

## INDUCTION OF SMALL BOWEL TRANSPLANTATION TOLERANCE BY DONOR-SPECIFIC BONE MARROW TRANSPLANTATION

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*Abstract* : Immune response after allogeneic small bowel transplantation is more vigorous compared to other organ transplantations. It is often difficult to control small bowel allograft rejection with conventional immunosuppressive therapy. While many experimental studies showed organ transplantation tolerance, there have been few reports of tolerance in allogeneic small bowel transplantation. Induction of bone marrow chimerism is a potent strategy of tolerance induction. We tried to induce tolerance in SBT using donor-specific bone marrow transplantation (BMT) with cyclophosphamide (CYP) and tacrolimus. BN and LEW rats were used as donors and recipients. LEW recipients received BMT from BN donors after injection of CYP. The recipients were further treated with 0.3 mg/kg/day tacrolimus on days 0-6 (n=5). Establishment of bone marrow chimerism in BMT recipients was evaluated by flowcytometry of peripheral blood mononuclear cells. These recipients received small bowel transplantation from BN donors on day 100 after BMT. All of these recipients accepted BN small bowel allografts indefinitely (>100 days), while untreated LEW controls rejected BN grafts within 8 days (n=6). Histologic signs of chronic rejection were not observed in small bowel allografts in these recipients. In conclusion, donor-specific BMT with a single dose of CYP and a short course of tacrolimus in the early phase successfully induced small bowel transplant tolerance across MHC-barriers. This strategy may lead to technical innovation of immunosuppressive treatment in clinical small bowel transplantation.

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**Key words** : small bowel transplantation, transplantation tolerance, chimerism

### INTRODUCTION

Immune response after allogeneic small bowel transplantation is more vigorous compared to other organ transplantations. The high proportions of lymphoid tissues in small bowel allografts are thought to be one of the underlying factors, which may induce allograft rejection by providing strong alloantigenic stimuli to the host immune system<sup>1)</sup>. It is often difficult to control small bowel allograft rejection with conventional immunosuppressive therapy. Operative techniques and management for recipients in clinical small bowel transplantation have been significantly improved by vigorous efforts<sup>2-4)</sup>. However, outcomes in clinical small bowel transplantation are not satisfactory primarily because of severe rejection and the high incidence of infectious complications<sup>5,6)</sup>. Tolerance induction is the best solution to overcome these problems. It may have great benefit especially in small bowel transplantation to induce tolerance, because of the difficulties in controlling rejection of small bowel allografts. Tolerance induction in small bowel transplantation was not as easy

even in experimental models of small animals as in other organ transplantations. There have been few reports of tolerance in small bowel transplantation, while many experimental protocols of tolerance have been reported in other organ transplantation models.

Induction of bone marrow chimerism is a potent strategy of tolerance induction. Ildstadt *et al.*<sup>7, 8)</sup> reported that animals with allogeneic mixed bone marrow chimerism accepted subsequent organ grafts from the bone marrow donor strain across MHC-barriers. However, the need for sublethal or lethal preconditioning including whole body irradiation of the recipient to establish bone marrow chimerism has been a major impediment to clinical application of this strategy. Recently, we established a protocol of inducing bone marrow macrochimerism and tolerance in a MHC-disparate rat model using a single dose of cyclophosphamide (CYP)<sup>9)</sup>. We applied this protocol to an allogeneic small bowel transplantation model with combined use of a short course of tacrolimus therapy.

## MATERIALS AND METHODS

**Experimental animals** Brown Norway (BN, *RT1<sup>n</sup>*) and Lewis (LEW, *RT1<sup>d</sup>*) rats 7–8 weeks of age were purchased from Charles River Laboratories (Yokohama, Japan), and used as donors and recipients respectively. The Institutional Animal Care and Use Committee approved all animal protocols according to the criteria outlined in the national guidelines for the care and use of laboratory animals. All procedures were carried out under sterile conditions and anesthesia.

