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ORIGINAL ARTICLE

Morphological and physiological changes of interstitial cells of Cajal after small bowel transplantation in rats

Toshiharu Matsuura, Kouji Masumoto, Satoshi Ieiri, Takanori Nakatsuji, Junko Akiyoshi, Yuko Nishimoto, Yukiko Takahashi, Makoto Hayashida and Tomoaki Taguchi

Department of Pediatric Surgery, Reproductive and Developmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

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Correspondence
Matsuura Toshiharu MD, Department of Pediatric Surgery, Reproductive and Developmental Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81-92-642-5573; fax: +81-92-642-5580; e-mail: matsuura@pedsurg.med.kyushu-u.ac.jp

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Summary

Intestinal dysmotility has been reported to be associated with a decreased number of interstitial cells of Cajal (ICCs). However, the chronological changes in ICCs after small bowel transplantation (SBT) have not yet been elucidated. In this study, we aimed to evaluate the chronological change of ICCs after SBT. Orthotopic syngeneic SBT was performed in rats. Graft specimens were obtained at postreperfusion, and on 1, 3, 7, 14, and 30 postoperative day (POD). Thereafter, immunohistochemical staining was performed and the spontaneous contractions measured. During the initial period after SBT, the temporal impairment of ICCs was found. In an immunohistochemical study, c-Kit-positive cells appeared to decrease on POD 0, 1, and 3. Thereafter, the number of cells increased gradually up to POD 7. In contrast, the recovery of the spontaneous contractile amplitude took more time. The frequency of the electrical signal was preserved at almost exactly the same levels throughout this experimental period. Although the network of ICCs was found to be temporarily impaired after SBT in an immunohistochemical examination, this change was reversible. Moreover, the recovery of the function of the intestinal motility associated with ICCs was delayed after the early postoperative period.

Introduction

In spite of the recent therapeutic advancements associated with organ transplantation, small bowel transplantation (SBT) recipients still often suffer from enteric dysfunction [1]. In SBT, several inevitable factors associated with the graft dysfunction have been reported [2]. However, the precise mechanisms which cause intestinal graft dysfunction in SBT have not yet been fully understood. Intestinal dysfunction is thought to result from immune reactions and the effect of immunosuppressive drugs, or it may also be associated with the transplantation procedure itself, being related to extrinsic denervation, interruption of enteric neural continuity of the graft, or ischemia–reperfusion (I/R) injury [2]. Intestinal smooth muscle cells are innervated by a network of extrinsic autonomic nerves controlled by both the central nervous system (CNS) and the enteric nervous system (ENS) [3]. Of these two systems, the investigation of ENS after SBT was considered to be important, because the CNS is completely interrupted during SBT.

The interstitial cells of Cajal (ICCs) are known to play a major role in regulation of smooth muscle activity in the gastrointestinal (GI) tract [4–6]. In the clinical setting, the intestinal dysmotility due to both congenital and acquired disorders, such as anorectal malformations [7], intestinal atresia [8], Hirschsprung's disease [9,10], infantile pyloric stenosis [11], inflammatory bowel disease [12,13], and stromal tumors [14], has been suggested to be influenced by the loss or lack of the ICC network. Moreover, W/W^{v} mutant mice [15], which have been shown to lack ICCs, are known to have no electrical slow waves.

In the small intestine, two populations of ICCs are known to morphologically exist: one located in the area of the myenteric plexus (ICC-MY), and the other in the area of the deep muscular plexus (ICC-DMP) [5]. ICC-MY form a network between the circular and longitudinal muscle layers, and these cells are thought to generate electrical slow waves to the smooth muscles as a pacemaker. On the other hand, ICC-DMP are located in close association with the varicosities of enteric motor neurons and form gap junctions with surrounding smooth muscle cells [4,5,16]. These ICCs can be identified immunohistochemically by c-Kit tyrosine kinase, which is expressed on the membrane of ICCs [4,6].

Although the network of ICCs is important in graft motility after SBT, their chronological changes have not yet been investigated. Therefore, the aim of this study was to evaluate the role of ICCs in the intestinal graft after orthotopic SBT in the rat model.

Materials and methods

Animals

Male Lewis (LEW, $RT1^l$) rats, weighing 200–250 g, were purchased from Charles River Japan, Inc. (Tokyo, Japan) and bred by providing a standard diet and water *ad libitum* in our animal center. The rats were randomly divided into two groups: donors and recipients. This experiment was approved by the Committee on the Ethics of Animal Experiments in the Graduate School of Medical Sciences, Kyushu University and was performed according to the Guidelines for Animal Experiments at Kyushu University.

Operation

The donar rats were anesthetized by ether inhalation and intraperitoneal injection of pentobarbital sodium salt (Nembutal®, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan, 40 mg/kg/body weight). A median incision was made and the vessels of the middle colon, left colon, and ileocolon were ligated and cut. The 15-cm ileum was resected for a graft. Regarding the vessels' procurement, the superior mesenteric vein (SMV) was first separated from the surrounding tissue and cut at the distal site of bifurcation of the splenic vein, and then the superior mesenteric artery (SMA) was cut at the origin of the aorta. Heparin® (100 U/kg body weight) was injected through the penile vein 1 min before graft harvesting. The graft vessel was flushed using 4 °C lactate Ringer's solution and then the graft was preserved using the same solution.

