Influence of liver nonparenchymal cell infusion combined with cyclosporin A on rejection of rat small bowel transplantation

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METHODS: The liver nonparenchymal cell suspension was prepared by density gradient centrifugation method with Percoll centrifugal solution. Heterotopic small bowel transplantation was performed. Then the rats were divided into four groups. Group one: homogenic transplantation (F344/N→F344/N), group two: allotransplantation (F344/N→Wistar), group three: allotransplantation (F344/N→Wistar) + CsA, with CsA 10 mg kg⁻¹ · d⁻¹ after transplantation, group four: allotransplantation + CsA (F344/N→Wistar) + liver nonparenchymal cell infusion + CsA (F344/N→Wistar), in which recipient Wistar rats had been injected with 2×10⁶ F344/N liver nonparenchymal cells 20 days before transplantation, and treated with CsA after transplantation. Finally, the survival time after small bowel transplantation, gross and histopathological examination, and IL-2 levels in serum were observed.

RESULTS: The survival time after small bowel transplantation was 7.14±0.33 d, 16.32±0.41 d and 31.41±0.74 d in group 2, 3, and 4, respectively. The survival time was significant longer (P<0.01) in group 4. The gross and histopathological examination showed that the rejection degree in group 4 was lower than those in groups 2 and 3. Serum IL-2 level in group 4 was also lower than those in groups 2 and 3 (P<0.01).

CONCLUSION: Liver nonparenchymal cell infusion combined with CsA can prolong the survival time of rat small bowel transplantation, and the anti-rejection effect is good.

INTRODUCTION

In clinical practice, rejection responses induced by organ transplants necessitate the use of potent immunosuppressive drugs. It should be noted, however, that excessive dosage of immunosuppressive agents may result in severe side effects such as hypertension and hepatic and/or renal toxicity. Moreover, prolonged usage of immunosuppressants often leads to severe infection and increased susceptibility to malignant tumors, thus critically affecting the health of recipients. It is therefore imperative to assess, as an alternative to immunosuppressants, the protective effect of induced immune tolerance on organ transplantation. The ideal strategy is to induce a immune tolerance state or a low reactive state toward donors’ grafts in the recipients, while preserving normal immunological functions for the recognition of tumor antigens and prevention of infection. Thus immunosuppressive agents can be avoided or used at a dramatically reduced dosage. The key steps toward a successful transplantation therefore include either attenuated immune reactions or induced immune tolerance to grafts.

Small bowel transplantation is an ideal method to treat short bowel syndrome and other end stage small bowel dysfunctions, and thus can free the patients from total parenteral nutrition, returning to normal life pattern. But because of the rich lymphatic tissue in small bowel and its mesentery, the mesenteric lymph nodes and lymphatic plexus are transplanted along with small bowel transplantation. So small bowel transplantation has more severe immune rejection compared with other organ transplantation, which is the main cause leading to failure of small bowel transplantation.

Liver is an immunologically privileged organ, and after liver transplantation, the incidence rate and degree of rejection are much lower than other solid organ transplantations. Liver transplantation can also induce tolerance in recipients to organs, such as the heart, kidneys, skin, etc, which are susceptible to be rejected. Both in experimental study and in clinical practice of recent years, liver nonparenchymal cells (including lymphocytes, dendritic cells, Kupffer cells, etc) play an important role in immune tolerance induction. In the present study, we took the advantage of liver nonparenchymal cell infusion combined with cyclosporin A (CsA) on rat small bowel transplantation. Some parameters were tested in order to confirm the anti-rejection effect of liver nonparenchymal cell infusion combined with CsA.

MATERIALS AND METHODS

Animals

Male FK344/N rats weighing 230-260 g as donors and male Wistar rats weighing 200-240 g as recipients were obtained from the Laboratory Animal Center of Beijing Medical University, and fed with standard rat chow.

