Mice with combined disruption of Gpx1 and Gpx2 genes have colitis

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Esworthy, R. Steven, Richard Aranda, Martín G. Martín, James H. Doroshow, Scott W. Binder, and Fong-Fong Chu. Mice with combined disruption of Gpx1 and Gpx2 genes have colitis. Am J Physiol Gastrointest Liver Physiol 281: G848–G855, 2001.—Glutathione peroxidase (GPX)-1 and gastrointestinal (GI) epithelium-specific GPX (GPX-GI), encoded by Gpx1 and Gpx2, provide most GPX activity in GI epithelium. Although homozygous mice deficient in either the Gpx1 or Gpx2 gene appeared to be normal under standard housing conditions, homozygous mice deficient in both genes, double-knockout (KO) mice, had symptoms and pathology consistent with inflammatory bowel disease. These symptoms included a high incidence of perianal ulceration, growth retardation that started around weaning, and hypothermia that resembled that observed in calorie-restricted mice, even though the double-KO mice in our study were allowed to eat ad libitum. The growth retardation and hypothermia were components of cachexia, which is fatal in a high percentage of mice. Histological examination revealed that the double-KO mice had a high incidence of mucosal inflammation in the ileum and colon but not in the jejunum. Elevated levels of myeloperoxidase activity and lipid hydroperoxides were also detected in colon mucosa of these homozygous double-KO mice. These results suggest that GPX is essential for the prevention of the inflammatory response in intestinal mucosa.

inflammatory bowel disease; lipid hydroperoxides; growth retardation; hypothermia; mitochondrial superoxide dismutase

Selenium-dependent glutathione peroxidases (GPXs) are the major selenoprotein-containing gene family in mammals. The GPXs include four selenium-dependent hydroperoxide-reducing isozymes: GPX-1, gastrointestinal (GI) epithelium-specific GPX (GPX-GI), the secreted plasma GPX (GPX-P), and monomeric phospholipid hydroperoxide GPX (PHGPX), which are encoded by the Gpx1, Gpx2, Gpx3, and Gpx4 genes, respectively. The GPX-1 and GPX-GI isozymes have very similar properties, such as substrate specificity and cytosolic localization (4, 9). They both reduce H₂O₂ and fatty acid hydroperoxides very efficiently and reduce lipid hydroperoxides poorly. Unlike the ubiquitous GPX-1, GPX-GI is expressed only in epithelium, most highly in the gastrointestinal epithelium. Although GPX-P and PHGPX are also present in the GI tract (5, 30), GPX-P is secreted extracellularly, and PHGPX has a substrate specificity distinct from these two cytosolic isozymes. GPX-1 and GPX-GI contribute almost all of the intracellular H₂O₂-reducing activity in the GI tract.

Little is known about the physiological roles of the GPX isozymes. The best available evidence points to GPX-1 having anti-inflammatory activity in animal studies. We observed that Gpx1-knockout (KO) mice were more susceptible to myocarditis than wild-type mice after infection with Coxsackie virus (3). Because the viral antibody titers in Gpx1-KO mice are about one-fifth of those found in wild-type mice, this suggests that, in addition to enhanced susceptibility to inflammation, humoral immune responses may also be impaired in homozygous Gpx1-KO mice. Additionally, the Gpx1-KO mice are more susceptible to neutrophil caused liver injury induced by combined treatment with lipopolysaccharide and galactosamine (21). The kidneys of Gpx1-transgenic mice are more resistant to renal ischemia/reperfusion injury, and these Gpx1-transgenic mouse kidneys have less neutrophil infiltration (20). Neutrophils, also called polymorphonuclear leukocytes, are often referred to as inflammatory cells because they play an important role in inflammation and natural immunity and function to eliminate microbes and necrotic tissues. These results suggest that GPX-1 in heart, liver, and kidney can prevent oxidative injury from the immune/inflammatory response.

