Experimental study of combined treatment with tacrolimus and donor splenocytes via the portal vein in small bowel transplantation

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Abstract : We previously reported that the combined treatment of perioperative administration of donor splenocytes via the recipient's portal vein (DSPV) and a short-course Tacrolimus significantly prolonged the survival of fully allogenic grafts in rat small bowel transplantation (SBTX). In the present study we examined whether this effect depended on the quantity of the administered alloantigens in DSPV. In addition, we examined the expression of the surface antigen on T cells of the splenocytes and the induced toleragenic factor, according to the tolerant recipients which in our previous report had shown the prolongation of allogenic transplant small bowel graft survival by the combined treatment of DSPV (1×10^8 donor splenocytes) and a short-course Tacrolimus. Donor splenocytes were prepared from Brown-Norway (BN ($RT1^n$)) rat spleens for Lewis (LEW ($RT1^l$)) recipients. The recipients (n=10), treated with a short course of Tacrolimus (0.5mg/kg, 0 to 3 days postoperatively) only showed graft rejection with an average of 6.3 ± 1.0 days postoperatively. However, the combined treatment, consisting of DSPV of 1×10^8 donor splenocytes and a short course Tacrolimus significantly prolonged graft survival to 12.7 ± 2.1 days (n=12, $P<0.01$). DSPV of less than 1×10^8 donor splenocytes (5×10^7 cells and 2.5×10^7) could not prolong the graft or animal survival under a short-course Tacrolimus treatment. In the tolerant recipients, the CD4 and CD8 percentages of splenocytes were not significantly different from those of control rats or recipients that were treated with short-course Tacrolimus alone. Neverthless, the percentage of Tcr-$\alpha\beta^+$ cells expressing IL-2 receptor (R) was significantly lower than in either control rats or the recipients with short-course Tacrolimus. In the suppression assay to one-way mixed lymphocyte response, a toleragenic factor was suggested to the present in the serum of the tolerant recipients. In the present study, it was suggested that the effects of the combined treatment of DSPV and short-course Tacrolimus for the prolongation of graft survival in the rat allogenic SBTX should depended on the quantity of the antigens administered into the portal vein. The beneficial effects of this treatment were reflected in the suppression of IL-2R on the recipient's splenocytes, and tolerogenic factor(s) might subsequently be induced in the tolerant recipient's serum. J. Med. Invest. 48 : 157-165, 2001

Keywords **:** *small bowel transplantation, portal vein, tacrolimus*

Received for publication April 26, 2001 ; accepted July 6, 2001.

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INTRODUCTION

Since the gut is the largest lymphoid tissue in the human body and has specific immunogenicity (1), graft rejection is the most important threat to suc-

cessful small bowel transplantation (SBTX) (2). Powerful immunosuppressive agents, such as Tacrolimus, have recently been exploited, and successful cases of SBTX have markedly increased in the recent years (3). However, complete prevention of graft rejection still remains an unresolved problem even during the early postoperative period, and the long prolongation of graft survival has not been achieved using Tacrolimus alone. Especially in the early period after SBTX, other immunosuppressive agents such as steroid, cyclophosphamide and prostaglandin are additionally administered in practical SBTX cases (4). These aggressive immunosuppressive treatments damage the host's immune system which might provoke the severe infectious diseases, such as cytomegalo virus infection (5).

