Amelioration of Chronic and Spontaneous Intestinal Inflammation with an Antisense Oligonucleotide (ISIS 9125) to Intracellular Adhesion Molecule-1 in the HLA-B27/β2 Microglobulin Transgenic Rat Model

Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada (M.B.B-Y., D.R.); Department of Medicine, Division of Gastroenterology, University of Alberta, Edmonton, AB, Canada (B.R.Y.); ISIS Pharmaceuticals, Carlsbad, CA (C.F.B.); Health Science Lab Animal Services, University of Alberta, Edmonton, AB, Canada (N.N.)

Received March 12, 2002; accepted May 3, 2002

ABSTRACT
Adhesion molecules are known to be an important part of leukocyte migration and extravasation in both homeostatic and inflammatory conditions. Intracellular adhesion molecule-1 (ICAM-1 or CD54) is constitutively expressed on endothelial cells and is up-regulated during acute and chronic inflammation. We investigated the efficacy and consequences of interfering with CD54 after administration of an antisense oligonucleotide to ICAM-1 (CD54) in the transgenic HLA-B27/β2 microglobulin rat model. One hundred percent of the HLA-B27 transgene animals will spontaneously develop chronic inflammation (some more severely than others) in the gastric mucosa, cecum, and colon. We carried out two studies, i.p. injection and rectal administration of antisense. Following i.p. and rectal treatment, there were significant decreases in colonic mucosal wall thickness, histologic inflammation, CD54 expression in the colon and peripheral blood, and the percentage of colon weight per end body weight. Furthermore, decreased expression of CD49d, CD18, and tumor necrosis factor-α was observed in antisense treated rats. Therefore, the HLA-B27 transgenic model of spontaneous and chronic inflammatory bowel disease, which has increased expression of adhesion molecules, responds to both routes of administration of ICAM-1 antisense oligonucleotides. These studies support the regulatory role of adhesion molecules in chronic intestinal inflammation, the need for an understanding of how the route of drug delivery can alter the dose and area affected, and finally the role of antisense oligonucleotides as a therapeutic modality in chronic spontaneous inflammatory bowel diseases.

The inflammatory bowel diseases (IBD), Crohn’s disease and ulcerative colitis are immunoregulatory disorders of the intestinal tract. They are a prolonged and inappropriately intense reaction to an undefined antigenic stimulation, which is primarily T-cell regulated (Elson and McCabe, 1995). Several animal models have been developed to study IBD. In the past, many of these models have involved the mechanical induction of colitis with di- or trinitrobenzene sulfonic acid, dextran sulfate sodium (DSS), or acetic acid (Yamada et al., 1992; Wong et al., 1995; Hamamoto et al., 1999; Yoshida et al., 2001). More recently, gene knock-out, transgenics, leaky gut, and adoptive transfer rodent models have been created that develop a spontaneous and chronic form of inflammation involving the colon and other parts of the intestinal tract (MacDonald et al., 2000; Neurath, 2000).

The recruitment and activation of inflammatory cells as a result of proinflammatory cytokine production (interleukin-12, interferon-γ, and TNF-α), with excess production of matrix-degrading enzymes and up-regulation of a spectrum of cell adhesion molecules, including intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule-1, selectins, and integrins, on mucosal endothelial and lamina propria mononuclear cells, are important immunological features of IBD (Jones et al., 1995; MacDonald et al., 2000; Yoshida et al., 2001). Our group and others have found a difference in the expression of adhesion molecules in Crohn’s disease compared with ulcerative colitis (Hemler, 1988; Maltz et al., 1991; Yacyshyn et al., 1994). Specifically, others have studied the role of ICAMs in gut inflammation (Wong et al., 1995; Hamamoto et al., 1999; Sans et al., 1999; Bendjelloul et al., 2000).

