Nitric oxide mediates increased P-glycoprotein activity in interferon-γ-stimulated human intestinal cells

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Dixit, Santosh G., Basilia Zingarelli, Donna J. Buckley, Arthur R. Buckley, and Giovanni M. Pauletti. Nitric oxide mediates increased P-glycoprotein activity in interferon-γ-stimulated human intestinal cells. Am J Physiol Gastrointest Liver Physiol 288: G533–G540, 2005.—Patients with refractory inflammatory bowel disease (IBD) exhibit increased expression of intestinal P-glycoprotein (P-gp) as well as elevated luminal interferon-γ and nitric oxide (NO) levels. Using the in vitro Caco-2 cell culture model, we investigated whether these pathological mediators associated with the etiology of IBD affect functional activity of intestinal efflux systems. Interferon-γ reduced cellular uptake of cyclosporin A (CysA) but not methotrexate (MTX) in a time- and concentration-dependent manner. Simultaneously, P-gp expression increased by approximately twofold. Coincubation with the inducible NO synthase inhibitor L-NIL (1-(iminomethyl)-lysinine (L-NIL)) dramatically reduced production of intracellular NO in response to interferon-γ stimulus. The presence of L-NIL also abrogated the cytokine-mediated increase in P-gp expression and function suggesting that NO is required for interferon-γ-mediated activation of this efflux system. Exposure of Caco-2 cells to the chemical NO donor S-nitrosoglutathione (SNAP) produced a concentration-dependent decrease in intracellular CysA accumulation that was paralleled by an increase in P-gp expression. Both interferon-γ and SNAP enhanced DNA binding of NF-κB, whereas inclusion of L-NIL dramatically decreased this cytokine-induced effect on NF-κB binding. These results suggest that NO mediates interferon-γ-induced increase in expression and function of intestinal P-gp in the human Caco-2 cell culture model by altering DNA binding of NF-κB, which may enhance transcription of the ABCB1 gene encoding for this efflux system.

Caco-2 cells; cytokines; efflux systems; signal transduction pathways
in the pathogenesis of IBD, was assessed in modulating intestinal P-gp activity after IFN-γ stimulation.

MATERIALS AND METHODS

Cell culture. Caco-2 cells originally obtained at passage 18 from ATCC (Rockville, MD) were routinely maintained at 37°C in a controlled atmosphere of 5% CO₂-90% relative humidity using DMEM supplemented with 1% t-glutaminate, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1% nonessential amino acids (Mediatech, Herndon, VA), and 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA). When ~80% confluent, cells were detached from 75-cm² flasks using PBS/EDTA (0.02%) and trypsin EDTA (0.025/0.05%) in PBS. Caco-2 cells between passages 25 and 35 were used in this study.

Cellular uptake studies. For uptake studies, cells were seeded in 12-well plastic plates (Costar, Cambridge, MA) at a density of 2.2 × 10⁵ cells/well. Medium was changed every other day, and cells were harvested on days 2, 4, 6, 8, 10, and 12-well plastic plates (Costar, Cambridge, MA). When ~80% confluent, cells were detached from 75-cm² flasks using PBS/EDTA (0.02%) and trypsin EDTA (0.025/0.05%) in PBS. Caco-2 cells between passages 25 and 35 were used in this study.

P-gp immunoblot analysis. Frozen control and cytokine-treated Caco-2 cells (~3.5 × 10⁵) were lysed in sample buffer (Invitrogen, Carlsbad, CA). Total protein in cell lysates was determined by using the Bradford assay (Bio-Rad Laboratories, Hercules, CA). To assess the effect of IFN-γ on intracellular solute uptake, Caco-2 monolayers were preincubated through 48 h with this cytokine using medium supplemented with 1–100 ng/ml of recombinant human IFN-γ (Leinco Chemicals, St. Louis, MO). Alternatively, Caco-2 cell monolayers were pretreated with 10 ng/ml of IFN-γ in the presence and absence of 1 mM L-Nω-(1-iminoethyl)lysine (L-NIL) and 100 μM aminoguanidine, respectively, or incubated for 6 h with 0.1–5 mM of S-nitroso-N-acetylpenicillamine (SNAP).