After the recipient rat was anesthetized by the same method as that used for the donor, laparotomy was performed. At first, the 15-cm native ileum without ileocecal valve was removed in order to close the abdominal wall easily. Then, the recipient's infrarenal aorta and vena cava were isolated. These vessels were clumped sequentially, both the graft SMA and SMV were anastomosed to the recipient infrarenal aorta and vena cava in an end-to-side fashion using interrupted and running 9-O nylon sutures, respectively, under operative microscopy (Wild M715, Wild Leitz, Gais, Switzerland). After anastomosing these vessels, they were declamped and reperfusion was performed. The graft ileum was anastomosed with the recipient's native intestine with 6-O silk sutures. In this experiment, we fixed the cold and warm ischemic time, at 2 h and 30 min, respectively. After the operation, these animals were allowed to have water and a standard diet *ad libitum* since the first POD.

Morphological examination

Samples were obtained from the ileum of the normal rats as control ($n = 5$). Only laparotomy was performed in the controls. The transplanted rats were killed at 30 min after graft reperfusion, and on 1, 3, 7, 14, 30 days after transplantation ($n = 5$). The sample obtained from graft was harvested at each point, immediately fixed in 10% buffered formalin and embedded in paraffin. The sections were cut at a thickness of 4 μm and stained with hematoxylin and eosin (H&E). In addition, immunohistochemical studies were performed on these samples. Each sample was immediately stretched and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) for whole-mount staining. The tissue specimens were incubated with 10% normal goat serum for 30 min in a moist chamber and were then incubated overnight at 4 °C with primary antiserum, a polyclonal antibody to c-Kit protein (C-19, Santa Cruz Biotechnology, Santa Cruz, CA, USA, diluted 1:200), as a marker of ICCs. The sections were then washed in PBS and incubated for 1 h at room temperature with biotinylated goat-antirabbit IgG antiserum. They were again washed in PBS and incubated for 30 min at room temperature with peroxidase-conjugated streptavidin. After the sections were washed with PBS, they were treated with diaminobenzidine tetrahydrochloride (Nichirei, Co. Ltd, Tokyo, Japan) with 0.6% hydrogen peroxide in PBS for 7 min at 25 °C, and then washed in distilled water. Thereafter, the sections were dehydrated and mounted on glass slides and cover-slipped. As an objective assessment, the length of the c-Kit-positive cells was measured in a 1-mm^2 area. The measurement was randomly performed in 10 selected areas for each sample.

Physiological examination

The smooth muscle strips were isolated from the intestinal graft specimens and rinsed in physiological salt

solution (PSS). The composition of the normal 5.9 mM K^+ PSS was as follows (in mM): NaCl 123, KCl 4.7, $NaHCO_3$ 15.5, $KHPO_4$ 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, and D-glucose 11.5. This solution was saturated with a 95% O_2 and 5% CO_2 mixture gas. For the measurement of contractility, the mucosa was removed in the PSS by using a binocular microscope. Next, the muscle tissue preparation was cut into strips along the circumference (longitudinal) muscle layer (1×15 mm). The muscle strips were connected to a force transducer (TB-612-T, Nihon Koden, Tokyo, Japan) and were mounted vertically in a quartz organ bath. In order to obtain spontaneous oscillatory contraction, the strips were equilibrated in the organ bath for 30 min. The frequency and amplitude of the spontaneous contractions were measured as intestinal motility parameters of ICCs. The frequency of spontaneous contraction was counted for 10 min, and the average number of contractions per minute was calculated. The contractile potency of the smooth muscle was determined for the response to exposure of 10 μM carbachol. Absolute force was measured as the contractile potency of smooth muscle in the intestinal graft.

Data analysis

All data from the simultaneous measurements of force were stored using a Macintosh computer and a data acquisition system (Power Lab; Analog Digital instruments, Castle Hill, Australia; Macintosh Computer, Cupertino, CA, USA). The representative traces shown were directly printed from the data using this system. All data are expressed as mean values ± SE (n = number of the experiments). A strip obtained from one graft specimen was used for each experiment, therefore the n value indicates the number of animals. A statistical analysis was performed using one-way ANOVA to determine significant differences between groups. When the P-values showed a significant difference, multiple comparisons with the Bonferroni t-test procedure were applied to determine which mean values differed from each other with a significant level of $P < 0.05$.

Results

Morphological examination

HE staining

Haematoxylin and eosin staining showed the mucosa to be denuded partially in the specimen at the time just after SBT in comparison with the control (Fig. 1a). Although on POD 1, the space within the villi was found to have widened with vaculation (Fig. 1b), the histological findings normalized after POD 3 (Fig. 1c). No significant infiltration of inflammatory cells was found in the muscle layers throughout the experimental period. A very few crypt apoptotic cells were found just after SBT (with 1–2 apoptotic bodies per 10 crypts). After POD 1, crypt apoptic cells were not found to have increased. Regarding the changes in the ganglionic cells, no pathological changes were evident throughout the experimental period.

c-Kit staining

Numerous c-Kit-positive cells were observed in the control intestinal whole-mount specimen (Fig. 2a). These multipolar cells formed networks with cell-to-cell contacts. In contrast, in the grafts, the c-Kit-positive cells markedly decreased during the early post-transplant