ABBREVIATIONS: IBD, inflammatory bowel disease; DSS, dextran sulfate sodium; TNF-α, tumor necrosis factor-α; ICAM, intercellular adhesion molecule; RT-PCR, reverse transcription-polymerase chain reaction; TGF-β, T-cell growth factor-β; SIS, severity of inflammation score; PBL, peripheral blood lymphocyte.
To evaluate the role of adhesion molecules in intestinal inflammation, we used the HLA-B27+/β2 microglobulin transgenic rat model. These rats, like humans, have a genetic component to their intestinal pathology (Hammer et al., 1990). In support of this fact is that HLA-B27− littermates do not develop inflammatory diseases (Hammer et al., 1990). Following one theory, IBD is associated with normal bacterial flora; it appears that bacteria trigger HLA-B27 transgenic rat intestinal inflammation because germ-free rats do not develop disease (Taurog et al., 1994; Rath et al., 1996). The profile of cytokine, biochemical markers and histology in HLA-B27 transgenic rats exposed to enteric bacteria, is similar to those found in human IBD and is consistent with T cell, NK cell, and macrophage-mediated inflammation (Taurog et al., 1994; Rath et al., 1996).

We sought to study the effect of an antisense oligonucleotide to ICAM-1 (ISIS 9125) in a placebo-controlled study in the HLA-B27 transgenic rat model of gut inflammation. In the past, most investigators studying ICAM-1 involvement in inflammation used intravenous, intraperitoneal, and colonic administration of monoclonal antibodies that bound to and blocked the effect of the surface protein ICAM-1 and its interactions taken out with cells (Wong et al., 1995; Hamamoto et al., 1999; Sans et al., 1999). i.p. and rectal administrations were used to determine whether blockade or dampening of ICAM-1 protein production could affect chronic inflammation using DNA-specific antisense for the 3′ portion of the mRNA of ICAM-1. Antisense molecules have been investigated in animal models of sepsis, neoplasm, organ transplantation, and inflammatory disease (Neurath et al., 1996). The study of antisense to ICAM-1 in the HLA-B27 transgenic rat determined the effects of specific intervention of CD54 on adhesion molecules and inflammation.

**Materials and Methods**

**Animals**

Female transgenic HLA-B27+/β2 microglobulin Fisher-344 rats and male, wild-type Fisher-344 rats were purchased from Taconic Farms (Germantown, NY) (Dr. Joel Taurog, University of Texas, Southwest Medical Center, Dallas, TX) and used as breeding stock. Breeding colonies were kept at the University of Alberta Transgenic Facility in pathogen-free barrier conditions. Tail biopsies were obtained from each pup and were tested between 14 to 21 days by PCR for the HLA-B27 transgene. Rat pups remained in a pathogen-free environment for 21 days, were weaned and moved into conventional housing, and allowed to further develop to maturity. This was the most cost-effective way to maintain the animals and provided the necessary exposure to normal environmental bacteria. All animal protocols were approved by the Health Sciences Animal Welfare Committee of the University of Alberta.

**RT-PCR Identification of HLA-B27+ Animals**

DNA was isolated from tail-tie biopsies using proteinase K digestion at 55°C, followed by a saturated salt protein precipitation, and then isopropanol DNA precipitation and a 75% ethanol wash. DNA samples were dissolved in Tris EDTA buffer and kept at ~70°C. RT-PCR was carried out by adding 2 μl (100 ng) of DNA to 20 μl of PCR buffer containing 100 mM Tris (pH 8.8), 1.5 mM MgCl2, 50 mM KCl, mixed dNTPs at 0.125 mM, 0.25% DMSO, 0.5% Tween 20, 0.5 mM spermidine, 1.0 μl of Taq polymerase, and 0.4 μM of HLA-B27-specific 5′ and 3′ primers (5′-GGG CCTG TCC AGG ACG T-3′ and 5′-GGG TCT CAC ACC CTC CAG ATT-3′), which were generously donated by Dr. Walter Maksymowych (Department of Medicine, University of Alberta, Edmonton, AB, Canada). Amplification was performed using a DNA thermal cycler (GeneAmp PCR System 9600; Applied Biosystems, Foster City, CA). After denaturing at 94°C for 5 min, the reaction mixture was subjected to 30 cycles consisting of 94°C for 30 s, 69°C for 30 s, and 72°C for 30 s. The positive control was DNA obtained from an HLA-B27+ female purchased from Taconic Farms and used for breeding. A negative water control and DNA from an HLA-B27− animal were used in all experiments. Products were electrophoresed on a 1.5% agarose gel stained with ethidium bromide and visualized with a UV transilluminator. Southern blots were run in parallel with initial PCR data to ensure that the methods were comparable. The HLA-B27 probe for the Southern blots was obtained from Taconic Farms.

