Neuromuscular Dysfunction in the Jejunum and Colon of Human Leukocyte Antigen B27 Transgenic Rats

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ABSTRACT

HLA-B27 transgenic rats are a model of spontaneous gastrointestinal inflammation associated with expression of human leukocyte antigen (HLA) B27 and β2-microglobulin. Our goal was to investigate in vitro enteric nerve regulation and contractile activity in isolated longitudinal muscles from the jejunum and colon of HLA-B27 rats. Nontransgenic age-matched Fisher 344 rats were used as controls. Intestinal inflammation and tissue injury, quantified histologically and through tissue myeloperoxidase activity, were evident in both the jejunum and colon of HLA-B27 rats. Although resting tension and spontaneous activity of the jejunal and colonic muscles from HLA-B27 rats did not differ significantly from controls, responses to both enteric nerve stimulation or direct muscle activation were significantly inhibited. In muscles from HLA-B27 rats, electrical field stimulation (0.5 ms, 0.5–20 Hz) induced low-amplitude contractions (maximal reduction 60–65%) compared with respective controls. In the presence of atropine and guanethidine, nonadrenergic and noncholinergic contractile responses to higher frequencies of stimulation (8–20 Hz) were also of lower amplitude. These changes were accompanied by a shift in neurally mediated contractions from predominantly cholinergic in the jejunum and colon of Fisher 344 rats to predominantly nonadrenergic and noncholinergic in HLA-B27 rats. Furthermore, maximal contractions to carbachol or KCl depolarization were reduced (up to 2.7-fold) compared with respective controls. In the jejunum of HLA-B27 rats the EC50 level for carbachol was decreased. The data indicate that gastrointestinal inflammation induced by expression of HLA-B27 is associated with hyporeactivity and inhibition of enteric cholinergic control of the longitudinal muscle in both the small and large intestine.

Gastrointestinal inflammation is often accompanied by alterations in intestinal motility. Clinical observations in patients with inflammatory bowel disease (IBD) showed abnormal colonic motor activity (Kern et al., 1951; Chaudhary and Truelove, 1961; Snape et al., 1991) and intestinal transit (Rao et al., 1987), which contribute to the symptoms of pain and diarrhea during exacerbation of the disease. More recent studies in IBD patients and in animal models of gastrointestinal inflammation have proven that mucosal inflammatory reactions trigger a cascade of events, including the interaction between immune mediators, enteric nerves, and effector muscle and epithelial cells (reviewed by Collins, 1996). Although it is evident that changes in smooth muscle (Vermilion and Collins, 1988; Blennerhassett et al., 1992) and enteric nerve function (Collins et al., 1989; Greenwood and Palmer, 1996; Venkova et al., 1999) are involved, the etiology of motility disorders in the inflamed gut is poorly understood.

However, several studies have reported an increased prevalence of IBD in patients expressing the human leukocyte antigen (HLA) B27 and β2-microglobulin (Cuvelier et al., 1992; Mielants et al., 1995; De Vos et al., 1996). Furthermore, a closer investigation revealed that patients with B27-associated spondyloarthritis, who do not complain of gastrointestinal disorders, still show changes in gut histology suggestive of silent IBD (Leirisalo-Repo et al., 1994; De Keyser et al., 1998). The clinical overlap between HLA-B27-dependent spondyloarthropathy and gut inflammation have led to a widely accepted assumption that B27-associated inflammation contributes to the pathogenesis of IBD.

With the recent advance in transgenic technology, a rat model of HLA-B27-associated human disorders has been developed, using the Fisher 344 rat strain, expressing high copy numbers of HLA-27 and β2-microglobulin genes (Hammer et al., 1990). HLA-B27 transgenic rats show a close relationship between the level of HLA-B27 protein expression and intestinal inflammation and are therefore considered a powerful tool in IBD research (Taurog et al., 1993). The inflammatory changes in the gut of transgenic rats are age-dependent and

ABBREVIATIONS: IBD, inflammatory bowel disease; HLA, human leukocyte antigen; EFS, electrical field stimulation; MPO, myeloperoxidase; NANC, nonadrenergic and noncholinergic; CSA, cross-sectional area.
are evident at 8 to 12 weeks of age, a period when the level of HLA-B27 protein expression is dramatically increased (Gough et al., 1994). Functional studies in HLA-B27 rats have demonstrated that chronic ileitis and colitis are associated with enhanced nitric oxide metabolism (Aiko and Grisham, 1995) and that the development of intestinal inflammation precedes changes in intestinal barrier function (Lundin et al., 1997). To date, no studies have been performed to investigate the relation between HLA-B27-induced inflammation and abnormal intestinal motility.