We have recently generated homozygous Gpx2-KO mice and have not observed any differential responses.
from wild-type mice under normal housing conditions and after exposure to limited types of treatment such as γ-irradiation to examine crypt regeneration (8). Because the epithelium of the GI tract has high-level coexpression of two very similar isozymes, GPX-1 and GPX-GI, the lack of phenotype in both unchallenged homozygous Gpx1-KO (7, 8, 16) and Gpx2-KO mice suggests a functional redundancy of these two genes in the GI tract. However, these two genes appear to be expressed differentially within the epithelium: the Gpx1 gene is predominately expressed in the vilius, whereas the Gpx2 gene is preferentially expressed in the crypt (6, 9, 30, 34). This suggests that expression of these two genes may be complementary rather than completely redundant.

To investigate the physiological functions of GPX in intestinal epithelial cells, we have generated homozygous double-KO mice deficient in both Gpx1 and Gpx2 gene expression. These double-KO mice have symptoms and histopathology consistent with inflammatory bowel disease (IBD). The three-fourths-KO mice with only a single Gpx1 allele are more prone to inflammation compared with the reciprocal three-fourths-KO mice with a single Gpx2 allele. These results suggest that these two genes are not functionally redundant. In this study, we have provided the first evidence suggesting that both GPX-1 and GPX-GI have anti-inflammatory activity in the intestinal mucosa. These homozgyous double-KO mice may provide a novel animal model in which to study the pathogenesis of IBD.

**EXPERIMENTAL PROCEDURES**

Generation and maintenance of double-KO mice deficient in both Gpx1 and Gpx2 gene expression. Gpx1-KO mice were generated by Ye-Shih Ho (Wayne State University, Detroit, MI) (16) as C57BL/6J (B6) and 129Sv/vj hybrids. The generation of Gpx2-KO mice as B6 and 129Sv hybrids at the City of Hope (COH; Duarte, CA) Transgenic Mouse Core has been described previously (8). These mice were housed at the COH Animal Resources Center in ventilated cage racks (Allentown Caging Equipment, Allentown, NJ). We have followed protocols approved by the COH Research Animal Care Committee, which abides by NIH guidelines, to perform all of the animal studies described in this paper. Sentinel mice housed in the cage containing bedding from the double-KO mice tested positive for Helicobacter hepaticus, H. typhlicus, and H. rodentium by PCR that was performed by the Missouri University Research Animal Diagnostic and Investigative Lab (Columbia, MO). The mice were free of parasites and microorganisms including Salmonella sp., Mycoplasma pulmonis, and Pasteurella pneumotropica. They had no serum antibodies to M. pulmonis or to known murine viruses including Sendai, mouse hepatitis, pneumonia of mice, and ectromelia, retrovirus type 3, GDVII (mouse polio), parvovirus, epidemic diarrhea of infant mice virus, and lymphocytic choriomeningitis. All mice had free access to water and a laboratory rodent diet (chow 5001, Purina Mills, Richmond, IN) that contained 23% protein, 4.5% fat, 6% fiber, and 0.28 ppm selenium as provided by the manufacturer (http://www.labdiet.com). If mice were emaciated, we were obligated to supplement the diet with Nutri-Cal (EVSCO Pharmaceuticals, Brenna, NJ), which has 0.7% protein, 34.5% fat, and 3.8% fiber and is enriched with vitamins A and E. One to two milliliters of Nutri-Cal paste was added on top of the chow every other day. This was a preferred diet judging from the immediate consumption on application.