In contrast to the progression of the chemical immunosuppressive agents, various investigators have studied the possibility of inducing a donor-specific tolerance using biological methods (6). Many authors have studied the administration of donor-specific allo-antigens via the portal vein of the recipient in experimental organ transplantations (6-8), and documented the beneficial effects for regulating the graft rejection. On the basis of these studies, we previously examined the effects of the combined treatment of perioperative administration of donor splenocytes via the portal vein (DSPV) and short-course Tacrolimus for the prolongation of allo-graft rejection in the rat fully allogenic SBTX. In brief, the perioperative administration of 1×10^8 Brown-Norway (BN ($RT1^n$)) rats splenocytes into the systemic circulation caused fatal graft-versus-host disease (GVHD) in Lewis (LEW ($RT1^l$)) recipients even when short-course Tacrolimus was combined. Without treatment, every allograft was rejected and the recipients died during the early postoperative days due to peritonitis caused by severe graft rejection. DSPV only caused fatal GVHD and the graft survival was not prolonged. A short-course Tacrolimus treatment (0.5 mg/kg BW, 0 day to 3 day postoperatively) prolonged recipient survival, but, graft survival was not prolonged. The combined treatment of DSPV and a short-course of Tacrolimus significantly prolonged both graft and recipient survival. However, all tolerant recipients that attained prolongation of transplant graft survival showed some signs of mild GVHD. The beneficial effects on the prolongation of allograft survival were confirmed to be donor specific because the perioperative administration of the third-party WKA rats splenocytes into the recipient's portal vein could not prolong the BN allograft survival. *In vitro*, donor-specific down-regulation of one-way mixed lymphocyte response (MLR) was also examined in tolerant recipients. Perioperative administration of the donor antigens via the systemic vein was very dangerous because it often induced fatal GVHD in the SBTX recipients (2). Grant *et al.* previously suggested that GVHD after SBTX damages host lymphoid tissues, producing profound immunosuppression and creating a risk of infectious complications (9). Several authors have also reported that the risk of GVHD after SBTX depends on the amount of administered alloantigens (10, 11). Therefore, in the present study, we investigated the optimal dose of splenocytes in order to assess the appropriate quantity of donor specific antigens for the effective and safe treatment for DSPV. We also examined the T cell surface antigens and the tolerogenic factors in the tolerant recipients to clarify the immunological conditions in tolerant SBTX recipients after DSPV.

MATERIAL AND METHODS

Animals

Inbred male BN ($RT1^n$) and LEW ($RT1^l$) rats were obtained from the Seiwa experimental animal farm (Fukuoka, Japan). The animals were bred under pathogen-free conditions and were used for experiments at a body weight of 200-300 g.

Intestinal transplantation

Operative procedures were performed under ether anesthesia. The small intestine was transplanted in the form of a Thiry-Vella fistula as described by Kobayashi *et al.* (12). In brief, after an overnight fast, a 10 cm segment of the proximal jejunum was harvested from the donor on a vascular pedicle that included the superior mesenteric artery and the portal vein. The donor was systemically anticoagulated with 200 U of heparin given intravenously immediately before graft removal. After excision, the intestinal lumen was flushed, using 10 ml of Ringer's lactate. The donor mesenteric artery and the portal vein were anastomosed to the recipient left renal artery and vein, using a cuff technique. Both ends of the graft were exteriorized as stomata, isolating the segment from the recipient native gastrointestinal tract.

Immunosuppressive agents

Tacrolimus, supplied in powder form by the Fujisawa

Pharmaceutical Company (Osaka, Japan), was diluted in normal saline and injected intramuscularly at a dose of 0.5 mg/kg body weight/ day from the day of surgery until the third postoperative day (POD). The properties and immunosuppressive activity of this drug have been previously reported (13, 14).

Administration of donor splenocytes via the portal vein (DSPV)

Following the experimental protocols, 1×10^8 million, 5×10^7 and 2.5×10^7 native splenocytes from BN rats were injected perioperatively through the recipient's portal vein, using a 27-gauge needle. Cell viability assessed by the trypan blue exclusion test was always greater than 95%.

Experimental groups

The protocol included five treatment groups as follows : group A (n=10) ; Recipients given a short-course Tacrolimus treatment (intramuscular doses of 0.5 mg/kg body weight/ day were injected intramusculary on the day of operation and postoperative days 1-3) ; group B (n=12) ; recipients given DSPV (1×10^8) and a short-course Tacrolimus treatment ; group C (n= 10) ; recipients given DSPV (5×10^7) and a short-course Tacrolimus treatment ; and Group D (n=8) ; recipients given DSPV (2.5×10^7) and a short-course Tacrolimus treatment. The details of the findings of the two groups, A and B have been described previously (2).

