BUTTERWORTH HEINEMANN

Decreased zinc absorption in guinea pig models of acute and chronic ileitis

Manuel Sandoval, Mark J.S. Miller,* Halina Sadowska-Krowicka,* Xiao-Jing Zhang,* and James M. Hempe

Department of Pediatrics, Divisions of GI/Nutrition and Neonatology, LSU School of Medicine, New Orleans, LA USA*

The biochemical defects underlying decreased Zn absorption in inflammatory bowel disease have not been identified. In vitro studies have suggested that reactive oxygen species (e.g., hypochlorous acid and superoxide), produced by phagocytic cells during the inflammatory response, mobilize Zn from metallothionein (MT) and other proteins that bind Zn via sulfhydryl groups. We evaluated the in vivo effects of intestinal inflammation on Zn absorption and Zn binding to intestinal MT and cysteine-rich intestinal protein (CRIP), possible components of a carrier-mediated intestinal Zn transport system. Guinea pig models of acute (NaOCl luminal perfusion) and chronic (2,4,6-trinitrobenzene sulfonic acid [TNBS] injection) ileitis were used. The rate of ^{65}Zn absorption from the isolated ileum and the molecular distribution of ^{65}Zn in mucosal cytosol determined by size-exclusion HPLC were measured after 1 hr of perfusion in the acute model and after 7 days in the chronic model. Zinc absorption was significantly lower in guinea pigs with either chronic or acute inflammation. In both models, decreased binding of ^{65}Zn to MT and CRIP was also observed. The results indicate that decreased Zn absorption during intestinal inflammation may be mediated by the effects of oxidants on the transport activity of intestinal proteins that bind Zn via sulfhydryl groups. (J. Nutr. Biochem. 6:534–539, 1995.)

Keywords: zinc absorption; metallothionein; cysteine-rich intestinal protein; inflammation; inflammatory bowel disease

Introduction

Despite extensive research on the immunological, biochemical, and epidemiological aspects of inflammatory bowel diseases (IBD), the etiology and pathogenesis of these diseases remain unclear.[1,2] Many characteristics of IBD-related growth failure, including delayed sexual maturity, dermatitis, hypogeusia, and lack of appetite are consistent with clinical symptoms of Zn deficiency,[3,4] such that Zn deficiency has been proposed as a contributing factor in IBD.[5] Also significant changes in Zn metabolism have been reported in patients with IBD, including ulcerative colitis[6] and Crohn's disease,[7,8] suggesting that intestinal inflammation adversely affects Zn absorption and may result in Zn deficiency. Naveh et al.[9] used a rat model of acetic acid–induced acute intestinal inflammation to show that inflammation reduced the rate of ^{65}Zn absorption from ligated intestinal segments. A cascade effect where inflammation adversely affects Zn transport leading to Zn deficiency which may in turn compromise gut repair or exacerbate the inflammatory response seems likely.

Lack of more specific information about the effects of intestinal inflammation on Zn absorption stems largely from a lack of information about the mechanism(s) of Zn transport. It has recently been proposed[10,11] that cysteine-rich intestinal protein (CRIP) may function as a diffusible intracellular carrier in transcellular Zn transport across the intestinal mucosa, and that Zn transport by CRIP is competitively inhibited by metallothionein (MT). Fliss et al.[12–14] using in vitro methods showed that oxidants produced by neutrophils during the inflammatory response (e.g., superoxide, hydrogen peroxide, hypochlorous acid) can mobilize Zn from MT and other metalloproteins that bind Zn via sulfhydryl groups. Since both MT[15,16] and CRIP[17] bind Zn via cysteine sulfhydryl residues, Zn transport may be altered during inflammation by the effects of oxidants on Zn

Address reprint requests to Dr. J.M. Hempe at the Department of Pediatrics, LSU School of Medicine, 1542 Tulane Avenue, New Orleans, LA 70112, USA.
Received 6 October 1994; accepted 19 April 1995.

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(95)00102-6

binding by components of the carrier-mediated transport system.

