painful distressing headache lasting 30 min 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 lumen. Marked mucosal hyperemia and engorgement with intermittent blanching and flushing (perfusion/reperfusion) was also noted. During periods of maximum engorgement, gentle movement of the proctoscope caused a superficial injury with hemorrhage. Nausea often accompanied visualized episodes of colonic spasm. This study indicates that stress can cause severe alterations in colonic function and predispose to colonic hypoxia and reoxygenation (perfusion/reperfusion) injury (sequential blanching and hyperemia with mucosal engorgement). Thus, local stress-induced colonic spasm is mediated via the enteric nervous system, which results in spastic contraction of colonic smooth muscle leading to transient local tissue hypoxemia with subsequent reoxygenation upon colonic smooth muscle relaxation.

XO is a prototypical example of an oxidase enzyme that is affected by perfusion/reperfusion-induced oxygen deprivation. XO catalyzes the conversion of hypoxanthine

to uric acid and in the process reduces molecular oxygen to hydrogen peroxide. During hypoxia, XO activity is significantly reduced due to unavailability of oxygen needed as an electron-accepting co-factor for the enzymatic conversion (oxidation) of hypoxanthine to uric acid. When oxygen is reintroduced (re-perfusion), an increased substrate load leads to increased hypoxanthine metabolism and hydrogen peroxide production. Stress-induced perfusion/re-perfusion, therefore, results in additional H₂O₂ due to increased metabolism of oxidase enzyme substrate which, after having accumulated during hypoxemia, undergoes amplified metabolism upon re-oxygenation with concomitant increases in hydrogen peroxide. Pre-treatment of mice with allopurinol (XO inhibitor) prior to experimentally induced colonic ischemia/reperfusion significantly attenuated leukocyte adhesion to colonic submucosal endothelium^[78]. Thus, in this model of murine colitis, inhibition of XO significantly reduces WBC endothelial adhesion, a crucial early step which is likewise present in the development of human UC^[79].

Stress-induced colonic smooth muscle spasm with hypoxia/re-oxygenation can also increase colonocyte electron transport activity with concomitant increases in H₂O₂. Rectal epithelial cells possess an ETC, which can become a source of excess H₂O₂ if subjected to hypoxia and sudden re-oxygenation^[60].

DECREASED HYDROGEN PEROXIDE NEUTRALIZATION

Decreased glutathione peroxidase activity

Hydrogen peroxide is metabolized via the enzymatic action of GPx, a selenium containing enzyme, which utilizes the anti-oxidant tri-peptide co-factor glutathione to neutralize intracellular H₂O₂. Genetic conditions which inhibit GPx or decrease glutathione availability will lead to increased hydrogen peroxide levels. Genetic research by Cho uncovered the existence of a "pathophysiologically crucial IBD susceptibility gene" located on the small arm of human chromosome 1 (1p36)^[80,81]. This genetic locus codes for two enzymes that exert control on intracellular H₂O₂.

One is methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20), which is a main regulatory enzyme of homocysteine metabolism^[82,83]. Molloy has reported that 17.5% of individuals with UC possess a polymorphic variant of the MTHFR gene *vs* 7.3% of controls^[84]. Polymorphic variants of MTHFR result in an elevation of serum homocysteine levels^[83]. Nagano has shown that children with UC have elevated serum homocysteine levels and concludes that elevated homocysteine may be associated with the underlying basic pathophysiology of the disease^[85]. Markedly elevated levels of tissue homocysteine have also been reported in colonic mucosa of individuals with UC^[86]. Elevated homocysteine will increase hydrogen peroxide production by several mechanisms.

Hydrogen peroxide is generated during the oxidation of homocysteine to homocystine^[82,87]. Homocysteine also increases levels of the enzyme SOD^[88]. SOD catalyzes the conversion of superoxide anion to hydrogen peroxide and increased activity of this enzyme will result in greater

hydrogen peroxide generation. Homocysteine has been reported to inhibit GPx activity^[87] by 10-fold. This epistatic inactivation of GPx will increase hydrogen peroxide levels and inhibition of GPx was shown to occur at physiologic (9 μmol/L) concentrations of free homocysteine^[89].