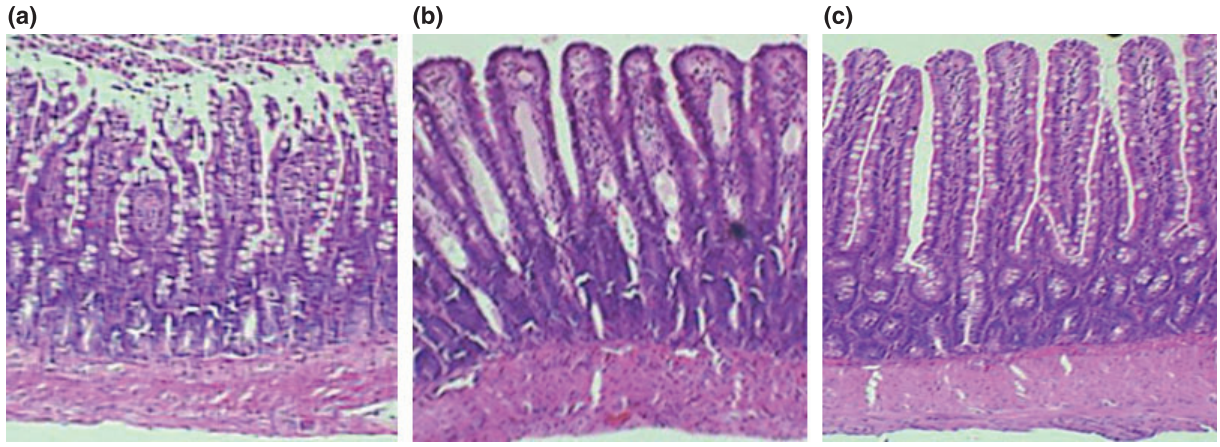

Figure 1 Histological findings of hematoxylin and eosin staining in the controls and the grafts after SBT (magnification ×200). The specimen at the time just after SBT showed that the mucosa was denuded partially (a). The specimen of POD 1 showed that the mucosa was flattened and the space in the submucosa was enlarged with vacuolation (b). The specimen from POD 3 showed that the structure of villi improved, compared with that from POD 1 (c).

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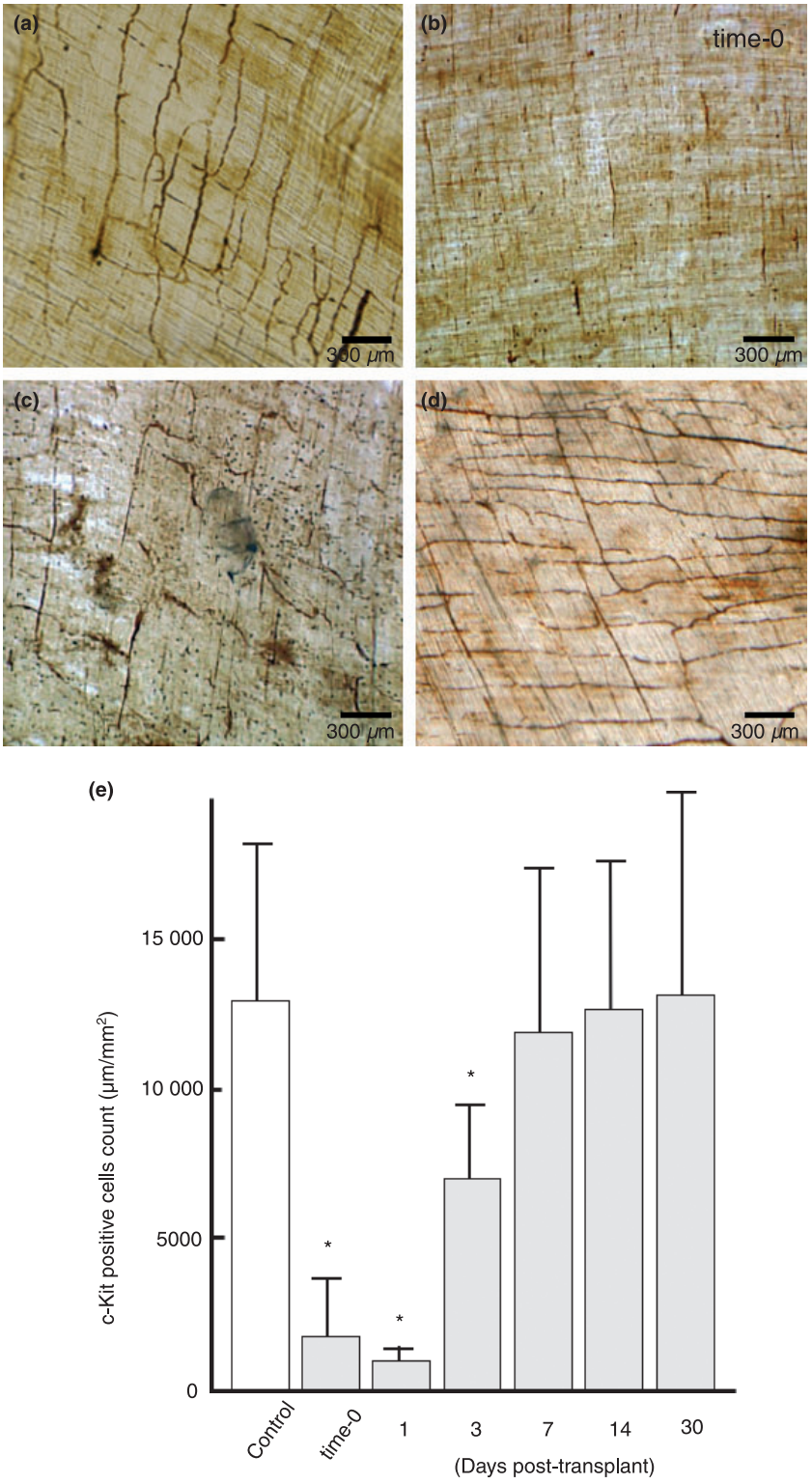