RESULTS

IFN-γ alters intracellular uptake of substrates for intestinal efflux systems. Initially, the relationship between extracellular IFN-γ concentrations, incubation time, and functional activity of intestinal efflux systems was determined by quantifying intestinal efflux systems. For these two membrane efflux systems, were verified by using the selective P-gp inhibitor GF-120918 and the MRP2 substrate methotrexate (MTX) (Fig. 1). Substrate specificity for selected efflux systems with HCl, and intracellular radioactivity was quantified by liquid scintillation counting. Substrate specificity for selected efflux systems was delineated by inclusion of 2 μM of the P-gp inhibitor GF-120918 (generous gift from Dr. Joseph Polli, GlaxoSmithKline, Collegeville, PA) and 50 μM of the MRP2 inhibitor MK-571 (LKT Laboratories, St. Paul, MN), respectively. Total protein content was determined by using the Bradford assay (Bio-Rad Laboratories, Hercules, CA). To assess the effect of IFN-γ on intracellular solute uptake, Caco-2 monolayers were preincubated through 48 h with this cytokine using medium supplemented with 1–100 ng/ml of recombinant human IFN-γ (Leinco Chemicals, St. Louis, MO). Alternatively, Caco-2 cell monolayers were pretreated with 10 ng/ml of IFN-γ in the presence and absence of 1 mM L-Nω-(1-iminoethyl)lysine (L-NIL) and 100 μM aminoguanidine, respectively, or incubated for 6 h with 0.1–5 mM of S-nitroso-N-acetylpenicillamine (SNAP).

P-gp immunoblot analysis. Frozen control and cytokine-treated Caco-2 cells (~3.5 × 10⁵) were lysed in sample buffer (Invitrogen, Carlsbad, CA). Total protein in cell lysates was determined by using the Bradford assay. After boiling samples for 5 min, 20 μg equivalents of total protein were subjected to 7.5% SDS-PAGE. Samples were then transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore, Bedford, MA), blocked in 5% nonfat dry milk prepared in TBS (0.1%/Tween 20 (TBST), and probed overnight with a monoclonal anti-P-gp antibody (F4, 1:2,000; Sigma, St. Louis, MO). Membranes were washed three times with TBST and incubated with peroxidase-conjugated goat anti-mouse secondary antibody (1: 10,000). After development with Western Blot Luminescence Reagent (Pierce Biotechnology, Rockford, IL), visible bands were quantified densitometrically using ImageQuant. Arbitrary densitometric values were normalized to vehicle-treated controls, and the means ± SD of at least three replicates is reported in addition to the scan of one representative immunoblot.

NO production. As an indicator of NOS activity, nitrate/nitrite production was colorimetrically measured in cell supernatant using the Griess reaction (46). Briefly, nitrate in the extracellular medium was reduced to nitrite by incubation for 3 h with nitrate reductase (670 μM/ml) and NADPH (160 mM) at room temperature. One hundred microliters of the Griess reagent (0.1% naphthalenediaminedisulfonic acid in water and 1% sulfanilamide in 5% concentrated phosphoric acid; volume 1:1) was added to 100-μl samples, and optical density was measured at 550 nm using a SpectraMax 250 microplate reader (Molecular Devices, Menlo Park, CA). Total nitrate and nitrite concentrations were calculated by using standard curves prepared with sodium nitrate and sodium nitrite, respectively.

NF-κB EMSA. Caco-2 cells (~10⁵) treated with 10 ng/ml of IFN-γ or vehicle (control) through 48 h were harvested by scraping and homogenized in 1.5 ml of a homogenizing buffer using a polytron. Homogenizing buffer was composed of (in mM): 10 Tris·HCl, pH 7.4, 1 EGTA, 2 EDTA, 5 NaNO₃, 10 β-mercaptoethanol, 0.2 PMSF, 50 NaF, and 1 sodium orthovanadate, plus 0.32 M sucrose, 20 μM leupeptin, 0.15 μM pepstatin A, and 0.4 mM microcystin.

Statistical analysis. Experiments were performed at least in triplicate, and the results are expressed as means ± SD. Significant statistical differences between control and experimental groups were evaluated by using one-way ANOVA followed by Newman-Keuls posttest at the significance level of P < 0.05.

G534

INF-γ ALTERS INTESTINAL P-GLYCOPIRTEN ACTIVITY VIA NO

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MRP2 are critically dependent on the availability of ATP (1), we conclude that IFN-γ treatments did not negatively impact the cellular energy status, which is consistent with results from 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) viability studies (data not shown).

