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<td>Author(s)</td>
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<td>Citation</td>
<td>Acta medica Nagasakiensia. 1995, 40(1-4), p.30-34</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1995-12-11</td>
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<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/16039">http://hdl.handle.net/10069/16039</a></td>
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Lung Xenotransplantation in the Rat

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The aim of this study was to investigate the functional and morphological aspects of orthotopic lung xenograft rejection in a concordant hamster-to-rat donor-recipient species combination. All transplanted lungs had infiltrates during the 3 postoperative days. Complement-dependent cytotoxicity assays revealed that anti-hamster lymphocytotoxic antibody titer increased to 5.2±1.1 (p < 0.05 vs. 1 day) 3 days after transplantation and reached 9.8±0.5 (p < 0.05 vs. 1, 3 and 5 days) 7 days after transplantation. Titers were not elevated 1 day after transplantation. The CD4+/CD8+ ratio in peripheral blood lymphocytes increased significantly at 3 days (p < 0.05, versus untransplanted). At 5th day, the ratio was lower than at 3rd day (p < 0.05). There was no significant difference in B-cell population between rats before grafting and each group. Histologically, xenotransplanted grafts were characterized by perivascular cellular infiltrates and edema 3 days after transplantation. The inflammatory infiltrate formed a dense cuff around the venules and arterioles. At 5th day, arteriolar luminal narrowing was observed, and air-space containing many alveolar macrophages and lymphocytes. Immunohistochemical analysis of these grafts showed strong IgM and C3 deposits in the vascular endothelium, without any IgG deposits. No IgM, C3 or IgG deposits were observed in normal hamster lung. This study indicates that both the cellular and humoral immune system mediate primary acute rejection in the hamster-to-rat orthotopic lung xenograft model.

Introduction

The development of immunosuppressive agents and surgical techniques has increased the number of patients eligible for organ transplantation. The shortage of donor organs, however, has become a serious problem. Xeno-transplantation, such as the baboon-to-human liver xenograft, recently reported by Starzl¹, may provide a solution to the shortage of donor organs. As with other organ xenografts², lung transplantation may soon be performed using xenogenic organs.

In a well-established model of hamster-to-rat heterotopic cardiac xenotransplantation, rejection of a concordant xenograft was found to be mediated mainly by humoral immunity³. The mechanism underlying lung xenograft rejection may differ, however, because the lung contains a considerable amount of lymphoid tissue, in contrast to most other organs⁴. Tavakoli⁵ has reported that in a guinea pig-to-rat discordant lung xenograft model, rejection occurred by a very rapid, predominantly humoral mechanism. However, there are no published papers on animal models of concordant lung xenograft. We have established a concordant hamster-to-rat orthotopic lung xenograft model to investigate the mechanism of lung xenograft rejection.

The aim of the present study was to investigate the functional and morphological aspects of orthotopic lung xenograft rejection in a concordant hamster donor to rat recipient model.

Materials and Methods

Animals.

Female Golden Syrian hamsters weighing 100-170 g and Fischer (F344/DuCrj) rats weighing 200-300 g were used as donors (SLC Inc, Japan), and inbred male Lewis (LEW/Crj) rats weighing 200-300 g were used as recipients (Charles River Inc, Japan).

Surgical procedure.

All animals were anesthetized with an intraperitoneal administration of 30 mg/kg of pentobarbital (Nembutal injection (R)), intubated, and ventilated at a tidal volume of 10 ml/kg and respiratory rate of 90 breaths/min using a respirator (Shinano-SN, Japan). Orthotopic lung transplantation was performed using a cuff technique, as previously described⁶. The pulmonary artery and pulmonary vein were anastomosed by the cuff technique and then the left main bronchi was anastomosed with a 9-0 polypropylene continuous suture for the cartilaginous ring and an interrupted suture for the membranous wall. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals of Nagasaki University".

Serial Chest Roentgenograms.

Chest roentgenograms were taken every day after
Experimental models

Rats were divided into four groups: group 1 (n = 4), animals were sacrificed 1 day after transplantation; group 2 (n = 6), animals were sacrificed 3 days after transplantation; group 3 (n = 7), animals were sacrificed 5 days after transplantation; group 4 (n = 4), animals were sacrificed 7 days after transplantation.

Complement-dependent cytotoxicity (CDC) assay.

The concentration of lymphocytotoxic antibodies in the serum of xenografted rats was determined using a CDC assay. After incubating the recipient's serum and complementing with hamster spleen cells (5x10^6/ml) for 45 min, the percentage of cells staining with trypan blue was calculated. A score of 50% cell death was considered positive. Guinea pig serum diluted 1:4 served as the source of complement.