Decreased 6-phosphogluconate dehydrogenase activity

A second enzyme located at this locus (1p36.3) is 6-phosphogluconate dehydrogenase (PGD) (EC 1.1.1.44). PGD is one of only two enzymes in the PPP, which are responsible for production of NADPH, which is crucial for the reduction of glutathione disulfide (GSSG) back to reduced glutathione (GSH) in order to neutralize the continuous production of H₂O₂ being generated within the cell. Without NADPH to regenerate reduced glutathione, intracellular enzymes would suffer irreversible oxidative damage from excess hydrogen peroxide and cellular function would cease in minutes as apoptosis is triggered. The PPP is the engine that drives H₂O₂ neutralization and there is no backup system. PGD exists in several polymorphic forms with decreased activity ranging from 22% to 79% of normal^[90-94]. Decreased levels of glutathione have been reported as a result of a PGD polymorphic enzyme^[95].

The phenotypic expression of both these genes supports Cho's conclusion of a pathophysiologically crucial IBD susceptibility gene located at 1p36. PGD activity is also lowered by exogenous factors, i.e. antibiotics, dietary fat and ageing^[96-98]. Studies of normal appearing colonic mucosa report significant inter-individual variation of enzymes involved in glutathione synthesis and metabolism^[99]. Individual variation was considerable at 8-fold for glutathione-S-transferase, 10 fold for GPx, 14-fold for gamma-glutamyl-transpeptidase and 5 fold for gamma-glutamylcysteine synthetase.

These large enzyme variations directly or indirectly affect intracellular glutathione concentrations which itself shows a 16-fold variation between individuals placing certain individuals at the very lowest range of H₂O₂ neutralizing capability.

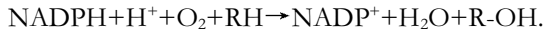
Decreased glucose-6-phosphate dehydrogenase activity

Epinephrine has been shown to stimulate H₂O₂ release by macrophages and to inhibit glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49)^[100]. G-6-PD is a crucial enzyme in the PPP, which produces NADPH needed to regenerate reduced glutathione, which is crucial in order to neutralize (reduce) H₂O₂. Inhibition of this enzyme by circulating epinephrine during stressful events reduces the amount of NADPH generated by the PPP, which may lead to increased intracellular H₂O₂ levels. Therefore, conditions of sustained stress can increase the concentration of circulating endogenous catecholamines and boost production of hydrogen peroxide by rectal epithelial cells by either direct production of H₂O₂ or reduction in NADPH needed for H₂O₂ neutralization.

Increased cytochrome P450 enzyme activity

The cytochrome P450 enzyme system is responsible for the majority of oxidation reactions of drugs and other xenobiotics^[101]. One study reports that 56% of over 300

drugs tested are metabolized via the cytochrome P450 (CYP) family of oxygenase enzymes present in the endoplasmic reticulum^[102]. CYP is mostly found in the liver but is also present in the intestine. A typical CYP catalyzed reaction is as follows:



This reaction consumes NADPH, which is also used in regeneration of reduced glutathione required to neutralize H_2O_2 . Excessive NADPH utilization in predisposed individuals with marginal anti-oxidant capacity may contribute to increased H_2O_2 levels and the development of colitis associated with certain drugs.

In a prospective cohort study, Jowett^[103] found that individuals who consumed the most alcohol tripled their risk of UC relapse compared to those who drank the least. After ingestion, alcohol is distributed to all cells of the body including the rectal epithelial cells. Alcohol is enzymatically converted to acetaldehyde by alcohol dehydrogenase. The acetaldehyde is enzymatically converted to acetic acid by aldehyde dehydrogenase. Both of these cytosolic enzymes utilize NAD^+ to oxidize their respective substrates and generate NADH that normally serves as an electron donor to the ETC. The increased availability of NADH can activate the ETC and generate excess hydrogen peroxide^[104,105].

Alcohol can also be metabolized in the endoplasmic reticulum by cytochrome P450 2E1 depleting NADPH needed for glutathione regeneration. Alcohol, thus, generates H_2O_2 and decreases production of glutathione needed for neutralization of hydrogen peroxide.

Alcohol inhibits GPx, a crucial enzyme that neutralizes H_2O_2 , and depletes mitochondrial glutathione^[104]. Glutathione is not synthesized within mitochondria and must be transported from the cytosol into mitochondria through mitochondrial membranes. Alcohol inhibits active transport of glutathione into mitochondria^[106,107] leading to mitochondrial depletion of glutathione and H_2O_2 accumulation.

A relationship exists, therefore, between UC and conditions that enhance H_2O_2 production. Furthermore, significant genetic variability in H_2O_2 neutralizing capacity confers greatest risk of developing UC to those individuals with genetically low H_2O_2 neutralizing capacity and co-existence of any of several conditions provoking increased production of H_2O_2 . Figure 1 illustrates this concept.