Preparation of rat liver nonparenchymal cells from donor liver

Rats were anaesthetized with intraperitoneal pentobarbital and the abdomens were shaved and cleansed with betadine solution. The peritoneal cavity was widely exposed, with the inferior vena cava cannulated, the portal vein divided, and the suprahepatic vena cava ligated. The liver was perfused at a rate of 3-4 ml/min at 37 °C in situ with a Hank’s calcium-free solution for 5 min followed by perfusion with a 0.05 % collagenase (Sigma, type V) solution for 15 min. Hepatic
attachments were divided and the liver was transferred to a Petri dish, where the liver substance was gently minced and filtered (100 µm) to remove large aggregates, followed by incubation for 45 min in 50 ml of Hank’s containing 0.05 % collagenase at 37 °C with continuous stirring. 0.5 mg DNAase in 1.0 ml of PBS was added 20 and 40 min after this incubation period. The cell suspension was filtered (40 µm) and nonparenchymal cells were separated by discontinuous density gradients of Percoll (Pharmacia Biotech) at 1.044 g/ml and 1.07 g/ml. The final cell suspension was prepared in PBS/15 % FCS at a concentration of 5x10⁶/ml. Cell viability counting (usually greater than 95 %) was done using trypan blue exclusion test, the cell suspension was used for infusion within 4 hours of preparation²²⁻²³.

Rat small bowel transplantation
Donor rats were fasted for 24 hours. All procedures were performed under inhalation anesthesia with ether. The entire small bowel from the ligament of Treitz to the ileocecal valve was isolated with the superior mesenteric artery on a segment of aorta and portal vein. After donor systemic heparinization (300 U), the graft was perfused with 20 ml of cold lactated Ringer’s solution via the aorta. The lumen was also washed in 20 ml of the same solution. In the recipient, end-to-side vascular anastomoses were performed between the graft aorta and recipient inferior vena cava with 10-0 sutures using the standard microsurgical technique. Superior extremity of transplanted small bowel was ligated and distal small bowel stoma was performed on left abdominal wall. Animals that died within 3 days were considered as technical failures and excluded from data collection²²⁻²⁰.

Experimental groups and postoperative care
The rats were divided into the following four groups. Group 1: homogentic transplantation group (F344/N→F344/N), Group 2: allotransplantation group (F344/N→Wistar), Group 3: allotransplantation group +CsA group (F344/N→Wistar), in which recipient rats received CsA 10 mg·kg⁻¹·d⁻¹ after transplantation, Group 4: allotransplantation +CsA+ nonparenchymal cell infusion group (F344/N→Wistar), in which nonparenchymal cell infusion was performed 20 days prior to transplantation, and CsA applied after transplantation. Animals were fasted with access to water on the day of surgery, fed with only sugar water (7 g/day) on day 1, and rat chow and water on day 2 and thereafter. The rats’ psyche status, appetite and ejection liquid of small bowel stoma were observed.

Graft histology
Rats’ small bowel allografts were excised from stoma or by laparotomy and fixed in 10 % formalin. The fixed tissue was paraffin embedded, and tissue sections were stained with hematoxylin and eosin (H-E). Rejection was evaluated according to the following scoring system: grade 0, intact mucosa with complete villi; grade 1, mucosa with shortened villi and initial cellular infiltration; grade 2, mucosa with incomplete and damaged villi or complete loss of villi, usually with cryptitis and lymphocyte infiltration; grade 3, no mucosa with extensive necrosis and fibrosis²⁰⁻²³.

Detection of interleukin-2 (IL-2)
The serum samples were collected on days 3, 5, and 7 after transplantation. Serum concentration of IL-2 was measured with trypan blue exclusion test, the cell suspension was used for infusion within 4 hours of preparation. The rats represented rejection of grade 0, with no rejection pathological finding but a few of lymphocytic infiltration in stroma. In group 2, rejection of grade 1 was found 3 days after transplantation, rejection of grade 2 was found 5 days after transplantation, and rejection of grade 3 was found 7 days after transplantation. In group 3, rejection of grade 1 was found 5 or 7 days after transplantation. In group 4, histopathologic examination showed similar results as in group 1.

Histopathologic examination
In group 1, the rats represented rejection of grade 0, with no rejection pathological finding but a few of lymphocytic infiltration in stroma. In group 2, rejection of grade 1 was found 3 days after transplantation, rejection of grade 2 was found 5 days after transplantation, and rejection of grade 3 was found 7 days after transplantation. In group 3, rejection of grade 1 was found 5 or 7 days after transplantation. In group 4, histopathologic examination showed similar results as in group 1.