Peripheral lymphocyte subpopulations

Peripheral lymphocyte subpopulations were analyzed with monoclonal antibodies (MAbs; SEROTEC LTD, Oxford). MAbs used in this study included phycoerythrin (PE)-conjugated anti-CD4 MAb (W3/25), fluorescein isothiocyanate (FITC)-conjugated anti-CD8 MAb (OX-8), PE-conjugated anti-B cell (RLN-9D3) and FITC-conjugated anti-CD3 MAb (Ir41). Fluorescence of individual cells was determined using a whole-blood labeling technique. Briefly, whole blood (100 μl) was incubated for 60 min on ice in the dark with 10 μl of FITC and PE conjugated MAbs. Erythrocytes were subsequently lysed by a 10 min incubation with FACS Lysing Solution (Becton Dickinson). Cells were suspended in 1.0 ml of 0.5% paraformaldehyde in phosphate buffered saline (0.5% PFA-PBS, PH 7.4). Counts of positively stained cells were computed as a percent of total lymphocytes by CONSORT 30 software on the fluorescence activated cell sorter (Becton Dickinson). Controls consisted of allografted (F344 to LEW) and untransplanted animals.

Histopathology.

At autopsy, the lung and heart were removed en bloc, and 4% paraformaldehyde in phosphate buffered saline (4% PFA-PBS, PH 7.4) was injected into the trachea until the alveolar spaces were fully expanded. Slices of approximately 0.5 cm thickness were cut from the lungs and stored at −80°C for immunohistochemical studies or fixed in 4% PFA-PBS overnight for hematoxylin-eosin staining.

Immunohistochemistry.

The presence of rat complement deposits was determined directly using FITC-conjugated anti-human C3 MAb. The presence of rat IgM and IgG deposits on the vascular endothelium and mononuclear subsets of perivascular infiltrative cells in the xenografts were determined by means of the labeled streptavidin-biotin (LSAB) technique. Five μm serial cryostat sections were cut with a cryostat microtome, air dried for 60 min, and incubated in normal goat serum to inhibit nonspecific binding. Mouse affinity-purified MAb to rat IgM (heavy chain) and rabbit F(ab')2, anti rat IgG (whole molecule) were added at room temperature as primary antibodies. Following three washes in 0.05 M Tris-HCl buffer, pH 7.2-7.6, sections were incubated for 10 min with diluted biotinylated antibody solution. Following three washes in 0.05 M Tris-HCl buffer, pH 7.2-7.6, sections were incubated for 10 min with streptavidin alkaline phosphatase reagent (Dako Co, Ca). Alkaline phosphatase was revealed by staining with the fast red substrate system (Dako Co, Ca). Sections were lightly counterstained with hematoxylin. Sections incubated with 0.05 M Tris-HCl buffer, pH 7.2-7.6, instead of primary antibody served as negative controls.

Statistical analysis.

Groups were compared by the Mann-Whitney U test to determine the level of significance of any difference. Any p value of less than 0.05 was considered to indicate statistical significance.

Results

In group 1 grafts, lung edema was observed, which was regarded as a reimplantation response of the transplanted lung. In group 2, 3 and 4, all transplanted lungs showed infiltrates during the 3 postoperative days. No evidence of significant change was observed in the native right lung of any of the rats.

Antibody titers are shown in Fig 1. Complement-dependent cytotoxicity assays revealed that anti-hamster lymphocytotoxic antibody titers increased to 5.2 ± 1.1 (loge) 3 days after transplantation (p < 0.05 vs. 1 day), 7.0 ± 0.8 (loge) 5 days after transplantation (p < 0.05 vs. 1 and 3 days) and reached 9.8 ± 0.5 (loge) 7 days after transplantation (p < 0.05 vs. 1, 3 and 5 days). Titers were not elevated 1 day after transplantation.

The ratio of CD4+/CD8+ in peripheral blood lymphocytes increased significantly 3 days after transplantation (p < 0.05 vs. preoperative, Fig 2). Five days after transplantation, the ratio was decreased less than that at 3rd day (p < 0.01). Furthermore, there was a highly significant depression when compared with the control allotrans-
Fig. 1. Anti-hamster lymphocytotoxic antibody titer of lung xenograft recipients, measured by complement-dependent cytotoxicity assay. Data given are means ±SD. (*P < 0.05 ; vs. group 1. **P < 0.05 ; vs. groups 1 and 2. ***P < 0.05 ; vs. groups 1, 2 and 3.)

In peripheral T, B cell populations, there was no significant difference between rats before grafting and each group (Fig 3).

Histologically, perivascular edema was present due to reperfusion injury in group 1 grafts, but there was no evidence of perivascular mononuclear infiltration, intraalveolar hemorrhage, or parenchymal necrosis. Group 2 grafts were characterized by perivascular cellular infiltrates and edema. The inflammatory infiltrate formed a dense cuff around the venules and arterioles (Fig 4A). Cytologically, the cells consisted of small round lymphocytes, monocytes and occasional neutrophils. Neutrophils were also detected in the vascular endothelium. Mild lymphocytic bronchiolitis and moderate alveolar macrophage accumulation was observed 3 days after transplantation. Group 3 grafts showed obvious perivascular and interstitial infiltrates of mononuclear cells. The diameter of arteriolar lumen was smaller and the airspace contained prominent alveolar macrophages and lymphocytes (Fig 4B). In addition, moderate peribronchiolar inflammatory cell infiltration, intraalveolar hemorrhage and hyaline membranes were observed. In group 4 rats, these signs of injury were more dramatic and were accompanied by parenchymal necrosis showed strong IgM and C3 deposits in the vascular endothelium (Fig 5A, B), but no IgG deposits in group 2 grafts. No IgM, C3 or IgG deposits were observed in normal hamster lung.