6. Is impaired beta oxidation and neoplastic transformation a consequence of excess H_2O_2 ? The preferred energy source for colonic epithelial cells is a short chain 4-carbon fatty acid known as butyrate (SCFA). Most butyrate is derived from colonic bacterial fermentation of unabsorbed dietary fiber^[113]. SCFAs are metabolized rapidly by beta oxidation and are the major respiratory fuels of colonocytes^[113]. Beta-oxidation is the anapleurotic process occurring within mitochondria by which fats are broken down into two carbon units to form acyl-CoA, which is the entry molecule for the Krebs (tricarboxylic acid) cycle. The Krebs cycle generates NADH, which is used as a fuel for ETC activity resulting in ATP production.

Inhibition studies carried out on beta oxidation led Roediger and Nance^[114], to conclude that "a suitable inhibitor of beta-oxidation would have unimpeded entry into mitochondria of colonic epithelial cells". Hydrogen peroxide

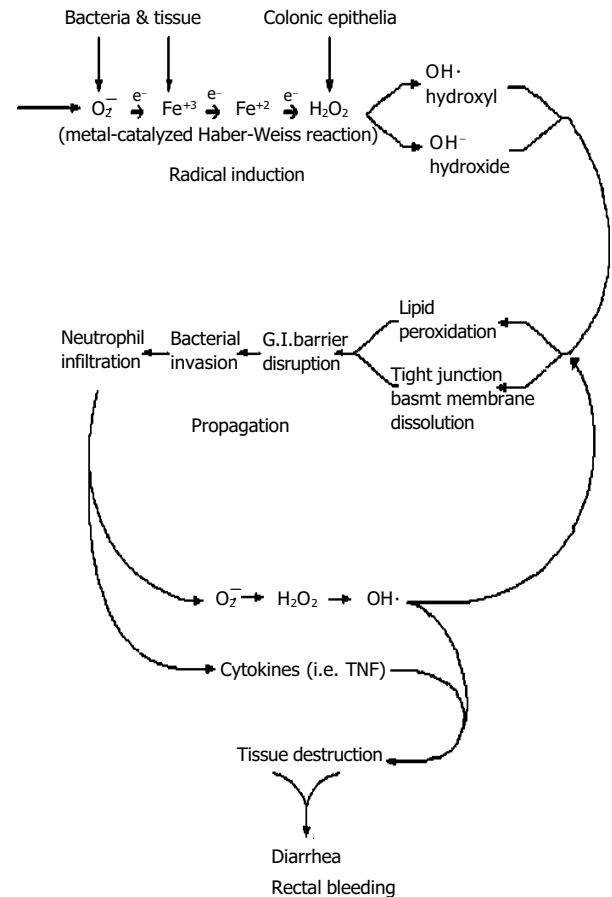


Figure 1 Pathogenesis of UC: Individuals with UC are basically normal. Polymorphic genes (MTHFR and PGD) and significant inter-individual variability in anti-oxidant capacity places certain individuals at the lower threshold of their physiological hydrogen peroxide reducing capability for any given environmental oxidant stress level. Oxygen radical production is induced by environmental oxidant stressors (xenobiotics, stress, smoking cessation, hypermetabolic states) interacting with cellular metabolism. This process is called "Radical Induction". Excess un-neutralized hydrogen peroxide generated during the radical induction phase diffuses from intracellular compartments of colonic epithelial cells, through the plasma membrane, to the extracellular space. Free extracellular hydrogen peroxide reacts with superoxide (O_2^-) in a transition metal catalyzed Haber-Weiss reaction to form hydroxyl radical (OH^\cdot) and hydroxide (OH^-) as follows: $\text{O}_2^- + \text{Fe}^{+3}$ (or Cu) $\rightarrow \text{O}_2 + \text{Fe}^{+2}$ (followed by) $\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \text{OH}^- + \text{OH}^\cdot$. Hydroxyl radical initiates oxidative damage to structures that comprise the gastrointestinal barrier (epithelial TJs, BM and epithelial lipid peroxidation) resulting in transient immune activation, which ceases when the damage is repaired. Intermittent immune activation by colonic bacterial antigens can lead to antibody formation (p-anca) and extra-intestinal manifestations. When oxidative damage to the GI barrier cannot be repaired in time to prevent subjacent endothelial adhesion molecule expression, WBCs (neutrophils) begin infiltrating into the damaged colonic epithelium in an effort to prevent systemic bacteremia, which would otherwise result in fatal sepsis. Resident mucosal neutrophils produce additional large quantities of diffusible H_2O_2 which causes oxidative tissue damage in adjacent colonic epithelial cells whose anti-oxidant (GSH) levels have already been previously compromised by radical induction. This results in a proximal "advancing edge" of oxidative tissue damage, TJ disruption and lipid peroxidation extending proximally from the rectum, a unique tissue with high oxidant exposure secondary to fecal generated oxygen radicals, maximum bacterial antigenic load and least anti-oxidant defense as compared to the rest of the GI tract. The accompanying intense neutrophilic diapedesis in a restricted area of epithelial TJ disruption is followed initially by microscopic erythrocyte extravasation eventually leading to frank hemorrhage as endothelial junctions fail to close leading to bloody diarrhea and further neutrophil infiltration characteristic of this disease. The process is only temporarily halted when sufficient anti-oxidant (GSH) capacity is encountered producing a clear line of demarcation between diseased and normal tissue. This self-perpetuating and auto-stimulating cycle is called propagation. Both iron (Fe) and superoxide (O_2^-) are plentiful within the colonic lumen^[108-110]. UC has been reported subsequent to oral iron supplementation for the treatment of anemia^[111] and in association with conditions of copper overload (i.e. Wilson's disease)^[112].