Figure 2 The c-Kit immunohistochemical staining in the controls and the grafts after SBT (magnification ×100). In the control native ileum, numerous immunoreactive cells for c-Kit were observed and these multipolar cells formed a network (a). In contrast, in the graft, at the time just after SBT (time 0), although a few c-Kit-positive cells were detectable, they were disrupted and appeared to show no connection with each other (b). In the graft on POD 3, the number of c-Kit-positive cells gradually increased and again they partially formed a network (c). In the graft on POD 7, the distribution of c-Kit-positive cells had completely recovered and formed the same network as that observed in the controls (d). The mean value in the sum of length of the c-Kit-positive cells network within the 1-mm^2 area is shown in (e). The mean length of c-Kit-positive cells during the early postoperative period (asterisks) was significantly less than that of the controls ($P < 0.05$, with Bonferroni's correction). Time 0 in the figure means the time just after SBT.

period. In the samples although a few c-Kit-positive cells were detectable just after SBT, they were disrupted and did not have any connection with each other (Fig. 2b). In the graft on POD 1, the distribution of c-Kit-immunoreactive cells was almost same as that of the graft just after SBT. However, in the POD 3 samples, the number of

c-Kit-positive cells increased and they began to form networks again (Fig. 2c). On POD 7, c-Kit-positive cells in the graft completely recovered and formed a network, similar to that of control (Fig. 2d).

The results of the measured total length of the network of the c-Kit-positive cells within the 1-mm^2 area are shown in Fig. 2e. From the time just after SBT to POD 3, the total length of the c-Kit-positive cells' network significantly decreased in comparison with that of the control ($P < 0.05$), but thereafter there were no significant changes between the graft after POD 7 and the intestine of control. Based on this result, c-Kit immunoreactivity was classified into the following three groups ±: <5000 μm/mm^2; +: 5000∼10 000 μm/mm^2; ++: ≥10 000 μm/mm^2.

Physiological examination (graft muscle strip contractility *in vitro*)

In this study, the contractile response to a cholinergic agonist was examined and the experiment was evaluated in terms of absolute force (*g*) (Fig. 3a i–iii). The contractile force was generated by 10 μM carbachol in the graft muscle strip (1 × 1.5 mm) at the designed time points. The generated contractile force of the graft during the initial period after SBT, especially at the time just after SBT and