The purpose of the present experiments was to evaluate the effects of inflammation on Zn absorption and Zn binding to CRIP and MT in two guinea pig models of intestinal inflammation. Acute inflammation was induced by perfusing guinea pig ileum with a solution of sodium hypochlorite (NaOCl), resulting in the production of hypochlorous acid, a reactive oxygen species produced by phagocytic cells during an inflammatory response.[18] Chronic inflammation was produced by luminal injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS), a hapten that stimulates the cell-mediated immune system[19] and induces an inflammatory response with morphological and biochemical changes typical of Crohn's disease.[20–23]

Methods and materials

Animals and diets

Female Hartley guinea pigs (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 250 to 300 g were housed in stainless steel cages in an environmentally controlled room with a 12-hr light/dark cycle. A commercial diet (Purina Guinea Pig Chow 5025, Ralston Purina, St. Louis, MO, USA) containing 70 mg of Zn/kg and tap water were supplied ad libitum. For all experiments, guinea pigs were anesthetized by intramuscular injection of ketamine (40 mg/kg of BW) and xylazine (4 mg/kg of BW), and anesthesia was maintained with methoxyflurane by inhalation as required. Care and treatment of experimental animals received prior institutional approval and followed recommended guidelines.[24]

Reagents

Unless otherwise noted, all chemicals were at least reagent grade and were obtained from Sigma Chemical Co. (St. Louis, MO, USA) or U.S. Biochemical Co. (Cleveland, OH, USA).

TNBS ileitis (chronic model)

The model used in these experiments was previously described in guinea pigs[23] as an adaptation of a rat colitis model.[20] Under sterile conditions, the distal small intestine of anesthetized guinea pigs was exteriorized after a midline laparotomy. A segment (10 cm) proximal to the ileo-cecal valve was cleared of residual luminal contents with sterile saline. Ileitis was induced by transmural injection of TNBS (30 mg/kg of BW in 50% ethanol) into the lumen using a 26-gauge needle. Control guinea pigs received the same treatment except that the intestine was injected with saline (0.9% NaCl). The ileum was then returned to the abdominal cavity and the peritoneum sutured layer by layer. Each guinea pig was placed in a "warm water bed" until recovery from the anesthesia, then returned to its cage for 1 week prior to measuring Zn absorption. Previous reports have shown that TNBS chronic ileitis in this model is characterized by hemorrhage and thickening of the intestinal wall, mucosal leakage of protein and nitrite, and development of a prosecretory condition.[23]

NaOCl ileitis (acute model)

Acute ileitis was induced in anesthetized guinea pigs by perfusion of the terminal ileum with sodium hypochlorite (NaOCl; J.T. Baker, Phillipsburg, NJ USA) as previously described.[25] Briefly, the distal small intestine was exteriorized after a midline laparotomy and a 10 cm segment of the terminal ileum was flushed with warm saline. After manual expression of the intestinal contents, polyethylene cannulae were inserted and tied into each end of the isolated segment. The intestine was then returned to the abdominal cavity and perfused at a rate of 0.5 mL/min with a buffered salt solution with or without NaOCl) (1 μmol/L). After 1 hr, the segment was rinsed with saline, and the rate of Zn absorption was measured as described below.

