IgG antibodies present in newborn sera are primarily directed against α Gal epitopes and may be implicated in the pathogenesis of the xenograft rejection process. In order to permit prolonged xenograft survival in newborn primates, therapeutic strategies may need to be developed that incorporate techniques to deplete IgG antibodies pretransplant, such as plasmapheresis, together with those which prevent induction of antibody production posttransplant, such as the use of anti–B cell chemotherapeutic agents.

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THE EFFECT OF SMALL BOWEL TRANSPLANTATION ON THE MORPHOLOGY AND PHYSIOLOGY OF INTESTINAL MUSCLE

A COMPARISON OF AUTOGRAFTS VERSUS ALLOGRAFTS IN DOGS[1,2]

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The effects of acute (AR) and chronic rejection (CR) on intestinal smooth muscle that are responsible for the dysmotility following small bowel transplantation (SBTX) are incompletely understood. Jejunal and ileal specimens from normal control dogs (n=7), and auto-transplanted dogs were examined at 7 days (n=6) and 1 (n=7), 3 (n=6), 6 (n=6), and 12 months (n=6). Allo-transplanted dogs that developed AR (n=8) and CR (n=5) were examined for gross and microscopic morphology (muscle thickness, the number and size of myocytes, and inflammatory infiltrate), and for contractile and intracellular electrical function in vitro. Auto-SBTX did not alter morphology at any period, but contractile function was impaired at 7 days (73.6%) compared with normal intestine. Acute rejection did not influence myocyte number or size, but was associated with a prominent infiltrate of neutrophils and lymphocytes, and severely impaired contractile function (20.6%) compared with auto-SBTX controls. Acute rejection also significantly inhibited the amplitude of slow waves and of inhibitory junction potentials. Chronic rejection caused thickening of muscularis propria by both hyperplasia (175.5%) and hypertrophy

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(202.6%) accompanied by moderate inflammatory cell infiltrate compared with auto-SBTX controls. We conclude that the marked inflammatory infiltrate into the muscularis propria indicates that the graft muscle is injured by both acute and chronic rejection; impaired function of intestinal smooth muscle following SBTX results from both rejection and the injury associated with transplantation, and chronic rejection following SBTX is associated with both hyperplasia and hypertrophy of the muscularis propria.

The advent of a new potent immunosuppressive agent, tacrolimus, has made small bowel transplantation clinically feasible (*1*), but persistent dysmotility of the intestinal graft after transplantation remains one of the important postoperative problems (*2*). Impaired motility results, in part, from injury to the neural control mechanisms of the graft. We have recently reported that this loss of neural regulation is caused not only from the extrinsic denervation associated with the grafting procedure, but is also due to immune mediated injury to the enteric nervous system (*3*, *4*). While it is clear that neuropathic changes play an important role in causing dysmotility, investigations of the effects of intestinal transplantation on intestinal muscle have been limited.

In a few experiments with intestinal transplantation in rats, a significant hyperplasia and hypertrophy of the intestinal muscle has been reported to develop during chronic rejection, and this was associated with severe functional deterioration (*5*, *6*). Importantly, they showed a 1.5-fold thickening of the intestinal muscularis externa of syngeneic grafts, although contractile properties and intracellular electrical activity were not significantly different from normals. If transplantation-induced abnormalities in intestinal muscle are found to be a common feature of the intestinal transplantation, this then will become the first major obstacle to be overcome for the future development of this procedure.

In this study, we analyzed morphological and functional changes in the intestinal muscle after auto-small bowel transplantation and after allotransplantation in dogs.

MATERIALS AND METHODS

Intestinal transplantation. Adult mongrel dogs of both sexes weighing 18 to 25 kg were used. The entire small intestine, except for short segments distal to the ligament of Treitz and proximal to the ileocecal valve, was isolated on a vascular pedicle of the superior mesenteric artery (SMA)* and vein (SMV). The graft was perfused via the superior mesenteric artery with one liter of cold lactated Ringer's solution, and the intestinal lumen was irrigated with one liter of the same solution.

The graft was then immediately reimplanted into the same dog for the autotransplant model (auto-Tx), or switched between two dogs for the allotransplant model (allo-Tx). The arterial anastomosis was performed using either an end-to-end SMA to SMA anastomosis or an end-to-side SMA to aorta anastomosis with a common iliac artery interposition graft. The venous reconstruction was completed using a simple end-to-end SMV to SMV anastomosis. Proximal and distal intestinal continuity was restored by end-to-end anastomosis using interrupted one-layer Gambee's suture.

For the acute and chronic rejection models, the grafts were orthotopically transplanted as described above by exchanging the intestine of two dogs simultaneously. In the acute rejection model, no immunosuppression was given after allo-Tx. For the chronic rejection model, 0.2 mg/kg of FK506 was given intravenously for the first 3 days and 0.15 mg/kg for the next 4 days; the dosage was maintained at 0.1 mg/kg thereafter. Prednisone 20 mg was given by mouth for 7 days.

All animals received 1 L of 5% dextrose in 0.25 N sodium chloride solution every 24 hr for 3 days postoperatively. Cefamandol nafate 1 g was given intramuscularly during surgery and then daily for 5 days. Dogs were allowed to drink and eat from the day after surgery.

