Paradoxical roles of different nitric oxide synthase isoforms in colonic injury


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Nitric oxide (NO) is a free radical that is largely produced by three isoforms of NO synthase (NOS): neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). NO regulates numerous processes in the gastrointestinal tract; however, the overall role that NO plays in intestinal inflammation is unclear. NO is upregulated in both ulcerative colitis and Crohn’s disease as well as in animal models of colitis. There have been conflicting reports on whether NO protects or exacerbates injury in colitis or is simply a marker of inflammation. To determine whether the site, timing, and level of NO production modulate the effect on the inflammatory responses, the dextran sodium sulfate model of colitis was assessed in murine lines rendered deficient in iNOS, nNOS, eNOS, or e/nNOS by targeted gene disruption. The loss of nNOS resulted in more severe disease and increased mortality, whereas the loss of eNOS or iNOS was protective. Furthermore, concomitant loss of eNOS reversed the susceptibility found in nNOS−/− mice. Deficiencies in specific NOS isoforms led to distinctive alterations of inflammatory responses, including changes in leukocyte recruitment and alterations in colonic lymphocyte populations. The present studies indicate that NO produced by individual NOS isoforms plays different roles in modulating an inflammatory process.

inducible nitric oxide synthase; endothelial nitric oxide synthase; neuronal nitric oxide synthase; dextran sodium sulfate

Nitric oxide (NO) is a free radical that regulates a variety of processes in the gastrointestinal tract, including blood flow, vascular permeability, mucosal defense, leukocyte recruitment, immune regulation, fluid secretion, and intestinal motility (26, 30, 35). NO is largely produced by three isoforms of NO synthase (NOS). Two isoforms, neuronal (nNOS) and endothelial (eNOS), are constitutively expressed and calcium dependent. nNOS is localized predominantly in the central and peripheral nerves but has also been detected in nonneuronal cells, including myocytes, epithelial cells, mast cells, and neutrophils (2, 13, 44, 56). eNOS was first identified in endothelial cells but is also present in epithelial cells, smooth muscle cells, platelets, and T cells (13, 47, 51, 53, 57). The third isoform of NOS is inducible NOS (iNOS) and is calcium independent. It is present in many cell types, including macrophages, neurons, and endothelial cells.

Although both eNOS and nNOS are constitutively expressed, their expression can be modulated by temperature, ischemia, and inflammation. Corticosteroids, bacterial lipopolysaccharide (LPS), and interferon (IFN)-γ decrease nNOS expression, whereas estrogen and testosterone enhance nNOS expression (9, 14). Regulation of eNOS appears to be even more complex. Hypoxia and LPS may increase or decrease expression depending on the cell type (8, 10). Estrogens, IFN-α/β, transforming growth factor-β (TGF-β), and fibroblast growth factor upregulate eNOS expression, and tumor necrosis factor (TNF-α) generally downregulates expression (21, 23). iNOS is markedly upregulated by numerous agents, including TGF-β, TNF-α, IFN-γ, and LPS (3, 16).

Altered regulation of NO has been implicated in many gastrointestinal disease states. More specifically, NO production was shown to be increased in ulcerative colitis (6, 22) and Crohn’s disease (6), toxic megacolon, diverticulitis, and diarrhea, as well as in animal models of intestinal inflammation. However, the relative contributions of the three different NOS isoforms in the regulation of inflammatory responses in the gastrointestinal tract are unclear.

Pharmacological manipulation of NO has yielded inconsistent and conflicting results. NO has been found to either increase or decrease water and electrolyte secretion and to either inhibit or promote inflammation (34). It has been suggested that some of these inconsistencies may be attributed to differential effects depending on dose, tissue, and the nature of the inflammatory/injury state. Some of these discrepancies may also result from differential effects on the activities of the three NOS isoforms. Laszlo et al. (29) found that NOS inhibitors exacerbated LPS-induced intestinal injury and that NO donors reduced the severity of injury. However, when NOS inhibitors were first administered 3 h after LPS they were found to be protective, and these authors speculated that this paradox was the reflection of differences in the temporal sequences of NOS isoform expression (29, 39). Thus it is possible that the NOS isoforms exert varied actions due to their differing location, cell-specific expression, and regulation.