**Antisense Oligonucleotide**

Phosphorothioate oligonucleotides were synthesized and provided by ISIS Pharmaceuticals (Carlsbad, CA). The sequences of the oligonucleotides used in this study were: ISIS 9125, antisense to rat ICAM-1, 5′-AGGGCCACTGCTGTTCCACA-3′; ISIS 12140-3, control mixed antisense, 5′-GAGGCTACTGCTGAGACC-3′.

**Experimental Design**

For the first study, rats were divided into five groups at 4 months (16 weeks). Each group was given antisense (ISIS 9125 drug) or placebo (vehicle control (saline) or control oligonucleotide) i.p. every other day from week 16 to week 20. Ten transgenic rats and 10 controls (HLA-B27− littermates) were given saline placebo (0.3 cc) intraperitoneally. Five transgenic and five littermate controls were given 5 mg/kg of the control oligonucleotide (ISIS 12140-3). Eleven HLA-B27 transgenic rats and 10 controls were treated with 0.5 mg/kg (0.3 cc) of antisense to ICAM-1 (ISIS 9125). Ten HLA-B27 transgenic rats and 10 controls were treated with 1.0 mg/kg (0.3 cc) of antisense to ICAM-1. Eleven transgenic rats and 10 controls were treated with 5 mg/kg (0.3 cc) of antisense to ICAM-1. Equal numbers of male and female rats were used in each of the groups. Rats were kept in polycarbonate cages lined with clipped wood bedding and had free access to water and rat chow.

A second set of animals was divided into four groups at 4 months (16 weeks). Ten HLA-B27+ and 10 littermate controls were in each group and were given antisense or control oligonucleotide rectally every other day from week 16 to 20. Equal numbers of males and females were used in each group. First, the rat was handled and allowed to pass at least seven fecal pellets and urinate. Next, a tomcat catheter was inserted 4 cm into the rectum of the animal; 0.5 ml of the treatment was slowly introduced, observed to stay in, and then 0.5 ml more was infused, for a total of 1 ml/animal. The dosage groups were the same as in the first study (0.5, 1, and 5 mg/kg). All groups in both the i.p. and rectal studies consisted of four to six males and four to six females. The control oligonucleotide placebo was added to the protocol of the second study for both the i.p. and rectal routes of administration.

At 5 months (20 weeks) of age, the animals were sacrificed using CO2 euthanization and bled by cardiac puncture for immunophenotyping (i.p. study only). Postmortem examinations and tissue sampling were performed by a veterinary pathologist (Dr. Nick Nation), who remained blinded to the treatment groups and recorded all measurements and descriptions. Some tissue samples were snap frozen in ornithine carbamyl transferase compound immersed in methanol and dry ice and stored in a ~70°C freezer, whereas other samples were fixed in 10% neutral buffered formalin and paraffin embedded. Snap-frozen samples were used for immunohistochemical evaluation.

**Histologic Measurements of Inflammation**

Paraffin samples were used in evaluating disease and inflammation severity. N.N. performed the evaluation, measurement, and recorded the results. N.N. was blinded to all treatment groups and
Immunohistochemistry of Rat Intestines. Four- to 6-μm thick cryostat tissue sections of rat intestine were fixed in ice-cold acetone and stained with mouse monoclonal antibodies (CD54, CD18, and CD49d from PharMingen, San Diego, CA; Cell cam-105 (CD66) from Accurate, Westbury, NY; and 3'/3-diaminoben-zidine tetrahydrochloride was used as a substrate (DAKO). The Dako Autostainer (DAKO, Carpenteria, CA), and 3'/H11032 or horseradish peroxidase-conju-gated antibodies were detected with horseradish peroxidase-conjugated rabbit polyclonal antibodies to detect a variety of antigens. Primary antibodies were detected with horseradish peroxidase-conjugated donkey anti-mouse IgG F (ab') 2 or horseradish peroxidase-conju-gated donkey anti-rabbit IgG F (ab') 2 that was absorbed for rat IgG (Jackson Laboratories, West Grove, PA). All slides were stained on a Dako Autostainer (DAKO, Carpenteria, CA), and 3'/3-diaminobenzidine tetrahydrochloride was used as a substrate (DAKO). The sections were counterstained with hematoxlyn, dehydrated, and mounted with permanent mounting medium for evaluation. The CD54 slides were read in a blinded fashion by pathologist (Dr. David Rayner) and scored on a scale of 0 to 3 for overall staining intensity. All other slides were rated on a scale of 0 to 4 for staining intensity and scored in a blinded manner.