Tissue procurement. Intestinal tissues from the jejunum and ileum were obtained from normal dogs to serve as controls (n=7). Auto-Tx dogs were sacrificed at five intervals: 7 days (n=6) and 1 (n=7), 3 (n=6), 6 (n=6), and 12 (n=6) months postoperatively. Acute rejection dogs (n=8) were sacrificed when they showed general weakness, severe diarrhea, or bloody stool, after 7 to 11 days. Chronic rejection dogs (n=5) were sacrificed when they had general weakness, continuous diarrhea, bloody stool, or more than 30% of body weight loss after 2 months (80 to 696 days). At the time of sacrifice, the dog was anesthetized with inhalation gas and laparotomy was performed. After macroscopic examination, intestinal tissue samples were obtained from the proximal and distal anastomoses, including both host and graft sides.

Histological examination. Intestinal tissue samples from the host jejunum and ileum, and from the graft jejunum and ileum were fixed in buffered formalin for 24 hr. Two or three longitudinal sections including parts of the mesentery were cut and stained with hematoxylin-eosin. Inflammatory cell infiltration was qualitatively assessed on a scale of 0 for none to 3 for severe.

Since it was very difficult to define acute rejection (AR) or chronic rejection (CR) on dog models by the clinical course alone, we used the clinical findings at laparotomy and histology to retrospectively confirm the diagnosis. Originally, allo-Tx without immunosuppression was performed in 25 dogs, of which 8 met our criteria for AR by the histologic presence of cryptitis, apoptosis, and lymphocyte infiltrate in the mucosa (*7–9*). Of 20 allo-Tx dogs who were given immunosuppression, 5 met criteria for CR. They were sacrificed at a wide range of postoperative days longer than two months (80 to 696 days). Those specimens showed obliterative arteriopathy as a hallmark (*10–12*). Other dogs were not included because an alternative diagnosis explained their clinical deterioration, including graft necrosis due to vascular thrombosis, severe peritonitis, perforation, or mechanical obstruction.

Histochemistry. Jejunal and ileal specimens from each graft were processed as described previously by Heeckt et al. (*5*). Briefly, each specimen was immersed in ethyleneglycol-diamine tetraacetate (1 mmol/L) Krebs' solution for 30 min to relax contractile elements and fixed in Zamboni's solution at 4°C for 24 hr. The tissue was immersed in cold 0.05 M phosphate-buffered saline containing 30% sucrose, and then embedded in OCT compound (Miles Inc., Elkhart, IN). Longitudinal sections of the intestine were cut at 14 micron with a cryostat, and mounted on Superfrost Plus treated microscopic slides (Fisher Scientific, Pittsburgh, PA). The sections were stained with the antibody against fibronectin, a compound of muscle basal lamina (Gibco/BRL, Gaithersburg, MD) to visualize muscle coats and to measure the cross-sectional area of circular muscle cell from camera lucida drawings of three sections per specimen and at five different locations. Muscle thickness and the number of myocyte nuclei in the longitudinal and circular muscles were measured using bisbenzimide method (Molecular Probes Inc., Eugene, OR), which stains DNA in nuclei of the cells. All sections were examined by an investigator

* Abbreviations: allo-Tx, allotransplantation; ANOVA, analysis of variance; AR, acute rejection; auto-Tx, autotransplantation; CM, circular muscle; CR, chronic rejection; EFS, electrical field stimulation; GI, graft ileum; GJ, graft jejunum; HI, host ileum; HJ, host jejunum; IFNγ, interferon γ; IJP, inhibitory junction potential; IL-1β, interleukin-1β; IL-6, interleukin-6; KRB, Krebs-Ringer's buffer; LM, longitudinal muscle; MHC, major histocompatibility complex; RMP, resting membrane potential; SBTX, small bowel transplantation; SMA, superior mesenteric artery; SMV, superior mesenteric vein.

blinded to the treatment received using a Nikon Microphoto-FXA epifluorescent microscope (Nikon Optical Co., Tokyo, Japan).

Mechanical activity. A segment of graft ileum was removed and immersed in preoxygenated Krebs-Ringer's buffer (KRB) as described previously (*5*). After cutting along the mesenteric border of an ileal segment, a strip of the gut wall was cut and placed in a dissecting dish containing KRB. The mucosa was removed and strips consisting of the full thickness muscularis externa (1 mm × 10 mm) were prepared by cutting parallel to the circular muscle layer. Each end of a muscle strip was then tied with 4-0 suture and then positioned in the organ chamber with one end fixed to a stationary post and the other attached to an isometric force transducer. Following equilibration of the muscle in the organ chamber for a period of 1 hr, each muscle strip was stretched to the point where maximal spontaneous phasic contractions were recorded (L_0).

Intracellular electrical activity and neuromuscular transmission. Full thickness muscle strips as described above were placed in a recording chamber, with the cross-sectional face upward, that was constantly perfused with warmed, preoxygenated KRB. One end was pinned down to record intracellular electrical activity and the other end attached to an isometric force transducer to record mechanical activity. Cross-sectional pinning exposed the entire thickness of the circular muscle layer and allowed for consistent impalement of cells within 0.5 mm of the myenteric plexus (*13*). Following equilibration and determination of L_0, smooth muscle cells were impaled with glass microelectrodes (20–50 MΩ) filled with 3 M KCl. Recordings were accepted when a sharp drop in voltage of greater than 55 mV and a stable resting membrane potential was observed. Intracellularly recorded potentials were amplified and displayed on an oscilloscope (5113; Tektronix Inc., Beaverton, OR). The analog output of electrical and mechanical activities was pulse code–modulated and recorded on a VCR (Sony Inc., Tokyo, Japan) for off-line analysis. Tetrodotoxin-sensitive responses were evoked by transmural nerve stimulation using two platinum wires placed in parallel to the long axis of the preparation. Electrical field stimulations were carried out using a range of voltages (10–150 volts) and frequencies (1–30 Hz).

