An ICAM-1 Antisense Oligonucleotide Prevents and Reverses Dextran Sulfate Sodium-Induced Colitis in Mice

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ABSTRACT

Mice treated p.o. with 5% dextran sodium sulfate develop a mild to moderate colitis characterized by focal areas of inflammation and crypt abscesses. Immunohistological analysis of colons from dextran sodium sulfate-treated mice revealed an increased expression of intercellular adhesion molecule 1 (ICAM-1) and infiltration of lymphocyte function antigen 1-positive cells. A murine-specific antisense oligonucleotide, ISIS 3082, was used to determine the role of ICAM-1 expression in the development of colitis. Prophylactic treatment of dextran sodium sulfate-treated mice with ISIS 3082 reduced the clinical signs of colitis in a dose-dependent manner, with maximal effects occurring at a dose of 1 mg/kg/day. Reductions in ICAM-1 immunostaining and infiltrating leukocytes were observed in colons of animals treated with 1 mg/kg ISIS 3082. Scrambled control oligonucleotides failed to modify the course of the disease. The ICAM-1 oligonucleotide also diminished the clinical severity of colitis in mice with established colitis. The toxicity of ISIS 3082 was assessed in normal CD-1 mice by administering the oligonucleotide intravenously every other day for 2 weeks. At pharmacologically relevant doses of ISIS 3082 (1 and 10 mg/kg), there were no signs of toxicity with respect to body and organ weights, clinical chemistry or hematology. At a dose of oligonucleotide 20- to 100-fold greater than maximal pharmacological doses, the oligonucleotide produced an increase in liver and spleen weights; a mild chronic inflammation in liver, lung and lymph nodes; monocytosis and an elevation of serum liver transaminases. These data suggest that an antisense oligonucleotide that reduces ICAM-1 expression could be effective in the therapy of inflammatory bowel disease in humans and that such an oligonucleotide would be safe at pharmacologically relevant doses.

Recruitment and retention of leukocytes at local sites of inflammation is a carefully orchestrated process involving both soluble and cell-associated molecules. Recently, much attention has been focused on the adhesion molecules responsible for the trafficking of leukocytes to sites of inflammation (Butcher, 1991; Bevilacqua, 1993; Springer, 1994; Albelda et al., 1994). These molecules include members of the selectin family (i.e., E-, L- and P-selectin), which mediate the initial rolling of leukocytes on vascular endothelium, and members of the immunoglobulin family (ICAM-1, ICAM-2 and VCAM-1) interacting with integrins expressed on leukocytes, resulting in firm adhesion and transmigration.

The endothelial cell adhesion molecules ICAM-1, VCAM-1, E-selectin and P-selectin normally either are not expressed or are expressed at low levels on the surface of capillary and venule endothelium. However, in response to inflammatory mediators such as autacoids and cytokines, endothelial cells markedly up-regulate expression of these molecules. In addition to facilitating the transport of leukocytes to sites of inflammation, ICAM-1 and VCAM-1 have a broader function in the immune response. They are induced on the surface of multiple cell types in response to cytokines, ICAM-1 exhibiting the broadest tissue distribution (Rothlein et al., 1986; Dustin et al., 1986; Bennett and Crooke, 1994). Expression of ICAM-1 and VCAM-1 on nonendothelial cells may play a role in local retention of inflammatory cells, but probably more important is the role of cell adhesion molecules in facilitating activation of cells of the immune system. Both ICAM-1 and VCAM-1 have been demonstrated to provide co-stimulatory signals to lymphocytes, resulting in increased responsiveness to antigen-specific stimulation (Van Seventer et al., 1990; Kuhlman et al., 1991; Damle et al., 1992; Damle et al., 1994; Poudrier and Owens, 1994; Koopman et al., 1994).

Increased expression of adhesion molecules has been noted in numerous diseases that have an inflammatory component (Griffiths et al., 1989; Adams et al., 1989; Hale et al., 1989;
DNA synthesizer (Millipore Inc., Bedford, MA) using modified phosphorodiamidite chemistries with β-cyanoethylphosphoramidites (Andrade et al., 1994). A crude product of approximately 70% purity was further purified by column chromatography using a Millipore HC18-HA column. The purified material was ethanol-precipitated, redissolved and further desalted by ultrafiltration. The samples were then deprotonated by ultrafiltration with endotoxin levels reduced to below detectable levels. Purity of the material was assessed by capillary electrophoresis, anion exchange HPLC and NMR. The oligonucleotides were found to be greater than 92% full-length material and to contain less than 0.3 mole% phosphodiester linkages. The sequences of the oligonucleotides used in this study were ISIS 3092, 5′-TCATCCCCAGGCCACCAT-3′; ISIS 4189, 5′-CAGCCTATGTTGCCCCCAAC-3′; and ISIS 8997, 5′-TGCATGACCCGCCCCTA-3′.

