Antisense Oligonucleotides to poly(ADP-ribose) Polymerase-2 Ameliorate Colitis in Interleukin-10-Deficient Mice

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Received June 5, 2002; accepted August 9, 2002

ABSTRACT

Poly(ADP-ribose) polymerase-2 (PARP-2) is a newly described member of the PARP family of nuclear enzymes. Previous studies have shown pharmacological inhibition of PARP activity to have a beneficial role in attenuating inflammation. We developed a chemically modified 2′-O-(2-methoxy)ethyl antisense oligonucleotide (ISIS 110251) inhibitor of PARP-2 and tested it for efficacy in the interleukin (IL)-10-deficient mouse. In tissue culture, ISIS 110251 reduced PARP-2 mRNA expression in a concentration- and sequence-specific manner. In 129 Sv/Ev mice, ISIS 110251 reduced PARP-2 mRNA in liver by 80%. This reduction was dependent upon treatment duration and was independent of the method of delivery. In interleukin-10-deficient mice with established colitis, treatment with ISIS 110251 normalized colonic epithelial barrier and transport function, reduced proinflammatory cytokine secretion and inducible nitric-oxide synthase activity, and attenuated inflammation. Our data demonstrate that selective inhibition of PARP-2 activity results in a marked improvement of colonic inflammatory disease in a mouse model of chronic colitis and a normalization of colonic function.

Inflammatory bowel disease is characterized by high mucosal levels of reactive oxygen and nitrogen species, which can damage intracellular DNA, causing strand breakage. One consequence of DNA strand breakage is the activation of a nuclear enzyme, poly(ADP-ribose) polymerase (PARP) (Le Rhun et al., 1998). Currently, the growing PARP family consists of five members (Smith, 2001). Both PARP-1 (113 kDa) and PARP-2 (62 kDa) respond to DNA-strand breaks with a rapid and transient poly(ADP-ribosylation) of numerous nuclear proteins involved in chromatin structure and/or DNA metabolism (Oei et al., 1997; D’Amours et al., 1999). However, although PARP-1 and PARP-2 are expressed in similar tissues, the expression of both genes is independently regulated (Ame et al., 2001), suggesting that these two proteins may have specific and/or complementary cellular functions. In addition, the DNA binding domain of PARP-2 is distinct from that of PARP-1 (Ame et al., 1999), suggesting the possibility of different substrate specificities for the two proteins.

Previous studies have shown the PARP family to play a critical role in the perpetuation of inflammation, and indeed, the PARP-2 gene is located in a region of chromosome 14 that contains a number of genes involved in apoptosis and the immune system (Ame et al., 2001). Cleavage of PARP-1 by caspase 3 is well documented in numerous models of apoptotic cell death (Duriez and Shah, 1997), and recent work has demonstrated that both caspase 3 and caspase 8 cleave PARP-2 (Benchoua et al., 2002). This would suggest that PARP-2 may also be involved in modulating cellular necrosis/apoptosis.

Antisense oligonucleotides are complementary to a specific RNA sequence within the cell. Upon binding to its complementary sequence, antisense oligonucleotides can reduce the abundance of specific RNA through multiple mechanisms, depending on the chemical composition of the oligonucleotide. These include RNase H-mediated degradation of target RNA, translation arrest, and altering RNA splicing (Kole, 1997; Taylor et al., 1999). In this study, we describe the characterization of an antisense oligonucleotide (ISIS 110251) containing 2′-O-methoxyethyl modification, which targets murine PARP-2. Efficacy of ISIS 110251 in the treatment of inflammation was assessed in the IL-10-deficient mouse model of colitis. IL-10-deficient mice receiving ISIS 110251 showed a significant improvement of histological disease in the colon that correlated with a reduction of proinflammatory cytokine secretion and inducible nitric-oxide synthase activity, and attenuated inflammation.
flamatory cytokine secretion and a normalization of epithelial transport and barrier function.

Materials and Methods

Animals

Homozygous interleukin-10 gene-deficient mice, generated on a 129 Sv/Ev background, and 129 Sv/Ev controls were housed under specific pathogen-free conditions. All provisions for the facility were autoclaved. Nonautoclavable supplies were sprayed with disinfectant and introduced through a HEPA-filtered air lock. Mice were housed in micro-isolator cages with tight-fitting lids containing a spun-polyester fiber filter. In sentinel BALB/c mice, bacterial cultures, parasitological examinations, serological tracking profiles, and histological stains were negative for known murine viral and bacterial pathogens, indicating that the barrier was intact. All experiments were performed according to the institutional guidelines for the care and use of laboratory animals in research and with the permission of the local ethics committee.