Data analysis. Statistical analysis was performed by one way analysis of variance (ANOVA) with $P<0.05$ for significant difference or compiled as mean ± SEM and analyzed for statistical significance using an unpaired or paired *t* test where appropriate.

RESULTS

Clinical course and macroscopic findings at sacrifice. Autotransplanted dogs that survived the surgical complications including vascular thrombosis, intestinal leakage, small bowel obstruction, or volvulus were essentially healthy throughout the study period, and had a good appetite. They experienced a 10.7%±1.3% loss of weight during the first 2 weeks— however, this was gradually regained over the next several months. Watery diarrhea developed within 3 days after transplantation, then improved gradually. Most of the auto-Tx dogs resumed the passage of formed stools 1 months after transplantation and thereafter until sacrificed. At the time of elective laparotomy 1, 3, 6, and 12 months following transplantation, the dogs had minimal or no ascites, no lymphadenopathy, and a healthy-appearing intestinal graft.

The early postoperative course of allotransplanted dogs not receiving immunosuppression (acute rejection model, AR) showed a course similar to that of the auto-Tx group. After 5 days, however, as clinical signs of rejection developed, the dogs became increasingly weak and progressively lost weight. Continuous diarrhea progressed to bloody diarrhea 7 to 11 days after transplantation. At laparotomy, the peritoneal cavity showed moderate to massive amounts of ascites,

FIGURE 1. Macroscopic findings of acutely rejected graft at sacrifice. The graft was dark with patchy necrosis and discoloration. The mesenteric lymph nodes were enlarged.

graft discoloration with patchy necrosis, and enlarged, dark mesenteric lymph nodes (Fig. 1).

The chronic rejection (CR) group had formed stool and demonstrated a good appetite during the early months following transplantation. After various periods following transplantation, ranging from 2.5 to 22 months, however, all of these dogs developed acute or chronic weakness, abdominal distention, hair loss, and/or more than 30% of body weight, meeting criteria for clinical chronic rejection. The survival durations of the dogs in this group were 80, 102, 116, 549, and 696 days. At the time of laparotomy, the stomach was typically distended to a moderate or severe degree. The graft intestine was characteristically pale with thickened walls, especially in the jejunum (Fig. 2). Frequently, a thickened mesentery associated with creeping fat and slightly swollen lymph nodes was seen. Ascites occurred uncommonly.

Histopathology. The mucosal surface and the longitudinal cut section through anastomoses of the autotransplanted intestine showed the same color and height of the mucosa (Fig. 3a) and the same thickness of muscle layer in both the graft and host (Figure 3b). The nearly complete resolution of postoperative changes made it difficult to locate the anasto-

FIGURE 2. Macroscopic findings of chronically rejected graft. The graft was characteristically pale, with thickened walls and prominent folds.

FIGURE 3. Mucosa view (left) and cut surface view (right) of the proximal anastomosis from on autotransplanted graft at 12 months (A), acute rejection (B), and chronic rejection (C). The autotransplanted specimen has a normal appearance of the mucosa on both host (left) and graft (right) jejunum. The nearly complete resolution of postoperative changes has made it difficult to locate the anastomosis (arrow). The mucosa and cut surface from acutely rejected grafts was thin, with areas of patchy necrosis. The chronic rejection grafts showed atrophic mucosa with a cobblestone-like appearance, and a noticeable thickening of the external muscle layers (double arrows).

mosis in some dogs at 12 months (Fig. 3b, arrow). The microscopic examination showed preservation of the mucosal architecture, normal or slightly increased inflammatory cells in the lamina propria, and no evidence of epithelial damage. There were some areas of mild, patchy neutrophillic infiltrate into the muscularis propria.

The mucosa from acutely rejected grafts was thin with areas of patchy necrosis (Fig. 3a), and cut surface showed muscle deterioration with scattered necrosis (Fig. 3b). Histologic evaluation showed moderate-to-severe changes extending throughout the intestinal wall. The mucosal damage was characterized by apoptosis of crypt cells, scattered sloughing and necrosis. There were increased inflammatory infiltrate consisting of mixed lymphocytes, eosinophils, macrophages, and neutrophils into the muscle layer accompanied by muscle cell degeneration. The mucosa from chronically rejected grafts often had an occasional cobble-stone appearance, with blunting of villi in a patchy distribution and ulceration (Fig. 3a). The cut section showed a decrease in the height of the mucosa and a noticeable thickening of the external muscle layers (Fig. 3b). Microscopically, the mucosa showed villus atrophy and fibrosis of lamina propria. Characteristic obliterative arteriopathy was observed in medium sized arteries in the accompanying mesentery and in medium sized vessels in the submucosal layer (Fig. 4a). A moderate lymphocyte infiltrate was widely distributed through the muscularis propria in association with scattered fibrosis and diffusely increased thickness (Fig. 4b).