Rate of Zn absorption

In both the chronic and acute model of inflammation, the rate of Zn absorption from the affected intestine was determined by measuring ^{65}Zn absorption from ligated segments as previously described.[11] The rate of absorption was measured 1 week after treatment with TNBS or saline in the chronic model and immediately after perfusion with NaOCl or saline in the acute model. Each guinea pig was anesthetized and the intestine exteriorized and cleansed as described earlier. The guinea pig was then placed on a warm heating pad to maintain surface temperature at approximately 37°C. In the TNBS experiment, ligatures were placed around the intestine immediately distal to the affected intestinal segment and 10 cm proximal to the first ligature. In the NaOCl experiment, each end of the perfused segment immediately adjacent to the cannulae was ligated. The isolated segment was then injected with 0.5 mL of saline containing 100 μmol/L of Zn as $ZnSO_4 \cdot 7H_2O$ and 200 kBq of ^{65}Zn (Du Pont NEN, Wilmington, DE USA) and returned to the body cavity. After 30 min, the segment was excised and flushed with cold homogenization buffer (HEPES, 10 mmol/L, pH 8.0; NaCl, 154 mmol/L; phenylmethylsulfonyl fluoride, 0.2 mmol/L; leupeptin, 0.6 mg/L; pepstatin A, 0.9 mg/L) into a test tube to collect quantitatively the ^{65}Zn remaining in the lumen. The ^{65}Zn content of the luminal fluid and the excised intestinal segment was determined by gamma spectroscopy using a Model 1272 Clinigamma (Pharmacia LKB, Gaithersburg, MD USA) multichannel analyzer using background adjustments corrected for individual detector efficiency. Due to the potential for intersample interference on this instrument, samples with high gamma activity (>3,000 cpm) were measured individually. Uptake was calculated as the disappearance of ^{65}Zn from the lumen, intestinal accumulation of luminal Zn as the amount of ^{65}Zn remaining in the tissue of the intestinal segment after flushing, and absorption by subtracting intestinal accumulation from uptake. Isotope data were converted to molar concentrations based on the Zn content of the injected radioisotope solution and converted to rates based on the length of the absorption period.

Molecular distribution of intracellular ^{65}Zn

After removing the luminal contents, the excised intestinal segment was placed on a cold glass plate, slit longitudinally, and the mucosal layer removed from the underlying submucosa by gently scraping the luminal surface with a glass microscope slide. Homogenates of intestinal mucosa were prepared 1:3 (wt/vol) with homogenization buffer using a Potter-Elvehjem glass-Teflon tissue grinder. After centrifugation at 40,000*g* for 30 min at 4°C (Model RC2-B, Sorval, Newtown, CT USA), the supernatant fraction was removed and filtered (Millex-GS, 0.22 μm, Millipore, Bedford, MA USA). Intracellular Zn-binding constituents were separated by size exclusion HPLC using a 1 × 30 cm Superdex 75 column (Pharmacia, Piscataway, NJ USA) and eluted at 0.5 mL/min (approximately 300 psi) with buffered saline (HEPES, 10 mmol/L, pH 8.0; NaCl, 154 mmol/L; NaN_3, 0.2 g/L). The ^{65}Zn content of the collected fraction (0.5 mL) was determined by gamma spectroscopy. The MT concentration of the intestinal mucosa of guinea pigs in the TNBS experiment was determined by ^{109}Cd-hemoglobin affinity assay as previously described.[26,27]

Table 1 Effect of intestinal inflammation on zinc absorption from guinea pig ileum

Treatment	Zn uptake (nmol)	Intestinal accumulation of luminal Zn (nmol)	Rate of Zn absorption (pmol/min)	Molecular ^{65}Zn distribution	
				MT (%)	CRIP (%)
Chronic model (n = 6)					
control	27.9 ± 1.5	22.7 ± 1.7	346 ± 57*	27.3 ± 1.9*	10.8 ± 1.7*
TNBS	21.6 ± 2.9	19.4 ± 2.5	153 ± 42†	13.8 ± 1.9†	4.3 ± 0.5†
Acute model (n = 5)					
control	23.0 ± 1.6	19.4 ± 1.8	239 ± 14*	26.6 ± 4.2	8.8 ± 0.6*
NaOCl	20.6 ± 1.6	19.1 ± 1.6	100 ± 23†	17.6 ± 2.6	5.1 ± 0.8†

Values are means ± SEM. Chronic intestinal inflammation was produced by transmural injection of TNBS into the ileum 7 days prior to measurement of zinc absorption. Acute intestinal inflammation was produced by ileal perfusion with NaOCl for 1 hour prior to measurement of zinc absorption. Molecular ^{65}Zn distribution was determined by size-exclusion HPLC and shows the proportion of radioisotope eluting in fractions containing MT or CRIP.
*†For each model, values in a column with different superscripts are significantly different, $P < 0.05$.

Statistical analysis

All data are expressed as the mean ± SEM and were analyzed by unpaired t-test with significance determined at $P < 0.05$. Variance homogeneity was confirmed by variance ratio test prior to statistical analysis.