Localization of oligonucleotide in colon. ISIS 3082 was labeled with rhodamine as previously described (Bennett et al., 1992). Briefly, ISIS 3082 synthesized with a 5′-amine was conjugated with a 5-fold molar excess of rhodamine isothiocyanate (Molecular Probes, Eugene, OR) overnight in 100 mM sodium carbonate. After quenching of the reaction with glycine, the oligonucleotide was separated from free rhodamine by gel filtration chromatography followed by reverse-phase HPLC. After injection of the oligonucleotide, the animals were sacrificed by perfusion with 4% paraformaldehyde plus 0.2% gluteraldehyde in phosphate buffered saline. Tissues were removed and fixed in perfusion buffer for 6 hr. The tissue was transferred into 15% sucrose in phosphate-buffered saline (PBS) for 12 to 16 hr and frozen in an isopentane dry-ice bath in OCT solution (Miles). Frozen 4-μm sections of the tissues were cut using a Leitz cryostat and mounted using GelMount (Biomeda Corp., Foster City, CA). The distribution of the rhodamine-labeled oligonucleotide was determined by fluorescence microscopy. The perfusion fixation technique was found to maintain the localization of the oligonucleotide and provides better tissue morphology than dry mounts of fresh-frozen sections (Butler et al., manuscript in preparation). Furthermore, the rhodamine label does not appear to change dramatically the disposition of the oligonucleotide in animals; similar patterns of disposition were observed using autoradiography of 3H-labeled and 35S-labeled oligonucleotide and immunohistochemical localization of oligonucleotide with a sequence-specific monoclonal antibody (Butler et al., manuscript in preparation). Furthermore, the label in the tissue does appear to be associated with the oligonucleotide as determined by capillary gel electrophoresis of tissue extracts (data not shown).

Induction of colitis. Female Swiss-Webster mice weighing 25 to 30 g were obtained from Ace Animals Inc. (Boyertown, PA). Standard mouse chow pellets and water were made available ad libitum. Mice were weighed and randomized into 10 groups of 10. Colitis was induced by replacing normal drinking water with distilled water containing 5% DSS (molecular weight 30,000–40,000) for 5 to 7 days as indicated.

Evaluation of colitis. Daily weight, physical condition, presence of gross blood in excreta and daily stool consistency were determined. On the last 2 days of the experiment, stools were tested for the presence of occult blood (Hemoccult strips, Smith Kline Diagnostics, San Jose, CA). At the end of day 6, mice were sacrificed by carbon dioxide inhalation, the colons were quickly removed and the length from the cecum to the rectum was measured. The DAI was calculated by assigning scores to changes in weight, Hemoccult positivity or gross bleeding and stool consistency, according to the system previously published by Murthy et al. (1993). This method of scoring was shown to correlate with more specific measures of inflammation and correlated with crypt score (Murthy et al., 1993). Data are summarized as means ± S.E.M. Significance of differences between means were assessed by analysis of variance (single-factor). Statistical significance of the difference between drug-treated and vehicle-treated groups was established at a probability of P < .05.

A histological lesion score was also used to evaluate evidence of
colitis in some groups of animals. The colon was removed from the mice 5 mm proximal to the anus and 5 mm distal to the ileocecal valve. It was trimmed longitudinally, processed through paraffin, sectioned in a longitudinal direction throughout the entire length of the colon segment (approximately 3 cm), stained with hematoxylin and eosin and evaluated microscopically. The individual lesions were scored as follows: 1, focal inflammatory infiltrate without disruption of the crypt epithelium; 2, inflammation with crypt epithelium disruption; 3, ulcer. An index was obtained by taking the score of the worst lesion in the colon segment and multiplying it by the amount of colon involved according to the following scale: 0, normal; 1, <25%; 2, 26–50%; 3, 51–75%; 4, 76–100%. The indexes of the groups were compared.

Drug treatment. For prophylactic treatment, mice were treated with test compounds starting the same day that administration of DSS began. The oligonucleotides were diluted in sterile 0.9% saline and administered daily by s.c. injection at the indicated doses. TGF-β2 diluted in saline was given daily for 6 days by intracolonic administration. Prednisolone diluted in saline was administered by daily s.c. injection. For therapeutic treatment, oligonucleotide administration began on the last day of treatment with DSS (5 days of DSS treatment) and continued for 7 days. Cyclosporin A was given by intracolonic administration in mineral oil, also starting on day 5.

Immunohistochemical determination of ICAM-1, LFA-1 and Mac-1 expression. Colon samples from the mice were frozen in OCT embedding medium (Miles, Elkhart, IN). Cryosections were prepared, fixed in acetone for immunohistochemical demonstration of ICAM-1, Mac-1 and LFA-1 and fixed in an ethanol-formaldehyde solution for staining with hematoxylin and eosin. Nonspecific binding of the antibodies was blocked by adding to the sections a solution of 0.5% casein, 1.0% BSA and 1.5% normal horse serum before the addition of primary antibodies. Endogenous peroxidase was blocked in the sections by hydrogen peroxide generated by the enzyme glucose oxidase on a glucose substrate. Endogenous biotin was blocked by the sequential addition of avidin and biotin to the tissues.