In this study we hypothesize that chronic gastrointestinal inflammation associated with HLA-B27 and β2-microglobulin expression induces functional changes at the neuromuscular junction, which may represent an important link in the chain of abnormal events contributing to the symptomatology of IBD. To test our hypothesis we used in vitro techniques to investigate the neuromuscular responses in isolated muscles from the jejunum and colon of HLA-B27 transgenic rats. Control experiments were carried out in muscles isolated from healthy Fisher 344 nontransgenic rats. Intestinal inflammation and tissue injury were quantified histologically and biochemically through evaluation of tissue myeloperoxidase (MPO) activity. The experiments were designed to establish alterations in cholinergic or noncholinergic enteric nerve control and muscle contractility that may contribute to neuromuscular motor dysfunction.

Materials and Methods

Experimental Animals. Male transgenic HLA-B27 rats, derived from the Fisher 344 strain, were obtained from Taconic Laboratory (Germantown, NY). Rats were purchased at 8 weeks of age and were housed individually under controlled conditions (21°C; 50 ± 10% humidity; 12-h light/dark cycle) until the age of 32 to 34 weeks. Control Fisher 344 rats were obtained from Charles River Inc. (Wilmington, MA) at 28 weeks of age and were housed under the same conditions for an additional 3 to 4 weeks. All animals were given water and laboratory rat chow ad libitum. The rats were observed for clinical symptoms of colitis manifested by loose stools or diarrhea. The use of animals in the study was approved by the Oklahoma City Veterans Affairs Medical Center Animal Study Subcommittee.

Constrictor Activity of Isolated Muscle Strips. Rats were euthanized and segments of the jejunum (approximately 5 cm distal to the ligament of Treitz) and the mid portion of the colon (4–5 cm distal to the ileocecal junction) were removed and placed in ice-cold oxygenated Krebs-bicarbonate solution. Longitudinal muscle strips were dissected from the intestinal segments by gently peeling the muscle in longitudinal direction. Both ends of the muscle strips were secured with silk surgical suture and were mounted vertically in 10-ml organ baths with one end fixed and the other attached to an isometric force transducer (Radnoti Glass Technology Inc., Monrovia, CA.) The baths were filled with Krebs-bicarbonate solution, maintained at 37°C, and aerated with 95% O2 and 5% CO2. During the experiments the Krebs’ solution in the baths was changed at 30-min intervals by perfusion of aerated solution preheated to 37°C to avoid changes in spontaneous contractility. Each strip was allowed to equilibrate at zero tension for 20 min, followed by consecutive loading with 0.20-g force increments until a level of optimal resting tension was achieved. Resting tension was considered optimal when the contractile response to 60 mM KCl ceased to increase with loading. Strips were allowed an additional 20-min equilibration. All experiments were performed at optimal tension, and isometric contractions were recorded using a MacLab data acquisition system (AD Instruments Ltd., Castle Hill, Australia).

Electrical Field Stimulation (EFS). Experiments were designed to study neurally mediated responses to stimulation of the enteric nerves. Electrical stimuli were delivered by a Grass S88 stimulator (Grass Instruments, Quincy, MA) and applied by pairs of platinum electrodes lying parallel to the muscle strips to provide EFS of enteric neurons. Rectangular pulses (0.5-ms pulse duration, 60 V) at frequencies ranging from 0.5 to 20 Hz were delivered in 5-s trains at 10- to 15-min intervals to provide sufficient time for recovery of resting tension levels. Under the conditions of our experiments, a constant voltage of 60 V applied at a frequency of 0.5 Hz was found to evoke responses ranging within 70 to 95% of the maximal response induced at 0.5-Hz stimulation in preparations isolated from control Fisher 344 rats. Thus, an electro-motor force of 60 V was used for the subsequent stimulation at higher frequencies to induce supramaximal responses yielding graded frequency-response curves for the muscles from both control and HLA-B27 rats.