A summary of the relative intensity of the inflammatory cell infiltrate in the muscularis propria of the host and graft jejunum and of the host and graft ileum is shown in Figure 5. In auto-transplanted grafts, occasional inflammatory cell infiltrate was seen in the muscle layer. Cellular infiltration in the graft jejunum was significantly increased by acute rejection compared with autotransplantation at day 7 (2.8±0.2 vs. 0.5±0.3,

FIGURE 4. Histopathology of a chronically rejected graft. At low-power magnification (a: ×40), the muscularis propria is thickened. A penetrating artery shows the obliterative arteriopathy (arrow). At high-power magnification (b: ×400), there is a moderate degree of lymphocytic infiltration into the muscle layer with intermittent fibrosis.

$P<0.0001$, mean±SEM). Interestingly, the host jejunum and ileum also showed a cellular infiltration, but to a much milder degree. These changes did not reach statistical significance for the host jejunum, but did for the host ileum (0.9±0.3, vs. 0.0, $P=0.017$). The scores of chronic rejection grafts (2.0±0.3 in graft jejunum, and 2.2±0.4 in graft ileum) were significantly higher than those of autotransplanted grafts (0.3±0.2 in graft jejunum, and 0.3±0.1 in graft ileum at 12 months).

Quantification of the mechanism of muscle thickening. Using a camera lucida technique with bisbenzimide histochemistry staining DNA in the nucleus of the cells, the thick-

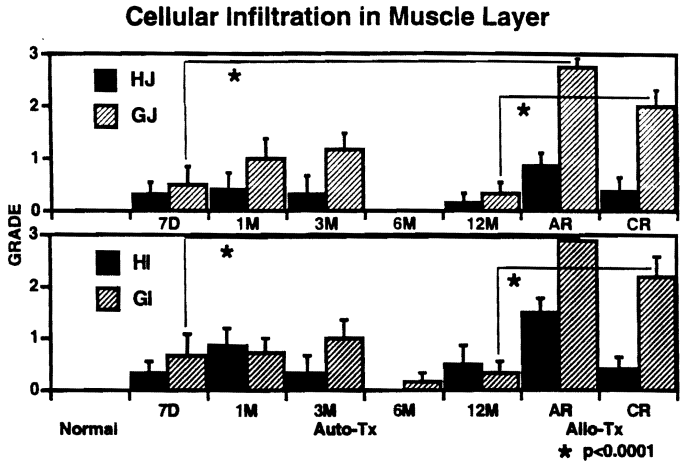

FIGURE 5. Summary of inflammatory cell infiltrate in muscle layer (HJ: host jejunum, GJ: graft jejunum, HI: host ileum, GI: graft ileum). The degree of infiltrate is graded semiquantitatively from 0 for none to +3 for severe. The autotransplanted grafts showed a slight infiltrate throughout the period. Acute rejection caused a significant increase on the graft side. Interestingly, the adjacent host intestines also were infiltrated by lymphocytes and neutrophils. Chronic rejection grafts showed a significant increase on the grafts compared with 3, 6, or 12 months autotransplanted dogs.

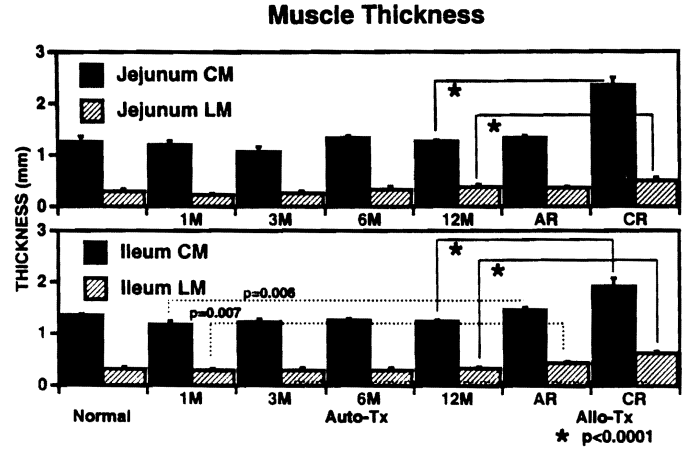

FIGURE 6. Summary of muscle thickness in jejunum (upper panel) and in ileum (lower panel). There is no significant difference in autotransplanted grafts compared with normal intestines. A trend toward increased thickness is seen in acutely rejected grafts, but this change reached significance only for ileal grafts compared with autotransplanted at 1 month. In contrast, chronic rejection significantly increased the thickness of all muscle layers in the grafts compared with autotransplanted grafts at 3, 6, or 12 months. (CM: circular muscle, LM: longitudinal muscle).

nesses of the circular and longitudinal muscle layers were measured, and these are summarized in Figure 6. There was no significant difference in the muscle thickness in autotransplanted grafts compared with normal controls.

A trend toward increased thickness was seen in the acutely rejected grafts, but this change reached significance compared with auto grafts at 1 month only for ileal circular (1.47±0.05 vs. 1.19±0.7 mm, $P=0.006$) and longitudinal muscle (0.44±0.02 vs. 0.29±0.05 mm, $P=0.007$) layers. In contrast, chronic rejection significantly increased the thickness of circular muscle compared with autotransplanted grafts, both in the graft jejunum (2.35±0.15 mm vs. 1.27±0.03 mm, $P<0.0001$) and ileum (1.92±0.16 mm vs. 1.25±0.03 mm, $P<0.0001$).

