[Frontiers in Bioscience E4, 1335-1344, January 1, 2012]

Oxidative stress and inflammatory bowel disease

Hazem A. Almenier[1], Hazem H. Al Menshawy[2], Maha M. Maher[3], Salah Al Gamal[4]

[1]Mansoura Emergency Hospital, Mansoura, Egypt, [2]Medical Specialized Hospital, Mansoura University,35516, Mansoura, Egypt, [3]Medical Specialized Hospital, Mansoura University,35516, Mansoura, Egypt, [4]Medical Specialized Hospital, Mansoura University,35516,Mansoura, Egypt

TABLE OF CONTENTS

1. ABSTRACT

Inflammatory Bowel Disease (IBD) is a chronic relapsing and remitting inflammatory condition of the gastrointestinal tract. The exact cause of IBD remains undetermined, the condition appears to be related to a combination of genetic and environmental factors. While many gaps in our knowledge still exist, the last two decades have witnessed an unprecedented progress not only in the etiology ; but mainly in the mechanisms underlying the chronic inflammatory response, immunologic and genetic aspects. We review some recent points of research in pathogenesis with special stress on oxidative stress and its correlations with disease activity.

2. INTRODUCTION

The aetiology of inflammatory bowel disease is likely multifactorial. Mostly involves the complex interaction of four elements: genetic susceptibility, host immunity, environmental factors and commensal enteric bacteria (1).

The most recent hypothesis on the pathogenesis of IBD states that individuals, who have a genetic predisposition, when confronted with unidentified aggressors from their natural environment, develop a loss of tolerance to luminal bacterial antigens (i.e. dysregulation of the enteric immune response) and initiate an uncontrolled inflammatory reaction targeted at the bowel wall (2).

3. PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

3.1. Genetics

The strongest evidence of a genetic influence for IBD is derived from twin studies. In three large European twin pair studies, approximately 6% to 16% of monozygotic twin pairs had concordant IBD compared with 0% to 5% of dizygotic twin pairs (3).

The quest for IBD-related genes has indicated that in some patients, deficiencies in the innate immune response can be linked to the development of IBD. The first susceptibility gene identified in CD (IBD1) on chromosome 16q12, originally known as NOD2 (nucleotide-binding oligomerization domain 2) but subsequently renamed CARD15 (caspase activation and recruitment domain 15) by the international Nomenclature Committee (4).

Mutations in CARD15 are seen in about 15-20% of Crohn's disease patients but are also seen in a smaller percentage of the general population, so mutations in CARD15 are neither necessary nor sufficient for the development of Crohn's disease. CARD15 variants are associated with younger age of onset, ileal disease and a tendency to develop strictures.

Other candidate susceptibility genes have been searched for. Putative loci have been mapped to chromosome 12(IBD2), 6(IBD3) and 14(IBD4) (5).

There are also genes that appear to influence disease behavior independently of susceptibility genes. The best studied of these are the human leukocyte antigen (HLA) alleles.

One allele of HLA-DR2 (DRB1*1502) appears to be involved in disease susceptibility in Japanese and Jewish populations. Several centers have reported an association between severe disease and a rare allele of HLA-DR1 (DRB1*0103).

In some studies, the HLA-DR3, DQ2 haplotype is associated with extensive colitis, especially among women. Among the Jewish population, the peri-nuclear antineutrophil antibody (pANCA) is a marker for the DRB*1502 allele of HLA-DR2, but in non-Jewish whites, this antibody is associated with the HLA-DR3 DQ2-tumor necrosis factor (TNF)-α2 haplotype (6).

3.2. Immunopathogenesis

Studies have shown that patients with IBD have reduced tolerance toward their own intestinal flora. It is suggested that in genetically susceptible patients, a dysregulation of the mucosal immune system leads to excessive immunologic responses to normal microflora with alterations in gut epithelial barrier function resulting in continuous stimulation of the mucosal immune system (7).

Crohn`s disease is associated with Th1-type T cell–mediated inflammation. This produces an excess IL-12, IL-17, IL-23, IFN-γ and macrophages derived cytokines, also there is overproduction of IL-1B, IL-2, IL-6, IL-8 and TNF-α. In comparison, ulcerative colitis is associated with Th2-type T cell–mediated inflammation with excess IL-4, IL-5, IL-10 and IL-13 production (8).

Macrophages in the inflamed colon in patients with active CD synthesize IL-1β, TNF and IL-6, whereas lamina propria T cells probably produce IL-2 and IFN-γ. This immune response can be up-regulated further by presentation of antigen to CD4 and lymphocytes (produced by cytokines) by colonic epithelial cells that express HLA class II antigens (9).

Nuclear factor kappa B (NF-κB) plays a central regulatory role by controlling the transcription of genes for these proinflammatory cytokines. There is also activation of other cells (eosinophils, mast cells, neutrophils and fibroblasts) which lead to excess production of chemokines (lymphokines, arachidonic acid metabolites, neuropeptides, leukotrienes, thromboxane, platelet-activating factor, nitric oxide, and free oxygen radicals), all of which can contribute to inflammation and mucosal injury, alter epithelial cell permeability, and interfere with iron transport, thereby further contributing to diarrhea (7).

Tumor necrosis factor alpha (TNF-α) is a potent proinflammatory cytokines that exert its stimulatory effect on cells which produce IFN-γ (immunomodulator and can cause direct tissue destruction). Over expression of TNF by a transgene is associated with a severe colitis and as proof of principle that TNF-α is important for the pathogenesis of IBD (10).