Postoperative monitoring

After the small bowel transplantation, all rats received standard rat chow and water ad libitum. The rats that died within two days were considered to be technical failures and excluded from this analysis. Autopsies were performed to confirm the cause of death. Graft rejection was evaluated by the clinical signs, previously described by Zhang *et al*, of progressive stomal ischemia, stomal closure and an abdominal mass (15). The severity of GVHD was estimated by clinical grading, as previously described by Saat *et al*. (16) : grade I (mild), light redness of ears, snout, and paws ; grade II (moderate), moderate redness of ears, snout, and paws, with slight hair loss and diarrhea;and grade III (fatal), severe redness of ears, snout and paws, with alopecia, generalized dermatitis, and profuse diarrhea. In the present study, signs of grade II (moderate) and grade III (fatal) GVHD were not detected in any animal.

Mixed lymphocyte culture and suppressor assay

Both splenocytes and serum were obtained from the SBTX recipients (n=5) with DSPV (1×10^8) and a short-course Tacrolimus treatment on the seventh postoperative day. Various cell numbers (1×10^5, 5×10^4, 2.5×10^4) of these SBTX recipient's splenocytes were added to the micro-titer culture plate wells of mixed lymphocyte culture (MLC) in which 5×10^5 native responder LEW splenocytes responded toward 5×10^5 mitomysin C treated stimulator splenocytes of BN or third-party WKA rats. The SBTX recipient sera were diluted, and then added into the wells of MLC at various concentrations (10%, 5%, 2.5%/200 μl total volume). After five days incubation at 37°C in a humidified atmosphere of 5% CO_2, in air, 0.5μCi of [^{3}H]-methylthymidine (N E N, Boston, MA) was added to each well. After incubating for another 18 hrs, the mixed lymphocyte culture proliferative response (MLR) was determined by measuring incorporation of [^{3}H]-methylthymidine by direct β counting (Packard Instrument B.V. Chemical Operations, The Netherlands). The results were expressed as the mean cpm of triplicate cultures. The percentage of suppression was calculated using the formula :
% suppression = (1-experimental cpm/positive control cpm)×100. Positive control MLC ; 5×10^5 native responder LEW splenocytes responded toward 5×10^5 mitomysin C treated stimulator splenocytes of BN or third-party WKA rats. This positive control MLC recieved neither tolerant SBTX recipients' splenocytes nor their serum in contrast to the experimental MLC which received either tolerant SBTX recipients' splenocytes or their serum.

Antibodies

Anti-rat CD4 mAb, W3/25, anti-rat CD8, mAb, OX8, and anti-rat IL-2R, mAb, OX39 were from Dr. M. Miyasaka, Tokyo Metropolitan Institute for Medical Science, Anti-rat TCR-αβ mAb-producing R73, hybridoma cell line was a gift from Dr. T. Hunig (University of Wurzberg, Germany).

Flow-cytometric analysis

Splenocytes were stained with combinations of FITC-conjugated mAb and biotin-conjugated mAb, followed by staining with PE-streptavidin. Analyses were performed with a FACScan and Consort 30 software (Becton Dickinson, Mountain View, CA). Dead cells were gated out using forward and side light intensities.

Statistical analysis

All comparisons were made using the Student's t test. Differences in values were considered signifi-

cant at P<0.05.

RESULTS

Observations of animals transplanted with intestinal allografts

After transplantation, recipients were monitored for rejection and GVHD (Table 1). All recipients treated with Tacrolimus alone (group A) showed graft rejection signs at 6.3±1.0 POD. Seven of 10 animals died from rejection with peritonitis within 24 POD. One animal died from an obstructed ileus due to the small bowel adhesion to the rejected graft. Two animals survived over 70 POD despite signs of graft rejection. In recipients treated with both DSPV (1×10^8) and Tacrolimus (group B), graft survival was significantly prolonged (12.7±2.1 days, P<0.01) compared with group A. In recipients treated with DSPV (5×10^7) and Tacrolimus (group C), graft survival was not prolonged (6.9±0.8 days) compared with group A. The period of animal survival was not longer than that of the recipients for short-course Tacrolimus alone : six of ten recipients died between 9 days and 33 days postoperatively. Four recipients survived for more than 70 days, even though signs of the graft rejection were confirmed within 7 days postoperative. Of note, these long surviving animals also showed signs of mild GVHD. The recipients treated with the lower dose DSPV (2.5×10^7) and short-course Tacrolimus (group D) also did not show prolonged the graft survival (6.3±0.7 days) or recipient survival : seven of 8 recipients died between 7 and 62 days postoperative. Only one recipient survived for more than 70 days despite of the evident clinical signs of mild GVHD.

