Inhibition of Interleukin-12 p40 Transcription and NF-κB Activation by Nitric Oxide in Murine Macrophages and Dendritic Cells*

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Nitric oxide (NO), an important effector molecule of the innate immune system, can also regulate adaptive immunity. In this study, the molecular effects of NO on the toll-like receptor signaling pathway were determined using interleukin-12 (IL-12) as an immunologically relevant target gene. The principal conclusion of these experiments is that NO inhibits IL-1 receptor-associated kinase (IRAK) activity and attenuates the molecular interaction between tumor necrosis factor receptor-associated factor-6 and IRAK. As a consequence, the NO donor S-nitroso-N-acetylpenicillamine (SNAP) inhibits lipopolysaccharide (LPS)-induced IL-12 p40 mRNA expression, protein production, and promoter activity in murine macrophages, dendritic cells, and the murine macrophage cell line RAW 264.7. Splenocytes from inducible nitric-oxide synthase-deficient mice demonstrate markedly increased IL-12 p40 protein and mRNA expression compared with wild type splenocytes. The inhibitory action of NO on IL-12 p40 is independent of the cytokine IL-10. The effects of NO can be directly attributed to inhibition of NF-κB activation through IRAK-dependent pathways. Accordingly, SNAP strongly reduces LPS-induced NF-κB DNA binding to the p40 promoter and inhibits LPS-induced iNOS phosphorylation. Similarly, NO attenuates IL-1-β-induced NF-κB activation. These experiments provide another example of how an innate immune molecule may have a profound effect on adaptive immunity.

Cytokines play an important role in the differentiation of naive T cells toward T-helper-1 (Th1) and T-helper-2 subtypes (1). IL-12 is a heterodimeric cytokine, composed of a 35-kDa (p35) subunit and a 40-kDa (p40) subunit, and plays a central role in the induction of a Th1 immune response. IL-12 is produced by macrophages and dendritic cells in response to infection with bacteria or exposure to bacterial constituents such as lipopolysaccharide (LPS) (2, 3). IL-12 production and Th1 cells are required for cell-mediated immunity and host defense against intracellular microbes (4), and overexpression of IL-12 has been implicated in the progression of chronic Th1-mediated inflammatory diseases like Crohn’s disease and rheumatoid arthritis (5).

IL-12 p40 and p35 are encoded by separate genes that form the biologically active p70 heterodimer (3, 6, 7). IL-12 p40 mRNA is exclusively detected in cells that produce bioactive p70 and is strongly induced by intracellular bacteria and bacterial products (1). For these reasons, studies of IL-12 transcriptional regulation have focused on the p40 gene. A cis-acting element from −132 to −122 with respect to the transcription start site in the murine p40 promoter binds Rel family members and is important for the induction of promoter activity by bacterial products (8–10). Although expression of IL-12 p40 is a proximal event in the development of a Th1 immune response, the induction and, importantly, the inhibition of IL-12 gene expression are still not fully understood.

Nitric oxide (NO) regulates a wide range of biological activities in the nervous, vascular, and immune systems (11–13). In activated macrophages, NO and its metabolites mediate a number of host defense functions that include anti-microbial and tumoricidal activity. Macrophages produce NO from l-arginine after activation of the inducible form of nitric-oxide synthase (iNOS). iNOS has been identified in a wide variety of cell types, and its expression can be activated by many immune stimuli (14). Due to its anti-microbial effects, NO is an important molecule of the innate immune system. Its role in regulating adaptive immune responses is less clear. Therefore, the purpose of this study is to determine whether NO may affect adaptive immune responses through a direct effect on IL-12 production by macrophages and dendritic cells.

We demonstrate that NO suppresses IL-12 p40 protein production, mRNA expression, and promoter activity in murine macrophages and dendritic cells. Splenocytes derived from iNOS-deficient mice produce increased IL-12 p40 protein and mRNA compared with wild type mice of the same strain. Down-regulation of IL-12 by NO is independent of the effects of IL-10. NO inhibits TLR and IL-1 receptor-dependent signal transduction through inhibition of IRAK activity and consequent attenuation of the molecular interaction between TRAF6 and IRAK. Accordingly, NO inhibits NF-κB DNA binding to the p40 promoter. These experiments suggest a role for NO in the regulation of Th1-type immunity through inhibition of IRAK activity, NF-κB, and consequently IL-12.