Results

TNBS ileitis (chronic model)

The rate of Zn absorption from isolated guinea pig ileum was approximately 2-fold lower in the group treated 7 days earlier with TNBS to induce ileitis than in controls (*Table 1*). Neither uptake nor intestinal accumulation of luminal Zn during the 30 min absorption period were significantly altered. The MT concentration of the distal ileum determined by Cd-hemoglobin affinity assay was similar in control (5.1 ± 0.6 μg/g of intestine) and TNBS-treated (5.8 ± 0.1 μg/g of intestine) guinea pigs, suggesting that reduced absorption is not attributable to the inhibition of Zn transport via the induction of MT by chronic inflammation. This is supported by the observation that the proportion of intracellular ^{65}Zn bound to MT was not elevated in the TNBS group (*Figure 1*) as is typically observed when intestinal MT levels are high. In contrast, proportionally less ^{65}Zn was bound to both MT and CRIP in the group treated with TNBS (*Table 1, Figure 1*) than in the control group, suggesting that chronic inflammation reduced the binding of luminal Zn by both proteins. Bowel wall thickening previously described in this chronic model of inflammation[23] was also observed.

NaOCl ileitis (acute model)

Results similar to those observed in the chronic model were also observed in the acute model. For example, the rate of Zn absorption from isolated guinea pig ileum immediately after perfusion with NaOCl was less than 50% of that observed in controls (*Table 1*), while cumulative uptake and intestinal accumulation of luminal Zn were not significantly different. Mucosal MT concentration was not measured in the acute model because of the short time course of the experiment. As in the TNBS-treated group, the proportion of intracellular ^{65}Zn bound to both MT and CRIP was decreased in guinea pigs treated with NaOCl to induce an acute inflammatory response (*Table 1, Figure 2*). Perfusion of the isolated intestinal segment with NaOCl resulted in the production of a highly viscous mucus that was not observed in control animals perfused with saline.

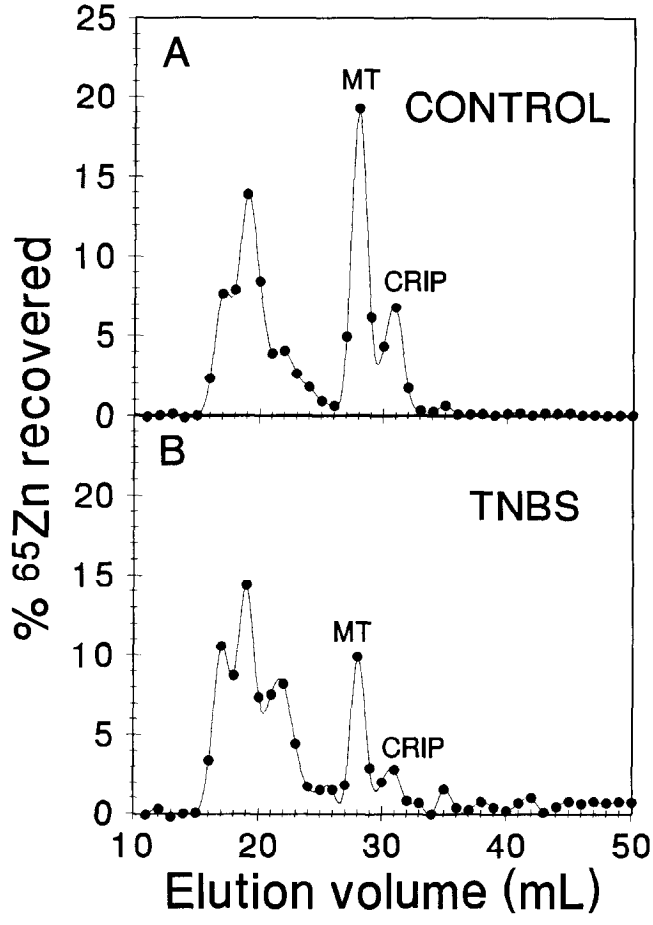

Figure 1 Molecular distribution of ^{65}Zn in homogenates of intestinal mucosa determined by size-exclusion HPLC: chronic model. Guinea pigs received a transmural injection of saline (A) or TNBS (B) into the terminal ileum. After 7 days in vivo isolated ileal segments were injected intraluminally with ^{65}Zn to measure Zn absorption and label intracellular Zn-binding constituents.