To further examine the mechanisms for the increased thickening, we sought to determine whether this was due to an increase intercellular edema, an increase in the number of myocytes (hyperplasia), and/or an increase in myocyte enlargement (hypertrophy). Bisbenzimide staining was used to count the number of myocyte nuclei through cross-section (Fig. 7a, 7b). A summary of our studies evaluating the presence of hyperplasia defined as the mean number of bisbenzimide stained myocyte nuclei counted in 15 cross sections is shown in Figure 8. There were no significant differences in the number of circular or longitudinal myocytes in the autotransplanted grafts at any of the periods compared with normals. Neither longitudinal or circular myocytes were increased in number in the acutely rejected grafts when compared with 1 month autotransplanted grafts. In contrast, the number of myocytes per cross-section in the graft jejunum was increased by 76% by chronic rejection compared with auto-transplanted at 12 months. The proliferative response was seen in both in circular (137.4±21.1 vs. 78.3±10.1, $P=0.001$) and longitudinal muscles (72.8±12.2 vs. 42.8±58, $P=0.003$). The graft ileum also showed a significant increase in the numbers of both circular myocytes (156.2±15.6 vs. 100.3±7.3, $P=0.0009$) and longitudinal myocytes (80.6±7.0 vs. 59.9±4.7, $P=0.005$) per cross-section.

Myocyte size determined using a fibronectin staining, which provided a prominent outline of the muscle coat (Fig. 9a, 9b), is summarized in Figure 10. In normal dogs, the size of myocytes in the circular muscle was 17.8±3.6 μ^2 in the jejunum and 14.2±2.6 μ^2 in the ileum. Autotransplantation had no effect on myocyte size. There was also no significant increase in myocyte cross-sectional area during acute rejection (18.7±3.8 μ^2 in graft jejunum, and 18.2±2.7 μ^2 in graft ileum). In contrast, following chronic rejection, the size of myocytes in the circular muscle was increased in the jejunum

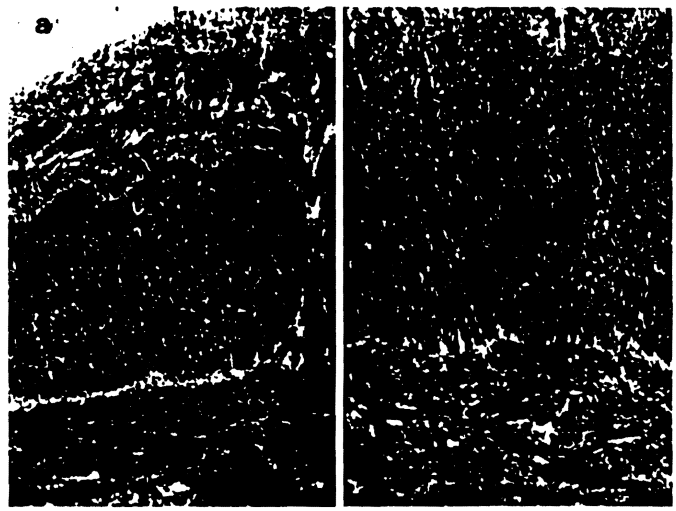

FIGURE 7. Bisbenzimide staining of autotransplanted grafts at 12 months (a), and of chronically rejected grafts (b). This staining was used to measure the muscle thickness and to count the number of myocyte nuclei through cross-section. The thickened muscle layer of chronic rejection grafts (b) has an increased number of myocyte nuclei in both the longitudinal and circular muscles (hyperplasia).

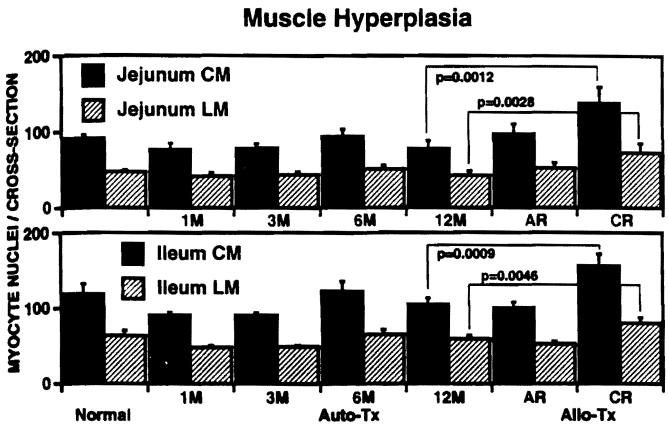

FIGURE 8. Summary of muscle hyperplasia. (CM: circular muscle, LM: longitudinal muscle). The number of myocyte nuclei per cross-section from the graft jejunum and ileum is summarized. There are no changes seen in autotransplanted grafts or acutely rejected grafts. In contrast, there is a significant increase in both grafts that underwent chronic rejection. This indicates that the increased muscle thickening results, at least in part, from a hyperplastic response.