Tail DNA was used for genotyping of Gpx1- and Gpx2-KO mice by either Southern blot or PCR analysis. For Southern blot analysis, 10 μg of DNA was digested with BanHI or ApaI to determine the genotype of Gpx1 and Gpx2, respectively. After overnight digestion, DNA was resolved on a 0.75% agarose gel, transferred to a ZetaProbe membrane (Bio-Rad, Richmond, CA), and probed with a 32P-labeled and random-primed 3′ EcoRI fragment of mouse Gpx1 cdNA and mouse Gpx2 exon 2 cdNA. The Southern blots were analyzed by phosphorimaging (Molecular Dynamics, Sunnyvale, CA) (8). The PCR primers for the wild-type Gpx1 allele were mPX101F (5′-AAGGGATTCGAGCGGCCTGTTGAGG-3′) and Gpx15 (5′-ACCGTTCACCTGTGACCTCT-3′) to amplify an ~600-bp DNA fragment. The primers for the Gpx1-KO allele were pPNTpgk1 (5′-CAGTTTCTAGCCTTGAAGAACGAG-3′) and Gpx15 to amplify an ~200-bp DNA fragment. The primers for the wild-type Gpx2 allele were MPX206 (5′-CCACCTGGTCTAGAGGACTTA-3′) and MPXin09 (5′-TCCATGGCAACCTAGTGAATT-3′) to amplify an ~600-bp DNA fragment. The primers for the Gpx2-KO allele were MPXin09 and pPNTpgk1 to amplify an ~400-bp DNA fragment. pPNTpgk1 is from the mouse phosphoglycerate kinase poly(A) signal that follows the neomycin phosphotransferase (neo) coding sequence. Both alleles were amplified in the same reaction tubes.

**Metabolic studies.** Rectal temperature was measured with a Thermalert mouse probe (TH-8; Physiomet Instrument, Clifton, NJ) between 6:00 and 8:00 AM for mice under normal housing conditions. To quantify the food and water consumption and feces and urine excretion, mice were placed in metabolic cages with wire grid floors for 24 h. This setting appeared to be stressful for the double-KO mice, as shown by a frequent hunched-over appearance and the presence of loose stools.

**Histology of small and large intestine from mice with altered Gpx1, Gpx2, or Sod2 gene expression.** The mice with altered Gpx1 and Gpx2 genes were killed by halothane overdose (Halocarbon Labs, North Augusta, SC). After the luminal contents were removed, sections of jejunum, ileum, colon, and rectum were rinsed with PBS and then fixed in 10% buffered formalin or Bouin’s fixative for 2–3 h. The fixed GI tissues from homozygous Sod2-KO [deficient in the Mn-superoxide dismutase (SOD) gene] B6D2F2 mice, 15–19 days of age, were obtained from Charles J. Epstein (University of California, San Francisco, CA) (18). The tissues were then dehydrated in ethanol, embedded in paraffin, and sectioned onto slides. The tissue sections were stained with hematoxylin and eosin alone or in combination with the periodic acid-Schiff reagent.

**GPX, myeloperoxidase, and lipid hydroperoxide assays.** The GPX activity was determined on intestinal and colonic epithelium from 42- to 47-day-old mice. Jejunal and ileal epithelium was isolated from the proximal and the distal one-third of small intestine as described previously (8). GPX activity was measured with 60 μM H2O2 and 3 mM GSH at pH 7.3. Myeloperoxidase (MPO) activity was determined with the enzyme that catalyzes the oxidation of 3′,3′,5′,5′-tetramethylbenzidine by H2O2 to yield a chromogen that can be measured at absorbance (A)655 nm (13). Elevation of lipid hydroperoxide (LPO) levels were measured colorimetrically with a commercial kit (LPO assay kit; Cayman, Ann Arbor, MI). This assay measures the hydroperoxide-driven production of thiocyanate ion, which is detected at A550, with a sensitivity of 0.25–5 nmol hydroperoxide. Mice 40 days of age...
were used for this assay. LPO levels were measured after the intestinal lumen was flushed with Ca\(^{2+}\)- and Mg\(^{2+}\)-free PBS. Two-tenths of a gram of jejunum, ileum, or colon was homogenized in 2 ml of ice-cold water. Fifty microliters were taken for protein assay. The remainder of the sample was extracted with methanol and chloroform and measured for LPO spectrophotometrically at \(A_{500}\), following the manufacturer’s recommendation. The protein concentration was determined with a BCA assay (Pierce Chemical, Rockford, IL), with BSA as the standard. Statistical analysis was performed with a two-tailed Student’s \(t\)-test using Excel (Microsoft Office 97, Professional Edition). A \(P\) value < 0.05 was considered significant.