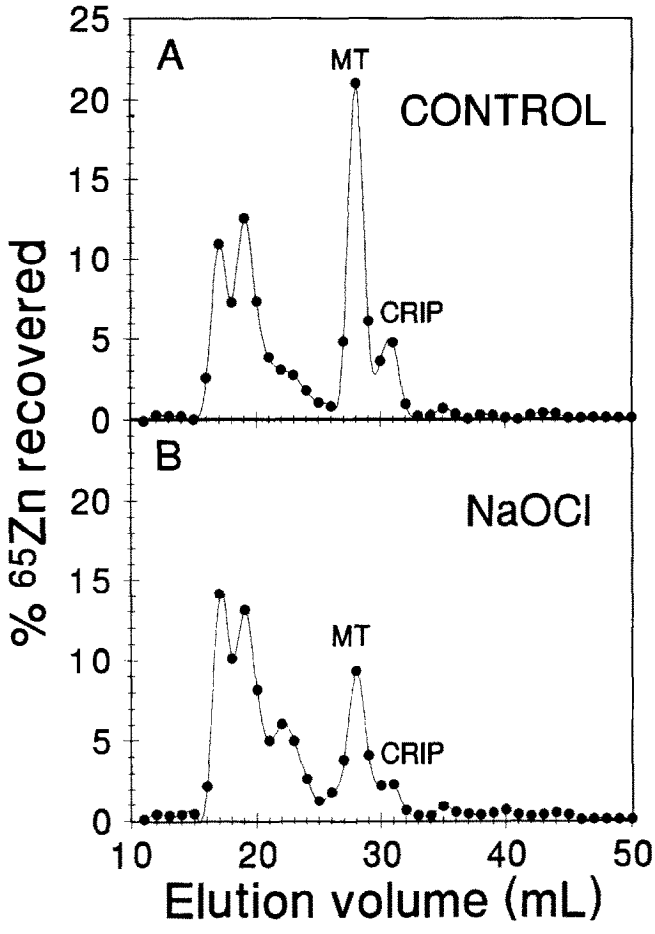

Figure 2 Molecular distribution of ^{65}Zn in homogenates of intestinal mucosa determined by size-exclusion HPLC: acute model. Guinea pig ileum was perfused with saline (A) or NaOCl (B) for 1 hr, after which the perfused segment was isolated and injected intraluminally with ^{65}Zn to measure Zn absorption and label intracellular Zn-binding constituents.

Discussion

Compared with controls, the rate of Zn absorption from the terminal ileum was more than 50% lower in guinea pig models of both chronic (TNBS) and acute (NaOCl) intestinal inflammation. A reduced rate of Zn transport indicates a reduced capacity to absorb ingested Zn, and thus a decrease in dietary Zn bioavailability due to intrinsic factors that alter components of the Zn transport mechanism in this disease state. Because the concentration of intestinal MT was unchanged by inflammation, the decreased rate of Zn absorption cannot be attributed to competitive binding by this known inhibitor of Zn transport. Lack of elevated intestinal MT in our animal models of ileitis agrees with data obtained from humans showing that the concentrations of both MT and SOD were lower in tissue obtained from intestinal resections in patients with Crohn's disease or ulcerative colitis.[28]

Although intestinal MT concentration was unchanged, the low rate of Zn absorption from ligated intestinal segments during inflammation was associated with decreased binding of luminal Zn to both MT and CRIP, putative components of the carrier-mediated Zn transport system. The similar relationship between the rate of Zn absorption and ^{65}Zn bound to CRIP in the soluble fraction of mucosal homogenates, i.e., both decreased during inflammation, adds to the circumstantial evidence that CRIP may have a functional role as a carrier in Zn transport. This relationship suggests a possible mechanism to explain the depressing effects of inflammatory bowel disease on intestinal Zn transport.

