Involvement of oxidative stress and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in inflammatory bowel disease

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The pathophysiology of inflammatory bowel disease involves excessive immune effects of inflammatory cells against gut microbes. In genetically predisposed individuals, these effects are considered to contribute to the initiation and perpetuation of mucosal injury. Oxidative stress is a fundamental tissue-destructive mechanisms that can occur due to the reactive oxygen species and reactive nitrogen metabolites which are released in abundance from numerous inflammatory cells that have extravasated from lymphatics and blood vessels to the lamina propria. This extravasation is mediated by interactions between adhesion molecules including mucosal addressin cell adhesion molecule-1 and vascular cell adhesion molecule-1 on the surface of lymphocytes or neutrophils and their ligands on endothelial cells. Thus, reactive oxygen species and adhesion molecules play an important role in the development of inflammatory bowel disease. The present review focuses on the involvement of oxidative stress and adhesion molecules, in particular mucosal addressin cell adhesion molecule-1, in inflammatory bowel disease.

Key Words: ROS, oxidative stress, MAdCAM-1, IBD

Although the etiology of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) remains uncertain, the interplay of environmental, genetic and immunological factors against bacterial flora is believed to underlie the generation of IBD.⁽¹⁾ Uncontrolled and excessive host immune responses, during which oxidative stress-like reactive oxygen species (ROS) and free radicals are produced from inflammatory cell infiltrates in the gut mucosa, are known to trigger mucosal injury and induce inflammation.

IBD is characterized by the extravasation of numerous inflammatory cells, including neutrophils and lymphocytes. Adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) mediate a series of immune responses and gut inflammation. Among the adhesion molecules that are upregulated in IBD, MAdCAM-1 is considered to be preeminent for the development of chronic gut inflammation. MAdCAM-1 expression is induced on the surface of lymphatic vessels by ROS and by inflammatory cytokines such as tumor necrotic factor (TNF)- α and interleukin (IL)-1 β . MAdCAM-1 has been implicated in the selective recruitment of lymphocytes to sites of inflammation in the gut. Thus, oxidative stress and MAdCAM-1 play important roles in IBD development by mediating the movement and accumulation of lymphocytes into gut interstitium and by causing mucosal injury.

The present review focuses on the involvement of oxidative

stress and MAdCAM-1 during the development of IBD.

Oxidative Stress

Oxidative stress primarily arises and causes tissue injury when the cytotoxic effects of ROS and free radicals overwhelm elimination of their cytotoxic effects by antioxidants. ROS, which are comprised of singlet oxygen, superoxide anions, hydroxyl radicals, and hydrogen peroxide including free radicals, are all generated as by-products of the normal metabolism of molecular oxygen. A broad definition of ROS includes hydroperoxyl, peroxyl and alkoxyl radicals, hydroperoxide, hypochlorous acid, ozone, nitric monoxide, and nitrogen dioxide. ROS can directly impair any oxidizable molecule.

Oxidative Damage by ROS

Excessive levels of ROS attack and impair almost all cellular components, including cell membranes, lipids, proteins, enzymes and DNA, and consequently cause apoptotic cell death. Regarding the effect of ROS on the cell membrane, it is known that the polyunsaturated fatty acids in the cell membrane lipid bi-layer have two or more carbon double bonds within their structure susceptible to oxidative attack.⁽²⁾ Sequential attack against these bonds by hydroxyl radicals ($\cdot\text{OH}$) converts the membrane lipids into oxidized phospholipids (lipid peroxidation). The accumulation of peroxidized lipids accelerates disruption of cell membrane integrity that occurs when the ability of the cell to remove excessive products of hydroxyl radicals and their precursors, in particular the products of hydrogen peroxide (H_2O_2), fails. This failure and the subsequent increase in ROS results in decreased function of transmembrane enzymes, transporters, receptors and other membrane proteins, which are consequently degraded.^(3,4) Moreover, colonic epithelia disintegrate because of the ROS-induced increase in mucosal permeability.^(5,6)

Next, proteins and enzymes, which are predominant constituents of the cells, are also the target of ROS and oxidative stress. Thus, the $\cdot\text{OH}$ radical also attacks, and abrogates many proteins and enzymes. The toxic oxidative effects of $\cdot\text{OH}$ include the induction of protein conformational change, which is a major cause of the partial or complete loss of protein function.⁽⁷⁾