Fig. 2. The CD4+/CD8+ ratio in peripheral blood lymphocytes was significantly greater 3rd day after transplantation than it was preoperatively (*P <0.05). Five days after transplantation, the ratio was decreased less than that at 3 days (**P < 0.01). Furthermore, there was a highly significant depression when compared with the control allotransplanted animals 5 days after transplantation after transplantation (**P <0.05). Data given are means ±SD.

Fig. 3. There was no significant difference in T or B cell populations between rats before grafting and each group.
Fig. 4. (A) Group 3 grafts were characterized by perivascular cellular infiltrates. The inflammatory infiltrate formed a dense cuff around the small venules. The cells consisted of small round lymphocytes, monocytes with occasional neutrophils (H & E ; ×480).

(B) Group 4 grafts showed an obvious perivascular and interstitial infiltrates of mononuclear cells. The arteriolar lumen was narrow and the airspaces contained prominent alveolar macrophages and lymphocytes. Moderate peribronchiolar inflammatory cell infiltration was present (H & E ; ×300).

Discussion

Previous research has indicated that antibody-mediated immunity plays an important role in graft destruction in the hamster-to-rat cardiac xenograft model and that it occurs within 3 days in untreated recipients. Liver xenografts, however, are more resistant to antibody-mediated injury and are rejected in about 7 days by a combination of humoral and cellular immunity.

In the present study, we demonstrated that rejection of a hamster-to-rat lung xenograft began within 3 days after transplantation. Although histologic examination of transplanted lungs revealed perivascular cellular infiltration, the infiltrating cells differed from those observed in allograft models, consisting not only lymphoid cells, but also of macrophages/monocytes. The perivascular infiltrates were associated with adherence of neutrophils to the vascular endothelium. In addition, recipient serum contained a moderate titer of cytotoxic antibodies as detected by a complement-dependent cytotoxicity assay.

Fig. 5. (A) Immunohistochemical analysis of grafted lung in group 3 rats showed heavy IgM deposits in the vascular endothelium (×300).

(B) C3 deposits were also observed in the vascular endothelium (×480).

and definite evidence of IgM and C3 deposits were observed in the vascular endothelium 3 days after transplantation. Beyond 3 days, perivascular and intraalveolar cellular infiltration and edema progressed, followed by hemorrhaging and destruction of the pulmonary parenchyma. This progression suggests that antibodies bound to the vascular walls, leading to infiltration of macrophages/macrophages into the xenograft and macrophages/macrophages-mediated endothelial cell damage. Fryer et al. have reported that antidonor antibodies and macrophages might interact through antibody-dependent cell-mediated cytotoxicity mechanisms to promote xenograft rejection. Our results agree with these findings.

Intraalveolar hemorrhage and increased number of intraalveolar macrophages indicate damage on the alveolar capillary endothelium. Although airway damage was very infrequent 3 days after transplantation, mild lymphocytic bronchiolitis and moderate alveolar macrophage accumulation was observed occasionally. Five days after transplantation, lymphocytic bronchitis associated with peribronchiolar inflammatory cell infiltration was observed, and airspaces contained many alveolar macrophages and lymphocytes. Although the role of alveolar macrophages in our model is not clear, it may be a response to xenogeneic MHC molecules.
The result of our study also indicated that cellular immunity played an important role in concordant lung xenograft rejection. The analysis of the peripheral lymphocyte subpopulations suggested that CD4+ T cell populations were elevated early to activate other lymphocytes, macrophages/monocytes and T cell dependent antibody production, and that CD8+ T cell populations then increased to complete the process of cellular rejection. But the B-cell population was not significantly increased in the peripheral lymphocytes during rejection. Thus, cell-mediated immunity also might play a role in lung xenograft rejection, as it does in allograft rejection. If humoral xenograft rejection can be conquered in concordant lung xenotransplantation, the T cell mediated response may be as great a problem as in allotransplantation.

In conclusion, our experimental results suggest that both the cellular and humoral immune system mediate primary acute rejection in the hamster-to-rat orthotopic lung xenograft model. Studies are underway to find a way to prevent lung xenograft rejection using immunosuppressive agents, and to thereby prolong xenograft survival.

Acknowledgement

I would like to express deep appreciation to Emeritus Professor Masao Tomita, the First Department of Surgery, Nagasaki University School of Medicine and other staffs of the Department for their cooperation.

References