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§ The abbreviations used are: Th1, T-helper-1; NO, nitric oxide; IL, interleukin; SNAP, S-nitroso-N-acetylpenicillamine; LPS, lipopolysaccharide; TLR, toll-like receptor; IFN, interferon; iNOS, inducible nitric oxide synthetase; NMMA, Nω-nitroso-L-arginine; BM, bone marrow; EMSA, electrophoretic mobility shift assay; IL-1R, IL-1 receptor; IRAK, IL-1 receptor-associated kinase; TNF, tumor necrosis factor; TRAP, TNF receptor-associated factor; MAP kinase, mitogen activated protein kinase; Hα, hemagglutinin; GM-CSF, granulocyte/macrophage colony-stimulating factor; rm, recombinant murine; DMEM, Dulbecco’s modified Eagle’s medium; ELISA, enzyme-linked immunosorbent assay; EM, electrophoretic mobility shift assay; RT, reverse transcriptase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MBP, myelin basic protein.
Experimental Procedures

Plasmid Constructs—IL-12 p40 promoter fragments were inserted into the luciferase reporter vector pGL2 (Promega) as described previously (10). A pair of distinct NF-κB elements was placed upstream of the luciferase reporter in the construct. The pGL2 vector was obtained from Adrian Ting. The toll-like receptor (TLR) 2 expression plasmid was provided by Paul Godowski (15), and hemagglutinin (HA)-tagged TLR4 was a gift of Felix Randow.

Cell Lines and Reagents—The human embryonic kidney 293T and the RAW 264.7 murine macrophage cell lines (American Type Culture Collection) were maintained in DMEM supplemented with 10% fetal bovine serum and penicillin-streptomycin. Lipo polysaccharide (LPS) from Salmonella enteridis was purchased from Sigma. S-Nitroso-N-acetylpenicillamine (SNAP) and N²-monomethyl-L-arginine monooacetate salt (NMMA) were obtained from Calbiochem. Antibodies against IκB, phospho-IκB, β-actin, MyD88, TRAF6, p38 MAP kinase, and HA were purchased from Santa Cruz Biotechnology. An IRAK antibody that detects IRAK1 was obtained from Upstate Biotechnology, Inc. GM-CSF, IL-1β, and IFN-γ were purchased from PeproTech.

Isolation of Bone Marrow-derived Dendritic Cells and Macrophages and Splenocytes—C57BL/6 and IL-10-deficient (C57BL/6 background) (The Jackson Laboratories), and iNOS-deficient (C57BL/6 background) (from Anthony Bauer) mice raised and maintained under specific pathogen-free conditions were used to obtain splenocytes, macrophage, and dendritic cells at 7–10 weeks of age. Bone marrow-derived dendritic cells and macrophages were cultured from C57BL/6 mice as described previously (16, 17). Mice were sacrificed and bone marrow (BM) cells harvested. At day 0, BM cells were seeded at 2 × 10⁶ per 100-mm dish in 10 ml of culture medium (RPMI 1640 supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), glucose (10 mM), and 10% heat-inactivated fetal bovine serum) containing recombinant murine (rm) GM-CSF. At day 10, cells were either used experimentally or cul tured inactivated fetal bovine serum (inactivated) containing recombinant (rm) GM-CSF, IL-1α, IL-1β, TNF-α, and IFN-γ were purchased from PeproTech. IL-12 p70 and rmIL-12 were obtained from R&D Systems. IRAK, MyD88, and TRAF6 antibodies were obtained from Santa Cruz Biotechnology.

Co-immunoprecipitation—RAW 264.7 cells were washed with cold phosphate-buffered saline and lysed for 15 min on ice in 0.5 ml of lysis buffer (1% Triton X-100, 0.1% SDS, 0.5 M NaCl, 0.2 mM EDTA, 2 mM EGTA, 10% glycerol, and 1 mM dithiothreitol) containing protease inhibitors. Cells lysates were clarified by centrifugation at 4 °C for 15 min at 14,000 rpm. Lysates were incubated with 2 μg of MyD88 or TRAF6 antibodies in the presence of 20 μl of 50% (v/v) protein G-agarose overnight at 4 °C with gentle rocking. After three washings with lysis buffer, precipitated complexes were solubilized by boiling in SDS buffer, fractionated by 10% SDS-PAGE, and transferred to nitrocellulose membranes. Western immunoblotting was performed using HA, IRAK, MyD88, and TRAF6 antibodies.