**Bone marrow transplantation (BMT)** BMT was performed from BN donors to fully-allogeneic LEW recipients as previously described<sup>9)</sup>. A single dose of CYP at a dose of 200 mg/kg was injected to the recipients. In addition, the recipients were further treated with 0.3 mg/kg/day tacrolimus intramuscularly on days 0–6 (n=5).

**Small bowel transplantation** Bone marrow transplant recipients underwent transplantation of small bowel allografts from BN donors 100 days after BMT. Six untreated LEW rats also underwent small bowel transplantation from BN donors as controls. Heterotopic small bowel transplantation was performed as described previously<sup>10)</sup>. Both ends of the graft jejunum were exteriorized as stomas in the form of Thiry–Vella fistula. The recipients were assessed by daily inspection and palpation for general state of health and graft survival. Rejection was determined by discoloration of the stoma and mass-formation of the graft by palpation of the abdomen and confirmed by histology with hematoxylin-eosin (H&E) staining as previously described<sup>11)</sup>.

## RESULTS

The protocols with CYP and tacrolimus used for the bone marrow recipients in the present study were revealed to be non-lethal in the separate experiment without BMT. Leukopenia was only transient and recovered quickly. All tested animals survived indefinitely without chronic deterioration of general conditions.

BMT recipients in the present study showed no signs of graft-versus-host disease. These recipients accepted BN small bowel allografts indefinitely (>100 days, n=5), while untreated LEW controls (n=6) rejected BN grafts by the 8th day showing typical histologic signs of acute rejection (Fig. 1A). The stoma of the small bowel allografts in bone marrow

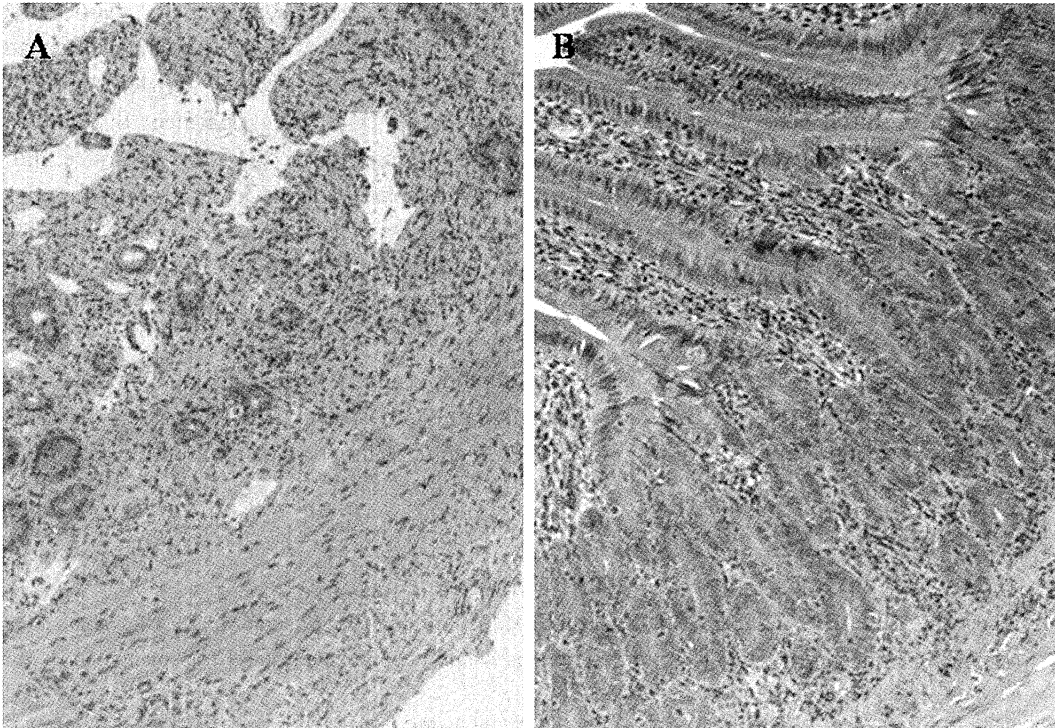


Fig. 1. Histology of the small bowel allograft (X 40). The mucosa of the small bowel allograft on day 8 was severely destroyed with infiltration of many mononuclear cells in untreated LEW recipients (A). The allograft on day 100 after small bowel transplantation in the recipients of bone marrow transplantation showed well maintained mucosal villi with minimum mononuclear infiltrates (B).