The present study explores the individual roles of the three NOS isoforms in intestinal inflammation to test the hypothesis that the functional effects of NO on inflammatory processes depend on the site of production. The impact of targeted disruption of iNOS (iNOS−/−), eNOS (eNOS−/−), nNOS (nNOS−/−), or both of the constitutive isoforms eNOS and
nNOS (e/nNOS−/−) was assessed by using a standard model of colitis.

**MATERIALS AND METHODS**

**Animals**

Mice with targeted deletion of iNOS were obtained from Jackson Laboratories (Bar Harbor, ME) and were of a C57B6 background (wild-type control animals of this background were obtained from the same supplier). The eNOS−/−, nNOS−/−, and e/nNOS−/− mice were generated as previously described (24, 30) and were of a 129-C57B6 background (wild-type control animals of the same background were generated at the same time and bred alongside the study animals). Animals were matched by age, sex, and body weight. All studies included like-matched wild-type control animals (C57B6 for mice). Wild-type control animals of the same background were obtained from the Jackson Laboratories (Bar Harbor, ME) and were of a C57B6 background (wild-type control animals of this background were obtained from the same supplier). The eNOS−/−, nNOS−/−, and e/nNOS−/− mice. Animals were ear tagged in a blinded fashion so that investigators assessing mice daily were unaware of the genetic background of each group. All animal experiments were performed in accordance with National Institutes of Health guidelines, and protocols were approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital and Harvard Medical School.

**Induction and Assessment of Colitis**

Colitis was induced by addition of dextran sodium sulfate (DSS) to drinking water (2.5% wt/vol in distilled water, mol wt 40,000, lot #3073B; ICN Biomedical, Aurora, OH) as described previously (36). Animals were assessed daily, and mean DSS/water consumption and body weights were recorded. The severity of diarrhea was assessed daily by using a 0-to-3 scale: 0 = normal, 1 = soft, 2 = very soft but formed, and 3 = liquid stool. Fecal blood was assessed by resuspending a fecal pellet in 400 μl of H2O2. Following brief centrifugation, 40 μl of supernatant was added to a 0.5 × 0.5-cm piece of SENSA paper (SmithKline Diagnostics, San Jose, CA), allowed to air dry, and developed with one drop of SENSA developer solution. The presence of blood results in a color change from white to blue that is proportional to the amount of blood present in the sample. The intensity of the SENSA color change was scored by observers blinded to animal group and treatment on a 0-to-4 scale: 0 = none, 1 = faintly blue, 2 = moderately blue, 3 = dark blue. Fecal blood visible to the eye was scored as a 4. The reproducibility of both the diarrhea and fecal blood scoring systems has been reported previously (1). Hematocrits were measured using a microhematocrit centrifuge and were centrifuged at 10,000 g for 10 min. Supernatant (1,000 μl) was added to the assay buffer, incubated for 20 min at room temperature, and then incubated with the final reaction solutions for 20 min at room temperature, and absorbance was then measured at 540 nm. The absorbance was plotted against standards and fit with linear regression, followed by calculation of NOx according to the manufacturer’s recommendations.

**Immunohistochemistry**

Paraffin-embedded sections were stained for eNOS, iNOS, and nNOS as follows. Slides were deparaffinized in a standard fashion, washed in tap water, and then microwaved in 10 mM citrate buffer for 2 cycles of 5 min, washed again in tap water, incubated in 0.3% H2O2-methanol solution for 10 min, and then washed again in tap water. Slides were then incubated for 1 h at room temperature in a blocking solution of 5% donkey serum, then rinsed and incubated with primary antibody at 4°C overnight. Rabbit polyclonal IgG for eNOS, mouse monoclonal IgG1 for iNOS, and mouse monoclonal IgG1 for nNOS were obtained from Santa Cruz Biotechnology. The Vectastain ABC kit (VINDA, P-6200) was used with biotinylated secondary antibodies and biotin-conjugated horseradish peroxidase and was developed with a 3,3′-diaminobenidine solution per the manufacturer’s instructions.