Oligonucleotide Design and Synthesis

Oligonucleotides that inhibit the expression of mouse PARP-2 were identified as previously described (Baker et al., 2001) using GenBank sequence AF072521.1 as the target mRNA. All oligonucleotides were synthesized as uniform phosphorothioate chimeric oligonucleotides, with 2′-O-methoxyethyl groups on bases 1 to 5 and 16 to 20. Modified oligonucleotides were synthesized on a Milligen model 8800 DNA synthesizer (Millipore Inc., Bedford, MA). The crude product was purified and desalted by column chromatography using a Millipore HC18-HA column. Final purity was assessed by capillary gel electrophoresis and electrospray mass spectrometry (Srivatsa et al., 1997).

PARP-2 oligonucleotides used in this study were as follows: ISIS 110251, CTTTTGCTTGTGAGGTGCA (position 418); ISIS 110261, TTTGCGCCACTGTCAGCTTT (position 709); ISIS 110262, GAGA GCTGGTACAACCGCCGT (position 732).

Control oligonucleotides used in this study were as follows: ISIS 113529 (6-base mismatch for ISIS 110251), CCTTACTCGTGCTGTGGACA; ISIS 113530 (6-base mismatch for ISIS 110261), TTGGCCTCCTCTATCGGCCT; ISIS 113531 (6-base mismatch for ISIS 110262), GATAAGCCTAGTCAAGCTT.

Cell Culture and Oligonucleotide Transfection

Mouse bEND.3 cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco’s minimal essential medium and 10% fetal calf serum (Invitrogen, Carlsbad, CA). Cells were plated and transfected with oligonucleotides in the presence of 3 μg/ml DOTMA/DOPE (Lipofectin, Invitrogen) in Optimem media (Invitrogen). The buffers were maintained at 37°C with 5% CO2. The plates were incubated for 48 h, and then grown in growth media.

RNA Isolation and Reverse Transcriptase-PCR Analysis

In Vitro. Total RNA from bEND.3 cells was isolated using an RNeasy Minikit (QIAGEN, Valencia, CA). Quantitation of RNA levels was determined by real-time quantitative PCR using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. TaqMan primer/probe sequences for murine PARP-2 were: forward, GATGATTGGATGAATGTGACACCAA; reverse, ACTGGTAAACCGGCCTTT; probe, CGCTTTGAGAAGCTTAGTGCC.

In Vivo. For analysis of PARP-2 inhibition in intestinal and liver tissue, adult 129 Sv/Ev mice were dosed (either by intraperitoneal injection, subcutaneously, or via colonic enema) for various time periods with doses of oligonucleotides ranging from 0.25 to 25 mg/kg. Mice were euthanized by sodium pentobarbital, and colonic and liver tissue was removed. Tissue (100 mg) was homogenized in 3 ml of guanidinium isothiocyanate solution to isolate RNA directly from the whole tissue. The RNA pellet was resuspended in 350 μl of RLT buffer (QIAGEN) and then further purified using the RNeasy Mini Kit (QIAGEN). Liver tissue (100 mg) was spiked with a known concentration of internal standard oligonucleotide and homogenized in a Bio Savant (Bio 101, Inc., Vista, CA). The material was then extracted with a phenyl-bonded solid phase extraction column (Supelco Inc., Bellefonte, PA). Samples were analyzed by capillary gel electrophoresis using a Beckman PACE model 5010 capillary electrophoresis instrument (Beckman Coulter, Inc., Fullerton, CA) with UV detection at 260 nm. The concentrations of ISIS 11025 and the oligonucleotide metabolites in liver samples were calculated from the ratio of the absorbances, based on the starting concentration of internal standard.

Effects of Oligonucleotides on Colitis

To determine the effect of PARP-2 inhibition in vivo, adult IL-10-deficient and control 129 Sv/Ev mice were administered either a specific PARP-2 antisense oligonucleotide (ISIS 110251) or a control mismatch nucleotide (ISIS 113529) at doses ranging from 0.25 to 25 mg/kg by s.c. injection for 14 days.

Histological Injury Grading

Mice were sacrificed using sodium pentobarbital (160 mg/kg). Colon weight was harvested and fixed in 10% phosphate-buffered formalin. Samples were paraffin-embedded in toto, sectioned at 4 μm, and stained with hematoxylin and eosin for light microscopic examination. The slides were reviewed in a blinded fashion by a pathologist and assigned a histologic score for intestinal inflammation as detailed in Table 1. Histological grades (ranging from 0 to 10) represent the numerical sum of four scoring criteria: mucosal ulceration, epithelial hyperplasia, lamina propria mononuclear infiltration, and lamina propria neutrophilic infiltration.