Antigen-specific suppressor factor in the serum from the torelant recipients

The sera obtained from the SBTX recipients with DSPV (1×10^8) and short-course Tacrolimus treatment at the seventh postoperative day, suppressed mixed lymphocyte culture proliferative response (MLR) of LEW responder cells towards BN, but not WKA stimulator cells, depending on the serum concentrations of these SBTX recipients in mixed lymphocyte culture wells ; Inhibition rate : 10% serum (46.7±6.7% *vs* 2.5±10.3% ; P<0.01), 5% serum (21.0±4.9% *vs* 1.5±9.1% ; P<0.01) and 2.5% serum (1.2±4.0% *vs* 4.4±12.2% ; NS), respectively. The same recipient sera, treated at 56℃ for 30 minutes, also suppressed MLR, depending on the concentration ; Inhibition rate : 10% serum (54.8±11.0% *vs* -0.0±8.6% ; P<0.01), 5% serum (16.2±4.0% *vs* -0.5±10.5% ; P<0.05) and 2.5% serum (-0.3±4.7% *vs* -0.5±9.8% ; NS), respectively (Fig. 1).

Failure to detect suppressor activity in splenocytes from tolerant recipients

1×10^5, 5×10^4, and 2.5×10^4 of splenocytes, obtained from the recipients treated with DSPV (1×10^8) and short-course Tacrolimus, could not suppress MLR of LEW responder cells toward either BN or third-party WKA stimulator cells ; Inhibition rate : 1×10^5 (-5.00±2.68% *vs* 0.06±3.01% ; NS), 5×10^4 (-0.74±5.21% *vs* 1.86±3.83% ; NS), 2.5×10^4 (-2.76±5.99% *vs* 1.47±5.43% ; NS), respectively (Fig. 2).

Flow-cytometric analysis

Fig. 3 shows the expression of $W3/25^+$ ($CD4^+$)

Table 1. Outcome after SBTX

Group	Cell numbers for DSPV	n	Animal survival days	Graft survival days (mean±SD)	GVHD mild	GVHD fatal
A	none	10	8,10,10,14,14,23, 24,25[a],>70×2	5,5,6,6,6,6,6,7,8,8, (6.3±1.0)	1	0
B	1×10^8	12	20,25,70×10	10,11,11,11,12,12, 12,12,13,15,16,17 (12.7±2.1)	12	0
C	5×10^7	10	9,10,10,21,30,33, >70×4	6,6,6,6,7,7,7,8,8,8, (6.9±0.8)	6	0
D	2.5×10^7	8	7,10,10,38,41,53, 62,>70×1	5,6,6,6,6,7,7,7, (6.3±0.7)	3	0

Underlining indicates mild GVHD in all experimental groups. [a] indicates death from volvulus. The results of group A and B have been already described in our previous report (2). All animals of these four experimental groups were treated with Tacrolimus at a dose of 0.5mg/kg body weight/ day from the day of surgery until the third postoperative day (POD).