**RNA analysis and RT-PCR**

Whole colonic tissue RNA was isolated by using TRIzol reagent (GIBCO BRL, Gaithersburg, MD). RT-PCR was performed as previously described (1). The PCR reaction was performed using 1 μl of the cDNA product produced by the RT reaction. This product was amplified in a final concentration of 1× PCR buffer (Perkin-Elmer-Cetus, Norwalk, CT), 0.8 μM each primer, 0.2 mM dNTPs, and 1 unit of Taq polymerase (Perkin-Elmer-Cetus) in a total volume of 50 μl. Primer sequences were as follows: 1) iNOS sense, ACA ACA GGA ACC TAC CAG CTC A; iNOS antisense, GAT GTT GTA GCG CTG TGT GTC A; product = 651 bp. 2) eNOS sense, GGG CCT CTC CCT TCC GGC TGC CAC C; eNOS antisense, GGA TCC CTG GAA AAG GCG GTG AGG; product = 258 bp. 3) nNOS sense, CCT TGT AGA GTA AGG AAG GCG GGC; nNOS antisense, GGC CCC ATC ATT GAC GGC GAG AAT G; product = 480 bp. 4) nNOS sense, GGC AAC CTG AGG TCG GCC ATC ACT; antisense, CTG CAG GCG TAC TCA TTC TCC; product = 623 bp. Conditions were 95°C, 1 min; 58°C, 1 min; and 72°C, 1.5 min × 30 cycles.

**Statistical Analysis**

All experiments represent a minimum of five animals per group. Data are presented as means ± SE. Parametric data were analyzed by using a one-way ANOVA, followed by a Dunnnett multiple-comparisons posttest. Nonparametric data (scoring) were analyzed by using a Kruskal-Wallis test (nonparametric ANOVA) followed by a Dunn’s multiple comparisons posttest. An associated probability (P value) of <0.05 was considered significant. Survival curves were
created by using the Kaplan-Meier method, and survival comparisons were performed by using the log-rank or Mantel-Haenszel test, which generates a two-tailed $P$ value. All statistical analysis was performed with GraphPad Instat and Prism 3.0 programs (GraphPad, San Diego, CA).

RESULTS

NOS Regulation During DSS Colitis

Studies were undertaken to assess the hypothesis that the effect of NOS in mucosal inflammation depends on the site of production. Initial studies were designed to assess NOS regulation in DSS-induced colitis in wild-type animals. All animals developed colitis after the addition of DSS to their drinking water. Wild-type mice (either C57BL/6 × 129/Sv or C57BL/6) developed diarrhea and fecal blood loss by day 5 of DSS and significant weight loss by day 7. DSS-induced colitis was associated with a significant increase in serum NOx levels. This increase appeared to be largely derived from iNOS (Fig. 1), since iNOS−/− animals exposed to DSS for 7 days had similar NOx to untreated matched wild-type controls. However, an increase in serum NOx concentrations was eventually observed in the iNOS−/− mice following 7 days of DSS compared with untreated iNOS−/− mice, suggesting that the later increase in NOx observed later in the course of colitis is due to NOx generated by the other NOS isoforms. Serum NOx in eNOS−/− and nNOS−/− mice did not differ from wild-type animals assessed at baseline nor following 7 days of DSS (data not shown).