RNA Protection Assay—Twenty μg of total RNA from primary cells or RAW 264.7 cells were analyzed by multiprobe RNA protection assay using the cytokine-specific set of probes. The probes were synthesized with T7 RNA polymerase and hybridized with RNA at 56 °C for 16 h. The samples were digested with RNase, purified by phenol-chloroform, and resolved by 6% denaturing PAGE.

Real Time RT-PCR—Fluorescence-labeled LUX primers (19) and their unlabeled counterparts were purchased from Invitrogen. LUX primers were designed utilizing a software program (LUX Designer) (www.invitrogen.com/lux). LUX primers were designed to produce amplification in size between 69 and 145 bp. Primer pairs for IL-12 p40 and GAPDH were selected to span an intron sequence. For IL-12 p40, the primers were 5′-GACCTGTGCAAAAGCTTACTCATGCGGAAT-3′ and 5′-GGAAGCACGGCAGCAGATTA-3′, and for GAPDH, the primers were 5′-GACATACAGCCGCGTGTAGTGTC-3′ and 5′-GGGGATGTAGCCCGCTT-3′. Total RNA (1 μg) isolated from spleen cells was reverse-transcribed (20 μl reactions), and cDNA was used for fluorogenic primer PCR. Plots of fluorescence versus PCR cycle were generated by the ABI PRISM 7700 SDS software. The cycle thresholds (CT), the cycle where the fluorescence rises above background (10 times the standard deviation of the background fluorescence), were between 15 and 35 cycles. Every 25-μl PCR mix contained 4 μl of cDNA, 1 μl of each gene-specific primer, and 12.5 μl of 2× Platinum Quantitative PCR SuperMix-UDG (Invitrogen) containing 80 units/ml Platinum TaqDNA polymerase, 40 mM Tris-HCl (pH 8.4), 100 mM KCl, 5 mM MgCl₂, 400 μM dNTPs, 0.2 μM dATP, 0.2 μM dGTP, 0.2 μM dCTP, 0.8 μM dUTP, 100 μM dUTP, and stabilizers, 1 μl of ROX reference dye, and 5.5 μl diethyl pyrocarbonate-treated water. Reactions were incubated at 50 °C for 2 min and 95 °C for 2 min, and then cycled (45 times) at 95 °C for 15 s and 60 °C for 30 s. Reactions were conducted in a 96-well spectrophotometric thermal cycler (ABI PRISM 7700 Sequence Detection System, Applied Biosystems). Fluorescence was monitored during every PCR cycle at the annealing or extension step and during the post-PCR temperature ramp. Quantification of IL-12 p40 mRNA was performed as a relative fold increase in transcript level with respect to unstimulated cells (3). In this method, the amount of target is calculated based on the difference (ΔΔCT) between the average CT of each reaction and the average CT of the housekeeping gene subtraction, both CT values are normalized by subtracting the average of the endogenous reference gene, GAPDH, from each. The CT values for each set of replicate lies within 2 Cq of one another.

In Vitro Kinase Assays—RAW 264.7 cells were pretreated with SNAP...
NO Inhibits IL-12 mRNA Expression and Protein Release in Primary Murine Macrophages, Dendritic Cells, and a Macrophage Cell Line—To determine whether NO affects IL-12 expression, murine bone marrow-derived dendritic cells, bone marrow-derived macrophages, and the macrophage cell line RAW 264.7 were pretreated with the NO donor SNAP. Additionally, the NO inhibitor NMMA was used in these experiments. Cells were subsequently activated with IFN-γ plus LPS for 4 (mRNA expression) or 24 h (protein determination). RNase protection assay was performed for the detection of IL-12 mRNA expression, whereas supernatants were collected for IL-12 p40 protein release (ELISA). SNAP inhibits both IL-12 p40 and p35 mRNA expression in primary murine macrophages and dendritic cells (Fig. 1A, upper and middle panel, respectively). In RAW 264.7 cells, SNAP inhibits IL-12 p40 mRNA expression (Fig. 1A, lower panel). IL-12 p35 mRNA is barely detectable in activated RAW 264.7 cells, so effects of NO are difficult to assess (data not shown). Furthermore, SNAP inhibits IL-12 p40 protein release from bone marrow-derived macrophages (Fig. 1B) and dendritic cells (Fig. 1C), as well as RAW 264.7 cells (data not shown).