RESULTS

Generation of double-KO mice. Homozygous Gpx1-KO and Gpx2-KO mice were bred to generate heterozygous double-KO mice. These heterozygous double-KO mice were bred to each other; one-sixteenth of the offspring were homozygous double-KO mice as determined by Southern blot (Fig. 1A). One-fourth of the mice were three-fourths-KOs, with either a single Gpx1 or Gpx2 allele. These double-KO and three-fourths-KO mice were then used as breeders to generate double-KO mice. The number of the double-KO mice agreed with the predicted value from Mendelian genetics, suggesting that there was no prenatal selection. Similar numbers of male and female offspring were obtained, suggesting that there was no gender selection. Fertile male and female double-KO mice were raised but were considered high-risk breeders because of their health problems.

The adult (45–47 days old) homozygous double-KO mice had background GPX activity in the mucosa of small and large intestine (Fig. 1B). In general, the GPX activity correlated with the gene dosage for both Gpx1 and Gpx2 genes. Deviations from this correlation were observed as follows: GPX activity in the jejunal mucosa corresponded to the dosage of the Gpx1 but not the Gpx2 gene as shown by the similar GPX levels in Gpx1-KO mice with 0 and 1 Gpx2 alleles. This agreed with our previous finding (9) that jejunal mucosa had a high level of GPX-1 and a low level of GPX-GI. In ileal mucosa, the dosage effect of the Gpx2 allele was evident only in the absence of Gpx1 gene expression. In colonic mucosa, the heterozygous double-KO mice had the same level of GPX activity as wild-type mice.

Weight gain and gross phenotypes of double-KO mice. The homozygous double-KO mice gained weight more slowly than mice of other genotypes or stopped gaining weight completely starting around 16 days of age. Figure 2A shows the typical growth of individual mice in a litter. Among the eight pups, two double-KO mice had the same body weight as their littermates until weaning. In a cohort of 33 double-KO mice, 32 showed growth retardation onset between 16 and 26 days of age. The last mouse started to show growth retardation at 30 days of age. Other symptoms often associated with these homozygous double-KO mice included perianal ulceration (shown in Fig. 2B) as well as anal mucus discharge, diarrhea, and hypothermia. One or more of these symptoms occurred as early as 14 days of age. Approximately 40% of the double-KO mice died or were euthanized because of presumed imminent death, which was judged by their emaciated body condition between 20 and 36 days of age. Autopsies revealed diarrheal stool in edematous colons. No macroscopic or microscopic abnormality was seen in other major organs.

To determine whether lack of food intake contributed to severe growth retardation despite normal intestinal growth in the homozygous double-KO mice, we moni-
stored the amount of rodent chow consumed by mice housed in the metabolic cages. The homozygous double-KO mice (24–49 days old) consumed similar amounts of food (0.16 ± 0.07 g chow·g body wt⁻¹·day⁻¹; n = 11 mice) compared with their littermates (0.10 ± 0.05 g chow·g body wt⁻¹·day⁻¹; n = 18 mice). It is possible that their slower weight gain was associated with cachexia but not anorexia. Mouse body temperature was measured with a rectal thermometer to determine if they were deficient in energy supply because mice on calorie-restriction diets are hypothermic (12, 22). We found that the double-KO mice were hypothermic compared with littermates who were maintained under normal housing conditions; they became more hypothermic after being housed in metabolic cages for 24 h. As shown in Fig. 3A, body temperatures of the younger (24–36 days old) and more mature (40–67 days old) double-KO mice were 37.0 ± 1.1 (mean ± SD) and 35.1 ± 2.2°C, respectively. The body temperatures of their littermates were 37.6 ± 0.6°C for all ages under normal housing conditions. After being housed in metabolic cages for 24 h, the body temperatures of 36-day-old double-KO mice had dropped from 36.2 ± 2.3 to 32.2 ± 1.8°C as shown in Fig. 3B. The control mice did not change their body temperatures significantly after being housed in metabolic cages.

Although the wild-type heterozygous double-KO and homozygous single-KO mice did not have any symptoms, the three-fourths-KO mice occasionally had some symptoms. Approximately 10% of the three-fourths-KO (2 of 22) mice with a single Gpx2 allele had diarrhea, and 17% of reciprocal three-fourths-KO (7 of 40) mice showed perianal ulceration and diarrhea. The Gpx2-containing three-fourths-KO mice had no mortality, but the Gpx1-containing three-fourths-KO had 10% mortality (4 of 40) before 35 days of age. However, none of these three-fourths-KO mice had cachexia, which was often observed in the homozygous double-KO mice.