Tissues sections were incubated with 5 μg/ml of the purified ICAM-1 monoclonal antibody YN1/1.7.4 (Takei, 1985) (ATCC, Rockville, MD), with 15 μg/ml of Mac-1 antibody (Pharmingen, San Diego, CA) or with 2.5 μg/ml of the LFA-1 antibody M174/4.11.19 (Sanchez-Madrid et al., 1983) (ATCC) for 1 hr at 25°C. The slides were washed in PBS to remove the primary antibody and incubated with biotinylated donkey anti-rat IgG (Jackson Labs., West Grove, PA) for 30 min, followed by ABC reagent (Vector Labs., Burlington, CA).Slides were dehydrated with 3,3′-diaminobenzidine as a peroxidase substrate (Sigma Chemical Co., St. Louis, MO). All sections were counterstained with hematoxylin. The coverslips were sealed with Permount, and the sections were examined microscopically.

Evaluation of oligonucleotide toxicity. Twenty male and 20 female CD-1 mice (6–8 weeks of age; Charles River, Wilmington, MA) were randomly assigned to three dose groups of five males and five females and a vehicle control group of equal size (PBS). Mice were housed individually in metal cages with suspended wire-mesh floors and were maintained in an environmentally controlled room (12-hr light/dark cycle) with ad libitum access to feed (Tekland rodent diet) and water.

Mice were treated every other day with 0, 1, 10 or 100 mg/kg ISIS 3082 via i.v. administration by caudal vein injection for 13 days (7 doses). The dose volume was 2 ml/kg. Mice were sacrificed 2 days after the last dose.

During the treatment period, all groups were observed daily for signs of toxicity. Additional antemortem observations included weekly determinations of body weight and food consumption. Blood samples were collected for clinical pathology determinations from the retroorbital sinus immediately before sacrifice. Hematology parameters included erythrocyte count, total hemocrit, platelet count, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration. Serum biochemistry parameters examined were total protein, albumin, globulin, albu-

min/globulin ratio, blood urea nitrogen, cholesterol, triglycerides, creatinine, sodium, potassium, chloride, calcium, total bilirubin, glucose and phosphorus. Enzyme activities were determined for alkaline phosphatase, AST and ALT.

At necropsy, complete macroscopic evaluation of all body cavities was performed. Selected organs (the adrenal glands, kidneys, liver, lungs, spleen and heart) were excised, trimmed of fat and connective tissue and weighed. Kidney, liver, lungs, pancreas, spleen, bone marrow, adrenal glands and injection site from all control and high-dose (100 mg/kg) animals were processed for histopathological analysis by fixation in 10% neutral-buffered formalin and staining with hematoxylin and eosin for standard microscopic evaluation. Differences between the 1–100 mg/kg ISIS 3082 dose group and the control group were evaluated. Statistical evaluation of clinical pathology, organ weight, body weight and food consumption was performed by using an appropriate one-way analysis of variance and a test for ordered response in the dose groups. For parametric data, a standard one-way ANOVA using F distribution to assess significance was employed. When significant differences among the means were indicated, Dunnett’s test was used to determine which treatment groups differed significantly from control. A standard regression analysis for linear response in the dose groups was also performed.

Results

ICAM-1 expression is increased in DSS-induced colitis. Treatment of mice with 5% DSS for 5 days produced a mild colitis, and none of the mice examined exhibited lesions involving greater than 25% of the colon (n = 10, fig. 1B). No lesions were observed in mice that were not treated with DSS (fig. 1A). The individual lesions ranged from a focal inflammatory infiltrate, without disruption of the crypt epithelium, to an ulcer (fig. 1B). Lesions were characterized by a mononuclear cell infiltrate, composed predominantly of macrophages with fewer lymphocytes and occasional eosinophils and neutrophils (fig. 1C). Endothelial cells adjacent to areas of inflammation, such as the tunica muscularis, were hypertrophic (fig. 1D).

The expression of ICAM-1 in mouse colons was determined by immunohistochemistry. LFA-1 staining in the colons was also determined, because it is one of the cell surface proteins expressed on leukocytes that bind ICAM-1 (Marlin and Springer, 1987). Staining intensity for ICAM-1 was greater in colon specimens from DSS-treated mice (fig. 2, C and D) than in controls (fig. 2, A and B). In normal colon, ICAM-1 was expressed at low levels on mucosal endothelial cells and in the germinal centers of lymphoid nodules (fig. 2, A and B). LFA-1-positive leukocytes were found to surround the germinal centers (fig. 2, E and F).