To study the effects of endogenous NO on IL-12, splenocytes were obtained from iNOS-deficient mice and IL-12 p40 protein and mRNA expression compared with splenocytes obtained from wild type mice of the same strain. Cells were activated with LPS and/or IFN-γ for 4 (mRNA expression) or 24 h (protein determination). In this experiment, IL-12 p40 mRNA accumulation was assayed by real-time RT-PCR. Consistent with a previous study (20), compared with wild type cells (Fig. 2A), iNOS-deficient cells demonstrate enhanced IL-12 p40 protein production particularly following activation with IFN-γ plus LPS. LPS and IFN-γ plus LPS IL-12 p40 mRNA expression were also markedly enhanced in iNOS-deficient splenocytes (Fig. 2B). Measurements of nitrite accumulation in supernatants confirm that activated iNOS-deficient splenocytes produce undetectable levels of endogenous NO (data not shown). This result suggests that endogenously produced NO may suppress LPS- and IFN-γ plus LPS-induced IL-12 p40 mRNA expression and protein release.

Down-regulation of IL-12 Expression by NO Is Independent of IL-10—IL-10 is a potent inhibitor of IL-12 gene expression and protein production (21). It is possible that down-regulation of IL-12 expression by NO is mediated through induction of IL-10. To address this question, bone marrow-derived macrophages were isolated from IL-10-deficient mice. SNAP significantly attenuates IL-12 protein release from IL-10-deficient macrophages (Fig. 3), suggesting that this inhibition is independent of IL-10. Furthermore, in IFN-γ plus LPS-activated wild type murine bone marrow-derived macrophages, SNAP inhibits IL-12 mRNA expression in a dose-dependent manner, although there are no significant changes in other cytokine mRNA levels (IL-1α, IL-1β, IL-1 receptor antagonist, IL-18; data not shown). Therefore, down-regulation of LPS-induced
**NO Inhibits IL-12 and IRAK**

**NO Inhibits NF-κB DNA Binding Activity**—A critical cis-acting element from -132 to -122 with respect to the transcription start site in the murine p40 promoter binds Rel family members and is important for the induction of promoter activity by bacterial products (8–10). Therefore, whether NO inhibits DNA binding of NF-κB to this site on the IL-12 p40 promoter was addressed. EMSAs were performed using oligonucleotide probes specific for the NF-κB element in the IL-12 p40 promoter. Using nuclear extracts from RAW 264.7 cells, SNAP reduces LPS-induced NF-κB DNA binding (Fig. 5A) in a dose-dependent manner. As a control, NO was demonstrated to have no effect on AP-1 DNA binding to the p40 promoter (data not shown). As described previously (9), the NF-κB family members c-Rel, p50, and p65 bind to the IL-12 p40 promoter (Fig. 5B). NO inhibits the lower EMSA complex, composed mainly of p50, as well as the upper complex that includes c-Rel and p65, suggesting that DNA binding of multiple NF-κB family members is attenuated (Fig. 5B). These results suggest that NF-κB is a key transcription factor target mediating NO inhibition of IL-12 p40 promoter activity and gene expression.

**NO Inhibits NF-κB Reporter Activity and IκB Phosphorylation**—The toll-like receptor (TLR) family of pattern recognition receptors mediates inflammatory gene expression in macrophages and dendritic cells in response to a wide range of microbial determinants including LPS (22). NF-κB is the best described transcription factor target of the TLR signaling pathway. As NO abrogates LPS-induced NF-κB DNA binding activity, it may directly inhibit the TLR signal transduction pathway. A multimerized NF-κB DNA-binding element luciferase-reporter was transfected into RAW 264.7 cells. In cells pretreated with SNAP prior to activation with LPS, NF-κB reporter activity is strongly reduced (Fig. 6A). To confirm that the TLR signal transduction pathway is modulated by NO, IL-1-mediated signal transduction, which shares common downstream targets including NF-κB, was next studied. An NF-κB luciferase reporter was transfected into the human embryonal kidney 293T cell line, and the cells were activated with IL-1β in the presence of SNAP or NMMA. SNAP dose-dependently inhibits NF-κB reporter activity induced by IL-1β (Fig. 6B). Next, TLR2 was expressed in 293T cells, as described previously (15), to confer responsiveness to LPS signaling, likely through contaminants in the LPS preparation. In this cell line model, SNAP inhibits LPS-induced, TLR2-dependent NF-κB reporter activity in a dose-dependent manner (Fig. 6C). Therefore, in 293T cells, IL-1 receptor (IL-1R), and TLR signal transduction is abrogated by NO.