Immunohistochemistry studies also revealed that iNOS was significantly upregulated following DSS exposure, with the highest expression present in areas of significant ulceration and inflammation (Fig. 2, b–d vs. e and f). More specifically, the majority of iNOS expression was present within the inflammatory cell infiltrate in the lamina propria and submucosa (Fig. 2, b–d). However, following DSS exposure there also appeared to be a small increase in intestinal epithelial cell iNOS expression (Fig. 2, b–d). Expression of eNOS was upregulated during DSS-induced colitis, with the majority of staining localized to endothelial cells (Fig. 3). There also appeared to be some specific staining for eNOS in epithelial cells during DSS exposure, but these findings were variable and less marked than iNOS (Fig. 3). No consistent significant increase in nNOS staining was noted during DSS exposure (Fig. 4). Occasionally, increased nNOS staining localized to neurons was noted in areas of inflammation (Fig. 4).

When mRNA expression was assessed by RT-PCR in wild-type animals, marked induction of iNOS mRNA was apparent by day 6 ($P < 0.001$) and day 9 ($P < 0.001$) of DSS (Fig. 5). No iNOS mRNA was detected in wild-type untreated animals, and it was only rarely detected in wild-type mice after 3 days of DSS (Fig. 5). In contrast, eNOS mRNA was detected in all mice studied, and there was an insignificant ($P = 0.08$) decrease in eNOS activity at day 3, followed by induction of eNOS mRNA at day 6 ($P = 0.05$) and day 9 ($P = 0.01$) (Fig. 5). No significant changes in nNOS mRNA were observed during DSS exposure.

Loss of iNOS was Associated With Reduction in the Severity of DSS-Induced Colitis

Subsequent studies were designed to assess the role of individual NOS isoforms in intestinal inflammation, addressing the hypothesis that individual NOS isoforms modulate the inflammatory response. Because the iNOS−/− line was of a different strain (C57B6) from the eNOS−/− and nNOS−/− lines (129-C57B6), the studies on the iNOS−/− mice did not differ from wild-type mice as determined by diarrhea score, bleeding score, macroscopic assessment, routine histology, and colonic tissue MPO activity ($P > 0.5$ for all parameters vs. wild-type).

The nNOS−/− mice were markedly more susceptible to DSS-induced colitis than wild-type mice, as evidenced by less severe weight loss, blood loss, macroscopic damage, and MPO activity (Fig. 6, A–D). These changes were most notable at day 7, although interestingly the iNOS−/− mice experienced a greater decrease in hematocrit and weight loss at day 3 than wild-type mice (Fig. 6, A–C). The reduced disease severity in the iNOS−/− mice was associated with significant improvement in survival (Fig. 6E).

Loss of eNOS was Associated With Reduction in the Severity of DSS-Induced Colitis, but the Loss of nNOS Increased Disease Severity

Subsequent to the study of iNOS−/− and matched wild-type mice, colitis was next assessed in nNOS−/− and eNOS−/− mice compared with matched control animals. No baseline colonic inflammation was observed in any of the NOS−/− mice as determined by diarrhea score, bleeding score, macroscopic assessment, routine histology, and colonic tissue MPO activity ($P > 0.5$ for all parameters vs. wild-type).

The nNOS−/− mice were markedly more susceptible to DSS-induced colitis than wild-type mice (Figs. 7 and 8). nNOS−/− mice developed significantly more severe diarrhea ($P < 0.001$) by DSS day 7 and experienced greater fecal blood loss by DSS day 2 ($P < 0.001$) compared with similarly treated wild-type mice (Fig. 7, A–B). This correlated with significantly greater reductions in baseline body weight by DSS day 3 (Fig. 1C). On death, nNOS−/− mice had more severe and extensive colitis as well as increased by colonic tissue MPO levels (Fig. 7, D–F). This increased disease severity led to reduced survival of the nNOS mice ($P < 0.0001$) (Fig. 7G). Interestingly, in contrast, the loss of eNOS resulted in a reduction in the severity of DSS-induced colitis, as indicated by reduced loss of