Peroxynitrite (ONOO^-) is a potent oxidant and nitrating species that is formed from a rapid reaction between the superoxide anion ($\text{O}_2^{\cdot-}$) and nitric oxide (NO).⁽⁸⁾ ONOO^- easily crosses biological

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membranes, and, despite a relatively short half-life (within 10 ms), it can interact with target molecules in an adjacent cell within one or two cell diameters.⁽⁹⁾ Interestingly, exposure to ONOO⁻ promotes the conversion of tyrosine residues into 3-nitrotyrosine, which cannot be readily phosphorylated. ONOO⁻ thus interferes with cellular signaling that is dependent on tyrosine phosphorylation by protein tyrosine kinases.⁽¹⁰⁾ Tyrosine nitration by ONOO⁻ can either prevent a protein from functioning as the phosphorylated form and/or can mimic the structural change induced by phosphorylation and thereby imitate the consequences of phosphorylation.⁽¹¹⁾ In contrast to 'OH, ONOO⁻ can up- or down-regulate signaling cascades by controlling the activities of protein kinases. This control is achieved by nitration of tyrosine residues, thereby resulting in gain- or loss of function of kinase activity.⁽¹²⁾

Finally, regarding the effect of ROS on DNA, both nuclear DNA⁽¹³⁾ and mitochondrial DNA⁽¹⁴⁾ are also known to be targets of oxidative attack, particularly from 'OH and ONOO⁻, which cause base and sugar hydroxylation⁽¹⁵⁾ as well as breaks in the double strand, leading to adenosine triphosphate depletion, gene mutations and mitochondrial DNA deletions.^(16,17) These changes ultimately induce malignant transformation and apoptotic cell death. Thus, oxidative stress thus harms almost all cellular components.

Noxious Involvement of ROS in IBD

Direct measurement of ROS in cells and tissues is quite difficult because of their short biological half-lives.⁽¹⁸⁾ However, direct quantification of ROS levels in colon biopsy specimens from UC and CD patients using chemiluminescence assays showed that ROS levels in these tissues are considerably increased compared to those in normal mucosa and positively correlate with IBD.^(19–22) Mounting evidences indicate that there are increased levels of reactive nitrogen metabolites (RNM) such as NO in the inflamed IBD mucosa based on analysis of nitric oxide synthase activity.^(23–26) Thus, increased levels of both ROS and RNM are closely correlated with the clinical development of IBD.

Relationship between Adhesive Molecules and Cytokines, and Inflammatory Cells Infiltration and Immune Responses

The consecutive events involved in the extravasation of inflammatory cells from lymphatics and blood vessels to the extravascular space include the following steps: 1, tethering and rolling of the inflammatory cells on the endothelial cell surface; 2, firm attachment to endothelial cells followed by transendothelial migration; and 3, migration toward chemoattractants produced in the lamina propria, which is mediated by the interaction between adhesion molecules on the surface of lymphocytes or neutrophils and their receptors on endothelial cells and *vice versa*. Various cytokines induce the tethering and rolling of neutrophils on vascular endothelial cells through modulation of the interactions between L-selectin and carbohydrate antigen on neutrophils, and P- and E-selectin on endothelial cells.^(27–29) On the other hand interactions between adhesion molecules on the surface of lymphocytes and the adhesion molecule MAdCAM-1 on lymphatic endothelial cells are responsible for lymphocyte tethering.⁽³⁰⁾ At a later stage, neutrophils and lymphocytes strongly adhere to endothelial cells through other adhesion molecules including ICAM-1, VCAM-1 and MAdCAM-1, and consequently transmigrate into lamina propria mucosae. ROS, lipopolysaccharide (LPS) and inflammatory cytokines such as IL-1 β and TNF- α induce the translocation of P-selectin, L-selectin and MAdCAM-1 from intracellular locations to the cell surface. Increased surface expression of P-selectin, ICAM, and MAdCAM-1 is observed in the colon mucosa of patients with IBD.^(31,32) Interaction between inflammatory cells and endothelial cells through these adhesion molecules is thus involved in the development of IBD.