MPO Activity. MPO is considered a specific marker of neutrophil infiltration, and MPO activity in tissue extracts is used as an index of inflammation. Full-thickness jejunal and colonic tissue samples (100–150 mg) were immediately frozen in liquid nitrogen. The samples were stored at −70°C and MPO activity was assayed simultaneously for the whole set of experiments. Tissue homogenization and extraction of MPO from the homogenate were carried out in hexadecyl-trimethylammonium bromide phosphate buffer (pH 6) using a modification of the procedure described by Castro et al. (1974). MPO activity was tested in 10-μl samples using a 3,3′,5,5′-tetramethylbenzidine microwell peroxidase substrate system (Sigma Chemical Co., St. Louis, MO) and horseradish peroxidase as a relative standard. MPO activity was expressed as an equivalent to the activity of the relative standard (nanograms of horseradish peroxidase) converting the same amount of 3,3′,5,5′-tetramethylbenzidine substrate for 10 min at room temperature. The total protein in the samples was measured using a protein assay (Bio-Rad, Hercules, CA) based on the method of Bio-Rad. All data were expressed as nanograms normal- ized per milligram of protein.

Histology. Segments of the jejunum and mid colon were fixed in 10% phosphate-buffered formalin and subsequently processed for embedding in paraffin. Tissues were sliced at 4-μm thickness, deparaffinized, hydrated, stained with H&E, and mounted with permount (Fisher Scientific, Pittsburgh, PA). The sections were examined and scored by a pathologist in a blinded fashion. An assessment of the degree of inflammatory mucosal injury was based on evaluation of the following histological features: ulceration, inflammation, depth of lesion, fibrosis, and granuloma. Each variable was graded separately from 0 to 5.

Solutions and Drugs. The modified Krebs-bicarbonate solution contained 120 mM NaCl, 6 mM KCl, 1.2 mM MgCl2, 1.2 mM NaH2PO4, 2.5 mM CaCl2, 14.4 mM NaHCO3, and 11.5 mM glucose. The solution was continuously gassed with 95% O2 and 5% CO2 (v/v), and the pH ranged from 7.2 to 7.3. The following drugs were obtained from Sigma: carbamylcholine chloride, atropine sulfate, guanethidine sulfate, and tetrodotoxin. All drugs were dissolved in distilled water and were added to the baths in volumes less than 1% of the total bath volume.

Data Analysis and Statistics. Contractions induced by KCl, carbachol, or EFS were analyzed using the MacLab data acquisition system. The amplitude of each response was measured as the maximal change in basal tension (mN) and normalized per mm² of cross-sectional area (CSA) for each muscle strip. The CSA was calculated using the following equation: CSA (mm²) = tissue wet weight (mg/tissue length (mm) × tissue density (mg/mm³)). The tissue length was measured at optimal tension at the beginning of each experiment, whereas tissue wet weight was measured on completion of the experiment. The specific smooth muscle tissue density was assumed to be 1.05 mg/mm³ (Gordon and Siegman, 1971).

Neurally mediated contractions in muscles from Fisher 344 or HLA-B27 rats yielded frequency-response curves to EFS (0.5–20 Hz), which were compared using analysis of covariance of multiple re-
gression lines (Kenakin, 1993). Concentration-response curves to carbachol were obtained to evaluate the efficacy and potency of a standard cholinergic agonist on smooth muscle M-cholinoreceptors. The concentration of carbachol producing 50% of the maximal effect (EC50) was calculated for each muscle strip by regression analysis of the linear portion (20–80% of maximal effect) of completed concentration-effect curves.

Histological damage was evaluated as a composite histological score, incorporating the individual scores for ulceration, inflammation, depth of lesion, fibrosis, and granuloma. The preparations were scored in a blinded fashion by a pathologist, assigning scores from 0 to 3 for each parameter (0 expressing no damage, 1 and 2 expressing slight and moderate damage, and 3 expressing severe damage). The composite score for each preparation was formed by adding the individual scores for ulceration, inflammation, depth of lesion, fibrosis, and granuloma. A mean composite histological index was calculated for each group (n = 6 animals).