3.3. Familial

Family history is one of the most important risk factors for developing IBD. Both CD and UC are more common amongst relatives of patients than in the general population. The figures vary widely among different studies, but about 10% to 20% of patients have at least one other affected family member.

This familial association generally occurs in first-degree relatives, and the incidence of IBD in first-degree relatives is 30 to 100 times that of the general population (6).

Although reports of IBD in both husband and wife are rare, a study of 30 conjugal instances of IBD found a higher frequency of husband-wife pairs, both of whom developed disease after cohabitation, thus suggesting a shared environmental exposure (11).

3.4. Environmental factors

Improved hygiene accompanied by a decrease in chronic infestations and reduced morbidity during childhood has been implicated as a possible cause of many autoimmune diseases especially IBD.

This is often referred to as the hygiene hypothesis (12). The hygiene hypothesis suggests that in a clean environment the intestinal immune system may not

exposed to pathogenic or non pathogenic microorganisms, particularly helminthic parasites, and therefore be untrained to confront minor infections without recruiting the full array of specific immune functions that lead to inflammation (13,14).

Recent large epidemiologic studies have documented in increase in the first presentation and subsequent flare-ups of IBD after documented gastrointestinal infections (15).

3.5. Nutritional factors

Many food and food components have been suggested as perhaps influencing the development of IBD include diet (wheat, maize, cow's milk, refined sugar, fat and alcohol), oral contraceptives, food additives (silicon dioxide) and toothpaste; none, however, has been shown conclusively to be associated with IBD.

Unfortunately the results of numerous studies have been equivocal so, as yet, there are no definitive data to support nutritional factors as a cause of either CD or UC (16,17).

3.6. Microbial Factors

A) Specific Infectious Agents

Several microorganisms have been proposed as having a potential etiologic role in the pathogenesis of IBD, such as Listeria monocytogenes, Chlamydia trachomatis, Escherichia coli, Cytomegalovirus, Saccha-romyces cerevisiae and others. Among those, the role of Mycobacterium Avium paratuberculosis (MAP) in CD has been the center of major controversy (18).

MAP was initially isolated from a few Crohn`s disease tissues and has found in the blood of patients with CD and further studies are awaited [19]. Also the role of measles virus infection in IBD is controversial (20).

B) Intestinal Commensal Flora

Over the past decade, there has been an exponential increase in notion that commensal enteric flora plays a key role in the development of IBD .Additionally, the following clinical observations support this hypothesis: (1) that the beneficial effect of antibiotics in the treatment of CD, and to a lesser extent UC, has been appreciated for years, (2) diversion of the fecal stream from inflamed bowel loops has been known to induce symptomatic improvement in CD patients, while relapse often occurs upon restoration of intestinal continuity, and (3) pouchitis, a chronic inflammation of a surgically constructed ileo-anal pouch, develops in a considerable proportion of UC patients after proctocolectomy, and is associated with a dysbiosis caused by the contact of the once near sterile small bowel mucosa with a rich colon-like flora repopulating the pouch after surgery (21).

Finally, and probably most convincingly, the majority of animal models of IBD fail to develop intestinal inflammation when kept in a germ-free environment (18).

3.7. Evidence for barrier dysfunction

Several lines of investigation indicate that disruption of the epithelial barrier may either instigate or perpetuate chronic intestinal inflammation. Abnormal intestinal permeability has been established among patients with Crohn`s disease and their healthy first-degree relatives, and may represent a primary abnormality predisposing to excessive antigen uptake, continuous immune stimulation, and eventually mucosal inflammation. Potential etiologic factors for barrier dysfunction in Crohn`s disease may be environmental or genetic.

Smoking and non-steroidal anti-inflammatory drugs both potentially affect gut permeability and are variably associated with IBD.

Mutations in two genes recently found to be potentially associated with Crohn`s disease (OCTN and DLG5) appear to affect epithelial permeability and may lead to inappropriate exposure of the mucosal immune system to luminal antigen (9).

3.8. Smoking

Patients with CD are more likely to be smokers, and smoking has been shown to exacerbate CD and worsens its course. In contrast, there is an increased risk of UC in non- or ex-smokers and nicotine patches have been shown to be an effective treatment of UC. Smoking cessation aggravates ulcerative colitis and improves CD .

The role of passive smoking, particularly in children, as either a risk factor or protective factor for CD and/or UC is still a matter of controversy with none of the studies having quantitatively assessed the passive smoke exposure (22).

The mechanisms underlying the differential effect of smoking in CD or UC remain obscure. However, smoking has been demonstrated to affect both systemic and mucosal immunity, as well as alter a wide range of both innate and adaptive immune functions (23).

3.9. Stress

Studies reporting that psychosocial stress increase the risk of relapse in patients with quiescent UC. Conversely, many of the psychological features observed in patients with UC are likely secondary to this chronic disease process. It is important for physicians to be aware of this phenomenon when managing these patients (24).

Stress is more likely to modulate disease manifestations, the clinical course and the response to therapy rather than being an initiating factor. Evidence that stress can modulate the course of IBD is provided by clinical observations, and studies of neuroimmune interactions in laboratory animals. Stress also augments intestinal permeability, and therefore, the entry of excessive amounts of luminal antigens could activate pre-sensitized mucosal T cells resulting in inflammation (25).

Experimental studies have helped to identify the mechanism of the proinflammatory potential role of stress

in animal models of colitis. When applied prior to the introduction of proinflammatory stimuli, stress has been shown to increase the severity of colonic inflammation in rats. (a) The similar neurohormonal and neurotransmitter peptide producing cells in the CNS and in the GIT, and (b) Extensive neuronal innervations of the bowel wall including the intestinal mucosa, indicate important pathways for psychologic neurologic -humoral - immunologic interactions between the brain and the gut (26).