MAdCAM-1 and Its Receptor, $\alpha 4\beta 7$ Integrin

MAdCAM-1 is a 58–66 kDa type 1 transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily, which is comprised of two amino-terminal Ig-like domains and shares a conserved amino acid sequence homology with VCAM-1.⁽³³⁾ The interaction of integrin $\alpha 4\beta 7$ and MAdCAM-1 is involved in cell homing, firm adhesion, and transendothelial cell migration when lymphocytes are recirculated to peripheral lymph nodes under normal conditions and when lymphocytes are recruited to sites of gut inflammation.⁽³⁰⁾ The homing of murine lymphocytes to intestinal mucosa was first discovered to be mediated by a molecule that bound to Peyer's patches high endothelial venules (HEV).⁽³⁴⁾ This molecule is known as lymphocyte Peyer's patches HEV adhesion molecule (LPAM)-1 and was ultimately identified as an integrin, which is a heterodimer of α - and β -subunits. The $\alpha 4$ -subunit of murine LPAM-2 has 84% homology at the amino acid level with the human integrin $\alpha 4$ -subunit.⁽³⁵⁾ An anti-rat $\alpha 4$ blocking antibody inhibited lymphocyte migration to Peyer's patches, indicating that $\alpha 4$ integrin plays an important role in mucosal homing.⁽³⁶⁾

Initially, the murine integrin β -subunit was believed to be a novel molecule. However, it is currently known that the human $\beta 7$ integrin subunit is the human homolog of the murine integrin β -subunit.^(37,38)

MAdCAM-1 is expressed in Peyer's patches HEV and in mesenteric lymph nodes, intestinal mucosal venules in the lamina propria, and lymphoid follicles in the normal murine gut. MAdCAM-1 directly binds to its receptor, $\alpha 4\beta 7$ integrin. Blocking antibodies against either $\alpha 4$ - or $\beta 7$ -subunits abrogate the binding of lymphocytes to MAdCAM-1 *in vivo* and *in vitro*.⁽³⁹⁾ Therefore, MAdCAM-1 binds to both $\alpha 4$ - and $\beta 7$ -subunits.

IBD and MAdCAM-1

Under normal conditions, MAdCAM-1 expression is limited to the endothelium of venules within the lamina propria and submucosa, and to the HEVs of Peyer's patches and mesenteric lymph nodes. In mouse models of IBD in which experimental colitis was induced with dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid (TNB), and also in the inflamed colon of human patients with UC and CD, MAdCAM-1 expression was reported to be increased in the lamina propria and submucosal venules within the inflamed sites of the colon compared to its expression in non-inflamed tissues.^(40–42) MAdCAM-1 transcription is activated through translocation of the activated p50/p65 nuclear factor kappa-B (NF- κ B) complex into the nucleus following proteosomal degradation of phosphorylated I κ B (inhibitor of κ B) in response to several cytokines including TNF- α and IL-1 β .^(43,44) Moreover, experimental studies using a SVEC cell line derived from axillary lymph nodes, and a colon-derived endothelial cell line established from transgenic mice bearing a temperature-sensitive SV40 large T antigen, have shown that TNF- α stimulates MAdCAM-1 expression through activation of tyrosine kinase, p38 and p42/p44 mitogen-activated protein kinase (MAPK), and NF- κ B/poly-ADP ribose polymerase (PARP) signaling cascades.^(45,46)

The addition of cytochrome P-450 (CYP450) 3A4 inhibitors such as bergamottin and 6',7'-dihydroxybergamottin (DHB) to cultured SVEC endothelial cells demonstrated that these CYP450 inhibitors blocked TNF- α -induced MAdCAM-1 expression and lymphocyte adhesion *in vitro*.⁽⁴⁷⁾ Interestingly, our very recent study showed that the angiotensin II type 1 receptor antagonist (AT1R antagonist), candesartan, can be used as a novel therapy for IBD. We demonstrated that this AT1R antagonist blocked intranuclear translocation of the activated p50/p65 NF- κ B complex in a p38 MAPK independent manner and thereby downregulated TNF- α -induced MAdCAM-1 expression, resulting in the amelioration of colitis.