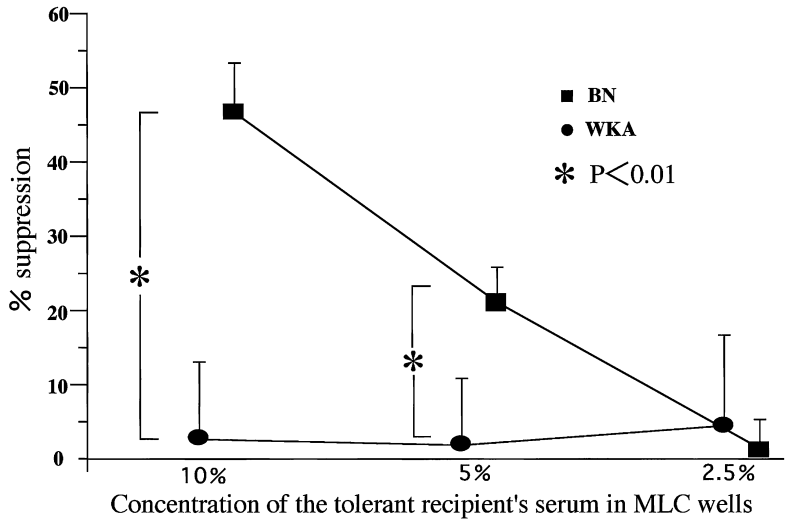

Fig. 1. Suppressor assay of mixed lymphocyte culture proliferative response by the tolerant SBTX recipient's serum. The sera were obtained from the five SBTX recipients that were treated with short-course Tacrolimus and DSPV (1×10^8) on 7 POD. The serum was diluted and added into mixed lymphocyte culture wells, in which 5×10^5 native LEW splenocytes responded toward 5×10^5 mitomysin C treated stimulator BN (■) or WKA (●) splenocytes at various concentrations (10%, 5%, 2.5%/200μl total volume). Results are presented as % suppression, calculated as described in Materials and Methods. The minimal spontaneous (LEW-to-LEW) cpm was 67.

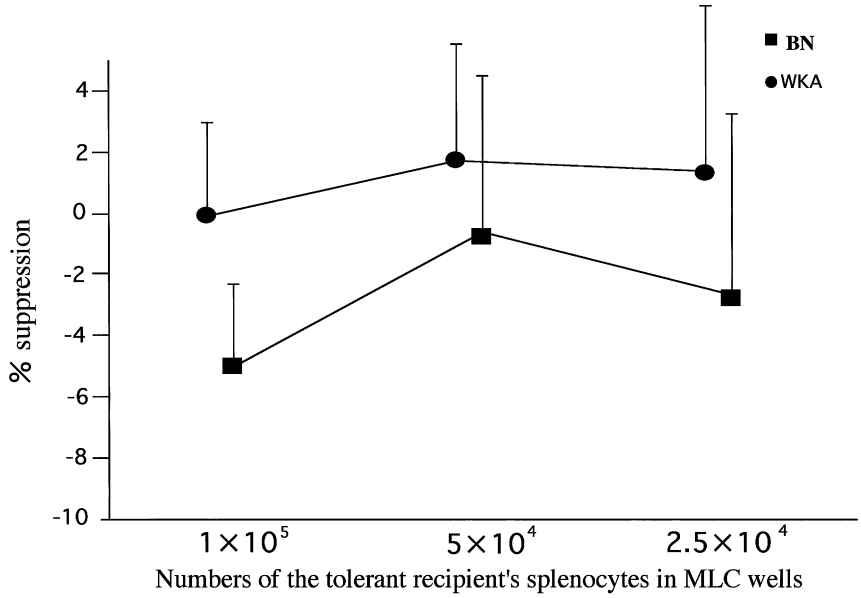

Fig. 2. Suppressor assay of mixed lymphocyte culture proliferative response by the tolerant SBTX recipient's splenocytes. Various number of splenocytes (1×10^5, 5×10^4, 2.5×10^4) obtained from the SBTX recipients with short-course Tacrolimus treatment and DSPV (1×10^8) on 7 POD, were added into mixed lymphocyte culture wells, in which 5×10^5 native LEW splenocytes responded toward 5×10^5 mitomysin C treated stimulator BN (■) or WKA (●). Results are presented as % suppression, calculated as described in Materials and Methods. The minimal spontaneous (LEW-to-LEW) cpm was 69.