In recent years, increasing attention has given to the role of reactive oxygen metabolites in the pathogenesis of inflammatory bowel disease.

In some recent studies it has been shown that increased number of granulocyte accumulation in inflammatory lesions of gut mucosa in patients with inflammatory bowel disease (27).

Those activated cells release a number of inflammatory mediators such as toxic oxygen metabolites, lysosomal enzymes, and derivatives of arachidonic acid metabolism. It has been proposed that, inflammation of mucosa causes impairment of antioxidant defense mechanism, and makes tissue more susceptible to oxidative damage (28).

In turn, superoxide anion and phagocytes accumulating in inflammatory lesion, cause impairment of cellular membrane stability and cell death by leading lipid peroxidation (29) Oxidative stress radicals, hydrogen peroxide, and hydroxyl radicals, secreted by neutrophils. Lih-Brody *et al.* found increased oxidative stress and decreased antioxidant defenses in colonic mucosal biopsies of patients with inflammatory bowel disease (30).

Metabolism of oxygen by cells generates potentially deleterious reactive oxygen species (ROS). Under normal conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination. However, an imbalance between prooxidants and antioxidants results in oxidative stress , which is the pathogenic outcome of oxidant overproduction that overwhelms the cellular oxidant capacity (31).

Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an overabundance of ROS from an environmental or behavioral stressor. If not regulated properly , the excess ROS can damage a cell's lipids, protein or DNA, inhibiting normal function. Because of this oxidative stress has been implicated in a growing list of human diseases (32). All cells have intracellular antioxidants (such as superoxide dismutase and glutathione) which are very important for protecting all cells from oxidative stress at all times (33).

4. FREE RADICALS

Free radicals can be described as highly reactive molecules, are generated during the normal oxidative process that occur in living cells (34). Free radicals is simply defined as any chemical species capable of independent existence that contains one or more unpaired electron in their outer orbital (35).

If the unpaired electron is not neutralized, it will induce damage by combining in a covalent bond with structural lipids and proteins and lead to cellular destruction (36).

The term "free radical species" summarizes a variety of highly reactive molecules that can be divided into different categories, e.g. reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS). The most prominent members of such categories include superoxide O^{-}_{2}, hydroxyl radical OH, peroxy radical ROO in the ROS group, and nitric oxide NO in the RNS group (37).

There is also accumulating evidence that increased free radical production under hyperglycemic conditions originates from mitochondrial respiration, cytochrome p450, xanthine oxidase and PKC-dependant activation of NADH/NADPH oxidase. The excess free radicals create oxidative stress ; a situation of serious imbalance between the production of free radicals and antioxidant defense , leading to potential tissue dysfunction and damage (34). There are many naturally occurring substances , which function to protect a against the harmful effect of pro-oxidants. These substances termed antioxidants must be present in biological systems in sufficient concentrations to prevent accumulation of pro-oxidant molecules.

Antioxidants are endogenous substances such as the enzymes superoxide dismutase , glutathione peroxidase , catalase and metal ion binding proteins as well as additional dietary antioxidants such as tocopherol, ascorbate, flavonoids and carotenoids (38).

It is now widely held that tissue damage in IBD is a result of abnormal mucosal immune reactions to bacterial products and other lumenal factors, reactions that initiate an inflammatory cascade (39). The inflammatory cascade begins by infiltration of inflammatory cells into the mucosa and release of proinflammatory mediators such as reactive oxygen metabolites (ROM) and reactive nitrogen metabolites (RNM) (40).

These mediators cause tissue damage and result in additional recruitment of inflammatory cells, a vicious cycle that sustains the inflammatory cascade. In this view, a disrupted intestinal barrier both initiates and perpetuates the cascade by exposing luminal factors to the mucosal immune system (41). When this vicious cycle is aborted, tissue damage can be repaired. Left unchecked, inflammation is sustained, resulting in intestinal tissue damage and symptoms of the active phase of IBD. Accordingly, it is now critical to identify the most important proinflammatory factors that maintain this vicious cycle and shift the disease from inactive to active phases. Such knowledge could have significant diagnostic, prognostic, and therapeutic impact (42) .

ROM and RNM represent one critical group of proinflammatory factors that could maintain the vicious cycle of IBD. And these oxidants can overwhelm antioxidant defences and, through protein oxidation, DNA strand breaks, and ATP depletion, cause tissue damage (40).

4.1. Evidences of oxidative injury in inflammatory bowel disease

There are several lines of indirect evidence which suggest that the chronically inflamed intestine or colon may be subjected to considerable oxidative stress and thus susceptible to oxidative injury: first, it is well known that phagocytes are activated by certain pro-inflammatory mediators such as leukotriene B4 (LTB4) or platelet activating factor (PAF) to release large amounts of potentially cytotoxic reactive oxygen metabolites into the interstitial compartment (43). Enhanced synthesis of LTB4 and PAF has been demonstrated in mucosal samples obtained from patients with active IBD (44).

Second, there are several reports that have demonstrated that phagocytic leukocytes (monocytes, neutrophils, macrophages) obtained from patients with active IBD respond to various proinflammatory stimuli with enhanced reactive oxygen metabolism when compared to cells obtained from healthy volunteers (45).

