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Intestinal Neurons in Acute and Chronic Rejection After Small Bowel Transplantation in Dogs

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THE mechanisms responsible for the altered neuromuscular activity that results in the impaired motility of small bowel transplant recipients are incompletely understood. Autotransplantation studies in small[1] or large[2] animals have shown that harvesting process, preservation, and reperfusion may impair the function of the enteric nervous system during small bowel transplantation. It is not known, however, whether the immunologic events and vasculopathy that occur during acute and chronic rejection cause further damage to the enteric nervous system.

MATERIALS AND METHODS

Jejunal and ileal specimens were obtained from seven normal dogs autotransplanted (auto-Tx) at five intervals: 7 days (n = 6), 1 (n = 7), 3 (n = 6), 6 (n = 6), and 12 (n = 6) months. In allotransplanted (allo-Tx) models, the entire length of jejunum and ileum was flushed and switched between two dogs simultaneously. For the acute rejection model (n = 8), no immunosuppression was given. A laparotomy was performed to remove specimens 7 to 11 days postoperatively, when the dog showed general weakness, persistent vomiting, severe diarrhea, or bloody stool. The other allo-Tx group survived more than 2 months with an FK 506 maintenance dose of 0.1 mg/kg. When they presented with clinical signs of rejection, general weakness, abdominal distention, persistent vomiting, severe diarrhea, or more than 30% loss of body weight, dogs underwent laparotomy with inhalation anesthesia and had specimens removed. The presence of chronic rejection was conclusively determined by histopathologic analysis including the presence of a hallmark change of obliterative arteriopathy.[3] Tissues in the studies were obtained from five dogs fit to this criteria, ranging from 80 to 696 postoperative days.

Indirect immunohistochemistry was used to examine the intensity of tyrosine hydroxylase (TH) as an example of an extrinsic neuron. Neuropeptide Y (NPY), substance P (SP), and calcitonin gene-related peptide (CGRP) staining of neurofibers had mixed extrinsic and intrinsic origins. Vasoactive intestinal peptide (VIP), galanin (GAL), gastrin-releasing peptide (GRP), L-enkephalin (L-ENK), and somatostatin (SOM) neurons were examined as examples of intrinsic neurons. Results were expressed on a qualitative scale of 0 to +3 in seven separate intestinal fields. Nitric oxide synthase histochemistry using the NADPH diaphorase technique was used to examine the intensity and morphology of the myenteric plexus, and expressed as the number of positive nerve cells per ganglion area evaluated.

RESULTS

TH immunoreactivity in the myenteric plexus and in the muscle layers was reduced to +1 in the auto-Tx 12-month group, and 0 to essentially absent in specimens showing acute rejection. One dog showing chronic rejection had scanty extrinsic reinnervation. The other extrinsic markers including NPY, SP, and CGRP perivascular fibers also showed a similar pattern. VIP reactivity in the myenteric plexus, muscle layers, submucosal plexus, and villi showed a similar pattern in normal and auto-Tx groups. Acute rejection severely reduced VIP expression. There was only +1 fibers in the myenteric plexus, and no evidence of VIP fibers in the muscle layers. In chronic rejection, VIP fibers in the myenteric plexus and muscle layers showed only +2 to +1 staining. Other intrinsic and mixed fibers reactive to SP, GAL, GRP, L-ENK, and SOM as well as VIP showed a similar decreased immunoreactivity. No differences were seen in the density of NO-containing neurons in the myenteric plexus in auto-Tx groups compared to normal dogs. During acute rejection, severe damage to NO neurons occurred, with most specimens showing no cells with NO (8.46 vs 66.64 in auto-Tx 7 days, $P < .0001$), and rare fibers. In chronic rejection, the number of NO-positive cells was also significantly decreased compared to auto-Tx dogs at 3, 6, or 12 months, which are the time consistent controls (37.5 vs 81.7 at 6 months, $P = .007$).

CONCLUSIONS

These findings indicate that acute and chronic rejection involve the enteric nervous system and cause significant widespread and nonspecific changes including damage to NO neurons when compared to autotransplantation. Thus, altered intestinal function in acute and chronic rejection is also mediated by damage to intrinsic neurons.

REFERENCES

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