Epithelial Functional Changes

A separate group of mice were killed by cervical dislocation and a segment of colon was used for transport studies. The mucosa was mounted in Lucite chambers exposing mucosal and serosal surfaces to 10 ml of oxygenated Krebs buffer (115 mM NaCl, 8 mM KCl, 1.25 mM CaCl2, 1.2 mM MgCl2, 2 mM KH2PO4, 225 mM NaHCO3, pH 7.4). The buffers were maintained at 37°C by a heated water jacket and circulated with CO2/O2. Fructose (10 mM) was added to the serosal and mucosal sides. The spontaneous transepithelial potential difference was determined, and the tissue was clamped at zero voltage by continuously introducing an appropriate short-circuit current (Isc) with an automatic voltage clamp (DVC 1000; World Precision Instruments, New Haven, CT), except for 5 to 10 s every 5 min when potential difference was measured by removing the voltage clamp (Clarkson and Toole, 1964). Isc was expressed as μA/cm2/h. Baseline Isc was measured after a 20-min equilibration period. Increases in Isc were induced by addition of the adenylate cyclase-activating agent forskolin (10−M) and the cholinergic agonist carbachol (10−M) to the serosal surface. Epithelial responsiveness was defined as the maximum increase in Isc to occur within 5 min of exposure to the secretagogues.

Intestinal Barrier Integrity Measurements

In Vivo Perfusion. On the day of the study, animals were administered atropine (0.2 mg/kg) 30 min before anesthesia. Anesthesia was induced via intraperitoneal injection of a cocktail containing Hypnorm (25 mg/kg) and midazolam (12.5 mg/kg). In vivo absorption was measured using a single-pass perfusion technique previously described (Miller and Schell, 1970). In brief, the colon was isolated and cannulated with flexible tubing at the proximal and distal ends. The gut was flushed with isosmotic Tyrode’s buffer (NaCl, 8 g/l; KCl, 0.2 g/l; NaH2PO4, 0.33 g/l; pH 7.4 at 37°C) to clear luminal contents. Neurovascular integrity was carefully maintained. The segment was then perfused with a test solution containing 5 g/l polyethylene glycol 4000 (nonabsorbable marker) and 1 mM Na-mannitol prepared...
in Tyrode's buffer, and radiolabeled with [14C] polyethylene glycol (10 μCi/ml) and D-[3H]mannitol (100 μCi/ml). The 37°C solution was perfused at a constant rate of 0.2 ml/min and kept at 37°C. Body temperature was monitored via a temperature probe and maintained at 37°C using a heating mattress and lamp. Intraluminal hydrostatic pressure was constantly monitored and maintained below 3 cm. After a 30-min equilibration period, eight consecutive 10-min perfusion samples were collected from the distal site. The samples were weighed, and 100-μl aliquots were taken for liquid scintillation counting. After completion of the procedure, animals were sacrificed, and the perfused segment of intestine was removed and its length measured. The segment was then dried for determination of dry weight.

**Intestinal Permeability.** Net water flux was calculated based upon the difference between initial and final volumes of perfusate and the differences between initial and final concentrations of [14C]PEG using the following equation: Pump Volume/1000 ((Experimental [14C]PEG/Initial [14C]PEG)Sample Volume)1000/10/Intestinal Length.

Mannitol clearance was calculated using the equation C_{probe} = (C_{i}V_{i} - C_{f}V_{f})/C_{avg}TW, where C_{i} is the measured initial mannitol concentration, C_{f} is the measured final mannitol concentration, V_{i} is the measured initial perfusate volume, V_{f} is the calculated final perfusate volume, C_{avg} is (C_{i} - C_{f})/ln(C_{i}/C_{f}), T is perfusion time in hours, and W is weight of gut in milligrams (Sadowski and Meddings, 1993).

**Mucosal Cytokine Secretion**

To measure mucosal cytokine secretion, colons were removed, flushed with cold phosphate-buffered saline, and cut into 2-mm squares. Each square was washed and suspended in tissue culture wells (Falcon 3046; BD Biosciences, San Jose, CA) in RPMI 1640 medium supplemented with 10% fetal calf serum, 50 mM 2-mercaptoethanol, penicillin (100 U/ml), and streptomycin (100 U/ml). Cultures were incubated at 37°C in 5% CO₂. Supernatants were harvested after 24 h and stored at −70°C for analysis of cytokine levels. Tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) levels in cell supernatants were measured using enzyme-linked immunosorbent assay kits (Medicorp, Montreal, QC, Canada).