Moreover, RNM such as NO may play an equally important or even bigger role in tissue injury (46). This evidenced by: High NO levels, which result from inducible NO synthase (iNOS) activation, are associated with tissue damage (47). iNOS is present in inflammatory cells such as neutrophils and macrophages and in intestinal epithelial and endothelial cells (48). iNOS is activated in the presence of proinflammatory factors such as cytokines—a milieu commonly found in active IBD (49). High iNOS activity has been found in experimental models of colitis and in the intestinal mucosa of patients with IBD (50) . High levels of the end products of NO metabolism, nitrates and nitrites, were found in the plasma, stool, and colonic lumen of IBD patients (51).

4.2. Mechanisms of free radicals Production and Tissue Injury in inflammatory bowel disease

Interaction of certain proinflammatory mediators (e.g. LTB4, PAF, immune complexes, bacterial products) with specific receptors on the neutrophil plasma membrane results in the dramatic increase in oxygen (O_2) consumption due to the activation of the latent, plasma membrane-associated NADPH oxidase (52).

Activation of this multicomponent, flavoprotein results in the production and release of large amounts of the superoxide anion radicals.

$$NADPH + O_2 \rightarrow NADP + +O_2 -.$$

Superoxide is very unstable at neutral pH and will spontaneously (or enzymatically) dismutate to yield hydrogen peroxide (H_20_2) and oxygen (O_2):

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2.$$

Some investigators have proposed that neutrophil-derived O_2 and H_2O_2 may interact with low molecular weight, redox-active iron (Fe) to yield the highly reactive hydroxyl radical (OH) via the superoxide-driven Fenton reaction:

$$O_2^- + Fe^{+3} \rightarrow O_2 + Fe^{+2}$$

$$H_2O_2 + Fe^{+2} \rightarrow OH^- + OH + Fe^{+3}$$

However, recent data suggest that neutrophils produce very little (if any) OH *in vitro*. The reasons for this lack of 'OH production are two fold: First, myeloperoxidase (and catalase) will consume most of the H_20_2 produced leaving little H_2O_2 to interact with O_2^- and iron (53).

Second, there is normally very little low molecular weight iron available *in vivo* with most of the metal chelated to proteins such as transferrin, lactoferrin and ferritin (54).

In addition to these reactive oxygen metabolites, the activated neutrophil secretes the green hemoprotein myeloperoxidase (MPO) into the extracellular medium. Myeloperoxidase catalyzes the oxidation of $C1^-$ by H_2O_2 to yield hypochlorous acid (HOCL; the active ingredient in Chlorox R bleach (55).

$$H_2O_2 + CL^- + H + \rightarrow HOCL + H_2O.$$

It is generally accepted that the myeloperoxidase-H_2O_2-CL- system is the most potent cytotoxic system of the neutrophil. HOCL is approximately 100-1000 times more toxic than either O_2^-or H_2O_2. HOCL is a nonspecific oxidizing and chlorinating agent that reacts rapidly with a variety of biological compounds including sulfhydryls, DNA, pyridine nucleotides, aliphatic and aromatic amino acids, and nitrogen-containing compounds (40).

Interestingly, HOCL does not appear to have the ability to peroxidize polyunsaturated lipids. HOCL also reacts very rapidly with primary amines (RNH2) to yield derivatives that contain the nitrogen- chlorine bond (N-chloramines; RNHCL) (56).

$$HOCL + RNH_2 \rightarrow RNHCL + H_2O.$$

RNHCL possesses the two oxidizing equivalents of H_2O_2 and HOCL however they may be more or less toxic than HOCL, depending on their lipophilicities (membrane permeabilities) (56). Because the sulfonic acid residue is negatively charged at physiological pH, TauNHCL is very hydrophilic (membrane impermeable), making it long-lived and relatively nontoxic. Intracellular (neutrophil) concentrations of taurine have been estimated to be approximately 26 mM ,although TauNHCL is a potent oxidizing agent which is capable of oxidizing certain compounds in free solution, it cannot gain access into the intracellular compartment of most cells and thus cannot mediate cytolysis (40).

A second class RNHCL are lipophilic in nature and are quite cytotoxic. These RNHCL are very short-lived owing to their ability to rapidly penetrate the membrane lipid bilayer and react with intracellular components. An example of this class of oxidants is monochloramine (NH_2CL), the reaction product generated from the interaction between HOCL and ammonia (NH_3) (40).

"$NH_3 + HOCL \rightarrow NH_2C1 + H_2O$.

NH_2CL has been shown to be produced by neutrophils and is significantly more toxic than HOCL toward bacteria and certain eukaryotic cells (40). The mechanisms by which myeloperoxidase-derived HOCL and RNHCL damage cells and tissue remain speculative. These oxidants may mediate toxicity directly via sulfhydryl oxidation, hemoprotein bleaching, protein and amino acid degradation and inactivation of essential metabolic cofactors (e.g. NADH) and DNA (40).

In addition, they found that these oxidants produced a concentration-dependent biphasic response with electrically-stimulated ileal smooth muscle strips that is characterized by a transient enhancement of neurally stimulated contraction followed by marked inhibition (57).

These data suggest that neutrophil-derived oxidants may play an important role in the pathogenesis of the depressed intestinal contractility observed in patients with IBD. Furthermore, Thomas and coworkers have demonstrated that sub lethal concentrations of neutrophil derived oxidants may be potent mutagens *in vitro* suggesting a possible link between chronic inflammation and neoplastic transformation (56).

4.3. Physiological consequences of uncontrolled oxidant and free radical production in IBD

In addition to their direct toxic effects mentioned above, reactive metabolites may also mediate epithelial and mucosal injury indirectly by altering the protease/antiprotease balance that normally exists within the intestinal interstitium. For example, neutrophil-derived oxidants (e.g., HOCL and possibly RNHCL) inactivate protease inhibitors such as α1-protease inhibitor and α2-macroglobulin present in the extracellular fluid (plasma, lymph), thus allowing for dysregulated proteolysis by phagocyte-derived elastase (55).

