EVALUATION OF THE IMMUNO-PROTECTIVE EFFECTS OF THE NEW-TYPE OF BAGS USING ELISA- AND FACS-ANALYSIS.

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Abstract

This study investigated the usefulness of modified polyurethane (MPU) coating micropore membrane bags for diminishing the immunological responses following organ or tissue transplantation in allogeneic setting. Spleen from Brown Norway (BN) rats (donor) were placed into the peritoneal cavity of Lewis rat (recipient) either directly or inside of MPU coated bags. Lewis rat with Sham's operation served as control. After 12 and 24 weeks, cytokines of IL-4, IL-13, TNF-α and IFN-γ, and flow cytometric evaluations for CD4+ and CD8+ cells of the recipient blood were carried out. TNF-α levels proved polyurethane coating effective in reducing inflammatory reaction at 12 weeks. Twelve week IFN-γ and, CD4+ and CD8+ cells indicated that graft-versus-host-reaction (GVHR) took place but polyurethane coated bag did not prevent or reduced this reaction. Thus, this study shows that MPU coating might be functional in preventing inflammatory reaction but is not useful for preventing GVHR.

1. Introduction

Polyurethanes form a versatile and useful class of polymers. As biomaterials, their uses have included the artificial heart, catheters, and synthetic blood conduits. Rejection and graft-versus-host disease are common as the solid organ transplantation and potential immunosuppressive drugs are routinely used after organ transplantation. Unfortunately, the risks of opportunistic infections, lymphoblastic malignancy and metabolic complications are frequently associated with immunosuppressive therapy. However, following most of the allotransplants, cell traffic seems to be a striking event with all transplants. Donor cells leaving the solid organ and recipient cell entering it include passenger leucocytes that were shown to be the main cause of allograft immunogenicity. TNF-α and IFN-γ are pro-inflammatory Th1-type cytokines, mediate cellular immune responses and have been shown to be involved in allograft rejection. On the other hand, Th2-cytokine IL-4 and IL-13 played their role in promoting graft survival has been suggested in animal models. Donor CD4+ and CD8+ cells that traffic to recipient, play an important role in the immunogenic outcome of the host tissue. The objective of this examination was to evaluate the effect of modified polyurethane (MPU) coated micropore membrane bag to eliminate or reduce the immunogenicity in an in vivo study using rat model.

2. Materials and Methods

2.1. Polyurethane coated bag

Bags with a size of 2 x 1.5 cm were made from MPU coated micropore membrane. The outer surface of the bag were coated by MPU. Bags were sterilized and turned into the hydrophilic characteristic bags by gradually dipping in 100 %, 90 %, 80 %, 70 %, 50 % and 25 % ethanol aqueous solution. Each step lasted for a half day and finally washed by distilled water and phosphate buffer saline.

2.2. Animals and Experimental Groups
Eight-week-old Lewis and Brown-Norway (BN) female rats were obtained from Charles River Japan Inc. Kanagawa, Japan. Rats were maintained in an air-conditioned animal facility at the national Institute of Health Sciences. The principles of laboratory animal care (according to NIH Publication No. 85-23, revised 1985) were carefully followed in this study. The rats were fed with a commercial diet and water ad libitum, pre- and post-implantation periods. Spleens from BN rats (donor) were placed into the peritoneal cavity of Lewis rats (recipient) either directly or inside of MPU coated bags. Lewis rat with Sham’s operation served as control. In another group, only MPU coated bag was placed into the peritoneal cavity.

2.3. Operative Procedures

The donor, under sedation with ether was anesthetized by intraperitoneal administration of pentobarbital sodium (20 mg/kg body weight). After proper sterilization, peritoneal cavity was opened through a ventral midline incision. From the BN rats spleens were removed and washed with PBS and immediately placed into the peritoneal cavity of Lewis rats, either directly or inside of a MPU coated bag containing 1 ml of RPMI 1640 medium (Dutch modification, Gibco BRL, Life Technologies Ltd., Paisley, Scotland). In bag group, only bag containing 1 ml of the medium was placed into the Lewis peritoneal cavity and in control group, Sham’s operation was performed. After the designated experimental period, rats were anesthetized in the same fashion as the initial operation. On opening the abdomen, spleens were collected (12 weeks post implantation only) and blood was collected from the descending abdominal aorta of recipient and then the rats were sacrificed.

2.4. Cytokines Assay

After 12 and 24 weeks of implantation, blood collected from the recipient was centrifuged and supernatant were stored at −80°C until cytokines were measured. Cytokines levels of IL-4, IL-13, TNF-α and IFN-γ were measured using conventional ELISA assay (Biosource International, Inc., CA, USA) according to the manufacturer’s instruction. Cytokine concentrations were calculated using manufacturer supplied cytokine standards and expressed in pg/ml.

2.5. Flow Cytometry

The analysis for CD4+ and CD8+ cells was carried out by flow cytometry. Anti-coagulant treated venous blood samples were analyzed with two-color flow cytometry to determine the percentages of CD4+ and CD8+ T cells. Briefly, 100 µl of heparin treated venous blood were incubated with 20 µl of the indicated FITC-labeled anti-CD4 and PE-labeled anti-CD8 mAb, vortexed vigorously and incubated at room temperature for 45 min. Following washing with 4 ml PBS two times, and further washing with 1 ml of immuno-lyse working solution and 250 µl of fixative was added within 30 sec to 2 min. After washing by PBS solution two times, the pelleted cells were analyzed with an EPICS XL II cytometer (Beckman-Coulter, Margency, France). The analysis was focused on lymphocytes, identified by their forward and right angle scatter features. At least 10000 events were collected in the lymphocyte gate and analyzed.

2.6. Antibodies

Rat mAb FITC –conjugated anti CD4 (IM3056) and PE –conjugated anti CD8 were used in this study. These mAb and their isotype-matched negative control mAb [mouse IgG1 –FITC (IM0639) and IgG1 –PE (IM0670)] were purchased from Beckman Coulter Immunotech (Marseille, France).

2.7. Statistical analysis

Data are presented as mean ± SD. Values of different experimental groups were analyzed with a paired t test. Differences were considered statistically significant at p < 0.05).

3. Results