**NO Synthase Activity**

Colonic mucosa was homogenized on ice in a buffer composed of 50 mM Tris-HCl, 0.1 mM EDTA, 0.1 mM EGTA, 12 mM 2-mercaptoethanol, and 1 mM PMSE (pH 7.4). The homogenate was incubated with a cation-exchange resin (AG 50W-X8, Na⁺ form) for 5 min at

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**Fig. 1.** Activity and sequence specificity of the murine anti-PARP-2 oligonucleotides. Relative levels of PARP-2 mRNA in bEND cells are shown 4 h after oligonucleotide treatment. ISIS 110251, ISIS 110261, and ISIS 110262 are specific for murine PARP-2, whereas ISIS 113529, 113530, and 113531 were used as mismatch control oligonucleotides. mRNA levels were measured by real-time quantitative PCR analysis and are depicted as percentages relative to the level of PARP-2 mRNA.
4°C to deplete endogenous L-arginine. Conversion of L-[3H]arginine to L-[3H]citrulline in homogenates was measured. Experiments in the presence of NADPH, without Ca2+ and with 5 mM EGTA, were performed to determine the Ca2+-independent NOS activity (Nathan and Xie, 1994). Protein concentration was determined using the Bradford method (Bradford, 1976).

Statistical Analysis

Data are expressed as means ± S.E.M., and statistical analyses were performed using the statistical software SigmaStat (SPSS Science, Chicago, IL). Differences between means were evaluated using analysis of variance or paired t tests where appropriate. Specific differences were tested using the Student-Newman-Keuls test.

Results

In Vitro Dose Response. Several antisense oligonucleotides targeted to PARP-2 were designed, synthesized, and evaluated for their ability to reduce PARP-2 mRNA expression in bEND cells (Fig. 1). The most active sequence identified, ISIS 110251, resulted in a dose-dependent decrease in PARP-2 mRNA expression (Fig. 1). The mismatch control oligonucleotide (ISIS 113529) had no effect on PARP-2 mRNA expression, suggesting that inhibition of PARP-2 was oligonucleotide sequence- and target-specific. Based upon these results, only ISIS 110251 and the mismatch ISIS 113529 were further characterized in vivo.

In Vivo Dose Response. We next determined whether ISIS 110251 was able to reduce PARP-2 mRNA in mouse liver or colon. Mice were treated with ISIS 110251 at different doses via a subcutaneous route once a day. PARP-2 expression in the liver was reduced in a dose-dependent manner (Fig. 2A). The maximal reduction in PARP-2 mRNA expression was obtained at doses of 25 mg/kg. This reduction was maximized by 14 days and did not show any further

![A - Liver](image)

![B - Colon](image)

Fig. 2. Reduction of PARP-2 mRNA in liver (A) and colon (B) in 129 Sv/Ev mice after 6 and 14 days of treatment with increasing doses of ISIS 110251. Oligonucleotides were administered subcutaneously once daily at the indicated doses. Results are expressed as mean ± S.D. where n = 4 for each time point. ISIS 110251 significantly reduced PARP-2 mRNA in liver in a dose- and time-dependent fashion. There was no significant reduction seen in colonic levels of PARP-2.
reduction up to 28 days (data not shown). Mice receiving the control mismatched oligonucleotide (ISIS 113529) did not demonstrate any reduction in PARP-2 expression (data not shown).

In contrast to the results seen in the liver, in the colon only the highest dose of ISIS 110251 resulted in a 20% reduction in PARP-2 expression (Fig. 2B). To determine whether this lack of response in colonic epithelium was due to the delivery method of the oligonucleotide, further experiments were performed using both an enema delivery and an intraperitoneal injection. Neither method resulted in any further reduction in PARP-2 expression in the colon (data not shown). Mice receiving the control mismatched oligonucleotide (ISIS 113529) did not demonstrate any reduction in PARP-2 expression in the colon (data not shown).