The results were expressed as mean ± S.E. for the HLA-B27 and Fisher 344 groups. Before selecting a statistical test for independent group comparison, the data were subjected to assumption testing of normality and equal variances to define whether a parametric or a nonparametric test was required. Differences between mean values obtained in control Fisher 344 and HLA-B27 rats were assessed for statistical significance using unpaired t test or an alternative Mann-Whitney rank sum test where appropriate. Statistical significance was inferred when P < .05.

Results

Intestinal Inflammation in the HLA-B27 Rat. All transgenic HLA-B27 rats used in our study showed clinical symptoms of intestinal inflammation manifested by loss of food or water were observed. However, the body weight of the HLA-B27 rats was lower (340 ± 20 g, n = 6) compared with the body weight of age-matched Fisher 344 rats (390 ± 10 g, n = 6, P < .05). Macroscopic examination of the gut of the HLA-B27 rats on laparotomy showed enlargement and dilatation of the cecum and colon and soft to watery intraluminal content without fecal pellet formation. The small intestine did not show notable changes, except for the intraluminal content, which was watery and yellow. The histological assessment of jejunal and colonic sections indicated abnormalities in the HLA-B27 rats, which were typical for mucosal inflammation. Tissue inflammation was characterized by abundant infiltration of inflammatory cells into the mucosa, submucosa, and lamina propria; increased crypt depth and hyperplasia of the crypt epithelial cells; and consistent thickening of the mucosa-submucosal and muscularis externa layers. The histological scores were significantly higher in both the jejenum and colon of the transgenic HLA-B27 rats than those for control Fisher 344 rats (Table 1). However, the high morphological score in the jejunum of the HLA-B27 rats was the composite of ulceration, fibrosis, granuloma, and a moderate increase in the infiltration of inflammatory cells, whereas in the colon the morphological score represented mainly a steep increase in the inflammatory infiltrate and depth of lesions. Accordingly, MPO activity in colonic tissue extracts showed a 7-fold increase relative to Fisher 344 controls (Table 1). Thickening of the external muscle layer was visible in the colonic sections on light microscopy examination. An increased thickness of the external muscle layer was also documented as an increase in the CSA of the isolated muscle strips (Table 1).

Table 1

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Histological scores, MPO activity, and muscle cross-sectional area in the jejunum and colon of transgenic HLA-B27 and control Fisher 344 rats</th>
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<tr>
<td>Values are mean ± S.E. Histological scores and MPO activity are obtained from six animals in each group. The numbers of muscle strips used to obtain the cross-sectional area are as follows: HLA-B27 rats (n = 20 jejunal and n = 18 colonic strips) and Fisher 344 rats (n = 18 jejunal and n = 18 colonic strips).</td>
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<tr>
<th></th>
<th>HLA-B27 Rats</th>
<th>Fisher 344 Rats</th>
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<tr>
<td></td>
<td>Jejunum</td>
<td>Colon</td>
</tr>
<tr>
<td>Histological score</td>
<td>5.7 ± 1.8*</td>
<td>5.0 ± 0.8*</td>
</tr>
<tr>
<td>MPO activity (ng/mg of protein)</td>
<td>0.41 ± 0.04</td>
<td>1.06 ± 0.22*</td>
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<tr>
<td>Cross-sectional area (mm²)</td>
<td>0.96 ± 0.05</td>
<td>1.72 ± 0.12*</td>
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* Significant difference (P < .05) compared to the same respective value in control Fisher 344 rats.
Neurally mediated contractions evoked by EFS (0.5 ms, 0.5–20 Hz, 60 V, 5-s trains) of longitudinal jejunal (A) and colonic (B) muscle strips isolated from HLA-B27 transgenic rats (n = 6; ●) or Fisher 344 control rats (n = 6; ○). Values are means ± S.E. (7 to 11 strips) for the responses to each frequency of EFS. Regression analysis of the frequency-response curves indicated linear relation (dashed lines) between stimulus frequencies and contractile responses within the range of 0.5 to 10 Hz (correlation coefficients, R ≥ 0.95). The slope and position of regression lines indicated a significant difference (P < .05) between the responses in muscles from HLA-B27 and Fisher 344 rats.