In DSS-treated mice, staining for ICAM-1 was most prominent in blood vessels of the tunica muscularis (fig. 2C) and on submucosal veins (fig. 2D); in both cases, expression was increased compared with control animals. ICAM-1 expression in lymphoid nodules and on mucosal leukocytes was similar in treated and control animals. In both control and treated mice, a similar incidence of LFA-1-positive cells was found in colon mucosa and lymphoid nodules. There was an increase in LFA-1-positive cells within and surrounding foci of inflammation of DSS-treated mice, and an increase in LFA-1-positive cells was observed within vascular spaces of the tunica muscularis (fig. 2G).

Localization of antisense oligonucleotides in colon. Previous studies examining the disposition of radiolabeled ISIS 3082 demonstrated that approximately 2% to 5% of the
Total dose administered accumulates in the small intestine (Crooke et al., 1996; Bennett et al., 1996). Published studies (Agrawal et al., 1991; Zhang et al., 1995), as well as our own unpublished data for ISIS 3082, have demonstrated that the concentration of phosphorothioate oligonucleotide in small intestine is similar to the concentration in large intestine. To obtain information about the localization of ISIS 3082 in the colon, we labeled ISIS 3082 with rhodamine and injected s.c., at 5 mg/kg every 24 hr for two doses, into either normal mice or mice treated with DSS for 5 days. The mice were sacrificed 24 hr after the second dose, and the oligonucleotide was localized in cryostat sections of the large intestine. There was minimal autofluorescence of colon tissue under these treatment conditions (fig. 3A). In normal mice, the antisense oligonucleotide localized predominantly to cells present in the lamina propria, though some material was detectable in epithelial cells (fig. 3B). With DSS treatment, the oligonucleotide was still detected in cells present in the lamina propria, but there was more accumulation of the oligonucleotide in the epithelial cells (fig. 3C). These data demonstrate that the antisense oligonucleotide does localize to sites in the bowel where ICAM-1 is expressed and that treatment with DSS promotes increased accumulation of the oligonucleotide within the mucosal epithelial cells. The cause for the increased accumulation of oligonucleotide in epithelial cells of mice treated with DSS is currently under investigation.

**Prevention of colitis with ISIS 3082.** To determine whether blocking ICAM-1 expression would prevent the development of colitis, mice were treated with the murine-specific ICAM-1 antisense oligonucleotide ISIS 3082 during treatment with DSS for 5 days. Daily administration of 1 mg/kg of ISIS 3082 by s.c. injection reduced by 40% the DAI in animals treated with DSS (fig. 4). ISIS 3082 (1 mg/kg/day) was as effective as 1 μg/day of TGFβ2, administered intra-colonically (fig. 4), which has been reported to be protective in this phase of the model (Murthy et al., 1992). ISIS 3082 given p.o. did not inhibit the development of colitis, probably because of limited oral bioavailability (data not shown).

Treatment of mice with DSS for 5 to 7 days produced a mild colitis with focal lesions as assessed by microscopic examination of hematoxylin- and eosin-stained tissue sections. The oligonucleotide reduced the number and severity of inflammatory lesions, although the reduction in histological score did not reach statistical significance: lesion scores were 1.9 ± 1.19 and 1.4 ± 1.07 for DSS-treated and ISIS 3082-treated animals, respectively (P < .1, n = 10). Concomitant with a reduction in inflammatory lesions was a reduction in ICAM-1 immunostaining in the colons from DSS-treated mice also treated with ISIS 3082 compared with the saline-treated controls (fig. 5). There was also a decrease in inflammatory infiltrates as measured by Mac-1 staining (fig. 5), a β2 inte-
Fig. 2. Expression of ICAM-1 and LFA-1 in colons of mice treated with DSS. Frozen sections of colons from normal and DSS-treated mice were stained either with ICAM-1 antibody (panels A–D) or with antibodies directed to murine LFA-1 (panels E–H). A) ICAM-1 expression in colon from a negative control mouse, ×90. B) Higher magnification of panel A, ×360. C) ICAM-1 expression in colon from a mouse treated with DSS for 5 days, demonstrating ICAM-1 expression on endothelial cells of all layers of colon, ×90. D) ICAM-1 expression in endothelium of submucosal vein from DSS-treated mouse, ×360. E) LFA-1 expression in cells surrounding germinal center of normal colon, ×90. F) Higher magnification of panel F, ×360. G) LFA-1 expression in cells in the inflammatory infiltrate of colons from mice treated with DSS, ×90. H) Background staining observed with isotype-matched control antibody, ×90.
n sense oligonucleotide at a dose of 0.3 mg/kg. Because ISIS 4189 provided approximately 31% protection in mice, the oligonucleotide localized mostly to cells in the lamina propria (panel B), whereas in mice treated with DSS, the oligonucleotide localized in lamina propria and mucosal epithelial cells (panel C).