Flow Cytometry. Peripheral blood lymphocytes were isolated using Ficoll-Hypaque density centrifugation and counted on a hemo-cytometer. Cells (5 × 10^5) were stained indirectly for ICAM-1 expression with primary antibody mouse anti-rat ICAM-1 (R&D Systems, Abingdon, UK) and secondary antibody goat anti-mouse IgG (H and L) RPE (Southern Biotechnology Associates, Inc., Birmingham, AL). Surface expression was determined and analyzed using the Cell Quest program on a BD FACScan flow cytometer (BD Biosciences, Franklin Lakes, NJ). Between 15,000 and 20,000 events were collected for each sample.

Weight and Colon Weights in Study 2 (Rectal). Animals were weighed on the initial day of treatment and once a week thereafter. On the day of sacrifice, a final weight was taken. Ten centimeters of distal colon (1 cm from anus was measured and then 10 cm above this was taken off and weighed) was removed by N.N. The lumen of the colon was washed out; then the piece of colon was weighed in a preweighed bowl, and a final wet weight of the colon was recorded.

Statistics. The unpaired, two-tailed Student’s t test was used (Microsoft Excel 98; Microsoft, Redmond, WA). The level of signifi-cance was set at <0.05. For the analysis of CD54 expression on the peripheral blood mononuclear cells, analysis of variance was per-formed (Microsoft Excel 98). The means and S.E.M. were plotted. Analysis of variance was also performed between the i.p. and rectal groups.

Results

Determination of Inflammation after Antisense Treatment to ICAM-1 (CD54). To determine whether i.p. or rectal treatment with antisense oligonucleotide altered inflammation, the pathology of the disease process was scored in two manners. First, N.N. scored the microscopic severity of inflammation score (SIS) using the criteria set out in Table 1. A cumulative score (stomach, duodenum, jejunum, cecum, and colon) and a tissue-specific score (stomach, cecum, or colon) were tabulated. There was no difference in inflammation severity and location between male and female HLA-B27+ rats. The disease is histologically indistinguish-able between the sexes. In Table 2, the overall total, stomach, and cecum scores are shown. There was no difference in the SIS scores between the male and female animals. There was only one significant difference seen in the stomach score, found in the rectal study at 1 mg/kg. There is a similar decrease in the stomach SIS in the i.p. study, but it does not reach significance.

In Fig. 1, the scores from the colon in the i.p. study (Fig. 1a)
and the rectal study (Fig. 1b) are shown. In Fig. 1a, there were significant changes in the SIS from the i.p. ISIS 9125 treatment at all doses ($p \leq 0.01$) when compared with the control antisense and saline placebos. In the cecum, there were significant decreases in all i.p. ISIS 9125 doses compared with the saline control ($p \leq 0.05$ for 0.5 and 5 mg/kg; $p \leq 0.01$ for 1 mg/kg) (Table 2). Although the antisense control showed differences, they were not significant ($p = 0.08, 0.06$, and 0.1 for 0.5, 1, and 5 mg/kg, respectively) (Table 2).

In the rectal study (Table 2; Fig. 1b), the greatest change was also seen in the colon. There appears to be a dose response in the colon with the 5 mg/kg dose providing the greatest alteration in inflammation. There were also significant changes ($p \leq 0.05$) in the cecum, albeit smaller than the i.p. treatment. There were no significant alterations in any of the tissues examined for the HLA-B27 littermates, and all were reported as histologically normal.

When comparing rectal (Fig. 1b) and i.p. (Fig. 1a) SIS scores in the HLA-B27$^+$ animals, there were differences in the histologic inflammation. These differences were significant in the colon at the 0.5 mg/kg ($p \leq 0.001$) and 1 mg/kg ($p \leq 0.01$) (Fig. 1), in the stomach (Table 2) at 0.5 mg/kg ($p \leq 0.001$) and the 5 mg/kg ($p \leq 0.001$), and in the cecum (Table 2) at 1 mg/kg ($p \leq 0.05$) and 5 mg/kg ($p \leq 0.05$).