**IFN-γ increases intestinal P-gp expression.** To assess whether changes in intracellular CysA measured in IFN-γ-stimulated cells were related to altered P-gp regulation, we determined protein expression of this efflux system using immunoblots (Fig. 2). Densitometric analysis revealed that preincubation of Caco-2 cells with IFN-γ (≥10 ng/ml) increased P-gp expression in a concentration- and time-dependent manner. Consistently, most dramatic increases in the expression of this efflux system (≥1.5-fold) were measured after 48 h with 10–100 ng/ml of IFN-γ. These results are in agreement with the functional data shown in Fig. 1A and suggest that IFN-γ decreases intracellular uptake of CysA through mechanisms that enhance P-gp expression in the apical membrane of Caco-2 cells.

**IFN-γ activation of iNOS and intestinal P-gp.** The clinical pathology of IBD is intimately linked to cytokine-induced upregulation of intestinal iNOS activity with increased production of NO (40). To investigate whether changes in iNOS protein activity after IFN-γ stimulus affect P-gp efflux function in Caco-2 cells, extracellular nitrate/nitrite concentrations were determined as an indirect measure of cytokine-induced, iNOS-mediated intracellular NO production. After a 12-h incubation with 1–100 ng/ml of IFN-γ, cell supernatant nitrate/nitrite increased by at least 95% (Table 1). Production of this reactive oxygen intermediate continued through 48 h with a maximum ninefold increase compared with control levels in cells exposed to 100 ng/ml of this cytokine. Addition of the irreversible iNOS inhibitor L-NIL (1 mM) significantly reduced IFN-γ-stimulated NO production (P < 0.05). These results suggest that IFN-γ stimulates iNOS activity in Caco-2 cells leading to

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**Fig. 1.** Effect of IFN-γ on functional activity of intestinal efflux systems. Caco-2 cells grown in 12-well plastic plates for 12 days were treated with 1–100 ng/ml of IFN-γ through 48 h. Intracellular uptake of cyclosporin A (CysA; A) and methotrexate (MTX; B) after 2 h was determined as nanomoles per milligram protein and normalized to vehicle-treated controls. Results are represented as means ± SD (n = 3). *Statistical significance of results was tested by using one-way ANOVA followed by the Newman-Keuls test (P < 0.05).

**Fig. 2.** Intestinal P-glycoprotein (P-gp) expression after IFN-γ stimulus. Caco-2 cells were treated with 1–100 ng/ml IFN-γ through 48 h. Cell lysate (20 μg total protein) was separated on a 7.5% polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane. The membrane was probed with the mouse anti-P-gp monoclonal antibody (F4, 1:2,000; Sigma) and the blot was developed by using Western Blot Luminescence Reagent (Pierce Biotechnology). Visible bands were quantified densitometrically using ImageQuant and normalized to vehicle-treated controls. *Statistical significance of results was tested by using one-way ANOVA followed by the Newman-Keuls test (P < 0.05).
increased NO production in a time- and concentration-dependent manner. To extend these observations, which are consistent with earlier reports (8, 9), we measured intestinal efflux activity of P-gp in Caco-2 cells treated with 10 ng/ml of IFN-γ. This cytokine concentration was selected because it corresponds to the pathophysiological concentration range reported in the intestinal lumen of IBD patients (32). Moreover, exposure of Caco-2 cells to a 10-fold greater IFN-γ concentration (i.e., 100 ng/ml) increased NO production only moderately (Table 1). Compared with control cells, intracellular CysA uptake was significantly decreased after IFN-γ stimulus (Fig. 3A), with the most dramatic effect measured after 48 h (5.15 ± 0.23 nmol/mg protein vs. 3.13 ± 0.18 nmol/mg protein, P < 0.05). Inclusion of the iNOS inhibitor L-NIL completely abolished the cytokine-induced increase in P-gp function, which is in agreement with previously observed effects on NO production (Table 1). Consistent results were obtained by using the chemically dissimilar iNOS inhibitor aminoguanidine (data not shown). Quantitative evaluation of immunoreactive protein revealed a 70% increase in P-gp expression after a 48-h incubation with 10 ng/ml of IFN-γ. Consistent with our functional activity data, expression of this efflux system was not significantly different from control when L-NIL was included together with IFN-γ (Fig. 3C). These results suggest that NO is required for IFN-γ-mediated effects on intestinal P-gp and may act as a biochemical regulator of ABCB1 gene transcription.