Next, bone marrow-derived macrophages were pretreated with SNAP for 2 h and activated with LPS for 20 min. In whole cell extracts, LPS-induced IκB phosphorylation is strongly reduced by SNAP (Fig. 6D). However, total protein levels of IκB were not altered (Fig. 6D) suggesting that NO inhibits IκB phosphorylation. Thus, NO inhibits TLR signal transduction, resulting in decreased IκB phosphorylation and decreased NF-κB activation. As LPS specifically induces NF-κB activation in murine macrophages through TLR4, most likely TLR4 is the relevant cell surface receptor for this phenomenon.
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NO Attenuates the Molecular Interaction of IRAK with TRAF6—LPS signaling through TLR4 utilizes an evolutionarily conserved signal transduction pathway that is common to the IL-1R. The IL-1 receptor-associated kinase (IRAK) is a downstream contributor to this pathway (23). After activation with LPS, an intracellular adaptor molecule, MyD88, is recruited to the cytoplasmic tail of TLR4, providing a platform for IRAK. IRAK subsequently undergoes phosphorylation and interacts with TNF receptor-associated factor-6 (TRAF6), a downstream transducer required for NF-κB activation. NO may affect any point along this cascade to inhibit IL-12 p40 expression. Therefore, RAW 264.7 cells were pretreated with SNAP for 2 h and then activated with LPS for 10, 30, and 60 min. Proteins were immunoprecipitated from whole cell extracts with TRAF6 and MyD88 antibodies, respectively, and immunoblotted with an IRAK antibody. The physical interaction between IRAK and TRAF6 is reduced in the presence of SNAP (Fig. 7A, 2nd panel, compare 2nd, 4th, and 6th lanes (−SNAP) with 3rd, 5th, and 7th lanes (+SNAP)). However, the interaction of IRAK with MyD88 is not affected (Fig. 7A, 1st panel, compare 2nd, 4th, and 6th lanes (−SNAP) with 3rd, 5th, and 7th lanes (+SNAP)). Furthermore, equivalent amounts of IRAK (Fig. 7A, 3rd panel), MyD88 (Fig. 7A, 4th panel), and TRAF6 (Fig. 7A, 5th panel) are present in whole cell extracts.

To determine whether NO inhibits formation of the TLR4-MyD88 complex, RAW 264.7 cells were transiently transfected with an HA-tagged TLR4 expression plasmid, pretreated with SNAP for 2 h, and then activated with LPS for 30 min. Transfection of an epitope-tagged TLR4 was utilized as several antibodies to murine TLR4 tested failed to detect endogenous TLR4 protein in Western immunoblot and immunoprecipitation experiments (data not shown). Proteins were immunoprecipitated from whole cell extracts with an anti-HA antibody and immunoblotted with a MyD88 antibody. This experiment demonstrates that formation of the TLR4-MyD88 complex is not inhibited by SNAP (Fig. 7B). These results, taken together, suggest that NO directly inhibits a molecular association between IRAK and TRAF6.

We next asked whether IRAK activity may be directly inhibited by NO using an in vitro kinase assay. RAW 264.7 cells were pretreated with SNAP for 2 h and activated with LPS for 30 min. LPS strongly activates IRAK in RAW 264.7 cells (Fig. 6C, 3rd lane) and kinase activity, assessed by phosphorylation of the substrate MBP, is markedly abrogated by pretreatment with SNAP (Fig. 7C, 4th lane). As a control, in vitro kinase assays show that SNAP does not affect MAP kinase p38 activity in LPS-activated RAW 264.7 cells (data not shown).

These results suggest that inhibition of IL-12 p40 gene expression and NF-κB activation by NO occur mainly through inhibition of signaling through the TLR/IL-1R pathway. The molecular mechanism for this phenomenon involves attenuation of IRAK activity with the consequent disruption of the molecular interaction between IRAK and TRAF6.

DISCUSSION

This study describes mechanisms of inhibition of IL-12 by the innate immune effector NO in murine dendritic cells and macrophages. NO inhibits LPS-induced IL-12 p40 gene expression and promoter activity by attenuating NF-κB DNA binding. The principal conclusion of these experiments is that NO inhibits IRAK activity.