![Image](https://example.com/image.png)

**Fig. 3.** Localization of rhodamine-labeled ISIS 3082 in normal and DSS-treated colon. Normal mice (panel B) and mice treated with DSS for 4 days (panel C) were injected s.c. two times with rhodamine-labeled ISIS 3082 24 hr apart. Twenty-four hours after the last injection, colon tissue was harvested and tissue sections prepared as described in “Materials and Methods.” Localization of rhodamine-labeled ISIS 3082 was evaluated by fluorescence microscopy. The absence of significant autofluorescence in the colon tissue is shown in panel A. In control mice, the oligonucleotide localized mostly to cells in the lamina propria (panel B), whereas in mice treated with DSS, the oligonucleotide localized in lamina propria and mucosal epithelial cells (panel C).

![Graph](https://example.com/graph.png)

**Fig. 4.** Prevention of colitis by an ICAM-1 antisense oligonucleotide. Swiss-Webster mice were administered 5% DSS in drinking water for 5 days to induce colitis. Mice were treated with either ISIS 3082 by daily s.c. injections or daily intracolonic administration of 1 μg TGF-β2, beginning at the same time DSS treatments were initiated. Clinical assessment of colitis was performed as described in “Methods” and was expressed as the DAI. Results are expressed as the mean ± S.E.M. (n = 10). * Significantly different from control at P < .05.

The effect of ISIS 3082 on the development of colitis was dose-dependent. In an experiment in which mice were given DSS and treated with oligonucleotide for 5 days, doses as low as 0.03 mg/kg of ISIS 3082 produced a statistically significant reduction in the severity of DAI, with maximal effects occurring between 0.3 and 1.0 mg/kg (fig. 6). At a dose of 5 mg/kg/day, ISIS 3082 was less effective than at 1 mg/kg/day (fig. 6), and at a dose of 10 mg/kg/day, ISIS 3082 reproducibly failed to prevent the development of colitis. ISIS 4189 is an active phosphorothioate oligodeoxynucleotide that inhibits murine protein kinase C-α expression both in vitro and in vivo (Dean and McKay, 1994). ISIS 4189 also serves as a scrambled control for ISIS 3082; it has the same base composition as ISIS 3082 with the bases arranged in a different sequence. At doses at which ISIS 3082 inhibited the development of colitis, ISIS 4189 failed to affect significantly the severity of colitis (fig. 6). At the highest dose level tested, 5 mg/kg/day, ISIS 4189 provided approximately 31% protection, compared with 64% achieved with the ICAM-1 antisense oligonucleotide at a dose of 0.3 mg/kg. Because ISIS 4189 had some effects that could be attributed to reduction in protein kinase C-α expression, a second scrambled control oligonucleotide, ISIS 8997, which has no known homology to any murine gene product, was also evaluated for activity in the model. This was done in a third set of experiments in which the DSS treatments were extended to 7 days. The scrambled control oligonucleotide, ISIS 8997, failed to reduce significantly the severity of the disease at a dose of 5 mg/kg, whereas the ICAM-1 antisense oligonucleotide, ISIS 3082, significantly reduced the DAI score (table 1). Prednisolone at a dose of 2.5 mg/kg/day did not prevent the development of colitis (table 1). Thus ISIS 3082 selectively reduced the severity of colitis in mice treated with DSS in a sequence-specific manner in three separate experiments, the optimal dose depending somewhat on duration of DSS treatment.

**Treatment of established colitis with ISIS 3082.** The colitis that develops after 5 days of treatment with DSS persists for several weeks after discontinuation of treatment with DSS (Okayasu et al., 1990; Cooper et al., 1993). Therefore, we evaluated ISIS 3082 as a therapeutic by initiating treatment after the disease was established. Treatment of mice with established colitis with 5 mg/kg/day of ISIS 3082 for 1 week resulted in an improvement in the severity of colitis (fig. 7), whereas 0.5 mg/kg/day failed to produce a significant effect (fig. 7). It should be noted that the animals were randomized to separate treatment groups before the beginning of DSS treatment; this accounts for the variability in DAI before treatment with the test agent was initiated (fig. 7). As previously reported (Murthy et al., 1993), cyclosporin A administered intracolonically also reduced the severity of the disease in this phase of the model. Animals treated with the antisense oligonucleotide exhibited a more profound weight loss than the other groups of animals, including the saline-treated control group, which failed to gain weight during the course of the experiment (fig. 8). The mechanism by which the oligonucleotide exacerbates weight loss in these animals is not known. These results demonstrate that ISIS 3082 not only prevents the development of colitis in mice treated with DSS when administered prophylactically but also decreases the severity of symptoms in mice with pre-existing disease.

**Toxicity of ISIS 3082 after subchronic administration in mice.** To evaluate the potential toxicity of ISIS 3082 in mice, the drug was administered by i.v. injection for 13
days at three dose levels: 1 mg/kg, 10 mg/kg and 100 mg/kg. The 1 mg/kg and 10 mg/kg represent pharmacologically relevant doses of ISIS 3082. There were no deaths in any of the ISIS 3082 treatment groups or vehicle control groups. There were no apparent clinical signs of toxicity in any dose group, and no effect on body weight (table 2) or food consumption (data not shown) was observed. Thus, in contrast to the results we observed in animals with colitis, ISIS 3082 does not cause weight loss in normal mice when repeatedly administered by i.v. injection. Liver and spleen weights were increased at the high dose level (table 2). No statistically significant changes in other organ weights followed treatment with the oligonucleotide (data not shown).