transplant recipients with CYP and tacrolimus in the early phase showed well maintained mucosa on macroscopic observation. This finding was confirmed on histology, and no signs of chronic rejection were observed in small bowel allografts on day 100 after small bowel transplantation (Fig. 1B).

## DISCUSSION

Donor-specific BMT with a non-lethal dose of CYP and a short course of tacrolimus successfully induced small bowel transplant tolerance across MHC-barriers. Use of tacrolimus in the early phase after BMT was effective on establishing stable acceptance of small bowel allograft without severe adverse effect.

Early studies revealed that hematopoietic chimerism was induced by whole body irradiation and subsequent donor-specific allograft acceptance<sup>7,8</sup>. While this tolerance status was stable, the toxicity of the preconditioning regimen for the recipients was an impediment to clinical application of this strategy. Many efforts have been made to reduce or omit the requirement of irradiation by using monoclonal antibodies, immunosuppressive drugs or other agents<sup>11-15</sup>. The effect of chemotherapeutic drugs as a substitute for of whole body irradiation has been also evaluated. Tomita et al.<sup>14</sup> reported induction of skin allograft tolerance by using CYP and donor-derived bone marrow cells in a mouse model, while the

degree of hematopoietic chimerism was not clearly demonstrated. Adams *et al.*<sup>16)</sup> reported the induction of macrochimerism by using busulfan in combination with costimulatory blockade. Colson *et al.*<sup>17)</sup> showed that simultaneous use of CYP and anti-lymphocyte globulin (ALG) reduced the required dose of total body irradiation to 300 cGy for engraftment of allogeneic bone marrow cells. Our previous study revealed that a single dose of CYP can induce stable hematopoietic macrochimerism without any other treatment in a MHC-disparate rat model<sup>9)</sup>. However, high incidence of lethal graft-versus-host disease was a problem to be solved. The combined use of tacrolimus in the present study successfully inhibited the development of lethal events and induced stable allogeneic macrochimerism and tolerance in small bowel transplantation.

In conclusion, the present study showed that induction of bone marrow chimerism with a non-lethal preconditioning protocol can induce small bowel transplantation tolerance across MHC-barriers. This strategy may lead to technical innovation of immunosuppressive treatment in clinical SBT.