The second manner used to substantiate an effect of antisense on the inflammation in this model, was to take measurements of mucosal wall thickness in the cecal or colonic tissue. These were taken and recorded (in millimeters) by N.N. The data are presented in Fig. 2 as the relative percentage of mucosal wall thickness in the sample groups compared with the control group. The mean thickness of the placebo group was recorded as 100%, and any change in the groups was compared with this number. The mean of the sample groups and standard error (S.E.) are represented in a bar graph with the numeric mean for each group placed in the center of each bar. In Fig. 2a, the greatest and most significant changes were seen in the colon in both the i.p. and rectal studies in the HLA-B27$^+$ rats. The 5 mg/kg dose decreased the mucosal wall thickness in the colon by 40% ($p = 0.03$) in the i.p. study and 27% ($p = 0.006$) in the rectal study. In the i.p. study, the saline placebo does decrease the colonic thickness when compared with the control antisense placebo, although not significantly. This suggested that simply rehydrating with 0.3 cc i.p. every other day with saline can have some effect on mucosal wall thickness. However, ISIS 9125 decreased the inflammation significantly over that of the saline placebo. Although the cecal wall thickness decreased in both the rectal and i.p. studies at 5 mg/kg, the differences were not significant ($p = 0.33$ and 0.22, respectively; data not shown).
With i.p. ISIS 9125 treatment, the HLA-B27\(^{−}\) rats showed a 10, 17, and 13\% decrease in colonic thickness with 5, 1, and 0.5 mg/kg, respectively. These animals also showed a 6\% decrease in mucosal thickness with the saline placebo (Fig. 2b). There were significant decreases in the colon at the 0.5- and 1-mg/kg doses in the B27\(^{−}\) rats, suggesting normal CD54 expression is affected with i.p. ISIS 9125. The HLA-B27\(^{−}\) rats had no significant decreases in the cecal wall thickness in either the i.p. or rectal protocols. Hence, it appears that either the rectal or i.p. administration of ISIS 9125 antisense to CD54 consistently decreased inflammation seen in the colon of the HLA-B27\(^{−}\) animals.

Although both drug routes decreased inflammation, it was apparent that the i.p. administration caused a greater decrease in mucosal wall thickness (Fig. 2a). When comparing equivalent dosage groups in the i.p. and rectal studies, there were statist difference in the 0.5-mg/kg (p = 0.03), 1-mg/kg (p = 0.008), and 5-mg/kg (p = 0.02) doses in the HLA-B27\(^{−}\) rats. These differences were not as apparent in the HLA-B27\(^{−}\) rats (Fig. 2b). The only significant difference was seen at the 0.5-mg/kg dose (p = 0.05).

In Fig. 3, a representative picture from the i.p. study is seen. Figure 3 shows colon (a) and cecum (d) tissues from a saline treated HLA-B27\(^{−}\) rat. Parts b and e of Fig. 3 are colon (b) and cecum (e) from a saline-treated HLA-B27\(^{−}\) rat. The HLA-B27\(^{−}\) tissues had substantially larger mucosal wall thickness, loss of goblet cells, mononuclear infiltrates, and hyperplasia (Fig. 3, b and e). Figure 3, c and f, represents the colon (c) and cecum (f) after ISIS 9125 (5 mg/kg) treatment of the rat. After treatment with ISIS 9125 to CD54, there appeared to be more goblet cells present and less hyperplasia in the colon (Fig. 3f). In the cecum, there appeared to be a few more goblet cells, less hyperplasia, and some development of more typical cecal architecture, although there still was mononuclear infiltrate (Fig. 3f). This histology further demonstrated that antisense treatment in this rat model was more effective in the colon.

**CD54 Expression in the Colon.** In both the i.p. and rectal studies, colon tissue was stained and examined for CD54 expression. A pathologist, D.R., blinded to the samples, read and scored (0–3) all the CD54 slides from all animals. Slides were scored for overall staining intensity of CD54 expression in the lamina propria and submucosa. In Fig. 4a, the overall scores for i.p. study are shown. There was a statistical difference between both placebos (saline and mixed antisense) and all antisense dose B27\(^{+}\) groups. Although there were some differences in the B27\(^{−}\) animals, the differences were not statistically significant. This decrease in staining was consistent with lower SIS scores and decreased mucosal cell wall thickness.