**Histological and Morphological Analysis.** To determine the effect of PARP-2 inhibition in vivo, adult IL-10-deficient and control 129 Sv/Ev mice were administered either a specific PARP-2 antisense oligonucleotide (ISIS 110251) or a control mismatch nucleotide (ISIS 113529) at doses ranging from 0.25 to 25 mg/kg by s.c. injection for 14 days. Over the 14-day treatment period, control mice gained an average of 0.15 g/day. The administration of either ISIS 110251 or the mismatch oligonucleotide, ISIS 113529, did not affect weight gain in control mice (Table 2). In contrast, whereas IL-10-deficient mice lost weight over the 14-day period, IL-10-deficient mice receiving ISIS 110251 gained weight (Table 2). Administration of the mismatch oligonucleotide, ISIS 113529, to IL-10-deficient mice had no effect on weight gain.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight</th>
<th>Weight Gain per Day</th>
<th>Colon Wet Weight</th>
<th>Colon Length</th>
<th>Weight/Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g/day</td>
<td>g</td>
<td>cm</td>
<td>%</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>26.6 ± 1.6</td>
<td>0.15 ± 0.08</td>
<td>0.16 ± 0.1</td>
<td>8.3 ± 0.1</td>
<td>1.9 ± 0.01</td>
</tr>
<tr>
<td>Control + ISIS 110251 (n = 4)</td>
<td>25.4 ± 1.8</td>
<td>0.17 ± 0.06</td>
<td>0.18 ± 0.2</td>
<td>8.1 ± 0.2</td>
<td>2.2 ± 0.02</td>
</tr>
<tr>
<td>IL-10-deficient (n = 8)</td>
<td>21.5 ± 1.5</td>
<td>-0.06 ± 0.09*</td>
<td>0.42 ± 0.6*</td>
<td>8.1 ± 0.3</td>
<td>5.1 ± 0.05*</td>
</tr>
<tr>
<td>IL-10-deficient + ISIS 110251 (n = 4)</td>
<td>23.7 ± 1.7</td>
<td>0.08 ± 0.05*</td>
<td>0.24 ± 0.4†</td>
<td>7.8 ± 0.2</td>
<td>2.5 ± 0.03†</td>
</tr>
<tr>
<td>IL-10-deficient + ISIS 113529 (n = 4)</td>
<td>22.6 ± 1.6</td>
<td>-0.03 ± 0.06*</td>
<td>0.39 ± 0.5*</td>
<td>7.9 ± 0.3</td>
<td>4.9 ± 0.05*</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with control.
† p < 0.05 compared with IL-10-deficient mice.

In contrast to the results seen in the liver, in the colon only the highest dose of ISIS 110251 resulted in a 20% reduction in PARP-2 expression (Fig. 2B). To determine whether this lack of response in colonic epithelium was due to the delivery method of the oligonucleotide, further experiments were performed using both an enema delivery and an intraperitoneal injection. Neither method resulted in any further reduction in PARP-2 expression in the colon (data not shown). Mice receiving the control mismatched oligonucleotide (ISIS 113529) did not demonstrate any reduction in PARP-2 expression in the colon (data not shown).

Histological and Morphological Analysis. To determine the effect of PARP-2 inhibition in vivo, adult IL-10-deficient and control 129 Sv/Ev mice were administered either a specific PARP-2 antisense oligonucleotide (ISIS 110251) or a control mismatch nucleotide (ISIS 113529) at doses ranging from 0.25 to 25 mg/kg by s.c. injection for 14 days. Over the 14-day treatment period, control mice gained an average of 0.15 g/day. The administration of either ISIS 110251 or the mismatch oligonucleotide, ISIS 113529, did not affect weight gain in control mice (Table 2). In contrast, whereas IL-10-deficient mice lost weight over the 14-day period, IL-10-deficient mice receiving ISIS 110251 gained weight (Table 2). Administration of the mismatch oligonucleotide, ISIS 113529, to IL-10-deficient mice had no effect on weight gain.