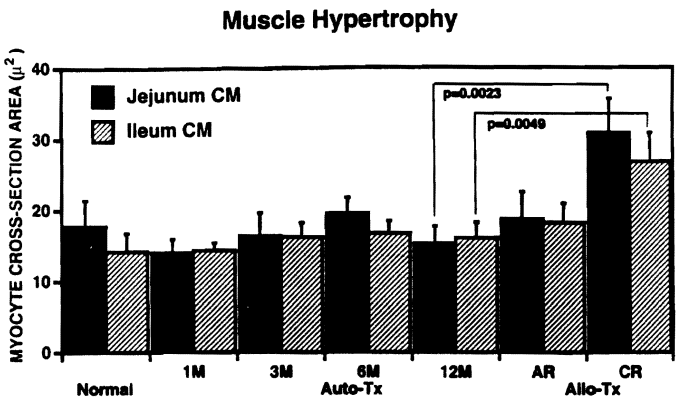

FIGURE 10. Summary of muscle hypertrophy (CM: circular muscle, LM: longitudinal muscle). The average area of myocytes (μ^2) in the circular muscle from the graft jejunum and ileum is summarized. There is no hypertrophy seen in autotransplanted or acutely rejected grafts, but it can be seen in chronic rejection grafts. This indicates that the increased muscle thickening results from hypertrophic response as well.

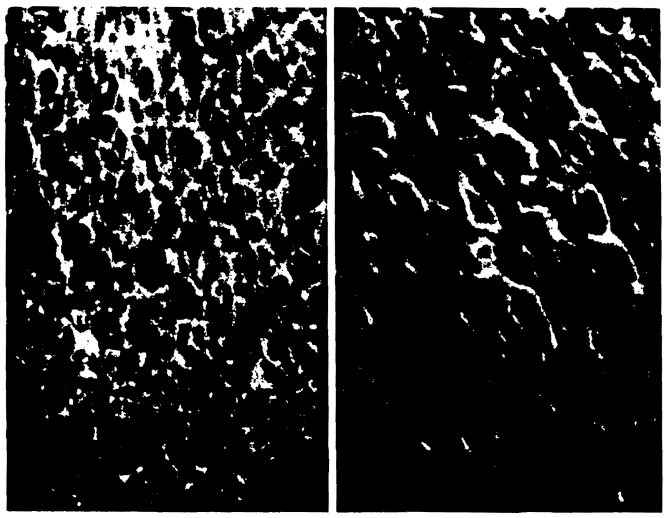

FIGURE 9. Fibronectin histochemical staining of the myocyte basement membrane in a cross-section from autotransplanted at 12 months (a), and from chronic rejection grafts (b). The chronically rejected graft shows an irregular distribution of enlarged cells mixed with some normal sized cells.

compared with auto. at 12 months (30.9±4.8 vs. 15.3±2.4 μ^2, P=0.002), and ileum (26.8±4.0 vs. 16.1±1.7 μ^2, P=0.005). Thus, chronic but not acute rejection was associated with an increased in myocyte size, suggesting an element of intracellular edema or hypertrophy.

Intestinal graft function. Mechanical activity was determined in response to increasing concentrations of bethanechol (0.1–300 μM) by recording from circular muscle strips of controls, autotransplanted grafts, and allotransplanted grafts on the 7th postoperative day. The phasic component of the contractile response was altered in both the autografts and allografts. Measuring the contractile area/mm^2/min demonstrates the combined changes of both tonic and phasic contractions of the muscles. As shown in Figure 11, muscle mechanical activity recorded from both autografts and allografts was markedly decreased compared with controls.

Threshold bethanechol doses—defined as the lowest dose that produced a 5% increase in spontaneously generated contractions—averaged 0.29, 1.33, and 1.21 μM, while the EC_{50} doses averaged 5.08, 20.69, and 13.77 μM, respectively, for muscles taken from controls, autografts, and allografts. In addition, the autotransplanted muscles produced an average of 73.6% of the maximum contractile activity recorded from normal controls and rejecting allotransplanted muscles produced only 20.6%.

We next sought to determine if these changes were isolated to the mechanical properties of the myocytes or whether intrinsic electrical control mechanisms of the muscles were affected. Resting membrane potential at day 7 was similar in controls, autografts, and allografts (−68±1.8, −64±1.0, and −68±1.4 mV, respectively). In contrast, the amplitude of electrical control activity (slow waves) was significantly decreased in both auto- and allotransplanted intestines compared to controls (18±2.4, 14±2.4 vs. 28±1.6 mV, respectively, P<0.01; Fig. 12). Furthermore, the inhibitory junction

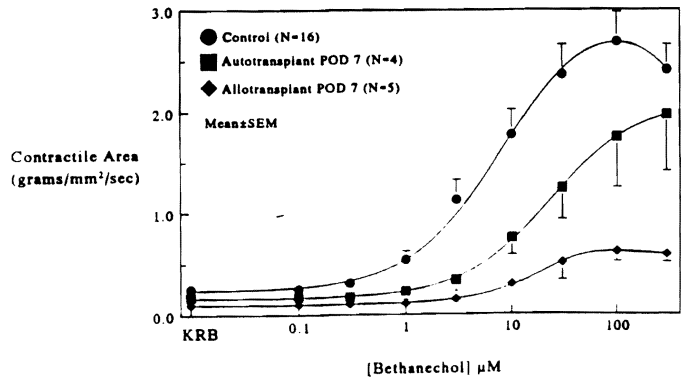

FIGURE 11. Mechanical response of the graft ileum to increasing concentrations of bethanechol in normal autotransplanted grafts at day 7 and from the acutely rejected grafts. The autotransplanted muscles produced 73.6% and rejecting muscles produced only 20.6% of the force generated by the normal intestines.