In the rectal study, only the 1- and 5-mg/kg dose groups were stained for CD54. Once again, there were decreases in the overall staining pattern and intensity of CD54 (Fig. 4b). Figure 5 shows pictures of representative immunostaining of CD54 in the colon of the HLA-B27\(^{−}\) (Fig. 5, a, c, e, and g) and HLA-B27\(^{+}\) (Fig. 5, b, d, f, and h) rats. The most intense stain is seen the HLA-B27\(^{+}\) placebo (mixed antisense), i.p. (Fig. 5b), and rectal (Fig. 5f). After 5 mg/kg of antisense to ICAM-1 was given, a substantial decrease in lamina propria and extracellular matrix staining intensity was observed (Fig. 5, d and b). Although the stain was not as light in the rectal study (Fig. 5f) after antisense treatment, the overall area of stain was decreased, and the endothelial stain associated with arterioles and venules was more distinctive. The darkest staining appeared to be associated with arterioles/venules in the mucosa in the antisense-treated animals, especially in the HLA-B27\(^{−}\) controls (Fig. 5, c, d, g, and h).

**Peripheral Blood Lymphocyte Expression of CD54.** At sacrifice, all rats in the i.p. study were bled by cardiac puncture, and their lymphocytes were stained with the monoclonal antibody to ICAM-1 (CD54) and analyzed by flow cytometry. In Fig. 6, the percentage of peripheral blood lymphocytes, which expressed the CD54 surface antigen, was plotted for each dosage group. Overall, the HLA-B27\(^{−}\) rats had a higher percentage of CD54 on their PBL than their HLA-B27\(^{−}\) littermates (54.5 versus 35\%). The 0.5-mg/kg HLA-B27\(^{−}\) 1.0-mg/kg groups expressed from 3 to 10\% more CD54 on their PBL compared with the placebo group. The 5-mg/kg dose decreased the CD54 expression on the PBL of the HLA-B27\(^{+}\) rats (p = 0.11). Hence, not only was colonic CD54 expression decreased but also CD54 PBL expression in HLA-B27\(^{+}\) was altered by the administration of i.p. antisense to ICAM-1.

**Macroscopic Changes after Antisense to ICAM-1.** Outwardly, at the beginning of both trials most B27\(^{−}\) animals appeared scuffy with thinning hair. Some animals had porphyrin staining around their eyes. Their stools ranged from soft but formed to loose and mucous laden but still
formed. After treatment with 5 mg/kg i.p. of antisense, the stools were observed to be consistently soft and formed, with some pellets appearing of normal consistency and shape. Very few of the pellets contained mucous. Within the rectal trial, all doses appeared to cause a firming of the expelled pellets. Visual appearance of blood was not consistently seen during the trial period, even in the placebo-treated animals. This was further verified by weekly measurements of blood occult in nontreated B27/H11001 animals from ages 4 to 20 weeks (data not shown). Watery diarrheal contents were particularly noted in the pathology at the time of sacrifice in the cecums of the placebo animals.

In the second study (rectal administration of ISIS 9125), we analyzed the change in weight during the treatment and wet colon weight at the time of sacrifice. Although the male animals weighed significantly more than the females, the changes in weight before and after antisense treatments were equivalent. All groups compared had equivalent numbers of male and female rats. As seen in Fig. 7, the weight change decreased with increasing doses of antisense. The control mixed antisense HLA-B27+ group weighed an average of 13% more by 20 weeks of age than the start of the treatment at 16 weeks of age. Interestingly, there was a steady decrease in the average weight gain, and by 5 mg/kg ISIS 9125, the mean weight change was −1%, or these animals weighed 99% of their start weights. In the 0.5- and 5-mg/kg doses of ISIS 9125, there were decreases, although not significant (p = 0.16 and 0.18). The percentage of colon weight per end body weight did not change significantly with any antisense dose in the HLA-B27+ littermate controls. Therefore, there was a specific decrease in percentage of colon/body weight with ISIS 9125 to CD54 in the HLA-B27+ rats but not in the HLA-B27− animals.