Inflammation of the small intestine, colon, and rectum. Histological analysis was performed on cross sections of stomach, jejunum, ileum, colon, and rectum after staining with hematoxylin and eosin as shown in Fig. 4. Cross sections from two representative 24-day-old littermates, a homozygous double-KO and a three-fourths-KO male (M, n = 3) and female (F, n = 3) mice, respectively. Double-KO male (♂, n = 1) and female (♀, n = 1) are also shown. Both female and male double-KO mice started to show growth retardation at 21 and 26 days of age. Bottom: perianal ulceration in a double-KO mouse.

Fig. 2. Gross phenotypes of double-KO mice. Top: body weights of a representative mouse litter of 6 three-fourths-KO mice with a single Gpx1 allele and 2 double-KO mice. Squares and circles, three-fourths-KO male (M, n = 3) and female (F, n = 3) mice, respectively. Double-KO male (♂, n = 1) and female (♀, n = 1) are also shown. Both female and male double-KO mice started to show growth retardation at 21 and 26 days of age. Bottom: perianal ulceration in a double-KO mouse.

Fig. 3. A: temperature of homozygous double-KO mice and their littermates (heterozygous double-KO or three-fourths-KO) in normal housing conditions. Values are means ± SD of 2–6 mice. B: hypothermia of double-KO mice before and after housing in metabolic cages. Body temperatures were measured from 36-day-old mice before and 24 h after being housed singly or doubly in metabolic cages. Values are means ± SD of 4 double-KO mice and 6 littermates with Gpx1+/−·Gpx2+/− and Gpx1−/−·Gpx2+/− genotypes.
fourths-KO with a Gpx2 allele, were compared. The three-fourths-KO mouse had normal histology in the GI tract. In contrast, the double-KO mouse had severe inflammation involving the ileum and colon, whereas the jejunum and stomach were unaffected. The inflammation was limited to the mucosa and was rarely transmural. Crypt abscesses were prevalent in ileum, colon, and rectum. These histological features of a mixed inflammatory cell infiltrate, mucin depletion, and occasional crypt distortion are features consistent with human ulcerative colitis.

The severity of infiltration in the distal GI tract was scored with histological parameters in five categories: 1) severity of the inflammatory cell infiltrate in the lamina propria, 2) epithelial cell reactive hyperplasia/atypia, 3) mucin depletion (colon and rectum only), 4) increases in intraepithelial lymphocyte numbers in crypts, and 5) number of inflammatory foci as defined previously (1). Periodic acid-Schiff staining was performed on some sections to confirm the depletion of mucin (data not shown). Table 1, top, demonstrates the progression of intestinal inflammation from distal to proximal bowel in 27 homozygous double-KO mice throughout development. Spontaneous colitis was limited to the distal colon as early as 11 days of age in 11 of the 12 mice analyzed. Colitis involving the proximal colon was only observed in three of twelve 11- to 14-day-old mice. Most mice 15 days of age and older had inflammation in both distal and proximal colon. Ileitis became evident and prevalent in mice at 20–27 days of age. No inflammation was seen in the stomach or jejunum in all animals up to 60 days of age (Table 1 and data not shown). Other major organs, including heart, liver, lung, kidney, testis, and brain, did not have any noticeable changes as determined by histological analysis (data not shown).

The inflammatory histology was negatively correlated with GPX activity level (Fig. 1 and Table 1). Although nearly all homozygous double-KO mice had inflammatory histology, only 6% (1 of 18) of heterozygous double-KO mice showed the same histology. Approximately 16% of three-fourths-KO (3 of 19) mice with a single Gpx2 allele and 50% of three-fourths-KO (16 of 32) mice with a single Gpx1 allele had inflammatory histology.