Treatment-related changes in hematology were limited to a statistically significant monocytosis (approximately a 5-fold increase compared with the control value) in the 100-mg/kg group (table 3). There was no incidence of anemia or thrombocytopenia in any dose group in this study. Treatment-related changes in clinical chemistry at the 100-mg/kg level included moderate to large increases in serum concentrations of liver transaminases (AST and ALT) and alkaline phosphatase, a slight increase in albumin and in the albumin/globulin ratio and a decrease in serum glucose. None of these alterations in hematology or chemistry were observed in the low-dose or middle-dose group.

Upon microscopic examination of organs and tissues, the primary histopathological finding was the presence, in the 100-mg/kg dose group, of mononuclear cell infiltrates that included liver, lung and lymph nodes (fig. 9). The liver appeared to be the most affected, cellular infiltrates being observed in 6 of the 10 mice in the high-dose group (100 mg/kg). Other histopathological lesions noted in the high-dose group included mild to severe chronic inflammation of the lung and severe hyperplasia of the follicular cortex in cervical lymph nodes. Treatment-related effects in the 10-mg/kg group were limited to small increases in mean liver and spleen (females
only) weight, mild inflammation of the liver and lung and moderate hyperplasia of cervical lymph nodes. No clearly treatment-related findings were observed in the 1-mg/kg dose group.

Discussion

Many studies have demonstrated increases in ICAM-1 expression in various human diseases with an inflammatory component, as well as in animal models of such diseases. The role of ICAM-1 in DSS-induced colitis was investigated first by demonstrating an association of ICAM-1 expression with disease and then by selectively blocking ICAM-1 expression with antisense oligonucleotides, which resulted in attenuation of clinical symptoms of the disease. By immunohistochemistry, it was also demonstrated that ICAM-1 is expressed on endothelium in normal colon. Expression of ICAM-1 was increased on vessels from mice treated with DSS. There did not appear to be an increase in ICAM-1 expression on epidermal cells of the crypt. In normal human colon tissue, ICAM-1 is expressed on capillaries and venules (Nakamura et al., 1993; Schuermann et al., 1993). In both ulcerative colitis and Crohn’s disease, ICAM-1 expression is increased on endothelium of venules, on inflammatory infiltrates and (rarely) on epithelial cells in crypt abscesses and mucosa adjacent to ulcers (Nakamura et al., 1993). These data demonstrate that, as in some human inflammatory bowel diseases, expression of ICAM-1 is increased in an experimental model of colitis and might contribute to the pathophysiology.

We demonstrated a role of ICAM-1 in the development of colitis in animals treated with DSS by using an antisense oligonucleotide, ISIS 3082, that selectively inhibits ICAM-1 expression in murine cells (Stepkowski et al., 1994). The ICAM-1 antisense oligonucleotide partially prevented the development of colitis when administered prophylactically and decreased the severity of symptoms in mice with pre-existing colitis. ISIS 3082 was more effective than, or as effective as,
other agents that have been reported to be active in both stages of this model (Murthy et al., 1992; Murthy et al., 1993). However, in both treatment regimens, the oligonucleotide failed to block completely or reverse clinical signs of colitis. This suggests that additional mediators contribute to the disease and/or that a more nearly complete inhibition of ICAM-1 expression is required for full therapeutic effect.

Previously we have demonstrated that ISIS 3082 blocked rejection of heterotopic cardiac allografts in a sequence-specific manner (Stepkowski et al., 1994). As reported for the heart allograft model, the effects of the oligonucleotide in the colitis model were sequence-specific in that two additional scrambled control oligonucleotides with the same base composition and length failed to modify significantly the development of the disease over the dose range that was effective for ISIS 3082. One of these oligonucleotides, which targeted murine protein kinase C-α, did not prevent the development of colitis except at the highest dose tested (5 mg/kg), which produced a 34% reduction in the DAI. The lowest dose of ICAM-1 antisense oligonucleotide tested (0.03 mg/kg) produced a 45% reduction in the DAI. It has previously been shown that the protein kinase C-α oligonucleotide selectively reduces expression of protein kinase C-α in murine tissue (Dean and McKay, 1994). Thus these data suggest that inhibition of protein kinase C-α expression does not dramatically affect the development of colitis in mice treated with DSS.