NO has a wide range of effects in the immune system (14). It has been reported that NO inhibited IL-12 synthesis from murine peritoneal macrophages (20). However, molecular
The TLR family of pattern recognition receptors mediates signal transduction and pro-inflammatory gene expression by a wide range of microbial determinants. LPS signals in macrophages and dendritic cells through TLR4. In our previous study, dominant negative signal transduction molecules downstream of TLRs (MyD88, IRAK, and TRAF6) inhibited LPS-induced activity of the IL-12 p40 promoter in RAW 264.7 cells (18). In the present study, NO inhibited IRAK activity and disrupted the interaction of IRAK with TRAF6 in activated macrophages. In the 293T cell line, SNAP inhibited both IL-1β and LPS-induced NF-κB activities. These data support the hypothesis that NO can disrupt both IL-1 and LPS signaling pathways by inhibiting IRAK activity. How NO specifically inhibits IRAK activation and blocks the interaction of IRAK with TRAF6 is a topic of future interest. One plausible hypothesis is raised by the description of the macrophage-specific inactive kinase, IRAK-M. IRAK-M negatively regulates TLR signaling through preventing the dissociation of IRAK from MyD88 and inhibits IRAK activation and blocks the interaction of IRAK with TRAF6 in activated macrophages (24). Thus, up-regulation of IRAK-M by NO could potentially explain the current findings.

As a consequence of IRAK inhibition, LPS-induced IκB phosphorylation and NF-κB reporter activity were strongly reduced by NO. Previous studies have shown that NO donor agents suppressed NF-κB activation in human endothelial cells by increasing the level of IκB (25). However, in another system, NO activated NF-κB in human peripheral blood mononuclear cells (26). The reasons for these differences are unclear. Perhaps cell type-specific and species-specific effects of NO explain the discrepant findings.

A systematic analysis of the murine IL-12 p40 promoter has been performed, providing a detailed understanding of the regulation of IL-12 p40 gene expression by bacterial products. In the present study, LPS-induced IL-12 p40 promoter activity...
was strongly inhibited by NO. The inhibitory effect was demonstrable even with a minimal inducible IL-12 p40 promoter containing a C/EBP and AP-1 element but lacking an NF-κB site (18). Although this study had focused upon NO-induced inhibition of NF-κB activation, these results also suggest that there may be NF-κB-independent pathways through which NO may inhibit IL-12 p40 transcription. There is a selective requirement for the NF-κB family member c-Rel in IL-12 p40 gene expression. Alterations in the ratios of NF-κB family members (27) may explain a phenomena observed in this study; NO profoundly inhibited IL-12 p40 expression, but other cytokines that are regulated through NF-κB, such as IL-1 receptor antagonist (28), were induced normally (data not shown). Activation of NF-κB has been well described through MyD88-independent signal transduction pathways (29), which may also explain why all NF-κB-regulated genes are not inhibited by NO to the same extent or with the same kinetics as IL-12 p40. Differential effects of NO on specific NF-κB family members and on MyD88-independent signal transduction pathways have not yet been determined.

Our results indicate that NO, an effector molecule for innate immunity, can deactivate the TLR signaling pathway, which results in the down-regulation of IL-12 release from antigen-presenting cells. The significance of this finding may be that during the late stages of infection with intracellular pathogens, NO could dampen the Th1 immune response through inhibition of IL-12, thus preventing excessive tissue damage to the host. However, in vivo, the effects of NO on adaptive immunity and inflammation are more complicated to assess (14). NO may have both deleterious and protective effects on different cell types during inflammation. However, the preponderance of experimental evidence suggests that NO can inhibit inflammatory Th1 responses. As NO has been shown to block T cell proliferation without affecting cytokine production (30, 31), it may inhibit a Th1 immune response at the level of the antigen-presenting cell. For example, in iNOS-deficient mice infected with herpes simplex virus, increased IL-12 production was detected compared with similarly infected heterozygous mice (32). Furthermore, exogenous NO inhibited the development of experimental autoimmune uveoretinitis in rats through inhibition of Th1 cells (33). In experimental autoimmunity encephalomyelitis, disease was exacerbated in iNOS-deficient mice, indicating that NO can inhibit Th1 immune deviation in vivo (34). Conversely, there are also experimental systems that implicate NO in the induction of a Th1 response, for example by up-regulating IL-12 β2 receptor expression on T cells (35).

In conclusion, this study demonstrates that NO inhibits IL-12 p40 protein production, mRNA accumulation, and promoter activation in murine macrophages and dendritic cells. At the molecular level, NO inhibits IRAK activation through the TLR/IL-1R pathways, with consequent NF-κB inhibition. These findings provide an example of how an innate immune effector, NO, may have a profound effect on adaptive immunity through the regulation of IL-12.
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