**Fig. 3.** Colonic histological scores in IL-10-deficient mice after 6 and 14 days of treatment with ISIS 110251 or ISIS 113529 with increasing doses. Mice receiving ISIS 110251 showed significant improvement in histological disease score at a dose of 25 mg/kg after 6 and 14 days of treatment. Mice receiving the mismatched oligonucleotide, ISIS 113529, showed no improvement in histological score at any dose or time point. n = 6 to 8 mice for each point. * p < 0.05 compared with untreated IL-10-deficient mice.
There was no difference in colonic length between the groups; however, colonic weight was significantly increased in IL-10-deficient mice compared with controls. ISIS 110251 reduced colonic weight in the IL-10-deficient mice, whereas ISIS 113529 had no effect. Wet weights of heart, liver, and kidney did not differ between groups (data not shown). Control mice receiving ISIS 110251 or ISIS 113529 for 14 days had no histological abnormalities in liver, kidney, or intestine (data not shown). Histological analysis of the colons showed that in the IL-10-deficient mice, the colonic epithelium was disrupted in a patchy fashion by either regions of erosion or deeper ulcerations beyond the level of the muscularis propria. Patchy transmural acute and chronic inflammation was evident, most notably in the regions of erosion and ulceration. Neutrophils and lymphocytes were present in the lamina propria and muscularis mucosa outside the realm of the erosions and ulcerations as well. Moderate to marked epithelial hyperplasia was present. IL-10-deficient mice receiving ISIS 110251 at the highest dosage (25 mg/kg) showed an improvement in histological disease by 6 days, with a greater improvement seen after 14 days of treatment (Fig. 3). There was a significant reduction in the number of neutrophils and lymphocytes in the lamina propria of the IL-10-deficient mice receiving ISIS 110251 at the highest dosage.

**Colonic Cytokine Secretion.** Previous studies have shown that colonic tissue from IL-10-deficient mice spontaneously produces higher levels of proinflammatory cytokines as compared with colons from wild-type mice due to the presence of activated T lymphocytes and macrophages in the lamina propria (Berg et al., 1996). To determine whether the improvement in histological score induced by ISIS 110251 was accompanied by an alteration in cytokine production, TNF-α and IFN-γ secretion were measured. As seen in Fig. 4, colons from IL-10-deficient mice spontaneously produced higher levels of TNF-α and IFN-γ compared with colons from control mice. In correlation with the attenuation of inflammation and reduction of lymphocytic infiltration, spontaneous colonic secretion of both TNF-α (Fig. 4A) and IFN-γ (Fig. 4B) were significantly reduced in those mice receiving ISIS 110251 for 14 days. IL-10-deficient mice receiving ISIS 113529 showed no reduction in colonic cytokine secretion. In control mice, there was no effect of ISIS 110251 on colonic cytokine secretion (Fig. 4).

**Nitric Oxide Synthase Assessment.** Nitric oxide is produced by two distinct isoforms in epithelial tissue. cNOS is a calcium-dependent constitutive enzyme, while iNOS is a calcium-independent isoform whose expression is induced in response to various insults, including TNF-α (Nathan and Xie, 1994). Colonic tissue from IL-10 deficient mice demonstrated high levels of iNOS activity in conjunction with low levels of cNOS activity (Fig. 5). After 14 days of treatment with ISIS 110251, cNOS activity was increased and iNOS activity reduced in IL-10 deficient mice. Again, as with cytokine secretion, ISIS 113529 did not affect either cNOS or iNOS activity in IL-10 deficient mice. NOS activity in control mice was not affected by ISIS 110251.

**Epithelial Function.** Under conditions of chronic inflammation, epithelial ion transport function is reduced (Madara and Stafford, 1989; Gardiner et al., 1995). To determine whether the improvement in histological disease observed in the IL-10-deficient mice was associated with improvements in epithelial function, we examined ionic responsiveness of colonic tissue in Ussing chambers. As seen in Fig. 6A, colons from IL-10-deficient mice demonstrated significant reductions in basal short-circuit current (I_{sc}) compared with age-matched controls. As a measurement of active calcium- and cAMP-stimulated chloride secretion, tissue response to carbachol and forskolin was determined. The I_{sc} response to carbachol and forskolin was greatly diminished in IL-10 deficient mice compared with control mice (Fig. 6B). Those IL-10-deficient mice receiving 14 days of treatment with ISIS 110251 showed an increase in baseline I_{sc}, whereas tissue response to both carbachol and forskolin was totally restored. IL-10-deficient mice receiving ISIS 113529 did not show any improvement in I_{sc} or response to secretagogues (Fig. 6). In control mice, ISIS 110251 had no effect on colonic I_{sc} or tissue response to carbachol or forskolin.