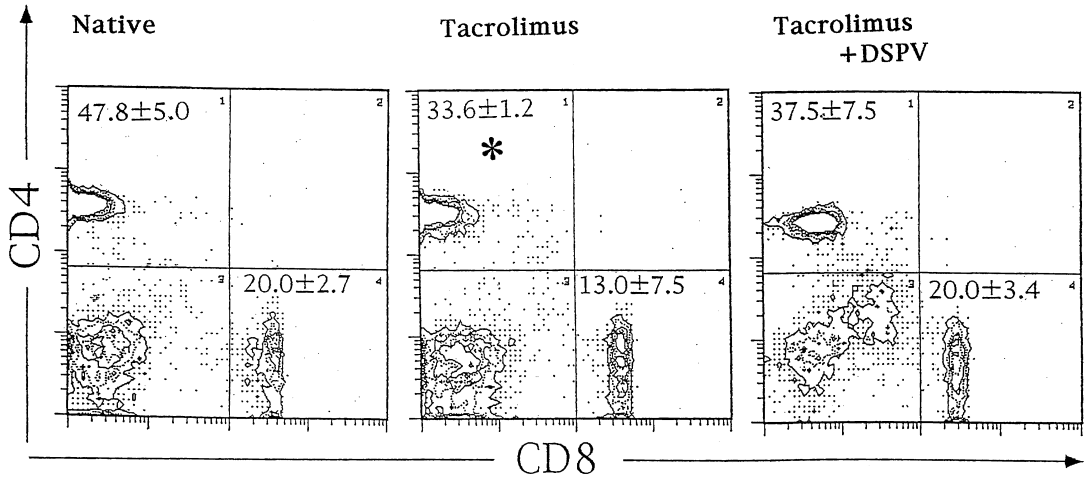

Fig. 3. Representative flowcytometric analysis of splenocytes from native rats(left), the recipient rats treated with short-course Tacrolimus only (middle), and the recipient rats treated with short-course Tacrolimus and DSPV (right). Cells prepared on 7 POD were stained with FITC-anti-CD 8 and biotin-anti-CD 4, followed by staining with PE-streptavidin. Numbers indicate percentage of each population (mean±SD). Symbol * means that in recipients treated with short-course Tacrolimus alone, the percentage of $CD4^+$ T cells population decreased significantly ($P<0.05$) compared with that of control rats.

and $OX8^+$ ($CD8^+$) cells in splenocytes from control and allografted animals at 7 POD (each experimental number of animals was five). The mean ratio of $CD4^+$ cells was 47.8±5.0% in control LEW rats ; in recipients treated with short-course Tacrolimus alone, the percentage of this population decreased significantly ($P<0.05$) to 33.6±1.2%. In the tolerant recipients treated with DSPV (1×10^8 cells) and short-course Tacrolimus, the mean ratio of $CD4^+$ cells was 37.5± 7.5%, which was not significantly different from the other two groups. The mean ratio of $CD8^+$ cells in splenocytes showed no significant difference between control rats (20.0±3.4%), recipients of Tacrolimus alone (13.5±7.5%), and recipients treated with DSPV (1×10^8 cells) and short-course Tacrolimus (20.0± 2.7%). Fig. 4 shows the expression of IL-2R on TCR-$\alpha\beta^+$ splenocytes. The proportion of IL-$2R^+$ cells in TCR-$\alpha\beta^+$ splenocytes from recipients of short-course Tacrolimus alone was 25.5±7.6%, which was higher than that for control rats (5.1±3.6%). The IL-2R positive T cells of recipients treated with the combination of DSPV (1×10^8 cells) and short-course Tacrolimus, was 1.7±0.5 %; significantly ($P<0.05$) lower than that of the recipients which were treated with short-course Tacrolimus alone but no significantly different from that of control rats.