Taken together, these data suggest that oxidative inactivation of important protease inhibitors coupled to the oxidant-mediated activation of metalloproteinases creates an environment favorable for elastase-, collagenase-, and gelatinase-mediated degradation of the mucosal interstitial matrix and epithelial cells (40). These data suggest that leukocyte-derived oxidants may play an important role in the pathophysiology of the depressed intestinal contractility observed in patients with IBD.

Finally, nontoxic concentrations of certain leukocyte-derived oxidants have been demonstrated to be potent mutagenic factors *in vitro*, suggesting a plausible correlation between chronic gut inflammation and neoplastic transformation in the distal bowel (56).

4.4. Estimating free radical production in inflammatory bowel disease

Measurement of oxidative injury in inflammatory bowel disease, has commonly relied on the assessment of oxidatively modified marker molecules (58).The increased levels of malondialdehyde and 4 hydroxynonenal, found in colonic biopsies from inflammatory bowel disease patients provide evidence for excess lipid peroxidation reactions (59). This is also true of the increased breath ethane and pentane excretion in these patients, which are non invasive markers of lipid peroxidation, and have been correlated with disease activity in most cases (60).

With regard to protein damage, the carbonyl content of cells / tissues has been widely used as a convenient marker of oxidative protein damage (61) . Where as the nitration of tyrosine residues in proteins to 3-nitrotyrosine is an indication of the presence of peroxynitrite modified proteins (62).

In colonic biopsies from Crohn's disease and ulcerative colitis patients, the protein carbonyl content has been reported to be increased (30), as has the immunohistochemical expression of 3-nitrotyrosine in mainly the lamina propria mononuclear cells of both Crohn's disease and ulcerative colitis mucosa (47).

Obvious correlations with disease activity, however, have never been established. Mucosal DNA oxidation in human inflammatory bowel disease has been evaluated in one study only, in which the DNA oxidation product, 8-hydroxy-2-deoxyguanosine, was found to be increased in Crohn's disease biopsies (30).

4.5. Molecular genetic alterations in IBD

The results of D'Inca *et al.* who analyzed the concentration of 8-oxo-7,8-dihydro-2,-deoxyguanosine (8-OH-dG)in colorectal biopsies, have shown that patients with UC and dysplasia had significantly increased mucosal 8-OH-dG concentrations. The concentrations were significantly higher in older patients with long standing disease, and dysplasia had a significantly increasing effect on 8-OHd-G levels. This confirms that oxidative DNA damage accumulates with the duration of the inflammation, and is mostly increased in dysplasias. Oxidative stress contributes to molecular carcinogenesis in IBD through DNA damage by targeting key genes for cellular homeostasis (63). Alterations of the cyclin-dependent kinase inhibitor p16 were found in about two thirds of dysplasias (64).

Furthermore, an up-regulation of cell adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule1 (ICAM-1) in intestinal tissues in both IBD patients and animal models of IBD has been reported Moreover, heatshock proteins, such as HSP70, also seem to protect colonic tissue against dextran sodium sulphate (DSS)-colitis by an inhibition of intestinal mucosal cell death induced by ROS

and suppression of pro-inflammatory cyokines, including TNFα , antioxidants significantly reduced TNFα induced genetic damage (65).

These results provide an excellent, detailed understanding of the molecular events in IBD-associated carcinogenesis. However, the more practical question is as follows: how pronounced are the molecular genetic differences between sporadic carcinogenesis and IBD-associated carcinogenesis in the colorectum, and how can the particular pattern of molecular alterations in IBD-associated carcinogenesis be applied to cancer surveillance in diagnostic routine (66). MSI is found in about 15% of sporadic colorectal cancer, caused by alterations in the DNA mismatch repair genes. It is interesting that principally the same two pathways were observed in IBD-associated colorectal carcinogenesis (66) .

6. SUMMERY

Inflammatory bowel disease (IBD) still presents major challenges to the understanding of its cause, mechanisms of inflammation, and therapeutic choices to control the damaged tissue.. While many gaps in our knowledge still exist, the last two decades have witnessed an unprecedented progress not only in the etiology but mainly in the mechanisms underlying the chronic inflammatory response .Immunological abnormalities triggered by genetic and environmental factors are thought to be important in its pathogenesis.

An abnormal response to normally occurring factors would suggest a genetic predisposition to the development of IBD. Genetic studies have indicated associations between IBD and major histocompatibility complex (MHC) class II antigens within some populations. The immune response of IBD patients is characterized by a predominance of TH2 cytokines, and the preferential activation of TH cells by epithelial cells.

Recently, the intestinal microflora have been thought to be an important and essential environmental factor in their etiology. There are two major hypotheses which explain the relationship between the intestinal microflora and the pathogenesis of IBD. The first hypothesis is that IBD are caused by abnormal immunological responses to the normal intestinal microflora. In this case, disorders of the mucosal innate immune systems, such as functional disorders of NOD, lead to excessive immune responses and cause mucosal inflammation of the gut. The second hypothesis is that IBD are caused by fundamental, abnormal changes in the intestinal microflora. In this case, the changes in the composition of the intestinal microflora lead to pathogenic responses and cause mucosal inflammation of the gut.

In recent years, increasing attention has given to the role of reactive oxygen metabolites in the pathogenesis of inflammatory bowel disease It has been proposed that, inflammation of mucosa causes impairment of antioxidant defense mechanism, and makes tissue more susceptible to oxidative damage increased oxidative stress and decreased antioxidant defenses in colonic mucosal biopsies of patients with inflammatory bowel disease.