is permeable through biomembranes including the cell membrane and both the inner and outer mitochondrial membranes. Hydrogen peroxide has been shown to inhibit the beta-oxidation enzyme system of enzymes^[115].

The last enzyme in the mitochondrial beta-oxidation process is acetyl-CoA C-acyltransferase (EC 2.3.1.16)(ACT) also known as 3-ketoacyl-CoA thiolase or thiolase I^[116]. This enzyme contains two active binding sites each of which includes a conserved cysteine residue, which is crucial for enzymatic activity^[117]. Cysteine is an amino acid which has a thiol (hydrosulfide or sulfhydryl) group. The thiol group is a univalent radical (-SH) which must be maintained in a reduced state in order for ACT to be functional. H₂O₂ is capable of oxidizing these cysteine residues and inactivating ACT.

Inhibition of beta-oxidation has been reported in macroscopically normal and clinically quiescent UC^[118]. Studies have shown that impairment of beta-oxidation is significantly associated with increased colonic permeability followed by clinical relapse to active UC within a few weeks. Remission was associated with normal beta-oxidation^[119].

This supports a separate and distinct diffusible intracellular oxidizing agent (i.e. H₂O₂) as the vehicle for both impaired beta-oxidation and subsequent increase in colonic permeability. Appearance of diffusible intracellular H₂O₂ explains the abnormalities of butyrate metabolism during active UC, which may resolve during remission and reappear prior to subsequent activation of the disease since H₂O₂ would accumulate within mitochondria and other sub-cellular organelles prior to extracellular diffusion and destruction of BMs and TJs.

NEOPLASTIC TRANSFORMATION

Patients with UC have an incidence of colorectal cancer (CRC) which is up to 20 fold higher and 20 years younger than CRC in the general population^[120]. UC associated CRC originates from dysplastic colonic epithelial cells^[121]. The mechanism of neoplastic transformation involves nuclear DNA damage within colonic epithelial cells^[122,123]. H₂O₂ is known to cause oxidative DNA damage and treatment of colonic epithelial cells with low concentrations of H₂O₂ has been shown to cause oxidative nuclear DNA damage^[124-126]. Cells whose nuclear DNA is damaged by H₂O₂ generated free radicals can undergo neoplastic transformation^[127,128]. Thus, active colonic inflammation will lead to cancer due to the high amount of H₂O₂ generated by infiltrating neutrophils; however, studies have shown that individuals with quiescent disease in “remission” have the same risk of developing CRC as those with a more active disease course^[129] suggesting an intracellular mechanism (i.e. excessive H₂O₂) which is present during “remission”. In fact, studies demonstrate that CRC in the setting of UC increases the risk of CRC in non-colitic relatives by 80%^[130] suggesting the antecedent presence of a genetically predisposing genotoxic mechanism or agent such as H₂O₂.