Detection of IL-2
Expression level of IL-2 was low in group 1, and increased in group 2 on days 3, 5 and 7 after transplantation. IL-2 level in group 3 was mildly increased, but was lower than that in group 2 on days 3, 5 and 7 after transplantation. IL-2 level in group 4 was significantly lower than those in group 2 and group 3 (P<0.01), and was slightly increased on days 5 and 7 after transplantation.

Table 1  IL-2 level after small bowel transplantation in rats (x±s, ng/ ml)

| Group | 3 d    | 5 d     | 7 d    |
|-------|--------|---------|--------|
| Group 1 | 1.46±0.02 | 1.73±0.01 | 1.61±0.05 |
| Group 2 | 2.44±0.07 | 5.15±0.31 | 5.83±0.52 |
| Group 3 | 1.72±0.11 | 2.17±0.09 | 2.43±0.06 |
| Group 4 | 1.62±0.08 | 1.81±0.05 | 2.06±0.13 |

P<0.01 vs group 2 and group 3

Survival time after transplantation
The survival time after small bowel transplantation was 7.14±0.33 d in group 2, 16.32±0.41 d in group 3, 31.41±0.74 d in group 4 which was significantly longer than that in other groups (P<0.01).
DISCUSSION

Small bowel transplantation is the ultimate therapy for patients with short-bowel syndrome or end stage intestinal function failure. But the small bowel is the maximal immunological organ in human body, the mesenteric lymph nodes and lymphatic plexus are transplanted along with the small bowel transplantation, thus the rejection of small bowel transplantation is much more fiercer than that of other organ transplantations. The failure of small bowel transplantation was more often due to severe rejection[13-34]. So rejection induced by small bowel transplantation necessitates the use of large potent immunosuppressive drugs. It should be noted, however, that excess dosage of immunosuppressive agents may result in severe side effects such as hypertension and hepatic and/or renal toxicity. Moreover, prolonged usage of immunosuppressants often leads to severe infection and increased susceptibility to malignancy, thus critically affecting the health of recipients. The ideal strategy is to induce a low responsiveness or irresponsiveness in recipients toward grafts from donors, while preserving normal immunological functions for the recognition of tumor antigens and prevention of infection. Thus immunosuppressive agents can be avoided or used at a dramatically reduced dosage. The key steps toward successful transplantation therefore should include either attenuated immune reactions or induced immune tolerance to grafts[35-38].

The liver is an immunologically privileged organ, the rejection incidence rate and degree of liver transplantation are much lower than other solid organs. Liver transplantation can also induce tolerance of other organ transplantations, such as the heart, kidneys, skin, etc. Experimental and clinical researches also showed that liver combined with small bowel transplantation could alleviate the rejection of small bowel transplantation. Investigations in recent years showed that liver nonparenchymal cells (including lymphocytes, dendritic cells, Kupffer cells, etc.) played an important role in inducing tolerance. Donor rat’s intrahepatic leucocytes were eliminated by rays before transplantation, acute rejection would happen after liver transplantation, and the recipients’ survival time was shortened. Yet after intrahepatic leucocyte infusion to recipients with rats treated donor liver, the recipients’ survival time was obviously prolonged[39-41]. So in our experiment, liver nonparenchymal cell transplantation combined with ciclosporin A was used to suppress the rejection of rat small bowel transplantation. It was observed that the rejection was effectively suppressed, indicating the feasibility of tolerance induction by this method. Compared with small bowel associated with liver transplantation, the method of donor liver nonparenchymal cell transplantation has the advantage of less demanding on manipulation and technique requirements. Its postoperative complications are relative less, yet the suppressive effect on rejection of small bowel transplantation is good. So the donor liver nonparenchymal cell transplantation is a simple and practical method, which has the prospect of becoming a new way to suppress rejection of small bowel transplantation in clinical practice. Further work is needed to reveal if chimerism is induced by liver nonparenchymal cells transplantation, and its possible influence on the immune system of graft recipients such as graft versus host reaction.

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