Inflammatory bowel disease is characterized by granulocyte infiltration and activation and the exaggerated release of inflammatory mediators.[29] Activated phagocytic cells (e.g., neutrophils and macrophages) produce toxic oxygen products like superoxide, hydrogen peroxide, and hypochlorous acid as part of the inflammatory response to invading microorganisms. Although toxic oxygen species derived from inflammatory cells are an important defense mechanism, the generation of free radicals and other reactive oxygen metabolites can also directly or indirectly lead to tissue injury. Fliss[30] showed that oxidants produced by activated neutrophils, especially hypochlorous acid, can oxidize methionine and cysteine residues in cellular proteins and suggested that the oxidation of protein sulfhydryl groups may constitute a primary cause of tissue injury at sites of inflammation. Since many proteins bind Zn via sulfhydryl groups,[31] some of the adverse effects of inflammation may be mediated by the effects of oxidants on protein-Zn interactions.

In a series of experiments, Fliss and colleagues[12–14] demonstrated, in vitro, that physiological concentrations of reactive oxygen species, especially hypochlorous acid, can mobilize cellular Zn from MT and other Zn-binding proteins. These investigators used both cultures of arterial cells and heart tissue sections as well as purified proteins to show that the effect of the oxidants was specific to proteins that bind Zn via sulfhydryl groups. This observation is particularly relevant to the possible effects of inflammation on Zn absorption since both MT[15,16] and CRIP[17] bind Zn via sulfhydryl groups. Also, both guinea pig models of inflammation used in the present experiments have been shown to result in the production of reactive oxygen species; NaOCl forms hypochlorous acid in solution[25] and superoxide and hydrogen peroxide are produced in TNBS-treated animals.[20] This suggests that oxidants produced in the intestine by granulocyte infiltration during an inflammatory response may decrease the binding of luminal Zn to CRIP and thereby reduce Zn absorption. Decreased binding of Zn to MT may reflect the proposed free radical scavenging properties of the protein.[15]

Although our data support previous observations or suggestions of depressed Zn absorption during intestinal inflammation,[6–9] it should be emphasized that other factors unrelated to oxidant interactions with CRIP or MT could be responsible for the observed changes in Zn absorption. For example, kinetic studies have indicated that Zn is absorbed by both carrier-mediated and passive diffusion pathways,[32] possibly representing transcellular and paracellular routes. Without kinetic data (i.e., measuring absorption at many different luminal Zn concentrations) it is impossible to de-

termine which pathway is affected in the inflammed guinea pig intestine. Since the TNBS model of chronic ileitis is characterized by hemorrhage and thickening of the intestinal wall, mucosal leakage of protein and nitrite, and development of a prosecretory condition,[23] some effects on passive Zn diffusion may be expected. Similarly, intestinal perfusion with NaOCl in the present study resulted in the obvious production of a highly viscous mucus secretion. Although no evidence of decreased uptake or accumulation of luminal ^{65}Zn in the mucosal cytosol was apparent in these studies, changes in gut morphology or secretory state may alter Zn absorption from inflamed guinea pig intestine in a manner unrelated to changes in Zn-binding by CRIP or MT.

It is also important to remember that evidence of a role for CRIP in carrier-mediated intestinal Zn transport remains largely circumstantial. CRIP gene expression is most prevalent in the small intestine and in mature villus cells,[33] and the protein is not detected in liver or pancreas.[16] This suggests a specific role for CRIP in nutrient absorption or intestinal metabolism. Induction of intestinal MT by high dietary Zn intake inhibits both the rate of Zn absorption and Zn-binding to CRIP in mucosal homogenates,[11] indicative of an antagonistic relationship between MT and CRIP in Zn transport. Kinetic studies[32,34,35] have shown that Zn is absorbed by both passive and carrier-mediated mechanisms and that the carrier-mediated component is up-regulated during short-term Zn deficiency. The binding of luminal Zn to CRIP determined by size-exclusion HPLC shows saturation at higher luminal Zn concentrations[11] and is therefore consistent with a carrier function. Although CRIP gene expression is not affected by short-term Zn deprivation,[36] MT gene expression is down-regulated by low Zn intake.[11,36] This indicates that carrier-mediated Zn transport may be homeostatically regulated through the effects of Zn status on MT rather than CRIP. Collectively, these data suggest antagonistic roles for CRIP and MT as carrier and inhibitor, respectively, in transcellular Zn transport. The depressing effect of inflammation on Zn transport and Zn binding to CRIP in the present study is consistent with the proposed model.