**Colonic Permeability.** In addition to reduced ion transport, chronic inflammation is also associated with increased intestinal permeability (Gardiner et al., 1995; Madsen et al.,

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**Fig. 4.** Effect of antisense oligonucleotide on colonic TNF-α (A) and IFN-γ (B) secretion in control and IL-10-deficient mice. Colons from IL-10 gene-deficient mice (n = 8) secreted significantly higher amounts of both TNF-α and IFN-γ compared with age-matched controls (n = 8). After 14 days of treatment with ISIS 110251 (25 mg/kg), both TNF-α and IFN-γ secretions were reduced. Administration of ISIS 113529 had no effect on cytokine secretion in IL-10-deficient mice. ISIS 110251 did not affect cytokine secretion in control mice (n = 6). *, p < 0.05 compared with IL-10 gene-deficient mice. **, p < 0.01 compared with age-matched control.
1999). Adult IL-10-deficient mice had significantly increased colonic permeability compared with age-matched control mice, whereas IL-10-deficient mice treated with ISIS 110251 for 14 days displayed normal colonic permeability (Fig. 7). Treatment of IL-10-deficient mice with ISIS 113529 had no effect. Likewise, in control mice treated with ISIS 110251, there was no difference in mannitol movement.

**Discussion**

Reduction of PARP-2 expression by specific antisense oligonucleotides results in a significant improvement in colonic inflammation in the IL-10-deficient mouse. This improvement in histological disease is accompanied by a restoration of epithelial transport function, a normalization of colonic permeability, and a decrease in proinflammatory cytokine secretion. Studies have demonstrated that systemic administration of antisense oligonucleotides will suppress target gene expression preferentially in liver (Dean et al., 1994; Zhang et al., 2000), fibroblasts (Musso et al., 1999), and kidney (Butler et al., 1997). In contrast, although antisense oligonucleotides can be absorbed across the intestine, epithelial cells of the gut show limited uptake of oligonucleotides (Butler et al., 1997). Data from this study confirm this finding. Although PARP-2 expression was reduced by up to 80% in liver tissue, gut mucosa showed only a minimal ~20% reduction in PARP-2 expression. This level of reduction was not affected by the delivery route of the oligonucleotide, because subcutaneous and intraperitoneal injection showed the same reduction in liver and intestine as compared with delivery of the oligonucleotide by rectal enema. The reason for this lack of effect of oligonucleotides in gut epithelial cells is unknown but may be due to the ability of epithelial cells to either degrade the oligonucleotides intracellularly, or to shuttle them across the cell and extrude them intact into the lamina propria. Evidence that oligonucleotides can traverse the gut wall intact and be found in the lamina propria supports the concept that epithelial cells can transport intact oligonucleotides with minimal target suppression (Butler et al., 1997; Khatsenko et al., 2000). This observation is of importance if antisense oligonucleotides are designed to target intestinal epithelial cell and, thus, their use may be limited in these types of applications.

A role for the PARP family in mediating acute inflammatory reactions has been reported in myocardial ischemia and reperfusion (Zingarelli et al., 1999), streptozotocin-induced diabetes (Pacher et al., 2002), and endotoxic shock (Szabo et al., 1997). Furthermore, the inhibition of, or the absence of, PARP-1 results in a resistance to neuronal damage deriving from ischemic injury, neurotoxin-induced parkinsonism, or traumatic brain injury (Eliasson et al., 1997; Mandir et al., 1999; Whalen et al., 1999). In addition, for a role for PARP in acute inflammatory reactions, studies have shown that inhibition of the PARP family may also be beneficial in the treatment of chronic colitis (Jijon et al., 2000). A selective reduction of PARP-2, while having no apparent effects in the absence of inflammation in normal control mice, is beneficial in reducing inflammation in the IL-10- deficient mouse model of chronic colitis. It is interesting that inhibition of

![Fig. 5. cNOS and iNOS enzymatic activity in colonic mucosa. cNOS activity was significantly decreased, whereas iNOS activity was significantly increased in colons from IL-10-deficient mice (n = 4) compared with control values (n = 4). ISIS 110251 treatment increased cNOS and decreased iNOS activity in IL-10-deficient mice but had no effect in control mice. ISIS 113529 treatment had no effect on either cNOS or iNOS activity in IL-10-deficient mice. * p < 0.01 compared with control mice. + , p < 0.01 compared with IL-10-deficient mice.](attachment:fig5.png)
PARP-2, which has been shown to contribute only a small amount of total ADP-ribosylation activity within cells (Le-Rhun et al., 1998), is effective in ameliorating inflammation. Indeed, Ame et al. (1999) failed to detect the synthesis of poly(ADP-ribose) polymers in PARP-1-deficient cells treated with low doses of DNA-damaging agents known to activate PARP-1. However, treatment of these same cells with high concentrations of these same agents revealed residual poly(ADP-ribose) polymerase activity corresponding to approximately 5 to 10% of total PARP activity. PARP-1 and PARP-2 share significant homology within their catalytic domains; however, it is unclear how structural differences between these enzymes relates to substrate specificity and cellular localization. Therefore, although PARP-2 may only contribute a small percentage of total cellular ADP-ribosylating activity, it is possible that it is the specific ribosylation of one or more unique PARP-2 substrates which underlies the therapeutic effect observed in this study.