**Immunohistochemistry of Other Adhesion Markers and Cytokines.** Cell Cam-105 is a 110- to 115-kDa cell surface glycoprotein and part of the CD66 (carcinoembryonic antigen) family. It has been shown to be involved in development, adhesion, prevention of tumors, signal transduction, receptor for virus and bacteria, as well as having an ectoAT-Pase function (27–39). An interesting difference in staining pattern between HLA-B27− and HLA-B27+ rats was observed. In the HLA-B27− animals (Fig. 9, a, c, and e), an intense staining was seen in the cytoplasm of epithelial cells in the villae and crypts. There was a weaker stain on the border of the epithelial cells at the luminal surface. This staining pattern was seen regardless of ISIS 9125 treatments. Figure 9, a, c, and e, represents the patterns seen in the HLA-B27− rats, the i.p. antisense control study, the 5-mg/kg ISIS 9125 i.p. study, and 5-mg/kg ISIS 9125 rectal study, respectively. The pattern found in the i.p. treated antisense control in the HLA-B27+ animals showed that...
crypt epithelial cells were completely devoid of cell cam-105 expression (Fig. 9b). At the luminal surface, there is a weak stain in the extracellular matrix, and throughout the lamina propria, there are individual cells that appear to stain (Fig. 9b). We have carried out two-color microscopy and did not find individual dual surface positive CD4, CD8, CD20 or CD11b cells (data not shown). We are currently trying to identify this cell type(s). After treatment with 5 mg/kg either i.p. (Fig. 9d) or rectal (Fig. 9f), there appeared to be more cell cam-105 staining on the epithelial cells in the crypts. The expression, however, appeared to be at the surface of the epithelial cells and not in the cytoplasm.

In the i.p. study, CD49d, CD18, fibronectin, TNF-α, and TGF-β were analyzed immunohistochemically, and the intensity of the expression was scored from 0 to 4 (no stain to most intense). The data are shown in Table 3. CD49d and CD18 staining was slightly, but not significantly, altered in the HLA-B27 rats. CD18 was significantly (p = 0.03) decreased at the 1-mg/kg i.p. dose in the HLA-B27 rats. Most significant was a decrease in the TNF-α staining of the colon in the 1-mg/kg (p = 0.02) and 5-mg/kg (p = 0.03) doses of ISIS 9125 seen in the B27 animals (Table 3). The TNF-α staining was not significantly altered in the B27 animals. TGF-β was also increased in both the HLA-B27 and HLA-B27 rats at 1 mg/kg. Neither group demonstrated a dose response. The reason for this is unknown but may potentially be related to this dose of antisense oligonucleotides.

We also stained for fibronectin. The only significant changes (decrease) were seen in the HLA-B27 rats. This suggested there was a consequence for effecting normal levels of CD54 expression in these animals, one that appeared to alter the extracellular matrix, at least fibronectin. This decrease in fibronectin was also seen in the HLA-B27 animals but was not significant at these concentrations of ISIS 9125 antisense.

Discussion

There was a therapeutic benefit of the first generation antisense ISIS 9125 against ICAM-1 (CD54) in the HLA-B27/β2 transgenic rat model of IBD. Rats entered into the study at 16 weeks of age, and 100% had moderate to severe inflammation of the colon, cecum, and the gastric areas of the intestinal tract. There was a 35 to 40% decrease in colonic mucosal wall thickness in the i.p. study and a 27% decrease in the colonic mucosal wall thickness in the rectal study. There was a significant change (p ≤ 0.05) for all i.p. doses and p = 0.04 for the 1-mg/kg rectal dose and p = 0.006 for the 5-mg/kg rectal dose. Furthermore, intense CD54 staining in the colon and the SIS index decreased significantly in both studies.

As a member of the immunoglobulin superfamily, ICAM-1 has an important role in gut inflammation. It is an inducible
transmembrane glycoprotein that is constitutively expressed in low levels on vascular endothelial cells and a subset of leukocytes, including antigen-presenting cells (Dustin et al., 1986). In response to proinflammatory stimulators, including TNF-α, many cells up-regulate ICAM-1 cell surface expression (Beutler, 1999). Tissue expression correlates with disease activity (Vainer and Nielsen, 2000).