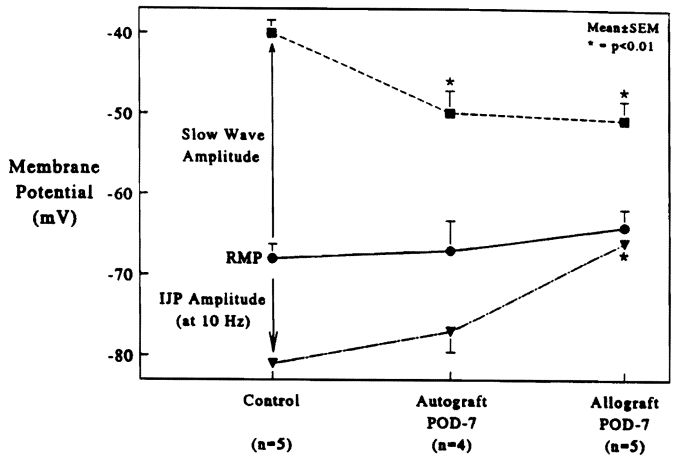

FIGURE 12. Electrical response of the graft ileum in normal autotransplanted grafts at day 7, and from the acutely rejected grafts. The Y axis shows a comparison of resting membrane potential (RMP), slow wave amplitudes, and inhibitory junction potentials (IJPs) in the three groups. RMP are not different in the three groups. However, autotransplanted grafts showed a significant decrease in slow wave amplitudes compared with normal intestines. Both slow wave amplitudes and IJPs were significantly decreased in acutely rejected grafts.

potentials generated by circular muscle responding to electrical field stimulation (EFS) were significantly reduced in the rejecting allografts, but not in autografts as compared with controls (2±0.5, 10±2.6, and 13±0.8 mV at 10 Hz EFS, respectively, $P<0.01$). Therefore, slow wave amplitude and the inhibitory neural inputs to the circular muscle were severely damaged by acute rejection, while the transplantation procedure alone was associated with a decrease in slow wave amplitudes (Fig. 12).

DISCUSSION

Small bowel transplantation is frequently complicated by impaired small bowel motility leading to distention, bacterial overgrowth, and an increased risk of sepsis. The evaluation of mechanisms responsible for this dysmotility could provide important insights into the factors that limit long-term graft and patient survival. A significant injury to the neural components that regulate intestinal motility has been reported previously by our laboratory (*3*, *4*) and others (*5*, *6*, *14*). The current study has compared normal controls to findings in a canine autotransplantation model with changes seen in two allotransplanted groups. These comparisons provide a mechanism to distinguish the injurious factors affecting the neuromuscular apparatus that are caused by the transplantation process from those due to acute and chronic rejection. This study has used such an approach in a large mammal (canine) model of transplantation that mimics the same surgical techniques used in human small bowel transplantation (*1*) and compared them to changes seen in animals treated chronically with the same immunosuppressive agents used in man (prednisone and tacrolimus [FK506]). While impaired motility has been previously reported by our group following both human (*15*) and canine small bowel transplantation (*16*), the mechanism responsible for these observation remain unclear. This dysmotility is associated with impaired contractile force and an irregularity of both fasting and fed contraction patterns (*15–17*). Our data indicate that transplantation, acute rejection, and chronic rejection each cause distinct, potentially clinically important alterations in the morphologic, mechanical, and/or electrical properties of normal muscle function.

First, it was surprising to find that autotransplantation was associated with a significant impairment of intestinal contractility induced by direct pharmacological stimulation (bethanechol) of the smooth muscle. Furthermore, there was a significant impairment of the intrinsic intestinal electrical activity as measured by a marked reduction in slow wave amplitude. These alterations were also associated with impairment of the inhibitory junction potential, an event that relies on both neural and muscular elements. Thus, transplantation alone, in the absence of rejection, has a direct inhibitory effect on intrinsic pharmacomechanical coupling, intrinsic electrical activity and on neuromuscular reflexes.

As we have reported elsewhere (*14*), these effects persisted well beyond the time frame that might be associated with typical postoperative intestinal dysmotility. This inhibition of mechanical function was noted despite the absence of any gross morphologic changes of the mucosa or muscularis propria. The only associated morphologic change was a mild inflammatory cell infiltrate. These findings suggest that adverse cellular events associated with the harvesting process—cold ischemia, warm ischemia, and reperfusion—have profound effects on intestinal muscle function and its innervation.

This study has also shown that, chronic, but not acute rejection, was associated with significant thickening of the longitudinal and circular muscle layers. Our group previously reported that morphologic and functional changes occur in the rat intestinal muscle after small bowel transplantation (*5*). In the current experiment, we have confirmed that muscle thickening occur in the dog treated with surgical techniques that imitate clinical transplantation.

The mechanisms responsible for the hypertrophy and hyperplasia of the intestinal muscle undergoing chronic rejection following long-term survival are unknown. Hyperplasia and/or hypertrophy of the intestinal muscle have been induced by stenosis (*18*), resection and anastomosis (*19*), inflammation due to *Trichinella spiralis* infection (*20*), intrinsic denervation (*21*), Hirschsprung's disease (*22*), Chagas' disease (*23*), cyclosporine A applied in vitro (*24*), and some cytokines in vitro (*25*). It was clear from our morphologic analysis that intestinal smooth muscles were infiltrated with leukocytes during both acute and chronic rejection, and that they were functionally affected by the rejection process. In contrast, however, the muscle of acutely rejected grafts showed only minimal thickening, no hypertrophy, and no hyperplasia. We have interpreted the minimal thickening observed in acute rejection to be the result of acute edema, and not due to specific myocyte changes.