The data generated to date suggest that ISIS 3082 acts in part by an antisense mechanism. This interpretation is supported by cell culture-based data, in which ISIS 3082 was identified as being the most effective of 18 different oligonucleotides in reducing ICAM-1 protein expression on murine endothelial cells (Stepkowski et al., 1994). ISIS 3082 reduced ICAM-1 protein expression by a mechanism consistent with RNase H-mediated hydrolysis of the target mRNA. The effects of ISIS 3082 were sequence-specific; scrambled control oligonucleotides failed to reduce ICAM-1 expression. At concentrations of ISIS 3082 that reduced ICAM-1 expression by 90%, expression of VCAM-1 or G3PDH were not affected (Stepkowski et al., 1994). Thus data obtained from cell culture experiments strongly suggest that ISIS 3082 inhibits ICAM-1 expression by an antisense mechanism of action. In the colitis model, ISIS 3082 was found to reduce ICAM-1 expression in the colon of animals treated with DSS and subsequent accumulation of Mac-1-positive leukocytes.

### TABLE 2
Effect of ISIS 3082 on whole-body and organ weights. CD-1 mice were treated with the indicated dosage of ISIS 3082 every other day for a total of 14 days by i.v. injection. Selected organs were excised, trimmed of fat and connective tissue and weighed. Values represent the mean ± S.E.M. of five mice per group.

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*P < .05 compared with control.

### TABLE 3
Effect of ISIS 3082 on blood chemistry and hematology
Effect of ISIS 3082 on serum chemistry and hematology. CD-1 mice were treated with the indicated dosage of ISIS 3082 every other day for a total of 14 days by i.v. injection. Serum or plasma samples were obtained before necropsy 2 days after the last dose. Values represent the mean ± S.E.M. of five mice per group.

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<tr>
<td>(mg/dL)</td>
<td>Female</td>
<td>116.8 ± 30.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>Male</td>
<td>2.32 ± 0.34</td>
</tr>
<tr>
<td>(g/dL)</td>
<td>Female</td>
<td>2.96 ± 0.17</td>
</tr>
<tr>
<td>Globulin</td>
<td>Male</td>
<td>2.48 ± 0.31</td>
</tr>
<tr>
<td>(g/dL)</td>
<td>Female</td>
<td>1.98 ± 0.08</td>
</tr>
<tr>
<td>Platelets</td>
<td>Male</td>
<td>790.4 ± 167.1</td>
</tr>
<tr>
<td>(× 1000/mm³)</td>
<td>Female</td>
<td>693.2 ± 136.2</td>
</tr>
<tr>
<td>WBC</td>
<td>Male</td>
<td>7.7 ± 1.29</td>
</tr>
<tr>
<td>(× 1000/mm³)</td>
<td>Female</td>
<td>6.82 ± 2.49</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Male</td>
<td>0.093 ± 0.064</td>
</tr>
<tr>
<td>(× 1000/mm³)</td>
<td>Female</td>
<td>0.078 ± 0.056</td>
</tr>
</tbody>
</table>

*P < .05 compared with control.
addition, two scrambled control oligonucleotides failed to modify significantly the development of colitis, which supports the hypothesis that ISIS 3082 works through an antisense mechanism of action.

In chronic disease models such as DSS-induced colitis, it is difficult to conclude unequivocally that the oligonucleotide is working by an antisense mechanism of action. ISIS 3082 could reduce cytokine production or other inflammatory signals by a nonantisense mechanism of action that led to decreased signals stimulating ICAM-1 expression. However, in an acute model of inflammation, in which bacterial endotoxin was used to stimulate ICAM-1 expression in lung directly, ISIS 3082 blocked the induction of ICAM-1 expression in a sequence-specific and dose-dependent manner, with maximal effects occurring at 30 mg/kg (Kumasaka et al., 1996). Furthermore, a decrease in ICAM-1 expression by ISIS 3082 correlated with a decrease in neutrophil emigration into airways. Monoclonal antibodies directed toward ICAM-1 blocked neutrophil migration to degrees similar to that seen with ISIS 3082 (Kumasaka et al., 1996). The dose of ISIS 3082 required to inhibit ICAM-1 expression in the lung was significantly greater than the dose required to inhibit ICAM-1 expression in the colon and reduce colitis. This may be explained in part by differences in the amount of oligonucleotide that accumulates in the two tissues. ISIS 3082, like other phosphorothioate oligodeoxynucleotides, distributes

Fig. 9. Histopathological changes occurring after treatment with ISIS 3082. CD-1 mice were treated by i.v. injection with 100 mg/kg ISIS 3082 every other day for 13 days. Mice were sacrificed, and tissues were weighed and fixed in formalin. Paraffin sections of liver (panels A and B), lung (panels C and D) and lymph node (panels E and F) were stained with hematoxylin and eosin. Shown are representative micrographs of normal tissues (panels A, C and E) and of tissues from animals treated with ISIS 3082 (panels B, D and F).
poorly to the lung, whereas intestine accumulates moderate levels of the drug (Agrawal et al., 1991; Cossum et al., 1993; Crooke et al., 1996; Bennett et al., 1996). Taken together, these data strongly suggest that ISIS 3082 is blocking inflammation by an antisense mechanism of action.