Fig. 1. Localization of NF- κ B p65 protein in the presence and the absence of Candesartan during TNF- α stimulation. Cells were pretreated with or without Candesartan and then stimulated with TNF- α . Immunofluorescent co-staining of NF- κ B p65 protein and cell nuclei stained using DAPI was subsequently performed. Quoted from ref. 48.

ration of colitis (Fig. 1).⁽⁴⁸⁾ It has also shown that there is a significant attenuation of MAdCAM-1 expression, inflammatory cell infiltration and mucosal damages during DSS-induced colitis in mice lacking AT1R gene compared to these parameters in wild-type mice.^(48,49) This result suggests that TNF- α can induce MAdCAM-1 expression by three different pathways.

Blocking the pathway by which TNF- α induces MAdCAM-1 expression is thus considered to be useful for IBD treatment. Several studies have been carried out to search for potent candidate blockers of TNF- α -induced MAdCAM-1 expression that could be used for IBD treatment. Such candidates include a component present in grapefruit and grapefruit peel oil, its derivatives; bergamottin and DHB,⁽⁴⁷⁾ CYP450 inhibitor, troglitazone,⁽⁵⁰⁾ which is a γ -subtype of a peroxisome proliferator-activated receptor (PPAR- γ) ligand that blocks phosphorylation of p65 NF- κ B, and candesartan,⁽⁴⁸⁾ which, as mentioned above, is an agent that blocks translocation of the activated p50/p65 NF- κ B complex into the nucleus.

Conclusion and Perspectives

It has been established that ROS and MAdCAM-1 play a critical role in the development of IBD by mediating enhanced extravasation of lymphocytes. Future development and study of neutralizing or blocking antibodies, and chemicals that target molecules involved in the development of IBD, (Table 1), will ensure that detailed molecular mechanisms that underlie the occurrence and perpetuation of gut inflammation will be elucidated in the future.

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Abbreviations

IBD	inflammatory bowel diseases
UC	ulcerative colitis
CD	Crohn's disease
ROS	reactive oxygen species
ICAM-1	intercellular adhesion molecule 1
VCAM-1	vascular cell adhesion molecule-1
MAdCAM-1	mucosal addressin cell adhesion molecule-1
TNF	tumor necrotic factor
IL	interleukin
NO	nitric oxygen
RNM	reactive nitrogen metabolites
LPS	lipopolysaccharide
Ig	immunoglobulin
HEV	high endothelial venules
LPAM	lymphocyte Peyer's patches HEV adhesion molecule
DSS	dextran sulfate sodium
TNB	trinitrobenzene sulfonic acid
NF- κ B	nuclear factor kappa-B
MAPKs	mitogen-activated protein kinases

Table 1. Therapeutic molecular target for IBD under clinical application and investigation

molecular targets and name		drug product	disease	efficacy for human	references
TNF- α	Infliximab	antibody	UC, CD	effective	51, 52, 53
	Adalimumab	antibody	UC, CD	effective	54, 55
	Certolizumab pegol	antibody	CD	effective	56
	Etanercept	antibody	CD	invalid	57
	Golimumab	antibody	UC, CD	effective	58
IFN- γ	Fontlizumab	antibody	CD	invalid	59
IL-6 receptor	Tocilizumab	antibody	CD	effective #	60
IL-12/23	Ustekinumab	antibody	CD	effective #	61
CD20	Rituximab	antibody	UC, CD	underway	
$\alpha 1\beta 1$ integrin	no name	antibody	DSS colitis	N/A for human	62
$\alpha 4$ integrin	AJM-300	chemical	CD	effective #	63
	Natalizumab	antibody	UC, CD	effective	64
$\alpha 4\beta 7$ integrin	Vedolizumab	antibody	UC, CD	effective	65, 66
$\beta 7$ integrin	rhuMab b7	antibody	UC	underway	
PSGL-1	no name	antibody	DSS colitis	N/A for human	67
ICAM-1	no name	antibody	DSS colitis	N/A for human	68
VCAM-1	no name	antibody	DSS colitis	N/A for human	69
MAdCAM-1	PF-00547659	antibody	UC	underway	

#: result in phase II trial, N/A: not applicable, DSScolitis: dextran sodium sulfate-induced experimental colitis
 PSGL-1: P-selectin glycoprotein ligand-1

PARP poly-ADP ribose polymerase
 CYP450 cytochrome P-450
 DHB 6',7'-dihydroxybergamottin

AT1R angiotensin II type 1 receptor
 PPAR- γ γ -subtype of peroxisome proliferator-activated receptor

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