## REFERENCES

- 1) Kanokogi, H., Ko, S., Kanehiro, H., Hisanaga, M., Tatekawa, Y., Kanamura, T., Okayama, J. and Nakajima, Y. Immune responses of graft mesenteric lymph node in small bowel transplantation. *J. Surg. Res.* **116** : 269, 2004.
- 2) Todo, S., Reyes, J., Furukawa, H., Abu-Elmagd, K., Lee, R.G., Tzakis, A., Rao, A.S. and Starzl, T.E. : Outcome analysis of 71 clinical intestinal transplantation. *Ann. Surg.* **222** : 270, 1995.
- 3) Langnas, A.N., Shaw, B.W., Antonson, D.L., Kaufman, S.S., Mack, D.R., Heffron, T.G., Fox, I.J. and Vanderhoof, J.A. : Preliminary experience with intestinal transplantation in infants and children: *Pediatrics* **97** : 443, 1996.
- 4) Abu-Elmagd K., Reyes, J., Bond, G., Mazariegos, G., Wu, T., Murase, N., Sindhi, R., Martin, D., Colangelo, J., Zak, M., Janson, D., Ezzelarab, M., Dvorchic, I., Parizhskaya, M., Deutsch, M., Demetris, A., Fung, J. and Starzl, T.E. : Clinical intestinal transplantation: a decade of experience at a single center. *Ann. Surg.* **234** : 404, 2001.
- 5) Grant, D. : Current results of intestinal transplantation: The International Intestinal Transplant Registry. *Lancet* **347** (9018) : 1801, 1996.
- 6) Kato, T., Ruiz, P., Thompson, J.F., Eskind, L.B., Weppler, D., Khan, F.A., Pinna, A.D., Nery, J.R. and Tzakis, A.G. : Intestinal and multivisceral transplantation. *World J. Surg.* **26** : 226, 2002.
- 7) Ildstad, S.T. and Sachs, D.H. : Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature* **307** : 168, 1984.
- 8) Ildstad, S.T., Wren, S.M., Oh, E. and Hronakes, M.L. : Mixed allogeneic reconstitution (A+B-A) to induce donor-specific transplantation tolerance: permanent acceptance of a simultaneous donor skin graft. *Transplantation* **51** : 1262, 1991.
- 9) Okayama, J., Ko, S., Kanehiro, H., Kanokogi, H., Hisanaga, M., Ohashi, K., Sho, M., Nagao, M., Ikeda, N., Kanamura, T., Akashi, S. and Nakajima, Y. : Bone marrow chimerism and tolerance induced by single dose cyclophosphamide. *J. Surg. Res.* **120** :102, 2004.
- 10) Monchik, G.J. and Russell, P.S. : Transplantation of small bowel in the rat: technical and immunological considerations. *Surgery* **70** : 693, 1971.
- 11) Sykes, M., Szot, G.L., Swenson, K.A. and Pearson, D.A. : Induction of high levels of allogeneic hematopoietic reconstitution and donor-specific tolerance without myelosuppressive conditioning. *Nat. Med.* **3** :

783, 1997.

- 12) **Monaco, A.P., Wood, M.L. and Russel, P.S.** : Studies on heterologous antilymphocyte serum in mice. III. Immunologic tolerance and chimerism produced across the H-2 locus with adult thymectomy and antilymphocyte serum. *Ann. N. Y. Acad. Sci.* **129** : 190, 1966.
- 13) **Qin, S., Cobbold, S.P., Benjamin, R. and Waldmann, H.** : Induction of classical transplantation tolerance in the adult. *J. Exp. Med.* **169** : 779, 1989.
- 14) **Tomita, Y., Nishimura, Y., Harada, N., Eto, M., Ayukawa, K., Yoshikai, Y. and Nomoto, K.** : Evidence for involvement of clonal anergy in MHC class I and class II disparate skin allograft tolerance after the termination of intrathymic clonal deletion. *J.Immunol.* **145** : 4026, 1990.
- 15) **Wekerle, T., Kurtz, J., Ito, H., Ronquillo, J.V., Dong, V., Zhao, G., Shaffer, J., Sayegh, M.H. and Sykes, M.** : Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Na.t. Med.* **6** : 464, 2000.
- 16) **Adams, A.B., Durham, M.M., Kean, L., Shirasugi, N., Ha, J., Williams, M.A., Rees, P.A., Cheung, M.C., Mittelstaedt, S., Bingaman, A.W., Archer, D.R., Pearson, T.C., Waller, E.K. and Larsen, C.P.** : Costimulation blockade, busulfan, and bone marrow promote titratable macrochimerism, induce transplantation tolerance, and correct genetic hemoglobinopathies with minimal myelosuppression. *J. Immunol.* **167** : 1103, 2001.
- 17) **Colson, Y.L., Li, H., Boggs, S.S., Patrene, K.D., Johnson, P.C. and Ildstad, S.T.** : Durable mixed allogeneic chimerism and tolerance by a nonlethal radiation-based cytoreductive approach. *J. Immunol.* **156** : 2820, 1996.