![Fig. 1. Serum nitric oxide (NO) metabolite (NOx) levels. Serum NOx levels in wild-type and inducible NO synthase (iNOS)−/− mice at baseline and at day 7 of dextran sodium sulfate (DSS)-induced colitis are shown. (Both mouse lines are of a C57BL/6 background).](http://ajpgi.org/pdf/10.2333/ajpgi.286.139a0b.png)
basal body weight (Fig. 7C), reduced disease severity scores (Fig. 7E), and improved survival (Fig. 7G). The loss of eNOS could confer reduced susceptibility to DSS-induced colitis in nNOS−/− mice, as evidenced by a decrease in most parameters assessed, and this resulted in disease severity that was similar to that of wild-type mice (Fig. 7).

mRNA Expression of NOS Isoforms During Colitis

As noted above, DSS-induced colitis in wild-type mice was characterized by induction of iNOS mRNA expression (Fig. 5). In contrast, nNOS mRNA expression did not change, but there was a rise in eNOS expression at days 6 and 9 in the wild-type mice. When iNOS−/−, eNOS−/−, nNOS−/−, and e/nNOS−/− mice were examined, there were no baseline differences in mRNA expression of iNOS, eNOS, or nNOS compared with wild-type mice (excluding the expected absence of the NOS isoforms that were disrupted) (Fig. 8). At day 3 of DSS, the most striking change was the marked induction of iNOS in nNOS−/− mice compared with the eNOS−/− and wild-type mice (expressed as percentage of GAPDH signal; nNOS−/− = 83.4 ± 17.1% vs. eNOS−/− = 0.0% and wild-type = 8.3 ± 5.6%; P < 0.001) (Fig. 8A). Although nNOS−/− mice exhibited decreased eNOS expression at days 3 and 9 compared with wild-type and iNOS−/− mice, these differences were not significant (P = 0.065–0.09) (Fig. 8). At day 9, iNOS−/− was detected in all wild-type and nNOS−/− mice and in most e/nNOS−/− and eNOS−/− mice. However, e/nNOS−/− and eNOS−/− mice had significantly less iNOS induction than wild-type and nNOS−/− mice (32.7 ± 8.1, 20.9 ± 16.2, 83.2 ± 18.0, and 94 ± 31.5% as percentage of GAPDH, respectively; P < 0.05–0.01) (Fig. 8B).

Effect of NOS Isoform Expression on Colonic Mucosal Leukocyte Populations

Because NO can alter leukocyte homing, flow cytometry was used to assess colonic lymphocyte populations. Mice lacking eNOS (both eNOS−/− and e/nNOS−/−) had reduced CD4/CD8 ratios both at baseline and at DSS day 6 compared with wild-type, iNOS−/−, and nNOS−/− mice (data are presented as results from pooling of isolated cells of 4–5 animals per group repeated twice; Fig. 9A). Previous studies have suggested that γδ-cells are involved in wound healing. In this model system, only wild-type and eNOS−/− mice showed an increase in the γδ/αβ ratio from baseline, whereas iNOS−/−, nNOS−/−, and e/nNOS−/− had a reduction in the γδ/αβ ratio, but most of these changes were minimal (Fig. 9B).
eNOS−/− mice also had reduced natural killer (NK) cells at baseline and DSS day 6 compared with the other NOS−/− lines, but again these changes were small. The loss of eNOS in the nNOS−/− background resulted in a decrease in the CD4/CD8 ratio and a decrease in NK cell numbers but conversely an increase in γδ-cells both at baseline and day 6 of DSS compared with the nNOS−/− mice (Fig. 9C). The iNOS−/− mice had a lower CD4/CD8 ratio but increased γδ-cells and increased NK cells at day 6 of DSS compared with wild-type mice; however, these changes were variable and failed to reach significance (Fig. 9).

DISCUSSION

The functional role of NO in the gastrointestinal tract is controversial. Increased NO is commonly associated with intestinal injury (e.g., Crohn’s disease, ulcerative colitis, and several animal models of colitis) (5, 6, 38) but can also be found in disorders characterized by minimal mucosal damage, e.g., collagenous and lymphocytic colitis (32, 46). By assessing the role of NO in colitis in mice lacking specific NOS isoforms, our studies suggest that the site of NO production is important in determining the biological effect of NO and its impact on intestinal inflammation.