**NO donor increases intestinal P-gp activity.** Because pharmacological inhibition of iNOS dramatically reduced the effect of IFN-γ on P-gp expression and functional activity, experiments were designed to identify the role of NO as a mediator of cytokine-induced regulation of this intestinal efflux system. Caco-2 cell monolayers were incubated with 0.1–5 mM of the NO donor SNAP, and NO production was measured over 6 h using the Griess reaction (Table 2). Cell viability throughout this time period was >95% determined by the MTT assay (data not shown). Supernatant nitrate/nitrite concentrations increased in parallel to greater extracellular SNAP concentrations. At maximum, the cells incubated with 5 mM of SNAP were exposed to a 13-fold increased NO level compared with controls (P < 0.05). Interestingly, total nitrite concentrations after a 6-h incubation with 1 mM of SNAP were comparable to the levels measured after a 48-h exposure of Caco-2 cells to 10 ng/ml of IFN-γ, which represents a pathophysiological reported cytokine concentration in the gastrointestinal lumen of IBD patients (32).

Effect of SNAP on intestinal efflux activity is shown in Fig. 4A. At all concentrations studied, intracellular accumulation of CysA decreased by at least 30% (P < 0.05). This indicates that a threefold increase in nitrate/nitrite concentrations compared

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**Table 1. Supernatant nitrate/nitrite following incubation of Caco-2 cells with IFN-γ in the presence and absence of the nitric oxide synthase inhibitor L-NIL.**

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Control</th>
<th>1 ng/ml IFN-γ</th>
<th>10 ng/ml IFN-γ</th>
<th>10 ng/ml IFN-γ + 1 mM L-NIL</th>
<th>100 ng/ml IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>22.1 ± 3.2</td>
<td>43.0 ± 5.5*</td>
<td>93.0 ± 16.5*</td>
<td>27.7 ± 2.3*</td>
<td>88.4 ± 1.9*</td>
</tr>
<tr>
<td>24</td>
<td>21.9 ± 0.8</td>
<td>67.0 ± 9.2*</td>
<td>93.0 ± 9.2*</td>
<td>30.1 ± 1.8*</td>
<td>103.0 ± 6.2*</td>
</tr>
<tr>
<td>48</td>
<td>22.9 ± 1.6</td>
<td>71.0 ± 4.3*</td>
<td>173.0 ± 6.5*</td>
<td>33.2 ± 1.8*</td>
<td>193.0 ± 3.4*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD; n = 6. Total nitrate/nitrite in cell supernatants in micrometers was determined using the Griess reaction. *Statistical significance between control and treatment groups was determined using one-way ANOVA followed by the Newman-Keuls test at the significance level of P < 0.05. L-NIL, L-6-(1-iminoethyl) lysine.

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**Fig. 3. Involvement of intracellular nitric oxide (NO) synthase (iNOS) in IFN-γ-mediated alterations of intestinal P-gp expression and function.** Caco-2 cells grown in 12-well plastic plates for 12 days were treated during 48 h with 10 ng/ml of IFN-γ in the presence and absence of L-NIL (1 mM). A: intracellular accumulation of CysA was determined and normalized to vehicle-treated controls. B: visible bands were quantified densitometrically using ImageQuant and normalized to vehicle-treated controls. All experiments were performed in triplicate. Data are presented as means ± SD. *Statistical significance of results was tested by using one-way ANOVA followed by the Newman-Keuls test (P < 0.05).
with controls significantly enhanced the functional activity of intestinal P-gp. In contrast, changes in immunoreactive P-gp protein were only measured after exposure of cells to SNAP at concentrations of ≥1 mM. These results strongly support a role for NO as a biochemical mediator in the IFN-γ-activated signaling cascade that alters functional activity of the P-gp efflux system.