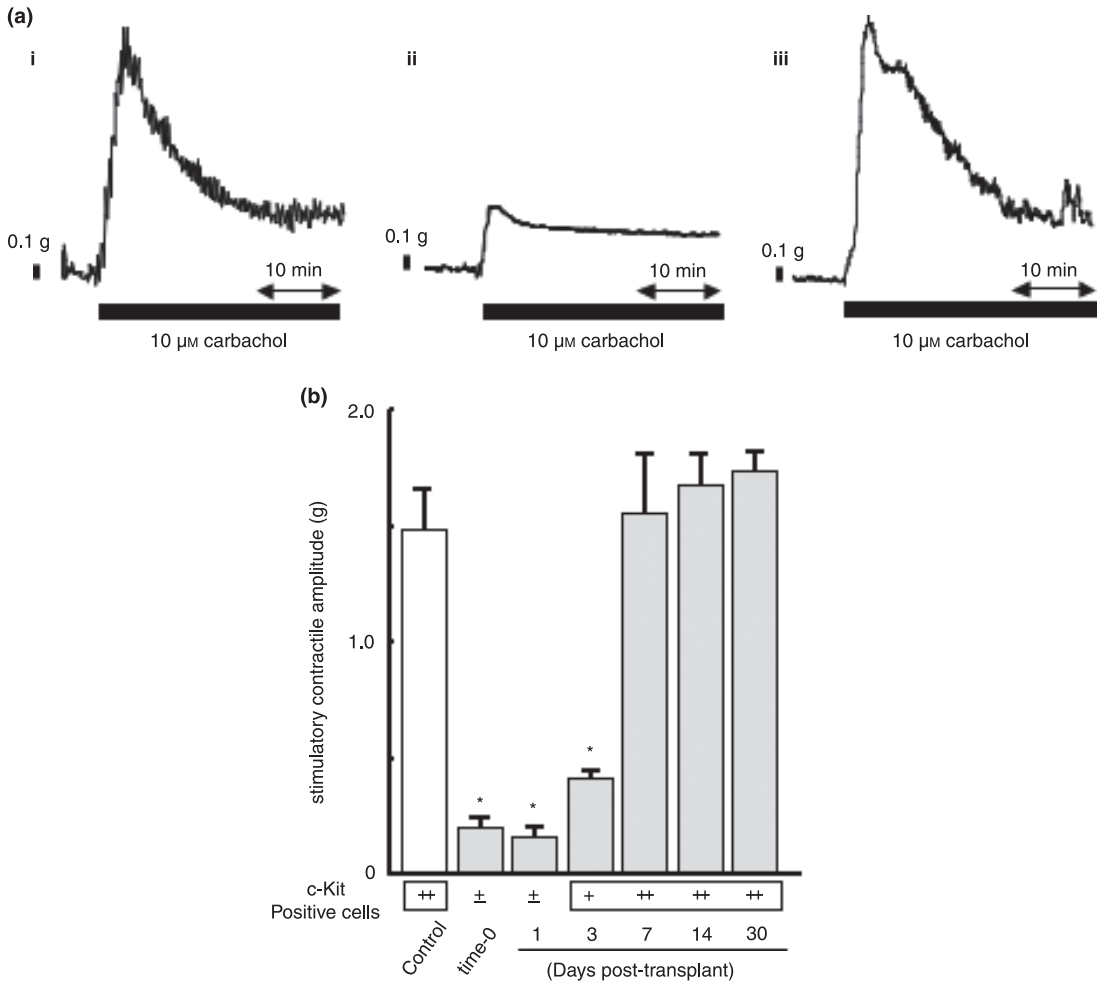

Figure 3 The contractile response to a cholinergic agonist in the controls and the grafts after SBT. The contractile force generated by 10 μM carbachol in the muscle strip (1 × 15 mm) *in vitro* in both the native ileum (a-i) and the graft on POD 1 and 7, respectively (a-ii, iii). The generated contractile force (*g*) during the early period after SBT, especially at the time just after SBT and on POD 1 was significantly smaller than that of control. The contractility recovered gradually from POD 3, and then it recovered to almost the same level as that of the control (b). The asterisks show the contractility during the early period after SBT to be significantly lower than that of the native control ($P < 0.05$, with Bonferroni's correction). The grade of c-Kit positivity was classified based on the mean value of the c-Kit-positive area as shown inFig. 2e. (±: <5000 μm/mm^2; +: 5000–10 000 μm/mm^2; ++: ≥10 000 μm/mm^2). Time 0 in the figure means the time just after SBT.

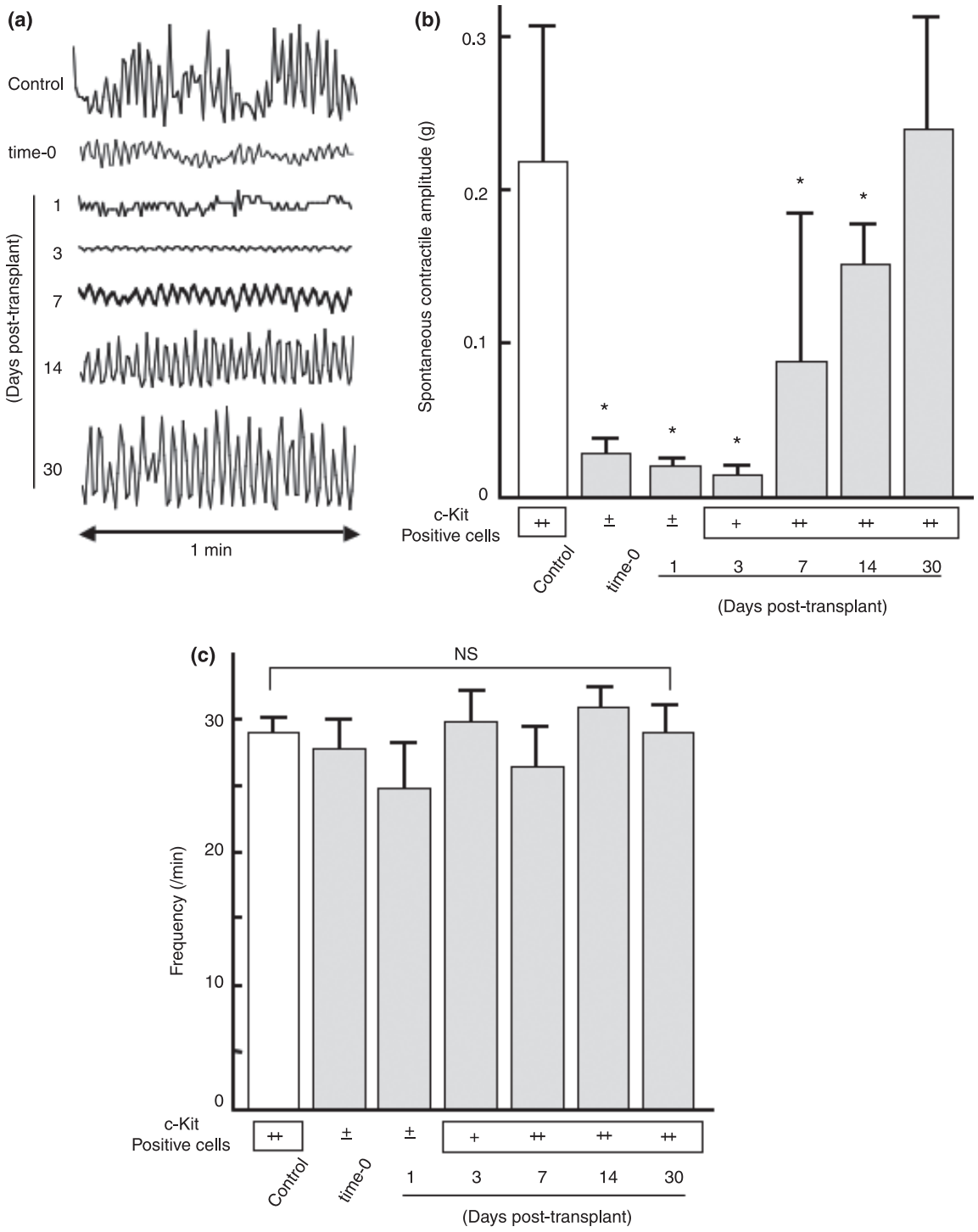

Figure 4 The amplitude and frequency of the spontaneous contractions in the controls and the grafts after SBT. The spontaneous electric waves of each time point were shown in (a). The mean value of spontaneous contractile amplitude (*g*) was also shown in (b). The mean value decreased during the early post-transplant period, and then after POD 7 gradually recovered to the same level as that of the native controls (b). However, the recovery tendency of the spontaneous contractile amplitude was slower than that of the distribution of c-Kit-positive cells. The asterisks show the mean value during the early period after SBT to be significantly lower than that of the native control ($P < 0.05$, with Bonferroni's correction). On the other hand, the contractile frequency (times/min) was not significantly different from the mean value at all the observation time points, in comparison with that of the controls (c). Time 0 in the figure means the time just after SBT.