In the present study, iNOS and to a lesser extent eNOS were found to be upregulated in DSS-induced colitis. Theoretically, NOS upregulation could be protective, injurious, or simply a marker of inflammation and tissue injury. Specifically, NO can protect the gastrointestinal tract against various forms of injury, including ethanol, ischemia-reperfusion, and endotoxin (26, 35, 55). Several mechanisms may contribute to these protective effects, including regulation of leukocyte recruitment, microvascular permeability, and wound repair (15, 26, 28). NO also shares many protective actions with prostaglandins (54). However, NO also has proinflammatory actions and NO inhibition is beneficial in several settings, including ischemia-reperfusion and LPS-induced intestinal injury, and several animal models of colitis (17, 40, 54). The mechanisms involved in the proinflammatory actions of NO are unclear but may involve the function of NO as a free radical and/or conversion of NO to more reactive nitrogen species (39) that can induce both cell damage and apoptosis (50). Of note, 5-aminosalicylic acid products used in treatment of inflamma-
tory bowel disease can reduce the production of reactive nitrogen species (39, 49).

The reduced susceptibility of iNOS−/− mice to DSS-induced colitis confirmed the previous findings of Krieglstein et al. (25). They found that both iNOS−/− and wild-type mice treated with the specific iNOS inhibitor 1400W had less severe DSS-induced colitis than wild-type mice. The mechanisms involved in protection afforded by a reduction or loss of iNOS activity is unclear, although altered responsiveness to intestinal bacteria that gain entry through disruption of the epithelial barrier as a result of DSS exposure may contribute. The susceptibility of iNOS−/− mice to LPS is controversial (30, 33). However, iNOS−/− mice appear to exhibit enhanced leukocyte-endothelium interactions during LPS endotoxemia (18).

The roles of constitutively expressed NOS (including both nNOS and eNOS) in intestinal inflammation are not fully understood. Targeted deletion of nNOS results in disruption of normal enteric inhibitory neurotransmission, and these mice exhibited gastric dilation and hypertrophy of the pyloric

Fig. 4. Immunohistochemical assessment of neuronal NO synthase (nNOS) expression in wild-type mice. a–c: nNOS immunohistochemical staining at day 7 of DSS colitis reveals significant neural cell staining. In general, there appeared to be an increase in staining of neural cells in areas of inflammation, but this was often patchy and inconsistent. d–f: nNOS immunohistochemical staining in untreated wild-type mice.

Fig. 5. Colonic expression of iNOS and eNOS mRNA at baseline and during DSS-induced colitis in wild-type mice. Colonic expression of iNOS, nNOS, and eNOS mRNA in wild-type mice either untreated or 3, 6, or 9 days following induction of DSS colitis is shown. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. corresponding values in untreated animals.
sphincter (19). Deletion of eNOS results in increased systemic and pulmonary pressures, whereas increased expression results in significant hypotension (20, 45). Both eNOS−/− and nNOS−/− mice have increased thrombin-stimulated leukocyte rolling and adherence (unpublished observations; see Ref. 20). However, in a murine model of asthma, no differences in ovalbumin-stimulated inflammatory cell infiltration were observed in the lung in eNOS−/−, nNOS−/−, e/nNOS−/−, and iNOS−/− mice compared with wild-type control animals, suggesting that leukocyte recruitment may not be affected by disruption of specific NOS isoforms (11).