In the IL-10-deficient mouse model, colitis begins to develop shortly after weaning and is well established by 8 weeks of age (Madsen et al., 1999). Colitis in this model is characterized by patchy mucosal ulceration, extensive neutrophilic and lymphocytic infiltration into the lamina propria, and epithelial hyperplasia (Berg et al., 1996; Jijon et al., 2000). Disease in IL-10 gene-deficient mice is initiated by an influx of activated CD4⁺ T cells into the lamina propria, and a resultant IL-12-directed excessive generation and activation of Th1 cells (Berg et al., 1996). As shown in this study, colonic inflammation in this model is associated with enhanced colonic permeability, high levels of intestinal mucosal IFN-γ and TNF-α, and increased nitric oxide production. Both IFN-γ and TNF-α have been shown to directly increase epithelial permeability (Madara and Stafford, 1989; Taylor et al., 1998). In addition, nitric oxide and peroxynitrite, a reaction product of nitric oxide and superoxide anion, and a common effector of tissue injury during inflammation, have been shown to break down the barrier function of the epithelium (Salzman et al., 1995; Beckman, 1996; Wiseman and Halliwell, 1996).

We have previously shown that IL-10-deficient mice have elevated levels of mucosal nitrotyrosine in colonic tissue, suggesting the presence of peroxynitrite and tyrosine residues (Jijon et al., 2000). Inhibition of PARP activity with 3-aminobenzamide was effective in reducing the levels of nitrotyrosine and restoring colonic permeability (Unno et al., 1995; Kennedy et al., 1998). IL-10-deficient mice receiving ISIS 110251 demonstrated significantly increased colonic permeability as compared with age-matched control mice (n = 6). After 14 days of treatment with ISIS 110251, IL-10-deficient mice had normalized colonic permeability, whereas ISIS 113529 had no effect. There was no effect of ISIS 110251 treatment in control mice. +, p < 0.05 compared with IL-10-deficient mice. *, p < 0.01 compared with age-matched control.
unlikely that a reduction in PARP-2 expression in colonic epithelial cells was responsible for the restoration of either barrier or transport function, because very little reduction was observed in colonic mucosa. Conversely, a reduction in either proinflammatory cytokine secretion or iNOS activity could result in a restoration of both barrier and transport function. Interestingly, it has been shown that an ADP-ribosylation reaction mediated by PARP occurs in macrophages and contributes to the activation of NF-κB and subsequent increase in proinflammatory cytokine secretion (Le Page et al., 1998). In macrophages, PARP activation precedes the up-regulation of iNOS activity and release of proinflammatory cytokines, and furthermore, inhibition of PARP prevents the release of these cytokines by attenuating NF-κB activation (Le Page et al., 1998). It is not known whether PARP-2 is also found in macrophages, or whether selective reduction of PARP-2 inhibits macrophage activity. However, in that there was no significant suppression of PARP-2 expression in the colonic mucosa, it is plausible that the anti-inflammatory effect may have been mediated by a PARP-2 reduction in macrophages/monocytes. Further experiments will be needed to resolve these questions.

The protective effect of inhibition of the PARP family seen in various experimental models of inflammation caused by oxidative or NO-induced stress indicates that clinical treatment with PARP inhibitors may provide therapeutic benefits. However, although it is clear that PARP-1 functions in genome protection (Ding et al., 1992; D’Amours et al., 1999), it remains to be shown what function PARP-2 has in maintaining genetic stability. However, the DNA binding domain of PARP-2 is distinct from that of PARP-1 (Ame et al., 1999), suggesting different substrate specificities and/or modes of activation for the two proteins. Inhibition of PARP-1 with pharmacological inhibitors or antisense RNA expression has been shown to increase the frequency of recombination, gene amplification, and sister chromatid exchanges after treatment with genotoxic agents (Waldman and Waldman, 1991; Ding et al., 1992; Kupper et al., 1996). It remains to be shown what effect the inhibition of PARP-2 alone has on these parameters or what the consequence of suppression of PARP-2 activity would be.

Taken together, these data support a role for PARP-2 in the perpetuation of inflammation and increasing colonic permeability in the IL-10-deficient mouse and supports the concept of selective PARP inhibition as a therapeutic tool in the treatment of inflammatory bowel disease.

References


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