on POD 1, was significantly smaller than that of the control muscle strip. The contractile force improved gradually over time from POD 3, and after POD 7, the contractile force of the graft generated by 10 μM carbachol was almost the same as that of the control (Fig. 3b).

The spontaneous contractile activity was also evaluated in the grafts at the various time points using isometric force measurements. The spontaneous electric waves in each time point are shown in Fig. 4a. The result of spontaneous contractile amplitude had the same tendency as that of contractile force generated by carbachol stimulation. In short, spontaneous contractile amplitude was lower during the early post-transplant period than that of controls, and after POD 7 it gradually recovered to the same level as that of the native muscle strip (Fig. 4b). There is a significant difference between the control and POD 7 muscle strips. Furthermore, even in the POD 14 muscle strips, the amplitude was significantly lower than that of the controls. In contrast, there was no significant difference between POD 7 and POD 14 muscle strips. The time needed to recover to a control level was 30 days after SBT in this study.

On the other hand, the frequency of the spontaneous contraction (contractile counts per minute) was 29.0 ± 0.33, 27.7 ± 0.84, 24.7 ± 1.37, 29.7 ± 0.93, 26.4 ± 1.10, 30.86 ± 0.64 and 28.86 ± 0.90 times/min in the controls and the grafts at the time just after SBT on POD 1, 3, 7, 14, and 30, respectively (Fig. 4c). Interestingly, the contractile frequency was almost the same and not significantly different in each sample during this experimental observation period.

Discussion

In the present study, we demonstrated an impairment of ICCs in the intestinal graft both morphologically and physiologically after SBT. In addition, we found this change to be temporary and the ICC network also gradually recovered within a short period. In this model, two factors were found to affect the changes of the ICC network and their functions: one was I/R injury and the other extrinsic denervation. Therefore, the effect of such factors on the change in ICCs was discussed at first, followed by the discrepancy between the immunohistochemical and physiological results.

In an I/R injury model, it was found that the GI dysmotility was related to the grade of I/R injury [17–19]. The tension of the muscle rings under hypoxia for a long time was demonstrated to be an irregular pattern during the reoxygenation period in the murine model *in vitro* [20]. Similarly, Hierholzer *et al.* demonstrated a significant decrease in the amplitude in circular muscle contractions in an I/R injury model using rats [17]. They also demonstrated the dysmotility to be associated with an inflammatory response that occurred in the intestinal muscle layers. Although such reports did not refer to the changes of ICCs, these muscle contractile changes may be associated with a disruption of the ICC network. Recently, Shimojima *et al.* reported on the correlation between the changes of ICCs and I/R injury [21]. In their study, the recovery of a transient impairment of ICCs was shown to occur at 4 days after only 80 min of ischemia followed by 12 h of reperfusion. They revealed patterns similar to those shown in our results. Therefore, the I/R injury might be one of the causes for the ICC impairment after SBT.

Small bowel transplantation causes extrinsic denervation, and a complete re-innervation of the gut wall does not occur. Our previous immunohistochemical study showed that the intrinsic peptidergic neurons were intact in the graft after SBT, while the extrinsic neurons were not distributed until 200 days after SBT and that they were sparsely innervated on the 400th day after SBT [22]. Kiyochi *et al.* also demonstrated that the re-innervation of the extrinsic sympathetic nerves was not found 27 weeks after SBT [23]. Therefore, the extrinsic nerves were not thought to be associated with the chronological change of ICCs after SBT. Whether or not the development and maintenance of the ICC network are required for gut nervous innervation remains a matter of debate. Several studies have suggested that the differentiation of ICCs depends on ENS [24], while other studies have shown no relationship between ICCs and the gut nervous innervation for the differentiation of ICCs [25]. Vanderwinden *et al.* demonstrated the existence of ICCs in the aganglionic segments in Hirschsprung's disease [9]. According to their study, some c-Kit-positive cells were found in the circular muscle layer, despite the lack of ganglionic cells. These findings may suggest that ICCs could develop without neurons. In contrast, the lack of ENS may influence the full differentiation of ICCs because the ICCs that they found were in immature form. Another study demonstrated that NO neurons, which are well known to be one of the inhibitory nerves, decreased markedly in number just after SBT but then completely recovered after POD 6 [26]. This recovery process of NO neurons is very similar to that of the ICCs shown in the present study. Some reports have shown that NO synthase is produced by the ICCs [27–29] in response to the intracellular Ca^{2+} concentration [5]. This means that both ICCs and NO neurons have some interactions for both the functional development and maintenance of each other. Therefore, based on the findings in the literature and our data, the process of the recovery of ICCs in the graft might be associated with that of the intramural nervous system, especially with that of NO neurons in the SBT model.