In the present study, nNOS appeared to play a protective role in DSS-induced colitis because nNOS−/− mice were markedly more susceptible to DSS-induced intestinal injury and death than wild-type mice. The mechanisms underlying the increased susceptibility of nNOS−/− mice to DSS-induced...
coliitis remain unclear. However, the inflammatory response in the nNOS−/− animals was characterized by an increase in neutrophils and NK cells as well as a reduced CD4/CD8 ratio compared with wild-type mice. DSS-induced colitis did not result in increased nNOS mRNA expression, suggesting that the loss of basal levels and not the failure to increase or decrease nNOS led to the observed increased susceptibility. nNOS may downregulate the neurally mediated inflammatory response by acting as an inhibitory neurotransmitter. Such inhibitory actions of nNOS have been previously demonstrated (8). Furthermore, neuronal regulation of mast cell function has been shown to be important in several models of intestinal inflammation (48). Because NO can downregulate the proinflammatory actions of mast cells and because nNOS is produced by neurons and mast cells, loss of nNOS may increase the proinflammatory activities of the neuronal mast cell-mediated response (27, 37, 48).

Expression of both iNOS and eNOS was upregulated in wild-type mice with DSS-induced colitis. However, in contrast to iNOS−/− and nNOS−/− mice, eNOS−/− mice were not more susceptible to DSS-induced colitis but actually experienced less severe colitis and significantly improved survival. Neutrophil infiltration was similar in the eNOS−/− mice compared with wild-type mice at day 9, but colonic γδ/αβ and CD4/CD8 lymphocyte ratios in eNOS−/− mice differed from...
wild-type mice both before DSS and at DSS day 6. In wild-type mice at day 6 of DSS, there was an increase in the CD4/CD8 ratio (suggestive of reduced suppressor T cell activity) and a reduction in the γδ/αβ ratio. Interestingly, the reverse pattern was observed in the eNOS−/− mice. Whether these changes simply reflect differences in the severity of inflammation or indicate possible mechanisms accounting for the differential susceptibility is unclear, but preliminary studies in this laboratory have demonstrated that mice deficient in mature T and B cells as well as those deficient in either γδ, αβ, or both γδ and αβ T cells are more susceptible to DSS-induced colitis (4).

In contrast to the intestinal injury examined in the present study, eNOS−/− mice have been found to have impaired cutaneous wound healing and angiogenesis (31) as well as increased susceptibility to limb ischemia (42). NO appears to interact with vascular endothelial growth factor (VEGF) in regulating angiogenesis and vascular permeability. Interestingly, VEGF increases angiogenesis in wild-type mice but not in eNOS−/− mice (42), and VEGF-induced increases in vascular permeability appear to be NO dependent (43, 52). VEGF has numerous proinflammatory effects, including the ability to increase expression of adhesion molecules, vascular permeability, and NK cell activity; whether these functions are NO dependent is unknown (12).

Further evidence that the eNOS-generated NO may actually promote colitis was obtained from mice deficient in both eNOS and nNOS. eNOS−/− mice and wild-type mice were similarly affected by DSS, but importantly eNOS−/− mice were less susceptible to DSS-induced colitis than mice only deficient in nNOS. The inflammatory cell infiltration was similar in eNOS−/−−/− vs. eNOS−/− mice. The main differences in eNOS−/− vs. nNOS−/−−/− were a reduction in neutrophil infiltration, CD4/CD8 ratios, and NK cell numbers but an increase in γδ T cells. These findings suggest that the proinflammatory effect of eNOS-derived NO may override the protective actions of NO generated by cells expressing nNOS.

These studies show that individual NOS isoforms can exert differential roles in intestinal inflammation. Because isoforms are present in numerous cell types, it appears that the cellular source of NO may have differential effects. These findings provide a context to reconcile previous studies that have shown that the timing of NO inhibition has differential effects on intestinal inflammation. Thus therapy directed at modulating NO may have mixed results depending on the NOS isoforms and cells affected as well as their roles in the inflammatory process. Furthermore, because NO has both pro- and anti-inflammatory actions, the timing of therapeutic modulation may be critical; that is, an adequate inflammatory response may be required following injury for effective healing, whereas chronic inflammation can result from failure to downregulate the inflammatory response.

In conclusion, it appears that the role of individual NOS isoforms in intestinal injury is complex and that the site, timing, and level of NO production as well as the type of injury and/or inflammation are all critical in determining its overall impact.

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