Recently, Krieg et al. (1995), reported that oligonucleotides with CpG motifs directly stimulate B cell proliferation and polyclonal immunoglobulin synthesis. This is probably not the mechanism of anti-inflammatory activity of ISIS 3082, because it does not contain CpG motifs. One of the inactive control oligonucleotides, ISIS 8997, contains three CpG motifs, and it did not affect the development of DSS-induced colitis. In our experience, most phosphorothioate oligodeoxyribonucleotides stimulate polyclonal B cell proliferation in rodents regardless of sequence (S. Henry, manuscript submitted), some sequences promoting a more robust response than others. In in vitro B cell proliferation assays, ISIS 3082 produced a degree of B cell proliferation similar to that produced by the control phosphorothioate oligodeoxyribonucleotides used in this study (data not shown). It is therefore unlikely that the beneficial effects of ISIS 3082 in this model are due to its immunostimulatory effects. However, the immunostimulatory effects of phosphorothioate oligodeoxyribonucleotides may explain why, at the higher doses of oligonucleotide examined (10 mg/kg and higher), ISIS 3082 appeared to lose its therapeutic benefit in the DAI scores. Accumulation of oligonucleotide in mucosal cells could nonspecifically increase inflammation, exacerbating the disease and abrogating the beneficial effects obtained by inhibiting ICAM-1 expression. It should be kept in mind that doses of ISIS 3082 as low as 0.03 mg/kg provide a statistically significant improvement in DAI, which makes for a 300-fold therapeutic window.

ISIS 3082 appears to be well tolerated at doses that produce pharmacological activity in the colitis and cardiac allograft models (0.3–5 mg/kg/day). High doses of ISIS 3082 (100 mg/kg) administered chronically produced enlargements of spleen and liver accompanied by inflammatory changes in these tissues as well as in the lung and lymph nodes. These effects, which occur primarily in rodents and have been observed with all phosphorothioate oligodeoxyribonucleotides we have examined (n = 7; D. Kornbrust and S. Henry, unpublished data), are probably related to the immunostimulatory effects of phosphorothioate oligonucleotides (Pisetsky and Reich, 1994; Krieg et al., 1995; Zhao et al., 1995). There was significant elevation in liver transaminases at the 100-mg/kg dose, which probably reflects inflammatory changes occurring in the liver. It should be noted that the inflammatory changes observed in rodents with ISIS 3082 appear to be less severe than those observed with other phosphorothioate oligonucleotides (S. Henry et al., manuscript submitted).

The two most common recurring inflammatory diseases of the bowel are ulcerative colitis and Crohn’s disease, both of which have unknown causes. Although the two disease have some similarities, they are histologically distinct. Typically, Crohn’s disease is characterized by a granulomatous lesion with transmural involvement of the bowel wall, which could involve any segment of the GI tract. In contrast, ulcerative colitis does not exhibit well-defined granulomatous lesions but is characterized by crypt abscess and ulcerations extending down to the muscularis and surrounded by a prominent mucosal infiltrate of inflammatory cells. The diseases differ with respect to the predominant cell types in the infiltrate; neutrophils and lymphocytes are found in ulcerative colitis, and macrophages and lymphocytes are more abundant in Crohn’s disease. There is also evidence that the two diseases differ immunologically, in particular with respect to IgG subclasses (Zhou et al., 1994). Local activation of inflammatory cells is evident in both diseases, increased early activation markers being evident in Crohn’s disease (Konttinen et al., 1987; Mullin et al., 1992). In addition, there is increased expression of HLA-DR in IBD, which can be attributed to local secretion of IFN-γ by activated T lymphocytes (Selby et al., 1983; McDonald and Jewell, 1987). Thus, although the diseases are somewhat different, they both exhibit leukocyte infiltration into the bowel tissue and increased expression of leukocyte adhesion molecules.

The mainstay of therapy for IBD is topical and systemic corticosteroids and sulfasalazine, both of which provide therapeutic benefit to some patients but are less than ideal. Steroids have numerous undesirable effects, and a significant number of patients do not tolerate sulfasalazine. Therefore, there is a need for improved therapeutic approaches. The data presented in this manuscript suggest that inhibitors of endothelial-leukocyte interactions may have a place in the therapy of IBD. Inhibition of ICAM-1 expression could inhibit leukocyte trafficking to inflamed regions of the bowel and attenuate activation of leukocytes within the tissue. The results of ongoing clinical studies with the human-specific ICAM-1 antisense oligonucleotide ISIS 2302 (Bennett et al., 1994) should help establish whether ICAM-1 antisense oligonucleotides are also effective in human inflammatory diseases.

References

Cosium, P. A., Sasmor, H., Dellinger, D., Tuong, L., Cummins, L., Owens, S. R., Markham, P. M., Shea, J. P. and Crooke, S.: Disposition of the 14C-labeled...


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