There are several lines of indirect evidence which suggest that the chronically inflamed intestine or colon may be subjected to considerable oxidative stress and thus susceptible to oxidative injury: first, it is well known that phagocytes are activated by certain pro-inflammatory mediators such as leukotriene B4 (LTB4) or platelet activating factor (PAF) to release large amounts of potentially cytotoxic reactive oxygen metabolites into the interstitial compartment .

Enhanced synthesis of LTB4 and PAF have been demonstrated in mucosal samples obtained from patients with active IBD. Second, there are several reports that have demonstrated that phagocytic leukocytes (monocytes, neutrophils, macrophages) obtained from patients with active IBD respond to various proinflammatory stimuli with enhanced reactive oxygen metabolism when compared to cells obtained from healthy volunteers. Third, a preliminary report suggests, using low level chemiluminescence as an index of active oxygen generation, that oxy radical formation is enhanced in mucosa of animals with experimental colitis when compared to normal controls.

Finally, it is well known that certain drugs (e.g. 5aminosalicylic acid) used clinically to attenuate the mucosal inflammation and injury associated with IBD are potent antioxidants and free radical scavengers.

Also oxidative stress and reactive oxygen species (ROS) play an important role in tumor progression and ulcerative colitis associated carcinogenesis through lipid peroxidation and DNA damage resulting in transforming, accumulating mutations, and genetic instability. In addition to these chemical effects on the DNA molecule, recent studies have shown that regarding their role as second messengers, ROS also have complex signaling effects on tumor growth, cellular survival, and metastasis formation.

Normally, most cells and tissue are protected from the injurious effects of reactive oxygen metabolites by the action of certain antioxidant enzymes such as superoxide dismutase (SOD), catalase and GSH peroxidase. However, it has recently been determined that the human colon contains relatively small amounts of these antioxidant enzymes when compared to tissue such as the liver. These data suggest that the oxidant defenses of the colon may be overwhelmed during times of chronic inflammation and thus susceptible to oxidative tissue injury.

7. REFERENCES

1. Chinyu Su and Lichtenstein GR: Epidemiology of inflammatory bowel disease. Feldman: Sleisenger & Fordtran's *Gastrointestinal and Liver Disease* 8; 109:2499-2500 (2006)

2. Assche GV, Vermeire S and Rutgeerts P: Focus on Mechanisms of Inflammation in Inflammatory Bowel Disease Sites of Inhibition: Current and Future Therapies. *Gastroenterology Clinics* 35; 4 (2006)

3. Orholm M, Binder V, Sorensen TI, Rasmussen LP, Kyvik KO: Concordance of inflammatory bowel disease among Danish twins: Results of a nationwide study. *Scand J Gastroenterology* 35:1075 (2000)

4. Heuschen UA, Hinz U, Allemeyer EH, Stem J, Lucas M, Autschbach F, Herfath C, Heuschen G : Backwash ileitis is strongly associated with colorectal carcinoma in ulcerative colitis. *Gastroenterology*; 120(4):841-7(2001)

5. Stenson WF: Genetics of inflammatory bowel disease. Goldman: Cecil Medicine; 23rd ed.; Chapter 144(2007)

6. Chinyu Su and Lichtenstein GR: Inflammatory bowel disease. Feldman: Sleisenger & Fordtran's *Gastrointestinal and Liver Disease* 8; 108, 109:2459-2538(2006)

7. Strober W, Fuss I and Mannon P: The fundamental basis of inflammatory bowel disease, Science in Medicine *The Journal of Clinical Investigation* 117: 514-521(2007)

8. Hanauer SB: Inflammatory Bowel Disease: Epidemiology, Pathogenesis and Therapeutic opportunities. *Inflamm Bowel Dis* 1: s3-9(2006)

9. Stoll M, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA, Rosenstiel P, Albrecht M, Croucher PJ, Seegert D, Nikolaus S, Hampe J, Lengauer T, Pierrou S, Foelsch UR, Mathew CG, Lagerstrom-Fermer M, Schreiber S: Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat Genet* 36:476-80 (2004)

10. Mamula P, Markowitz JE, Baldassano RN: Pediatric Inflammatory Bowel Disease, cytokines and Inflammatory Bowel Disease 3; 59-69 (2008)

11. Laharie D, Debeugny S, Peeters M, Van Gossum A, Gower-Rousseau C, Belaiche J, Fiasse R, Dupas JL, Lerebours E, Piotte S, Cortot A, Vermeire S, Grandbastien B, Colombel JF: Inflammatory bowel disease in spouses and their offspring. *Gastroenterology* 120:816-9 (2001)

12. Lashner BA and Loftus JE: true or false? The hygiene hypothesis for Crohn`s disease. American Journal of *Gastroenterology* 101:1003-4 (2006)

13. Amre DK, Larnbrette P, Law L, Krupoves A, Chotard V, Costea F, *Grimard G*, Israel D, Mack D, Seidman EG: Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn`s disease: a case control study. *American Journal of Gastroenterology* 101:1005-11(2006)

14. Bemstein CN, Rawsthorne P, Cheang M and Blanchard JF: A population based case control study of potential risk factors for IBD. *American Journal of Gastroenterology* 101:993-1002(2006)

15. Garcfa RLA, Ruigomez A and Panes J: Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease. *Gastroenterology* 130:1588-94 (2006)

16. Moum B, Ekbom A, Vatn MH, Aadland E, Sauar J, Lygren I, Schulz T, Stray N, Fausa O : Inflammatory bowel disease: Re-evaluation of the diagnosis in a prospective population-based study in southeastern Norway. *Gut* 40:328 -32(1997)