In the present study, c-Kit positivity in the intestinal graft deteriorated from just after SBT to POD 3, in com-

parison with that of the controls, and thereafter it recovered gradually. In addition, the same tendency was shown for the recovery pattern of the response to carbachol stimulation in the graft muscle strip *in vitro*. These findings imply that the injury to the smooth muscle cells caused by I/R injury or transplant procedure itself improved on POD 3. However, it took a long time for the recovery of the spontaneous contractile amplitude to the normal level after SBT, in comparison with the duration of both the c-Kit positivity and the recovery of response to carbachol stimulation (about 30 days). Thus, there was a discrepancy between the recovery process of c-Kit staining and that of the spontaneous contractile amplitude. Although we did not clarify the mechanisms responsible for this discrepancy, it may be associated with the consequence of the inflammatory changes in the intestinal muscle layer after SBT. Several reports have shown a correlation between the inflammatory changes and the change in the ICC network in the intestine [6,30–32]. Wang *et al.* demonstrated an intestinal infection to lead to a disruption of ICC network, damaging the smooth muscle cells with the infiltration of immune cells, including lymphocytes, neutrophils, and macrophages [32]. Won *et al.* also demonstrated a reduction of the c-Kit-positive cells to be dependent on the increased number of ED2-positive macrophages in the muscle layers of the rat intestinal obstruction model [33]. ICCs are known to come in contact with each other, with the ends of nerve fibers and with smooth muscle cells, and thus form gap junctions among them [5]. In addition, some inflammatory cytokines are known to modulate both the connexin expression and the junctional coupling strength [34]. Considering these findings, the present results concerning the discrepancy of recovery suggest that even after the recovery of the c-Kit positivity, the connection to smooth muscle cells through gap junctions might be poorly reconstructed. Therefore, the c-Kit-positive cells in the graft during the early postoperative period might not always have their unique functions. In addition, the connection to the surrounding cells through gap junctions might need more time than that of the recovery of c-Kit immunoreactivity after SBT. Unfortunately, this study did not show the evidence for the impairment of gap junction. To clarify this hypothesis, the gap junction needs to be evaluated using electron microscopy or immunohistochemistry in future.

We also demonstrated that the frequency of the electrical signals generated from ICCs was preserved at almost the same levels throughout the entire experimental period. Recently, Kit signaling has been suggested to contribute to both the development of ICCs and the stabilization of the ICC phenotype [35]. When the passway of Kit signaling is blocked, then ICCs are shown to revert to the smooth muscle-like phenotype cells. Although it remains unclear as to whether or not these changed ICCs have functions that adequately correspond to those of regular ICCs, based on our results, the changed ICCs retain the electrical function. In other words, SBT may lead to a minor phenotype change in ICCs while they still partially maintain their normal electrical functions.

In summary, after SBT, the network of ICCs was impaired and demonstrated changes in the electrical spontaneous contractile amplitude of the graft intestine, although the rhythmic slow waves generated by ICCs was intact. In addition, these changes in the ICCs were shown to be reversible during the short period after SBT. These changes might be caused by either a disruption of the connection between ICCs and smooth muscle cells, mainly because of I/R inflammatory injury, or they may be the result of phenotype changes in the ICCs. Further investigations are needed to confirm these hypotheses regarding the mechanism of changes which are observed to occur in the graft after SBT.

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References

1. Mousa H, Bueno J, Griffiths J, *et al.* Intestinal motility after small bowel transplantation. *Transplant Proc* 1998; **30**: 2535.
2. ZyromskiHirst NJ, Duenes JA, Sarr MG. Inhibition by nitric oxide and nonadrenergic, noncholinergic (NANC) nerves is preserved in a canine model of extrinsic denervation: implications for small bowel transplantation. *Surgery* 2005; **138**: 905.
3. Ruhl A. Grial cells in the gut. *Neurogastroenterol Motil* 2005; **17**: 777.
4. Hirst GDS, Ward SM. Interstitial cells: involvement in rhythmicity and neural control of gut smooth muscle. *J Physiol* 2003; **550** (pt 2): 337.
5. Camborova P, Hubka P, Sulkova I, Hulin I. The pacemaker activity of interstitial cells of Cajal and gastric electrical activity. *Physiol Res* 2003; **52**: 275.
6. Huizinga JD. Neural injury, repair, and adaptation in the GI tract. Pathophysiology of GI motility related to interstitial cells of Cajal. *Am J Physiol Gastrointest Liver Physiol* 1999; **275**: 381.