**IFN-γ increases DNA binding of transcription factor NF-κB.** The clinical course of intestinal inflammation is paralleled by increased production of proinflammatory cytokines and subsequent activation of signaling molecules, including NF-κB (37). The same transcription factor is implicated in elevating expression and functional activity of P-gp in drug-resistant tumor cells and renal epithelium (5, 26, 42). DNA binding activity of NF-κB in nuclear extracts from Caco-2 cells incubated with 10 ng/ml of IFN-γ was increased as early as 15 min posttreatment and gradually decreased after 60 min (Fig. 5A). Specificity of the labeled oligonucleotide consensus sequence was validated by using nuclear extracts of cells incubated with 10 ng/ml of IL-6 that activates NF-κB binding in Caco-2 cells (43) and addition of 50- and 100-fold excess of the unlabeled oligonucleotide probe (Fig. 5A, lanes 5 and 6). Interestingly, enhanced DNA binding activity of this transcription factor in nuclear extracts of IFN-γ-stimulated cells appears to undergo time-dependent changes and shows increased binding again at 24 h (Fig. 5B, lane 2). Coincubation with i-NIL dramatically reduced NF-κB binding to a level similar as in nuclear extracts of control cells (Fig. 5B, lane 3). i-NIL alone, however, did not alter NF-κB binding (Fig. 5B, lanes 4 and 5). Consistent results were obtained by using the chemically dissimilar iNOS inhibitor aminoguanidine (data not shown). These observations suggest that cytokine-induced changes in intracellular NO levels of Caco-2 cells impact DNA binding of NF-κB, which, consequently, may affect transcription of target genes, including the P-gp encoding *ABCB1*. Additional support for this conclusion was obtained by evaluating the NF-κB binding pattern in cells that were treated with 1 mM of SNAP (Fig. 5C). Within 15 min of exposure to this chemical NO donor DNA binding of the transcription factor was activated and remained strong until 90 min. These results provide the first evidence that NO may contribute to IFN-γ-dependent regulation of intestinal P-gp by modulating nuclear binding activity of NF-κB.

**Table 2. Nitric oxide production in Caco-2 cells following incubation with SNAP**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrate/Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium only</td>
<td>3.0±0.5</td>
</tr>
<tr>
<td>Vehicle-treated Caco-2 cells</td>
<td>22.4±1.1*</td>
</tr>
<tr>
<td>SNAP, 0.1 mM</td>
<td>63.0±1.6*</td>
</tr>
<tr>
<td>SNAP, 1 mM</td>
<td>188.5±15.6*</td>
</tr>
<tr>
<td>SNAP, 5 mM</td>
<td>287.1±21.2*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD; n = 6. Total nitrate/nitrite in micrometers in cell supernatants was determined using the Griess reaction. Caco-2 monolayers were incubated with 0.1–5 mM of SNAP for 6 h. *Statistical significance between control and treatment groups was determined using one-way ANOVA followed by the Newman-Keuls test at the significance level of P < 0.05. SNAP, S-nitroso-N-acetylpenicillamine.

**DISCUSSION**

Biochemical and molecular pathways that alter expression of membrane efflux systems in enterocytes of IBD patients are largely unknown. We have demonstrated that exposure of human, colon-like, intestinal Caco-2 cells to apical IFN-γ concentrations corresponding to cytokine levels measured in the gastrointestinal lumen of IBD patients (32) significantly decreased intracellular accumulation of CysA but not MTX. In accordance with established substrate specificity, reduced cellular uptake of CysA suggested that this cytokine primarily alters functional activity of P-gp. In addition, it may modulate activity of other members of the ABC transporter superfamily, including MRP2 and BCRP (12, 45), although less effectively. Earlier, significant increases in *ABCB1* mRNA and P-gp protein levels were reported in differentialed, small intestine-like Caco-2 cells after incubation with 10 ng/ml of IFN-γ (3, 6). Apparently, those molecular changes did not augment func-

![Fig. 4. NO modulates intestinal P-gp expression and function. Caco-2 cells grown in 12-well plastic plates for 12 days were treated for 6 h with 0.1–5 mM of SNAP. Intracellular accumulation of CysA (A) and P-gp protein expression using immunoblots followed by densitometric analysis were normalized to vehicle-treated controls (B and C). All experiments were performed in triplicate.](http://ajpgi.physiology.org/)

Data are presented as means ± SD. *Statistical significance of results was tested by using one-way ANOVA followed by the Newman-Keuls test (P < 0.05).
Fig. 5. NO influences DNA binding of NF-κB. Total nuclear proteins obtained from Caco-2 cells treated with IFN-γ (10 ng/ml) were incubated with 32P-end-labeled NF-κB consensus binding sequence for 30 min. A: DNA-protein complexes through 120 min were resolved by PAGE, and nucleoprotein complexes were detected by autoradiography. Competition studies using a 50- and 100-fold molar excess of unlabeled, NF-κB-specific oligonucleotide probe were performed at 30 min (lanes 5 and 6). Nuclear extract from IL-6-treated cells (10 ng/ml for 120 min) served as a positive control (lane 9). B: results from similar experiments performed in the presence and absence of L-NIL (1 mM) using nuclear extracts 24 and 48 h posttreatment, respectively. Finally, binding activity profile of the radiolabeled, double-stranded NF-κB oligonucleotide probe through 120 min was determined by using nuclear extracts from Caco-2 cells incubated with 1 mM of SNAP (C). Individual panels depict representative EMSA results that were confirmed in at least 2 additional experiments. Sample loading was adjusted to equal protein concentration.