To determine whether oxidative stress alone could trigger inflammation in mice, we examined the histology of homozygous Sod2-KO mice on a B6D2F2 background. Sod2 encodes mitochondrial SOD, which appears to play a vital role in the reduction of superoxide generated from electron transport, thus maintaining the integrity of mitochondrial enzymes susceptible to direct inactivation by superoxide. Homozygous Sod2-KO mice on different genetic backgrounds die from midgestation to ~19 days after birth (18). Sod2-KO mice on a B6D2F2 background, hybrids of C57BL/6J (B6)
Table 1. Severity of inflammation in mice deficient in Gpx1 and Gpx2 genes

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Fig. 5. Myeloperoxidase (MPO) activity levels in mouse colon. Values are means ± SD. MPO activity was assayed from 8 Gpx1+/−, Gpx2+/−, 6 Gpx1+/+−, Gpx2+/−, 8 Gpx1+/−, Gpx2+/−, and 15 Gpx1+/−, Gpx2−/− mice. *Significantly different from other samples, P < 0.02. One unit/mg of protein is estimated to be equivalent to 4.6 U/g wet tissue.

Fig. 6. Lipid hydroperoxide (LPO) levels in jejunum, ileum, and colon. Heterozygous double-KO (n = 2), three-fourths-KO (n = 1 Gpx1−/−, Gpx2+/− and 4 Gpx1+/−, Gpx2−/−), and homozygous double-KO (n = 5) mice were assayed. Values are means ± SD. * and #Significant difference between columns by Student’s t-test, P < 0.05.

DISCUSSION

We have generated a line of mice deficient in two isozymes that are present in intestinal epithelial cells, Gpx1 and Gpx2, and have provided evidence indicating that these two isozymes contribute nearly all of the GSH-dependent, H2O2-reducing activity in the distal gastrointestinal epithelium. This was demonstrated by the decreasing GPX activity in the intestinal epithelium in those mice with a decreasing number of Gpx1 and Gpx2 alleles. The homozygous double-KO mice had almost no GPX activity in the mucosa of their distal GI tracts.

The expression pattern of Gpx1 and Gpx2 genes differs in different regions of the GI tract. Although both Gpx1 and Gpx2 mRNA are present in jejunal epithelium (8), unlike Gpx1 mRNA which is translated into GPX-1 efficiently, the Gpx2 mRNA does not appear to be translated into GPX-GI efficiently in this region. Also, GPX-1 may be compensating for the lack of GPX-GI in the epithelium of the small intestine but not the large intestine. We conclude this from the following observations: 1) the same level of GPX activity was detected in mice with heterozygous and homozygous deletions of the Gpx2 allele, 2) a higher level of GPX-1 was detected in homozygous Gpx2-KO intestine compared with that in wild-type mice as determined by immunoprecipitation (8, 9), and 3) the same level of GPX-GI was detected in Gpx1-KO and wild-type mouse intestinal mucosa. These observations sug-
suggest that the Gpx1 gene can compensate for the lack of Gpx2 gene expression but not vice versa. Alternatively, this implies that Gpx2 mRNA may be competing for the limiting translational machinery for selenoprotein synthesis because GPX-GI has approximately one-third the specific activity of GPX-1, and the same GPX level is detected in colonic mucosa of wild-type control and heterozygous double-KO mice.

The first sign of abnormality that we observed in these double-KO mice was growth retardation. Weight loss can be caused by either decreased caloric intake or an increase in cachectic cytokine levels, such as tumor necrosis factor-α, interleukin (IL)-1, or IL-6, during inflammation (19). Because these double-KO mice ingested amounts of food similar to those ingested by the control mice, the weight loss was most likely due to an increase in inflammatory cytokines. Small mammals, including mice, that are experiencing caloric restriction can lower body temperature to conserve energy, inducing hypothermia (12, 22). Therefore, cachexia and hypothermia are related pathophysiologically.

GPX activity in the crypt epithelium appears to be most important in the prevention of inflammation. The frequency of IBD-like pathology is inversely correlated with the GPX activity level; nearly all of the homozygous double-KO mice had classic symptoms of IBD, and very few of the wild-type and heterozygous double-KO mice had IBD-like symptoms. Because the three-fourths-KO mice with one Gpx1 allele are more prone to IBD-like pathology compared with those with one Gpx2 allele, this suggests that GPX-GI, which is predominantly expressed in the crypt epithelium, is more protective than GPX-1, which is predominately expressed in mature epithelial cells.