7. Kenny SE, Connell MG, Rintala RJ, Valliant C, Edgar DH, Lloyd DA. Abnormal colonic interstitial cells of Cajal in children with anorectal malformations. *J Pediatr Surg* 1998; **33**: 130.
8. Masumoto K, Suita S, Nada O, Taguchi T, Guo R. Abnormalities of enteric neurons, intestinal pacemaker cells, and smooth muscle in human intestinal atresia. *J Pediatr Surg* 1999; **34**: 1463.
9. Vanderwinden JM, Rumessen JJ, Liu H, *et al*. Interstitial cells of Cajal in human colon and in Hirschsprung's disease. *Gastroenterology* 1996; **111**: 901.
10. Taguchi T, Suita S, Masumoto K, Nagasaki A. An abnormal distribution of c-kit positive cells in the normoganglionic segment can predict a poor clinical outcome in patients with Hirschsprung's disease. *Eur J Pediatr Surg* 2005; **15**: 153.
11. Piotrowska AP, Solari V, Puri P. Distribution of heme oxygenase-2 in nerves and interstitial cells of Cajal in the normal pylorus and in infantile hypertrophic pyloric stenosis. *Arch Pathol Lab Med* 2003; **127**: 1182.
12. Rumessen JJ. Ultrastructure of interstitial cells of Cajal at the colonic submuscular border in patients with ulcerative colitis. *Gastroenterology* 1996; **111**: 1447.
13. Porcher C, Baldo M, Henry M, Orsoni P, Jule Y, Ward SM. Deficiency Of interstitial cells of Cajal in the small intestine of patients with Crohn's disease. *Am J Gastroenterol* 2002; **97**: 118.
14. Furuzono S, Ohya S, Inoue S, Nakao A, Imaizumi Y, Nakayama S. Inherent pacemaker function of duodenal GIST. *Eur J Cancer* 2006; **42**: 243.
15. Nakagawa T, Misawa H, Nakajima Y, Takaki M. Absence of peristalsis in the W/W^{v} mutant mice that are selectively deficient in myenteric interstitial cells of Cajal. *J Smooth Muscle Res* 2005; **41**: 141.
16. Torihashi S, Kobayashi S, Gerthoffer WT, Sanders KM. Interstitial cells in deep muscular plexus of canine small intestine may be specialized smooth muscle cells. *Am J Physiol* 1993; **265** (4 Pt 1): G638.
17. Hierholzer C, Kalff JC, Audolfsson G, Billiar TR, Tweardy DJ, Bauer AJ. Molecular and functional contractile sequelae of rat intestinal ischemia/reperfusion injury. *Transplantation* 1999; **68**: 1244.
18. Ballabeni V, Barocelli E, Bertoni S, Impicciatore M. Alterations of intestinal motor responsiveness in a model of mild mesenteric ischemia/reperfusion in rats. *Life Sci* 2002; **71**: 2025.
19. Lindestrom LM, Ekblad E. Structural and neuronal changes in rat ileum after ischemia with reperfusion. *Dig Dis Sci* 2004; **49**: 1212.
20. Bielefeldt K, Conklin JL. Intestinal motility during hypoxia and reoxygenation in vitro. *Dig Dis Sci* 1997; **42**: 878.
21. Shimojima N, Nakaki T, Morikawa Y, *et al*. Interstitial cells of Cajal in dysmotility in intestinal ischemia and reperfusion injury in rats. *J Surg Res* 2006; **135**: 255.
22. Hirose R, Taguchi T, Hirata Y, Yamada T, Nada O, Suita S. Immunohistochemical demonstration of enteric nervous distribution after syngeneic small bowel transplantation in rats. *Surgery* 1995; **117**: 560.
23. Kiyochi H, Ono A, Yamamoto N, Ohnishi K, Shimahara Y, Kobayashi N. Extrinsic sympathetic reinnervation after intestinal transplantation in rats. *Transplantation* 1995; **59**: 328.
24. Torihashi S, Ward SM, Sanders KM. Development of c-kit-positive cells and the onset of electrical rythmicity in murine small intestine. *Gastroenterology* 1997; **112**: 144.
25. Lecoin L, Gabella G, Le Douarin N. Origin of the c-kit-positive interstitial cells in the avian bowel. *Development* 1996; **122**: 725.
26. Taguchi T, Guo R, Masumoto K, *et al*. Chronological change of distribution in nitric oxide and peptidergic neurons after rat intestinal transplantation. *J Pediatr Surg* 1999; **34**: 341.
27. Altdorfer K, Bagameri G, Donath T, Feher E. Nitric oxide synthase immunoreactivity of interstitial cells of Cajal in experimental colitis. *Inflamm Res* 2002; **51**: 569.
28. Tomita R, Fujisaki S, Park E, Kimizuka K. Physiologic studies on nitric oxide in rat small bowel isografts. *World J Surg* 2003; **27**: 734.
29. Zyromski NJ, Duenes JA, Sarr MG. Inhibition by nitric oxide and nonadrenergic, noncholinergic (NANC) nerves is preserved in a canine model of extrinsic denervation: Implications for small bowel transplantation. *Surgery* 2005; **138**: 905.
30. Faussone-Pellegrini MS, Gay J, Vannucchi MG, Corcani L, Fioramonti J. Alterations of neurokinin receptors and interstitial cells of Cajal during and after jejunal inflammation induced by *Nippostrongylus brasiliensis* in the rat. *Neurogastroenterol Motil* 2002; **14**: 83.
31. Der T, Bercik P, Donnelly G, *et al*. Interstitial cells of Cajal and inflammation-induced motor dysfunction in the mouse small intestine. *Gastroenterology* 2000; **119**: 1590.
32. Wang XY, Vannucchi MG, Nieuwmeyer F, Ye J, Faussone-Pellegrini MS, Huizinga JD. Changes in interstitial cells of Cajal at deep muscular plexus are associated with loss of distention-induced burst-type muscle activity in mice infected by *Trichinella spiralis*. *Am J Pathol* 2005; **167**: 437.
33. Won KJ, Suzuki T, Hori M, Ozaki H. Motility disorder in experimentally obstructed intestine: relationship between muscularis inflammation and disruption of the ICC network. *Neurogastroenterol Motil* 2006; **18**: 53.
34. Chandross KJ. Nerve injury and inflammatory cytokines modulate gap junctios in the peripheral nervous system. *GLIA* 1998; **24**: 21.
35. Torihashi S, Nishi K, Tokutomi Y, Nishi T, Ward S, Sanders KM. Blockade of kit signaling induces transdifferentiation of interstitial cells of Cajal to a smooth muscle phenotype. *Gastroenterology* 1999; **117**: 140.