Fig. 1. Neurally mediated contractions in the presence of atropine (1 μM) and guanethidine (30 μM) evoked by EFS (0.5 ms, 0.5–20 Hz, 60 V, 5-s trains) of longitudinal jejunal (A) and colonic (B) muscle strips isolated from HLA-B27 transgenic (n = 6; ●) or Fisher 344 rats (n = 6; ○). Values are mean ± S.E. (8 to 12 strips) for each stimulus frequency. Regression analysis of the frequency-response curves indicated linear relation (dashed lines) between contractile responses and stimulus frequencies (correlation coefficients, R ≥ 0.93). In the jejunum, the slope and position of regression lines showed no significant difference (P > .05) between muscles from HLA-B27 rats and control rats. In the colon, there was a significant difference (P < .05) between the slopes but no significant shift in the position of the regression lines for HLA-B27 rats.

Effect of Atropine and Guanethidine on Neurally Mediated Contractions. Although a detailed pharmacological analysis of neurally mediated responses was out of the scope of this investigation, we aimed to establish whether the abnormalities observed in the HLA-B27 rats are confined to cholinergic responses. When applied alone, atropine (1 μM) abolished the responses to stimulation at 0.5 to 1 Hz and reduced the responses to higher frequencies. In contrast, guanethidine had no effect at any frequency of stimulation. Responses to EFS were induced in the absence of drug treatment and repeated after subsequent application of atropine (1 μM) and guanethidine (30 μM) to distinguish between cholinergic components and those that are nonadrenergic and noncholinergic (NANC). In the jejunum and colon from control Fisher 344 rats, NANC contractions developed in response to stimulation at frequencies higher than 2 Hz and achieved maximum at 20 Hz in the jejunum (Fig. 1A) and at 8 to 10 Hz in the colon (Fig. 2B). A common characteristic of the NANC contractions was that they developed after a poststimulus delay ranging with the increase in frequency from 2 to 8 s. In both jejunal and colonic muscles from HLA-B27 rats, NANC contractions were of lower amplitude than those induced in muscles from control Fisher 344 rats (Fig. 2, A and B).

Alterations in neurally mediated responses in HLA-B27 rats were additionally characterized by evaluating the cholinergic and NANC components as a fraction of the total response obtained before treatment. We found that, in addition to the decrease in amplitude, the relative proportion of cholinergic (atropine-sensitive) components and NANC (atropine- and guanethidine-resistant) components of the responses is changed dramatically in the HLA-B27 rats. In both the jejunum and colon from control animals responses to low frequencies (0.5–2 Hz) of stimulation were predominantly cholinergic, whereas high frequencies (15–20 Hz) induced predominantly NANC contractions (Fig. 3, A and B).

In contrast, the muscles isolated from HLA-B27 rats responded to all frequencies of stimulation with predominantly NANC contractions. In the colon of transgenic animals EFS-induced contractions virtually lacked cholinergic components, except for responses to the very low stimulus frequencies of 0.5 to 2 Hz. A similar phenomenon was found in the jejunum of the HLA-B27 rats, where the appearance of predominantly NANC contractions was shifted from 20 Hz in controls to 1 Hz in the transgenic rats (Fig. 3A).
Contractile Responses to Carbachol and High KCl.

Carbachol-induced concentration-dependent contractions of the longitudinal muscle of the jejunum and colon were resistant to pretreatment with tetrodotoxin (1 μM) (data not shown). In jejunal and colonic muscles from HLA-B27 rats, the amplitude of contractile responses was reduced compared with responses in control Fisher 344 rats (Fig. 4). In the jejunal muscle from HLA-B27 rats, the decrease in amplitude was associated with a significant decrease in the EC_{50} for carbachol (HLA-B27 rats: 73 ± 12 nM, n = 13 versus Fisher 344 rats: 246 ± 33 nM, n = 11; P < .05). Although the EC_{50} for carbachol in some of the colonic muscles from HLA-B27 rats was lower than the EC_{50} in control Fisher 344 rats, the difference did not prove significant for the complete set of experiments (HLA-B27 rats: 192 ± 37 nM, n = 12 versus Fisher 344 rats: 315 ± 94 nM, n = 12; P > .05).