5-Aminosalicylic acid (5-ASA) has been proposed as a maintenance therapy for the prevention of CRC^[131-133]. However, results are conflicting and recent studies show that maintenance with 5-ASA does not protect against the development of colonic cancer^[134]. Once dissolved in the

near neutral pH of colonic fluids 5-ASA becomes a zwitterion with a positive charge on the protonated amino group at one end and a negative charge on the dissociated carboxylic group at the other end of the molecule. This promotes attraction between the positively charged amino terminal of 5-ASA and negatively charged surface membrane proteins favoring retention of 5-ASA on the exterior surface of colonic epithelial cells^[135,136]. *In vitro* studies of 5-ASA have shown that the site of action of 5-ASA is extracellular^[137]. Its mode of action is that of an extracellular tetravalent reducing agent capable of donating four electrons per molecule for H₂O₂ and oxygen radical neutralization^[138]. 5-ASA also sequesters ferrous ions (Fe⁺²)^[139] possibly by electrostatic attraction to its (5-ASAs) negatively charged terminal thereby inhibiting the hydroxyl generating extracellular Haber-Weiss reaction.

5-ASA's extracellular site and mode of action precludes it from affecting the intracellular pathogenetic mechanisms leading to neoplastic transformation during disease quiescence. While in “remission” H₂O₂ generated during the radical induction phase and exiting the cell will be neutralized by luminal 5-ASA reducing the incidence of clinical reactivation; however, excessive intracellular H₂O₂ is free to diffuse into the nucleus causing oxidative DNA damage resulting in CRC in this clinically quiescent stage of disease.

CONCLUSION

The evidence presented in this paper points to excess hydrogen peroxide diffusing out of colonic epithelial cells as the initiating etiology of UC. Hydrogen peroxide (H₂O₂), a highly toxic oxidizing agent and by-product of normal aerobic cellular metabolism, is constantly being generated within all cells including colonic epithelial cells and must be immediately neutralized in order for the cells to survive.

The intracellular generation of H₂O₂ is determined by the metabolic and respiratory activity of the cell; however, the anti-oxidant capacity required to neutralize H₂O₂ resides within a genetically determined fixed range for each individual with significant inter-individual variability being expressed. This places a sub-group of individuals at the lowest end of anti-oxidant (H₂O₂ neutralizing) capacity. It is this sub-group of individuals that have the highest risk of developing UC when exposed to oxidant stressors.

Under appropriate conditions excess H₂O₂ generated due to the effect of oxidant stressors on cellular metabolism may overwhelm the genetically predetermined anti-oxidant/reducing capacity available within the cell resulting in intracellular H₂O₂ accumulation. This process is called “Radical Induction”. Radical induction will initially manifest in the rectum which has the lowest anti-oxidant defense (H₂O₂ neutralizing) capacity of the entire GI tract coupled with the highest bacterial exposure^[140-143]. This makes rectal epithelium especially vulnerable to oxidant injury.

Hydrogen peroxide is freely permeable through cell membranes and essentially forms one intracellular compartment. H₂O₂ generation is biochemically coupled to fundamental metabolic processes such as ATP (energy) production and ETC activity in addition to the enzymatic activity of nearly 100 intracellular enzyme systems. H₂O₂

production is therefore very sensitive to and will fluctuate with environmental influences that affect respiratory activity (tobacco use, stress, hyperthyroidism) and availability of specific enzyme substrate (i.e. xenobiotics, alcohol, vitamin B-6). Excess un-neutralized H_2O_2 will diffuse from any intracellular location to the extracellular space where it can be converted to the highly destructive hydroxyl radical via a metal catalyzed Haber-Weiss reaction causing significant oxidative damage to colonic epithelial TJs, BM and epithelial biomembranes, which are micro-anatomical structures that comprise the colonic barrier function. This in turn increases colonic mucosal permeability to luminal antigens resulting in the *initial* influx of WBCs (neutrophils) from the subjacent vasculature to the mucosal surface. This is a normal and expected immune response to antigenic exposure; a normal response to normal colonic flora.

Once present on the colonic mucosal surface, exposure to high concentrations of fecal bacterial antigens stimulates neutrophils to secrete their own tissue damaging oxygen radicals. Neutrophil mediated tissue damage attracts additional neutrophils from the subjacent intravascular compartment to the mucosal surface. This process is repeated until sufficient tissue anti-oxidant (glutathione) levels are encountered to temporarily halt its progression, sharply delineating diseased from normal tissue.

This self-perpetuating process of tissue destruction is called propagation and results in a proximally advancing edge of contiguous oxidative tissue destruction in adjacent epithelium whose anti-oxidant defense capacity has already been previously compromised during the radical induction phase. Continued inflammatory tissue destruction results in rectal bleeding and bloody diarrhea characteristic of this disease.