Rodent models of intestinal inflammation have shown ICAM-1 involvement in inflammation using monoclonal antibodies (Wong et al., 1995; Hamamoto et al., 1999; Sans et al., 1999). McCafferty, using double knockout mice (P-selectin and ICAM-1) and inducing colitis in two different ways, demonstrated potentially differing roles of ICAM-1 in the ability to protect against inflammation and leukocyte recruitment that depended on the method of colitis induction (McCafferty et al., 1999).

Antisense works at the level of mRNA by hybridizing to a sequence in the 3′-untranslated region of ICAM-1 mRNA. Early work using the DSS mouse model showed that antisense affected both the induction and established (5-day treatment with DSS, followed immediately by a 7-day s.c. treatment with antisense) phases of colonic inflammation (Bennett et al., 1997). Their results showed that the s.c administration of antisense both ameliorated and prevented inflammation.

Both routes of administration in this rat model ameliorate the disease. However, the i.p. route appeared to be more efficacious based on the SIS scores and relative mucosal wall thickness (Figs. 1 and 2) when given in adequate dosage. In both colon and cecum, the mucosal wall thickness, microscopic index of SIS, and the CD54 expression in the colon were altered with increasing i.p. doses of antisense. In Fig. 6, PBL expression of placebo (saline)-treated HLA-B27+ rats was approximately 20% greater than the placebo-treated HLA-B27− control littermates. This increase was consistent with ongoing intestinal inflammation and an alteration in cell migration and gut homing. Increasing the dose of antisense from 0.5 to 5 mg/kg in the B27+ rats first increased the PBL expression of CD54...
expression of CD49d (H9251 oligonucleotides. Coupled with effects on cytokines, cell adhesion molecules, and regulatory proteins such as cell cam-105, we demonstrated its importance in intestinal immune involvement.

Acknowledgments
We thank the excellent technical help provided by Christine Cook, Norma Yachimec, Kasia Matejko, and Kim Stecker.

References


Elson CO and McCabe RP (1995) *Immunology of Inflammatory Bowel Disease*, Williams & Wilkins, Baltimore.


table 3

<table>
<thead>
<tr>
<th>Dose</th>
<th>CD49d</th>
<th>CD18</th>
<th>TNF-α</th>
<th>TGF-β</th>
<th>Fibronectin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HLA-B27</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1.17 ± 0.4</td>
<td>1.91 ± 0.28</td>
<td>1.91 ± 0.28</td>
<td>0.38 ± 0.26</td>
<td>2.67 ± 0.42</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>1.0 ± 0.4</td>
<td>1.63 ± 0.32</td>
<td>2.2 ± 0.32</td>
<td>1.6 ± 0.56</td>
<td>N.D.</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>0.33 ± 0.21</td>
<td>1.14 ± 0.14*</td>
<td>1.88 ± 0.44</td>
<td>0.4 ± 0.24</td>
<td>1.5 ± 0.28*</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>0.2 ± 0.2</td>
<td>1.75 ± 0.36</td>
<td>1.88 ± 0.35</td>
<td>0.0 ± 0.0</td>
<td>1.4 ± 0.24*</td>
</tr>
<tr>
<td><strong>HLA-B27</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>2.0 ± 0.0</td>
<td>3.5 ± 0.17</td>
<td>3.4 ± 0.16</td>
<td>0.57 ± 0.2</td>
<td>3.43 ± 0.2</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>2.38 ± 0.32</td>
<td>3.38 ± 0.26</td>
<td>3.5 ± 0.22</td>
<td>1.75 ± 0.41*</td>
<td>N.D.</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>2.4 ± 0.24</td>
<td>3.11 ± 0.26</td>
<td>2.4 ± 0.37*</td>
<td>0.38 ± 0.26</td>
<td>3.0 ± 0.57</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>1.89 ± 0.26</td>
<td>3.0 ± 0.25</td>
<td>2.7 ± 0.26*</td>
<td>0.44 ± 0.33</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

N.D., not determined.
*0.01 ≤ p ≤ 0.05.


Address correspondence to: Dr. Mary Beth Bowen-Yacyshyn, Gastroenterology Section, 111 E (W), VA Medical Center, 10701 East Blvd., Cleveland, OH 44106. E-mail: bruce.yacyshyn@med.va.gov