After equilibration, each muscle strip was exposed to high concentrations of KCl (20–80 mM) to induce receptor-independent membrane depolarization and muscle contraction. All muscles required a concentration in the range of 60 to 80 mM KCl to achieve a maximal response. However, in the jejunal and colonic strips from HLA-B27 rats, the magnitudes of maximal contractions in response to either KCl or carbachol were significantly lower (P < .001) than those of the maximal contractions in muscles isolated from control Fisher 344 rats (Table 2). Furthermore, a comparison between maximal responses to high KCl-induced membrane depolarization or activation of muscarinic cholinoreceptors showed that in both transgenic and control rats maximal contractions induced by carbachol were of higher amplitude than those of the maximal responses induced by KCl (Table 2). This difference was most prominent in the jejunal muscle from control Fisher 344 rats, whereas only a moderate dif-
HLA-B27 or six control Fisher 344 rats. The presence of atropine, indicating that the suppression in enteric neurotransmission affects mainly the cholinergic component of the responses. Because guanethidine had no effect on EFS-evoked contraction, the difference between responses to EFS obtained before and after administration of atropine and guanethidine was interpreted as a cholinergically mediated component, whereas the rest of the responses were NANC. A decrease in cholinergically mediated contraction in the HLA-B27 rats is in agreement with the impairment of acetylcholine release in the myenteric plexus, found in a model of intestinal inflammation caused by a parasitic nematode (Collins et al., 1989). Furthermore, our results demonstrated that chronic inflammation in the HLA-B27 rats induced changes in the balance between cholinergic and NANC neurally mediated contractions, shifting the responses from predominantly cholinergic in healthy tissue to predominantly NANC during inflammation. This observation is supported by the finding that, in a rabbit model of ileitis, changes in the activity of small intestinal circular muscle were characterized by enhancement of noncholinergic excitation related to Substance P-mediated pathways (Goldhill et al., 1995).

At the level of the smooth muscle, abnormalities in jejunal and colonic contractility in the HLA-B27 rats were identified as an impaired ability of the longitudinal muscle to contract in response to either direct stimulation of muscarinic cholinceptors or receptor-independent depolarization induced by high concentrations of extracellular K+*. A general conclusion from these results is that spontaneous gut inflammation in HLA-B27 rats, is accompanied by a decreased ability of intestinal longitudinal muscle to generate active tension. Moreover, our results indicate a specific involvement of cholinergically activated contractile pathways and the existence of regional differences. For instance, the amplitude of carbachol-induced contractions in the jejunum of HLA-B27 rats was 2.7 times lower than those of the healthy Fisher 344 rats, suggesting dysfunction of a smooth muscle signal-transduction pathway, which couples activation of muscarinic cholinceptors to contractions. Jejunal muscles isolated from HLA-B27 rats also proved to be more sensitive to cholinergic activation showing a lower EC50 for carbachol. The shift of responses to lower concentrations could reflect a lowering of the resting potential toward the contraction threshold of the muscle caused by inflammation-induced suppression of sodium pump activity in the smooth muscle membrane (Muler et al., 1989; Khan and Collins, 1993). There were also some regional differences, showing that in HLA-27 rats responses to carbachol are suppressed to a greater extent in the jejunum and to a lesser extent in the colon. In contrast, KCl-induced contractions in the jejunum and colon of HLA-B27 rats were reduced to a similar extent (see data in Table 2). Our data disclose two distinct sides of the impaired muscular function—one being a specific dysfunction of cholinergically driven contractions and the second representing the malfunction of a receptor-independent contractile mechanism. As a whole, the results confirm the existence of regional differences in the functional response to inflammation in the small and large intestine (Collins, 1996; Collins et al., 1996). However, it is evident that both small and large intestine in the HLA-B27 rat show similar disruption in basic mechanism(s) that maintain contractility.

The mechanisms by which human B27 molecules induce an inflammatory response are presently under investigation, and evidence is accumulating to support the role of T-cells.

### TABLE 2

Maximal contractions (mN/mm²) induced by high KCl depolarization or activation of muscarinic cholinceptors by carbachol in the jejunum and colon of transgenic HLA-B27 rats and control Fisher 344 rats.

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* Significant difference (P < .05) compared with the same respective value in control Fisher 344 rats.