Increased MPO activity and LPO levels in the double-KO mice suggest that GPX prevents neutrophil-activated tissue injury. Neutrophils are the predominant granulocytes in the mediation of mucosal injury, and MPO is a granulocyte-specific enzyme (13, 29). A significantly higher amount of MPO activity was detected in the double-KO mice than in those mice that had at least one copy of the Gpx1 or Gpx2 gene. Because the cytotoxic activity mediated by neutrophils is produced by the respiratory burst that generates reactive oxygen species, we have also determined the level of lipid peroxidation in the mouse GI tract. Significantly higher levels of LPO were detected in the ilea of homozygous double-KO mice than in three-fourths-KO mice. Higher LPO levels were also found in the colons of homozygous double-KO mice than in heterozygous double-KO mice. These results suggest that the inflammatory injury is contributed by activated neutrophils and that GPX protects against such injury. It is possible that mice deficient in both Gpx1 and Gpx2 genes and phagocyte NADPH oxidase activity will not have colitis because this NADPH oxidase generates a large number of oxidants, including H₂O₂, in neutrophils (17, 31).

Hydroperoxides, but not superoxide, appear to mediate the IBD-like symptoms. Mice deficient in the Sod2 gene were found to be hypothermic and very short-lived (18, 24). These Sod2-KO mice had impaired mitochondrial enzymes in the oxidative phosphorylation reaction chain (26, 33) and thus were not able to convert food into metabolic energy efficiently. However, these mice did not have an IBD histology. This shows that there is a unique role for GPX activity as a major mediator of inflammation in the GI tract. However, whether H₂O₂ or fatty acid hydroperoxides mediates the inflammation is not known because GPX can reduce both.

In addition to the weakened host anti-inflammatory response, bacterial colonization appears to play an important role in the pathogenesis of IBD-like symptoms. Our mouse colonies were naturally infected with several Helicobacter species. These pathogen-associated IBD symptoms have been observed mainly in immune-deficient mice (11, 23), and the IBD symptoms present in immunocompetent mice are infrequent and affect only older mice (10, 11, 32). The progression of disease in the double-KO mice at the time of weaning is consistent with the timing of bacterial colonization. Mammals are born without microorganisms. Increases and alterations in intestinal bacterial species occur during the first month after birth (25). Changes in bacterial flora are likely to result from decreases in protective IgA levels and other bactericidal components present in milk or solid food, which alter luminal pH (14, 25, 28). To confirm this hypothesis regarding the role of bacteria, germ-free double-KO mice must be derived to further examine IBD pathology.

Among many existing IBD animal models, the most unique feature of our GPX double-KO mice is the early onset and prevalence of colitis. The most aggressive IBD symptoms to date have previously been reported in IL-10 deficient mice, which develop symptoms at 4 wk of age. Other mouse models, such as those expressing a dominant-negative N-cadherin or mice deficient in the multiple drug resistance gene mdr1a, develop colitis-like symptoms after 12 and 20 wk of age (15, 25, 28). Histological analysis shows that homozygous GPX double-KO mice have inflammation starting in the distal colon as early as 11 days of age, which was the youngest age analyzed. After weaning, the inflammatory changes progressed from distal to proximal colon and then to ileum.

Although reactive oxygen species are clearly involved in inflammation, current views of the function of antioxidant enzymes suggest that these proteins are passively involved in dissipating toxic oxidative species to avoid damage to DNA, proteins, or membrane lipids. Our double-KO mice provide the first clear evidence supporting a role for cytosolic GPX activity in the prevention of IBD-like pathology. Because IBD patients often have selenium deficiency, dietary supplementation with selenium has been recommended. Understanding the mechanism for GPX protection against IBD-like symptoms may provide new ideas for therapeutic reagents, such as the use of GPX-mimic compounds in addition to standard therapies.

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