In summary, these experiments provide further evidence that intestinal inflammation reduces Zn absorption. Moreover, the data suggest that the mode of action may be mediated by the interaction between reactive oxygen species produced as part of the inflammatory response and intestinal Zn-binding proteins with proposed roles in carrier-mediated Zn transport. These in vivo results support in vitro reports[12–14] that oxidants alter the interactions between Zn and proteins that bind Zn via sulfhydryl groups. Many proteins bind Zn in this manner, including DNA transcription factors (e.g., zinc-finger proteins), protein kinase C, and alcohol dehydrogenase.[31,37] This suggests that many of the adverse effects of inflammation, or beneficial effects like feedback regulation of inflammatory processes, could be mediated by similar interactions between oxidants and Zn-binding proteins.

Acknowledgments

This work was funded by the Crohn's & Colitis Foundation of America, Inc. and NIH grant R01 HD31885 to MJSM.

References

1 Apgar, J.T. (1991). Newer aspects of inflammatory bowel disease and its cutaneous manifestations: a selective review. *Sem. Dermatol.* **10,** 138–147

2 Shanahan, F. and Targan, S. (1992). Medical treatment of inflammatory bowel disease. *Am. Rev. Med.* **43,** 125–133

3 Cousins, R.J. (1985). Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol. Rev.* **65,** 238–309

4 Hambidge, K.M. (1989). Mild zinc deficiency in human subjects. In *Zinc in Human Biology*. (C.F. Mills, ed.), p. 281–296, Springer-Verlag, Berlin

5 Hendricks, K.M. and Walker, W.A. (1988). Zinc deficiency in inflammatory bowel disease. *Nutr. Rev.* **46,** 401–407

6 Ainley, C.C., Cason, J., Carlson, L.K., Slavin, B.M., and Thompson, R.P.H. (1988). Zinc status in inflammatory bowel disease. *Clin. Sci.* **75,** 277–283

7 Fleming, C.R., Huizenga, K.A., McCall, J.T., Gildea, J., and Dennis, R. (1981). Zinc nutrition in Crohn's disease. *Dig. Dis. Sci.* **26,** 865–870

8 Kruis, W., Rindfleish, G.E., and Weinzier, M. (1985). Zinc deficiency as a problem in patients with Crohn's disease and fistula formation. *Hepato-gastroenterology* **32,** 133–134

9 Naveh, Y., Lee-Ambrose, L.M., Samuelson, D.A., and Cousins, R.J. (1993). Malabsorption of zinc in rats with acetic acid-induced enteritis and colitis. *J. Nutr.* **123,** 1389–1395

10 Hempe, J.M. and Cousins, R.J. (1991). Cysteine-rich intestinal protein binds zinc during transmucosal zinc transport. *Proc. Natl. Acad. Sci. USA* **88,** 9671–9674

11 Hempe, J.M. and Cousins, R.J. (1992). Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. *J. Nutr.* **122,** 89–95.

12 Fliss, H., Menard, M., and Desai, M. (1991). Hypochlorous acid mobilizes cellular zinc. *Can. J. Physiol. Pharmacol.* **69,** 1686–1691

13 Fliss, H. and Menard, M. (1992). Oxidant-induced mobilization of zinc from metallothionein. *Arch. Biochem. Biophys.* **293,** 195–199

14 Fliss, H. and Menard, M. (1991). Hypochlorous acid-induced mobilization of zinc from metalloproteins. *Arch. Biochem. Biophys.* **287,** 175–179

15 Sato, M. and Bremner, I. (1993). Oxygen free radicals and metallothionein. *Free Radical Biol. Med.* **14,** 325–337

16 Vallee, B.L. and Auld, D.S. (1990). Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* **29,** 5647–5659

17 Kosa, J.L., Michelsen, J.W., Louis, H.A., Olsen, J.I., Davis, D.R., Beckerle, M.C., and Winge, D.R. (1994). Common metal ion coordination in LIM domain proteins. *Biochemistry* **33,** 468–477