DISCUSSION

The phenomena in which the intra-portal and oral administration of antigen induce immune tolerance have been known as the Chase-Surzberger effect (17). On the basis of this advantage for inducing immunological tolerance, many studies have examined the effects of administration of donor-specific antigens into the portal vein (DSPV) for protection against graft rejection in the experimental organ transplantations and reported the beneficial effects. Previous studies, according to DSPV, have proposed several possible mechanisms for the induction of tolerance : the inability of Kupffer cells to present the antigen (18) ; the preferential generation of T suppressor cells due to the slow release of antigen by the liver (19);the presence of a factor in the serum that mediates tolerance (20) ; and selective activation of TH1/Th2 cells (21). We previously studied the effects of DSPV in SBTX, using fully allogenic rat strains (BN-to-LEW), and demonstrated that DSPV has the advantage of prolonging the BN small bowel graft survival in the LEW recipient under short-course Tacrolimus treatment. However, the findings gave rise to another important problem ; GVHD, which might be a threat to the successful treatment for SBTX. Indeed, short-course Tacrolimus proved to be useful for ameriolating lethal GVHD, which was induced in recipients treated with DSPV by 1×10^8 splenocytes alone. However, all tolerant recipients showed signs of mild GVHD. It was suggested that this mild GVHD in tolerant recipients was caused by the splenocytes, which entered the recipient's systemic circulation without liver trapping (2). According to the tolerance of organ transplantation, Starzl *et al.* postulated a two-way paradigm, i.e. a

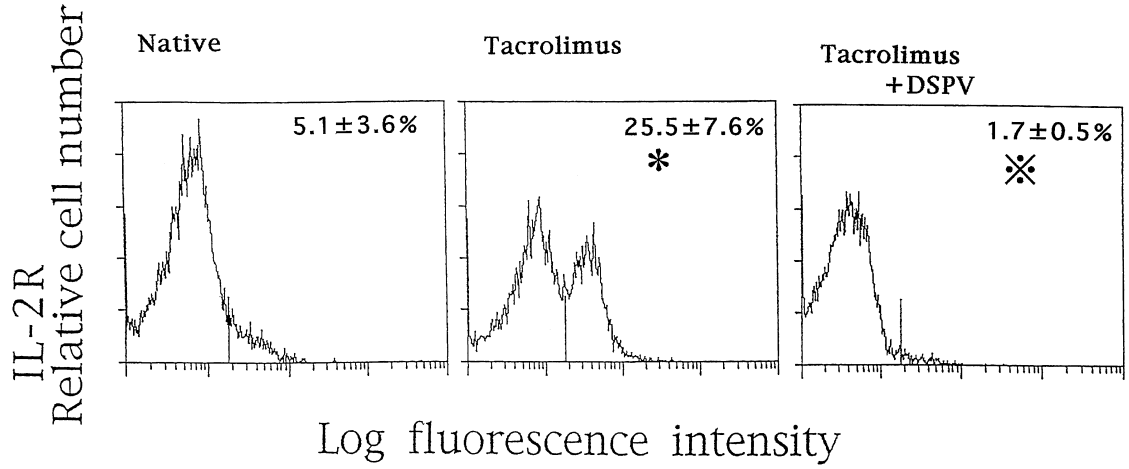

Fig. 4. IL-2R expression on TcR-$\alpha\beta^+$ splenocytes from native rats (left), the recipient rats treated with short-course Tacrolimus alone (middle), and the recipient rats treated with short-course Tacrolimus and DSPV(right) on 7 POD. Splenocytes were stained with FITC-anti-TcR-$\alpha\beta^+$ and biotin-anti-IL-2 R, followed by staining with PE-streptavidin. Results represent single histogram pattern of gated TcR-$\alpha\beta^+$ cells. Numbers indicate percentage of IL-$2R^+$ cells. Symbol * means that IL-2R relative cell number was significantly higher than that of control rats ($P<0.05$). Symbol ※ means that IL-2R positive T cells of recipients treated with the combined treatment of DSPV (1×10^8) and short-course Tacrolimus was significantly ($P<0.05$) lower than that of the recipients treated with short-course Tacrolimus alone and showed no significant difference from that in control rats.

balance of opposing immunologic forces between rejection and GVHD in organ transplantation (22). Following SBTX, there is a large bi-directional transfer of lymphoid cells between the graft and the recipient (23). Murase *et al*. described that the incidence of lethal GVHD depends on either the dose or quality of the passenger leukocytes, and reported that in the transplantation of intestine, spleen or lymphocytes enriched with T cells, 5%～7% of the donor-derived cells were found in the recipient's organs, and that the proportion of T cells was higher than that of other lineages of donor cells. In a chimeric recipient's tissues, over-representation of donor T cells was associated with GVHD (24). Kenick *et al*. have suggested that the induction of tolerance following DSPV might depend on the quantity of allogenic cells trapped by the liver (25). Kamei *et al*. described that the initial effective uptake of alloantigens by Kupffer cells appeared to be essential for their presentation of antigen in the liver (26).