Discussion

In this study, gut inflammation in the HLA-B27 transgenic rats was found to induce alterations in neuromuscular function of the external longitudinal muscle in the jejunum and colon. Although inflammatory infiltration (high activity of tissue MPO) was concentrated mainly in the large intestine of the transgenic animals, functional alterations were expressed in both the large and small intestine, indicating that HLA-B27-associated neuromuscular dysfunction is a generalized rather than a local phenomenon. Our results demonstrated that contractions in response to both neurally mediated or direct activation of jejunal and colonic muscle were reduced in HLA-B27 rats. In contrast to the observations made by others in the colonic muscularis mucosae layer in a rabbit model of colitis (Percy et al., 1993), we found that chronic inflammation in HLA-B27 rats has no effect on the basal tone of the longitudinal muscle in the jejunum and colon, whereas the development of active tension is significantly inhibited. Although relevant under experimental conditions, such observations should be considered cautiously because they may be limited to the low levels of basal tone maintained by the isolated muscles.

The presynaptic or postsynaptic localization of abnormalities causing impairment of contractile activity were characterized at the level of the neuromuscular junctions by analyzing neurally evoked responses to EFS. The contractile responses to EFS increased with stimulus frequency (0.5–20 Hz) and were abolished by tetrodotoxin in both HLA-B27 and control Fisher 344 rats, demonstrating that the intestinal muscles retain their ability to respond with increasing contractions to gradually increasing stimuli under the conditions of chronic inflammation. However, in comparison to healthy Fisher 344 rats, the neurally mediated responses in muscles from HLA-B27 rats were significantly decreased in amplitude at all frequencies of stimulation. Moreover, the dynamics of the frequency-response relation has been altered by the inflammatory process, because regression lines representing the frequency-response relationship in jejunal and colonic muscles from HLA-B27 rats had different slopes and positions than those of the Fisher 344 controls.

Abnormalities in contractile responses may reflect changes in cholinergic and noncholinergic components of the contractile response in the inflamed tissue. The decrease in amplitude was more obvious in untreated muscles than in the presence of atropine, indicating that the suppression in enteric neurotransmission affects mainly the cholinergic component. The difference between carbachol and KCl-induced responses was found in the jejunum from HLA-B27 rats.

**Neuromuscular Function in HLA-B27 Rats**

*Values are mean ± S.E. Each group includes 11 to 18 muscle strips isolated from six HLA-B27 or six control Fisher 344 rats.*

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**TABLE 2**

Maximal contractions (mN/mm²) induced by high KCl depolarization or activation of muscarinic cholinceptors by carbachol in the jejunum and colon of transgenic HLA-B27 rats and control Fisher 344 rats.

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* Significant difference (P < .05) compared with the same respective value in control Fisher 344 rats.
interacting with the B27 molecules expressed on antigen-presenting cells (Breban et al., 1993, 1996). A putative role for proinflammatory cytokines in the development of HLA-B27-associated inflammation has been suggested based on the report of an increased expression of interferon γ, interleukin 1α, and interleukin 6 mRNA in the inflamed colon (Feltkamp et al., 1996). Interestingly, neither colitis nor arthritis occur when HLA-B27 transgenic rats are raised under germ-free conditions, inferring that the source of antigens driving the inflammatory reaction is normal enteric bacterial flora (Taurog et al., 1994).

In conclusion, this study has established that the rat model expressing HLA-B27 and human β2-microglobulin genes displays pathophysiological changes similar to human IBD, including the development of spontaneous inflammation and neuromuscular dysfunction of the small and large intestine. There is a correlation between the decreased intestinal muscular contractility observed in the HLA-B27 rats and the reduction of motor activity found in the colon of patients with active ulcerative colitis (Rao et al., 1987; Snape et al., 1991). Furthermore, it is likely that the inhibition of cholinergic responses and the prevalence of NANC excitation in the neurally mediated contractions of intestinal muscles from HLA-B27 transgenic animals may correspond to the alterations of the enteric nervous system and to possible changes in Substance P content, similar to those found in the gut of patients with IBD (Koch et al., 1987; Goldin et al., 1989).

Finally, this study supports the use of HLA-B27 transgenic rats in experimental studies of the pathophysiological changes that occur in response to chronic intestinal inflammation associated with the expression of the human class I HLA-B27 molecule.

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References


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