17. William J, Sandborn WJ, Colombel JF, Enns R, Feagan BG, Hanauer SB, Lawrance IC, Panaccione R, Sanders M, Schreiber S, Targan S, Deventer SV, Goldblum R, Despain D, Hogge GS, D.V.M., Rutgeerts P: Natalizumab induction and maintenance therapy for Crohn's disease. *N Engl J Med* ; 353:1912-25 (2005)

18. Saeed S and Kugathasan S: Epidemiology of Pediatric Inflammatory Bowel Disease, *Pediatric Inflamm Bowel Dis* 4; 83:102 (2008)

19. Ellingson JL, Anderson JL, Koziczkowski JJ, Radcliff RP, Sloan SJ, Allen SE and Sullivan NM: Detection of viable Mycobacterium avium subsp. paratuberculosis in retail pasteurized whole milk by two culture methods and PCR. *J Food Prot* 68:966-72 (2005)

20. Ghosh S, Armitage E, Wilson D, Minor PD and Afzal MA: Detection of persistent measles virus infection in Crohn disease: current status of experimental work. *Gut* 48:748-52 (2006)

21. Katz JA: Prevention is the best defense: Probiotic prophylaxis of pouchitis. *Gastroenterology* 124:1535-8 (2003)

22. Lakatos PL, Szamosi T and Lakatos L: Smoking in inflammatory bowel diseases: Good, bad or ugly? *World Journal of Gastroenterology* 14; 13(46):6134-9 (2007)

23. Rubin DT and Hanauer SB: Smoking and inflammatory bowel disease. *European Journal of Gastroenterology Hepatology 12:855-62 (2000)*

24. Riddell RH, Kirsner JB and Shorter RG: Pathology of idiopathic inflammatory bowel disease. In: Kirsner JB, ed. *Inflamm Bowel Dis* 427 (2000)

25. Stadnicki A and Colman RW: Experimental models of inflammatory bowel disease. Arch Immunol .*Ther. Exp* (Warsz) 51:149-55(2003)

26. Drossman DA and Ringel Y: Psychosocial factors in ulcerative colitis and Crohn`s disease. In: Sartor RB, Sandborn WJ, ed. Kirsner's Inflammatory Bowel Diseases, 6th ed. Philadelphia : WB Saunders 340 (2004)

27. Kirsner JB, Shorter RG. : Recent developments in "nonspecific" inflammatory bowel disease (first of two parts) *N Engl J Med*;306:775–85(1982)

28. Fantone JC, Ward PA.: Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am J Pathol* 107:395–418 (1982)

29. Flohe L, Beckmann R, Giertz H. : Oxygen-centered free radicals as mediators of inflammation. In: Sies H, editor. Oxidative stress. London: Academic Press 403–36 (2006)

30. Lih-Brody L, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, Weissman Gs, Katz S, Floyd RA, McKinley MJ, Fisher SE, Mullin GE.: Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease . *Dig Dis Sci* 41: 2078–86 (1996)

31. Rhian MT: Reactive oxygen species, Vascular Oxidative Stress, and Redox Signaling in Hypertension *American Heart Association*, Inc.44:248-252 (2004)

32. Radhika D ,John T, Hancock ,Steven JN :Oxidative stress signaling. *Diabetes Metab Res Rev*;4: 121 -149 (2003)

33. Medina S, Martinez M, Hernanz A: Antioxidants inhibit the human cortical neuron apoptosis induced by hydrogen peroxide , tumour necrosis factor alpha, dopamine and beta- amyloid peptide 1- 42. *Free Radic Res* 36:1179-84 (2002)

34. Dickinson1 PJ, Carrington AL, Frost GS, Boulton AJM : Neurovascular disease , antioxidants and glycation in diabetes. *Diabetes Metab Res Rev*18:260-272 (2002)

35. Gutteridge C : Lipid peroxidation and antioxidants as biomarker of tissue damage. *Clin. Chem* 41(12):1918-28 (1995)

36. Soloway RD and Weinmman SA: Oxidative stress as a cause of biliary lithiasis . New possibilities for medical therapy of stones or a false alarm . *Hepatology* 32(3):670-1(2000)

37. Vincent AM and Feldman EL: New Insights Into The Mechanisms of Diabetic Neuropathy. *Reviews in Endocrine & Metabolic Disorders* 5:227-236 (2004)

38. Tepel M, Echelmeyer M, Orie NN , Ziedek W: Increased intracellular reactive oxygen species in patients with end-stage renal failure : effect of haemodialysis . *Kidney Int* 58:2,867-72 (2000)

39. Simmonds NJ, Allen RE, Stevens TR, Van Someren RN, Blake DR, Rampton DS: Chemiluminescence assay of mucosal active oxygen metabolites in inflammatory bowel disease. *Gastroenterology* 103:186–96 (1992)

40. Grisham MB : Effect of 5-aminosalicylic acid on ferrous sulfate-mediated damage to deoxyribose. *Biochem Pharmacol* 39 : 2060-2062 (1990)

41. Hollander D. The intestinal permeability barrier: A hypothesis as to its regulation and involvement in Crohn's disease. *Scand J Gastroenterol* 27:721–6 (1992)

42. Gardiner KR, Anderson NH, Rowlands BJ, Barbul A: Colitis and colonic mucosal barrier dysfunction. Gut 37:530–5 (1995)

43. Ingram LT, Coates T, Aden J, Higgens C, Baebner R, Boxer L: Metabolic, membrane and functional responses of human polymorphonuclear leukocytes to platelet activating factor. *Blood* 59:1259-66 (1982)