In the present study, we examined the optimum dose of the donor splenocytes for DSPV. Yoshimura *et al*. described that 2×10^8 splenocytes might give rise to emboli, which could cause recipient death (27), and therefore we determined that the 10^8 splenocytes was the maximum dose for DSPV. DSPV of 5×10^7 and 2.5×10^7 splenocytes could not prolong graft survival. These findings strongly suggest that the induction of tolerance after DSPV might depend on the quantities of allogenic cells trapped by the liver. Of note, a lower quantity of splenocytes, even at a dose of 2.5×10^7, was shown to have a risk of inducing GVHD. In respect to this point, Kobayashi *et al*. recently described that the major SBTX damages the host lymphoid organ, especially thymic T cells, and increases host sensitivity to GVHD (28). In the present study, it is suggested that the signs of mild GVHD provoked by a low dose of DSPV might support their finding.

Qian *et al*. described that the generation of serum factor might be able to transfer alloantigen-specific tolerance for DTH by DSPV in the murine system (6). In the present study, suppressor activity was not identified in splenocytes from tolerant recipients, while antigen-specific suppressor factor(s) was suggested in the serum of the tolerant recipients. From these findings, it was suggested that DSPV might produce suppressor factors, which could regulate the graft rejection in SBTX.

We have already described that the beneficial effect of DSPV in vitro was reflected in the donor specific downregulation of one-way MLR (2). According to the surface antigens of tolerant recipient T cells, the findings of the present study suggested that DSPV could suppress T cell activity against donor-specific antigens, because IL-2R is known to be expressed on activated T lymphocytes (29). According to the ratios of $CD4^+/CD8^+$ cells among splenocytes, there was no significant difference between the tolerant recipients and non-tolerant recipients. Recently, helper T cells have been divided into two groups, Th1 and Th2, according to their cytokine production (30). IL-2, a Th1-derived cytokine, produced after T cell, B cell stimulation during the amplification of immune response, may provide the environment necessary for anergy induction (31). Based on the non-responsiveness (T cell tolerance or anergy) produced by administration of alloantigens via the portal vein, Gorczynski *et al*. described that presentation of multiple minor cell surface antigens by hepatic antigen presenting cells leads to anergy induction in IL-2 producing T cells (Th1), and also that subsequent activation of Th2 cells with further feedback inhibition of Th1 cells may play an important role in the long-term allograft survival (21). In the present study, it was suggested that donor-specific serum factor(s) might suppress IL-2R expression on the splenocytes in the tolerant recipients. Sullivan *et al*. recently reported that the liver may regulate the rejection of vascularized allografts (in particular small bowel and renal allografts) due to the altered migration of lymphoid cells into and out of the graft after portal drainage. They also reported that the altered ratios of $\gamma\delta$TcR+/$\alpha\beta$TcR+ cells, altered cytokine production (IL-2, INF-γ, IL-4, IL-10), and/or other functional activities may play key roles in determining successful engraftment (32). Gorczynski *et al*. have recently reported that after the portal venous drainage of small bowel graft, type-2 cytokine (IL-4, IL-10, TGFβ) producing $\gamma\delta$TcR+ cells may play an important role in regulating graft rejection (33). In contrast, Starzl *et al*. reported that microchimerism could be important for inducing immunological tolerance in organ transplantations (22), including SBTX. However, we did not study chimerism in the present study. The detailed mechanisms of the tolerance after DSPV still remain obscure.

In conclusion, the effective advantages of combined treatment of DSPV and short course Tacrolimus for the prolongation of graft survival in rat SBTX depended on the quantity of donor-specific antigens administered via the recipient's portal vein. In the present study, the effects of the protection of the SRTX allo-grafts might be reflected in the suppression

of IL-2R expression on the recipient's splenocytes. Furthermore, the recipient's serum factor(s), which were induced in the tolerant SBTX recipients, might play an important role in the protection of small bowel allo-grafts.

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