SUMMARY

Since Hale-White first coined the term "ulcerative colitis" in 1888^[144] we have learnt that the clinical phase of this disease which begins with rectal bleeding is characterized by colonic mucosal inflammation mediated by the accumulation of WBCs (mainly neutrophils) within the colonic epithelium. Based on the histological findings in this phase, UC is classified as an inflammatory bowel disease. The mechanism of neutrophil-mediated tissue injury responsible for colonic bleeding has been well described^[145].

To date however there is no satisfactory answer for why neutrophils accumulate within the colonic mucosa to begin with. The Radical Induction Theory of UC provides an explanation for this initial influx of neutrophils.

Radical Induction Theory states that H_2O_2 originating from colonic epithelial cells diffuses to the extracellular space resulting in oxidative damage and dissolution of intercellular TJs and BM, which are micro-anatomical structures that maintain the GI barrier function. Once compromised, the GI barrier can no longer exclude highly antigenic bacterial antigens from invading the normally sterile deeper layers of the colonic wall resulting in the initial influx of neutrophils to the mucosal surface. Continued accumulation of neutrophils results in extensive tissue damage and bleeding characteristic of this disease.

Radical Induction Theory implies two distinct phases of UC. The first phase is operational prior to any colonic bleeding. During this initial preclinical "Radical Induction" phase colonocytes are induced to generate excessive un-neutralized H_2O_2 due to effects of oxidant stressors upon cellular metabolism. Hydroxyl radical and H_2O_2 have very short half lives (nanoseconds and seconds to minutes respectively) which limits their destructive activity to intracellular molecules (i.e. DNA, enzymes) and local extracellular structures immediately adjacent to the cell (i.e. TJs and BM). Pro-inflammatory cytokines, however, may be carried to distal sites to exert their effect. Thus, initial intermittent extracellular diffusion of H_2O_2 from epithelial cells causes short lived local barrier compromise and transient immune activation resulting in cytokine production and distal extra-intestinal manifestations such as arthritis, uveitis, skin manifestations (i.e. pyoderma gangrenosum) and p-anca type antibodies. Continued oxidative insult to colonic barrier function culminates in neutrophilic infiltration.

A second (clinical) phase begins with rectal bleeding and signals further destructive GI barrier compromise secondary to neutrophil-mediated tissue damage. Continued stimulation of mucosal neutrophils by fecal bacteria converts the condition into an auto-stimulating/self-perpetuating process termed propagation.

Patients will normally present for treatment during the propagation phase, which will continue inexorably to the inflammatory destruction of the colon without outside intervention. Although clinical remission (cessation of rectal bleeding), endoscopic remission (normal macroscopic mucosal appearance) and histologic remission (no mucosal neutrophils) are important milestones, the lack of metabolic remission precludes complete reversal of this condition and predisposes to future reactivation and neoplastic transformation.

Fifty years of research has not demonstrated any antecedent immune vulnerability in patients with UC^[146]. However, rather than a local mucosal immune dysfunction, high levels of H_2O_2 found in other cell lines in individuals with UC^[147] suggests that this condition is a systemic disease of oxidant stress whose primary pathological manifestation is in the rectum, a unique body site with minimal anti-oxidant defense, high continuous oxidant stress and maximum bacterial antigenic exposure.

Thus, the crucial element required for mucosal integrity mentioned earlier consists of the biochemical machinery needed to detoxify hydrogen peroxide which, if allowed to accumulate, can oxidize and disintegrate TJ proteins leading to dissolution of barrier integrity and UC. Neutralization of intracellular hydrogen peroxide, therefore, constitutes a vital process whose dysfunction results in physical disintegration of gastrointestinal barrier function.

GPx (E.C. 1.11.1.9) in conjunction with co-factor glutathione, a self-replenishing tripeptide reducing agent, is responsible for 91% of H_2O_2 neutralization. Factors that decrease the activity of GPx or decrease the amount of available reduced glutathione will lead to increases in intracellular hydrogen peroxide which upon diffusion to the extracellular space will result in oxidative disruption of TJs and BM whose integrity is required for GI barrier function.

The oxidative stress to which UC patients are exposed

has exceeded their physiological antioxidant defense mechanisms. The significant inter-individual variability in oxidant neutralizing capacity places certain individuals at the lower threshold of their physiological (H₂O₂) reducing capability for any given environmental oxidant stress level. As the environment becomes increasingly toxic more individuals will succumb to its effects. UC, a purely descriptive term, may be more accurately described by its pathophysiology as oxidative colitis.

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