Important differences were noted in the two animal models. Syngeneic transplantation in the rats showed hypertrophy and hyperplasia, but retained normal function (*5*). This observation in the rat suggested that transplantation played the crucial role in causing muscle thickening. As noted, our findings have demonstrated a possible species difference. However, it must be noted that the rat graft intestines were cold preserved for a period of 20 min before implantation.

In the canine model, autotransplantation alone for up to 12

months or acute rejection did not cause significant hypertrophy or hyperplasia. In contrast, chronically rejected grafts demonstrating immunological infiltrate over a prolonged period may be necessary for significant morphological changes to occur in the muscle layers.

Intestinal muscle hypertrophy in chronic rejection may be related to mechanisms seen in other organ transplants associated with obliterative arteriopathy. In this process, intimal thickening results from the deposition of macrophage and myointimal cells combined with myofibroblast proliferation in the liver (*10*) in heart and kidney allografts (*12*). Unlike acute rejection, continuing cellular rejection and chronic ischemia would be the contributing factors (*26*).

Not surprisingly, acutely rejected grafts showed severe impairment on both mechanical and electrical activity in vitro. Our findings indicate that threshold response to bethanechol, maximal contractile force, slow wave amplitudes, and inhibitory junction potentials are impaired during acute rejection. This suggests the presence of injury both to the pharmacomechanical coupling and the electrical-mechanical coupling mechanisms. During acute rejection a prominent infiltrate of lymphocytes, neutrophils, and macrophages was observed not only in the mucosa and submucosa but also in the muscle layers. This suggests that the production and release of cytokines and other immune modulatory factors contribute to the neuromuscular dysfunction. Previous reports have demonstrated the suppressive effects of norepinephrine release in myenteric plexus by IL-1β and IL-6 (*27*). Heeckt et al. (*5*) reported prominent decrease in contractile and intracellular electrical activity in chronically rejecting allografts in the rats.

We are unable to state with any certainty whether the adverse effects we have documented in these intestinal muscles result from the direct or indirect effects of immune injury. It is important to note that intestinal smooth muscle cells infected by *T spiralis* can express class II MHC molecules, an effect very likely due to IFNγ (*28*). Salomon et al. (*29*) reported that cultured smooth muscle cells contained in human aortic homografts could induce not only class I but also class II MHC antigen after exposure to IFNγ. Expression of these antigens would render myocytes immunogenic and susceptible to rejection by the recipient's immune system. Taken together, these observations suggest that intestinal smooth muscle cells appear to be another target tissue for immune mediated rejection injury.

While these finding have provided important insights into the mechanisms responsible for posttransplantation induced intestinal dysmotility, it must be acknowledged that there are limitations associated with our techniques. There are no sensitive or specific markers of either acute or chronic intestinal rejection. Every effort was made to identify clinical symptoms of rejection in a timely fashion to permit elective animal sacrifice according to our protocol (*1*, *2*). All specimens were obtained under general anesthesia to avoid damage due to prolonged tissue procurement procedures. Nevertheless, it was difficult to develop effective rejection models in dogs that permitted the accurate judgment of clinical symptoms, and timing of sacrifice. In many cases the definitive diagnosis was uncertain until it could be retrospectively confirmed based on the pathological hallmark, obliterative arteriopathy. Only five dogs, therefore, were included in the chronic rejection group. Further examination of the muscle that were performed in the electively sacrificed allotransplants were not completed on all chronically rejecting animals.

In summary, we have shown that the transplantation process alone causes significant impairment of graft function despite the absence of only a mild cellular infiltrate, and no gross changes. Acute rejection causes profound inflammatory infiltration of the muscularis and an associated inhibition of mechanical and electrical intestinal smooth muscle function. Lastly, we noted that chronic rejection, but not acute rejection, causes myocyte hypertrophy and hyperplasia after small bowel transplantation in dogs. Further studies will be needed to delineate the precise mechanism responsible for the damage that occurs during preservation and reperfusion of the graft, and its potential for recovery. Our observations also suggests that marked changes in mechanical and electrical muscle function can be observed when there is still minimal mucosal changes, and suggest that intestinal smooth muscle participates in the immune events associated with both acute and chronic rejection.

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RENOPROTECTIVE EFFECTS OF THE 21-AMINOSTEROID U74389G IN ISCHEMIA-REPERFUSION INJURY AND COLD STORAGE PRESERVATION[1,2]

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Free radical mediated lipid peroxidation (LPO) has been implicated in the pathogenesis of ischemic-reperfusion injury (IRI). To address the renoprotective effect(s) of LPO inhibition, the efficacy of the 21 aminosteroid U74389G was evaluated in three IRI models. In Model 1 51 unilateral nephrectomized rats that underwent 60 min of warm ischemia followed by a 72-hr reperfusion interval were treated with the test vehicle only, or 3, 6, or 12 mg/kg of U74389G intravenously, 5 min pre- or postischemia. In Model 2 Sprague-Dawley rats underwent sham operation (n=9), or 45 min of warm ischemia and 10 min of reperfusion with U74389G (6 mg/kg; n=10) or test vehicle only (n=10) administered intravenously over 10 min beginning 5 min prior to clamp release. After reperfusion, LPO was determined by assay of snap frozen tissue for thiobarbituric acid (TBA) concentrations (nmol/g tissue weight). In Model 3 domestic lean maid

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