intestinal activity of this efflux system as determined by transepithelial transport of rhodamine 123 from the basolateral to the apical direction. In our experiments, P-gp activity was quantitatively assessed by using cellular uptake of the P-gp substrate CysA. As a consequence, reduced intracellular accumulation of this solute directly correlates with increased activity of this efflux system expressed in the apical membrane of Caco-2 cells. In contrast to bidirectional transport studies, this experimental approach eliminates confounding cytokine effects on tight-junction barrier properties of cell monolayers, which may increase paracellular solute flux (29, 47). From the results presented in this study, we concluded that increased P-gp protein levels and enhanced efflux activity most likely reflect molecular consequences of greater P-gp protein synthesis, which is in agreement with the earlier reported increase in IFN-γ-induced ABCB1 gene transcription (3, 6). Nevertheless, reduced proteosomal P-gp degradation and/or altered intracellular sorting of this efflux system cannot be excluded.

IFN-γ has been implicated in causing a global phenotypic switch in intestinal epithelial function during inflammation (10, 34). Some of these cytokine-induced changes contribute to progression of intestinal inflammation, whereas other effects are more immunosuppressive. This ambivalent role of IFN-γ in inflammation is cell type specific and appears to result from selective regulation of various target genes after activation of a complex interplay of cell signal transduction pathways (31). In IBD patients, iNOS expression and enzymatic activity dramatically increase in response to IFN-γ and other proinflammatory cytokines. Consequently, they are clinically employed as biochemically increased in response to IFN-γ in IBD patients, iNOS expression and enzymatic activity dramatically increase in response to IFN-γ and other proinflammatory cytokines. Consequently, they are clinically employed as biologically increase in response to IFN-γ and other proinflammatory cytokines. Consequently, they are clinically employed as biologically increase in response to IFN-γ and other proinflammatory cytokines. Consequently, they are clinically employed as
ABCB1 gene may predispose human subjects to development of UC (39).

With the use of the Caco-2 cell culture model, we demonstrated that IFN-γ-induced changes in P-gp expression and functional activity critically depend on the presence of NO. Inhibition of iNOS completely abrogated cytokine-induced effects on this efflux system. NO regulates a wide range of physiological responses and has traditionally been associated as an effector of fast-acting signaling cascades that rapidly subside after the disappearance of the original stimulus [e.g., Ca**⁺ influx (21)]. In addition, NO can stimulate signaling pathways that regulate gene expression and, consequently, elicit long-lasting changes in the cell. Recent microarray data in fibroblasts identified three distinct kinetic waves of gene activation by NO (19). The authors of this study also applied pharmacological and genetic approaches to determine the signaling pathways used by NO to regulate gene expression. Specific groups of genes were identified that require the activity of phosphatidylinositol 3-kinase, protein kinase C, or NF-kB. Activation of some genes, however, was sensitive to more than one inhibitor, suggesting the possibility of cross talk among various pathways in transducing the NO signal to the transcriptional machinery. In our study, incubation of Caco-2 cells with SNAP dramatically changed nuclear binding of NF-kB. The susceptibility of this Cys-dependent transcription factor to nitrosylation and/or oxidation and the consequences arising for transcriptional reprogramming have been evaluated in various cell systems (22). Whether NO may also affect JAK/STAT-mediated signaling events deserves further investigations.

Recently, NF-kB was reported to contribute to transcriptional regulation of the ABCB1 gene (5, 26, 42). The dramatic effects of SNAP-inducing activation of DNA binding of NF-kB, which augmented P-gp activity and protein expression to a similar extent as IFN-γ, suggest that the interplay between NO and events upstream of nuclear NF-kB binding control transcription and/or translation of this efflux system in colon-like enterocytes. However, NO could also alter intestinal efflux activity by modulating directly or indirectly intracellular protein sorting, ATPase activity, and/or protein phosphorylation status. Consequently, additional studies will be required to unveil the molecular mechanism underlying IFN-γ-induced upregulation of intestinal P-gp activity.

In conclusion, results presented in this study demonstrate that IFN-γ increases protein expression and functional activity of intestinal P-gp in the human, colon-like, Caco-2 cell line in a time- and dose-dependent manner. Furthermore, our data suggest that cytokine-induced release of NO alters DNA binding of NF-kB, which may enhance transcription of the ABCB1 gene encoding for this efflux system.

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