18 Schraufstatter, I.U., Browne, K., Harris, A., Hyslop, P.A., Jackson, J.H., Quehenberger, O., and Cochrane, C.G. (1990). Mechanisms of hypochlorite injury of target cells. *J. Clin. Invest.* **85,** 554–562

19 Kim, H.S. and Berstad, A. (1992). Experimental colitis in animal models. *Scand. J. Gastroenterol.* **27,** 529–537

20 Grisham, M.B., Volkmer, C., Tso, P., and Yamada, T. (1991). Metabolism of trinitrobenzene sulfonic acid by the rat colon produces reactive oxygen species. *Gastroenterology* **101,** 540–547

21 Yamada, Y., Marshall, S., Specian, R.D., and Grisham, M.B. (1992). A comparative analysis of two models of colitis in rats. *Gastroenterology* **102,** 1524–1534

22 Morris, G.P., Beck, P.L., Herridge, M.S., Depew, W.T., Szewczak, M.R., and Wallace, J.L. (1989). Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* **96,** 795–803

23 Miller, M.J.S., Sadowska-Krowicka, H., Chotinaruemol, S., Kakkis, J.L., and Clark, D.A. (1993). Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmacol. Exp. Ther.* **264,** 11–16

24 National Research Council, Committee on Care and Use of Laboratory Animals, and Institute of Laboratory Animal Resources Commission on Life Sciences. (1985). *Guide for the Care and Use of Laboratory Animals*. National Institutes of Health, Bethesda, MD

25 Miller, M.J.S., Zhang, X.J., Barkemeyer, B., Grisham, M.B., Sad-

owska-Krowicka, H., Eloby-Childress, S., and Clark, D.A. (1992). Rabbit gut permeability in response to histamine chloramines and chemotactic peptide. *Gastroenterology* **103,** 1537–1546

26 Eaton, D.L. and Toal, B.F. (1982). Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. *Toxicol. Appl. Pharmacol.* **66,** 134–142

27 Hempe, J.M., Carlson, J.M., and Cousins, R.J. (1991). Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1 alpha. *J. Nutr.* **121,** 1389–1396

28 Mulder, T.P., Verspaget, H.W., Janssens, A.R., de Bruin, P.A., Pena, A.S., and Lamers, C.B. (1991). Decrease in two intestinal copper/zinc containing proteins with antioxidant function in inflammatory bowel disease. *Gut* **32,** 1146–1150

29 Ward, P.A., Warren, J.S., and Johnson, K.J. (1988). Oxygen radicals, inflammation, and tissue injury. *Free Radical Biol. Med.* **5,** 403–408

30 Fliss, H. (1988). Oxidation of proteins in rat heart and lungs by polymorphonuclear leukocyte oxidants. *Mol. Cell Biochem.* **84,** 177–188

31 Vallee, B.L. and Falchuk, K.H. (1993). The biochemical basis of zinc physiology. *Physiol. Rev.* **73,** 79–118

32 Steel, L. and Cousins, R.J. (1985). Kinetics of zinc absorption by luminally and vascularly perfused rat intestine. *Am. J. Physiol.* **248,** G46–G53

33 Birkenmeier, E.H. and Gordon, J.I. (1986). Developmental regulation of a gene that encodes a cysteine-rich intestinal protein and maps near the murine immunoglobulin heavy chain locus. *Proc. Natl. Acad. Sci. USA* **83,** 2516–2520

34 Hoadley, J.E., Leinart, A.S., and Cousins, R.J. (1988). Relationship of 65Zn absorption kinetics to intestinal metallothionein in rats: effects of zinc depletion and fasting. *J. Nutr.* **118,** 497–502

35 Davies, N.T. (1980). Studies on the absorption of zinc by rat intestine. *Br. J. Nutr.* **43,** 189–203

36 Levenson, C.W., Shay, N.F., Hempe, J.M., and Cousins, R.J. (1994). Expression of cysteine-rich intestinal protein in rat intestine and transfected cells is not zinc dependent. *J. Nutr.* **124,** 13–17

37 Coleman, J.E. (1992). Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu. Rev. Biochem.* **61,** 897–946