44. Sharon P, Stenson WF : Enhanced synthesis of leukotriene B 4 by colonic mucosa in inflammatory bowel disease.*Gastroenterology* 86:453- 60 (1984)

45. Kitahora T, Suzuki K, Asakura H, Yoshida T, Suematsu M, Watanabe M, Aiso S, Tsuchiya M :Active oxygen species generated by monocytes and polymorphonuclear leukocytes in Crohn's disease. *Dig Dis Sci* 33:951-5(1988)

46. Alican I, Kubes P: A critical role for nitric oxide in intestinal barrier function and dysfunction. *Am J Physiol* 270:225–37(1996)

47. Singer II, Kawka DW, Scott S, Weidner JR, Mumford RA, Riehl TE, Stenson WF: Expression of inducible nitric oxide synthase and nitrotyrosine in colonic epithelium in inflammatory bowel disease. *Gastroenterology* 111:871–85 (1996)

48. Whittle BJ: Nitric oxide in gastrointestinal physiology and pathology. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. New York: Raven 267–94(1995)

49. Roediger WE, Lawson MJ, Nance SH, Radcliffe BC: Detectable colonic nitrite levels in inflammatory bowel disease—mucosal or bacterial malfunction? *Digestion* 35:199–204 (1986)

50. Kimura H, Hokari R, Miura S, Shigematsu T, Akiba Y, Kurose I, Higuchi H, Fujimori H, Tsuzuki Y, Serizawa H, Ishii H: Increased expression of an inducible isoform of nitric oxide synthase and the formation of peroxynitrite in colonic mucosa of patients with active ulcerative colitis.*Gut* 42:180–7 (1998)

51. Oudkerk Pool M, Bouma G, Visser JJ, Kolkman JJ, Tran DD, Meuwissen SG, Pena AS: Serum nitrate levels in ulcerative colitis and Crohn's disease. *Scand J Gastroenterol*. 30:784–8 (1995)

52. Tauber AI, Babior BM : Neutrophil oxygen reduction: the enzymes and the products. *Adv Free Rad Biol Med* 1 : 265-307 (1985)

53. Winterbourn CC : Myeloperoxidase as an effective inhibitor of hydroxyl radical production. Implications for the oxidative reactions of neutrophils. *J Clin Invest* 78 : 545-550 (1986)

54. Britigan BE, Coffman TJ, Buettner GR : Spin trapping evidence for the lack of significant hydroxyl radical production during the respiration burst of human phagocytes using a spin adduct resistant to superoxide-mediate destruction. *J Biol Chem* 265:2650-6 (1990)

55. Weiss SJ : Tissue destruction by neutrophils. *N Engl J Med* 320 : 365-376 (1989)

56. Thomas EL, Grisham MB, Jefferson MM: Myeloperoxidase- dependent effect of amines on functions of isolated neutrophils. *J Clin Invest* 72:441-454 (1983)

57. Moummi C, Gullikson GW, Grisham MB, Gaginella TS: Differential effects of reactive oxygen metabolites on neurally stimulated and nonstimulated guinea pig ileum. *J Pharmacol Exp Ther* 265:266 271 (1991)

58. Rumley AG, Paterson JR : Analytical aspects of antioxidants and free radical activity in clinical biochemistry. *Ann Clin Biochem* 35: 181–200 (1998)

59. Chiarpotto E, Scavazza A, Leonarduzzi G, Camandola S, Biasi F, Teggia PM, Garavoglia M, Robecchi A, Roncari A, Poli G: Oxidative damage and transforming growth factor beta 1 in pretumoral and tumoral lesions of human intestine. *Free Rad Biol Med* 22: 889–94 (1997)

60. Sedghi S, Keshavarzian A, Klamut M , Eiznhamer D, Zarling EJ: Elevated breath ethane levels in active ulcerative colitis: evidence for excessive lipid peroxidation. *Am J Gastroenterol* 89: 2217–21 (1994)

61. Chevion M, Berenshtein E, Stadtman ER: Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Rad Res* 33: S99–108 (2000)

62. Ischiropoulos H, Al-Mehdi AB: Peroxynitrite-mediated oxidative protein modifications. FEBS Lett 364: 279–82 (1995)

63. R. D'Inca, R. Cardin, L. Benazzato, I. Angriman, D. Martines, G.C. Sturniolo : Oxidative DNA damage in the mucosa of ulcerative colitis increases with disease duration and dysplasia. *Inflamm. Bowel Dis* 10:23–27 (2004)

64. F. Fogt, A.O. Vortmeyer, H. Goldmann, T.J. Giordano,M.J. Merino, Z. Zhuang: Comparison of genetic alterations in colonic adenoma and ulcerative colitis associated dysplasia and carcinoma, *Hum. Pathol* 29:131–136 (1998)

65. Y. Yuan, F. Li, M.W. Dewhirst, C.Y. Li,: Tumor necrosis factor alpha is a potent endogenous mutagen that promotes cellular transformation. *Cancer Res* 66: 11565–11570 (2006)

66. R.A. Risques, P.S. Rabinovitch, T.A. Brentnall : Cancer surveillance in inflammatory bowel disease: new molecular approaches. *Curr. Opin. Gastroenterol* 22: 382–90 (2006)

Key Words: Inflammatory Bowel Disease, Pathogenesis, Oxidative Stress, Review

Send correspondence to: Hazem Almenier, Mansoura Emergency Hospital, Mansoura, Egypt, Tel: 0105826828 E-mail: Hazem_almenier@yahoo.com

http://www.bioscience.org/current/vol4E.htm