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Increased Expression of Proliferating Cell Nuclear Antigen in Rejecting Rat Lung Allografts

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The aim of this study was to investigate the expression of proliferating cell nuclear antigen (PCNA) as an index of cell proliferation in the Brown Norway (BN) to Lewis (LEW) rat lung allograft model.Following transplantation of BN left lungs into LEW recipients, counts of PCNA-positive cells in the perivascular cellular infiltrate and bronchus-associated lymphoid tissue (BALT) were compared with the histological grade of rejection. Lungs were excised on postoperative days 3 and 5. LEW-to-LEW donor-recipient transplantation was performed as a control. Routinely processed, paraffin-embedded sections were selected and stained with PCNA. The PCNA index (% of nuclei positive for PCNA) in the BALT was significantly higher in allograft (19.1%, $p < 0.05$) compared with isograft (4.2%) at 3 days following transplantation. Similarly, the PCNA index was also greater in the perivascular cellular infiltrates of rejecting lungs (23.9% at 3 days, 31.6% at 5 days). These findings indicate that the cells stimulated by the rejection reaction could be increase the expression of PCNA, and the increasing severity of rejection was paralleled by an increase in the number of PCNA-positive cells. In conclusion, PCNA may be a useful marker of acute cellular rejection in lung allografts.

Introduction

In lung transplantation, open or transbronchial lung biopsies are still important in the screening of graft condition. However, the diagnosis of acute cellular rejection may be difficult in biopsies taken at the first sign of lung allograft function. Perivascular mononuclear infiltrates are a well known as the diagnostic feature in acute lung rejection, but they are not exclusive to acute rejection. Pathologic problems in the immediate postoperative period include harvest injury, reimplantation response, and infection[1]. These complications may cause diagnostic difficulties. To resolve these difficulties, the immunophenotypes of the infiltrating cells has been widely studied but the results are not conclusive[2].

It is well documented that the lung possesses its own intrinsic lymphoid tissue-the bronchus associated lymphoid tissue (BALT) whch is involved in local antigen processing and preparation of antigens for systemic presentation. Prop et al. in animal studies has noted that BALT magnifies the interaction between lung graft and recipient host, accelerating the induction of the rejection response both locally in the graft and systemically in the recipient's lymphoid organs[3].

Recently, proliferating cell nuclear antigen (PCNA), a 36-kDa nuclear protein that is a cofactor for polymerase delta during replication of DNA, has been used as a marker of proliferating cells because it is expressed during late G1 and S phases of the cell cycle but disappears during early G2[4]. PCNA has an advantage over other methods of assessing cell proliferation.

In this study, we investigated the perivascular cellular infiltrates and BALT cells using PCNA marker as an index of cell proliferation in the Brown Norway (BN) to Lewis (LEW) rat lung allograft model in comparison with LEW-to-LEW rat lung isograft.

Materials and Methods

Inbred male Brown Norway (BN/Crj) rats weighing 200-300 g and Lewis (LEW/Crj) rats weighing 200-300 g were used as donors (SLC, Japan), and inbred male Lewis rats weighing 200-300 g were used as recipients (Charles River, Japan). 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 described[5]. The pulmonary artery and pulmonary vein were anastomosed by the cuff technique and then the left main bronchus 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".

The rats were divided into the following four groups : group 1 ($n = 4$), isotransplanted recipient rats sacrificed

on postoperative day (POD) 3 ; group 2 (n = 4), isotransplanted animals sacrificed on POD 5 ; group 3 (n = 4), allotransplanted animals sacrificed on POD 3 ; and group 4 (n = 4), allotransplanted animals sacrificed on POD 5. 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 in thickness were cut from the lungs fixed in 4% PFA-PBS for 4 hours for hematoxylin-eosin and immunohistochemical staining.

PCNA staining.

The labeled streptavidin-biotin (LSAB) system (Dako Co, Ca) was used to detect bound antibody[6]. After deparaffinization, 4-μm sections were prepared, and incubated in normal goat serum to inhibit nonspecific binding. Monoclonal murine antibody PC 10 (IgG2a ; DAKO, Glostrup, Denmark) at a dilution of 1 : 100 was 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 counter stained with hematoxylin. Sections incubated with 0.05 M Tris-HCl buffer, pH 7.2-7.6, instead of primary antibody, served as negative controls. The PCNA index (percentage of PCNA-positive nuclei) was determined from counts of more than 1,000 cells in the perivascular cellular infiltrates. Whole cells were counted in the infiltrating cells of BALT.

Statistical analysis.

Groups were compared by the Mann-Whitney U-test to determine the level of significance of any difference. A p value of less than 0.05 was considered to be statistically significant.

Results

Histologically, perivascular edema occurred due to reperfusion injury in the group 1 grafts, but there was no evidence of perivascular mononuclear infiltration, intraalveolar hemorrhage, or parenchymal necrosis. Group 2 grafts also showed normal structure. In the group 3 grafts, perivascular mononuclear infiltrates are identified, and there was no extension of the inflammatory infiltrate into alveolar septae. The perivascular infiltrates showed a dense cuff which consisted mostly of small round and blastic lympyocytes. Mild peribronchial infiltration was also detected. Group 4 grafts showed diffuse perivascular and peribronchial cellular infiltrates. The alveolar spaces were filled with prominent alveolar macrophages and lymphocytes.

In the BALT of the group 3 grafts, the size of this area increased markedly compared with the group 1 grafts. However, it was impossible to distinguish disseminating BALT cells from the cellular infiltrates in group 4 grafts. The PCNA index (% of nuclei immunostaining for PCNA) of the BALT cells (Fig. 1) in the group 1 and 2 grafts was 4.2±6.2% (range 0 to 11.3%) and 2.95±1.72% (range 1.25 to 4.69%). Conversely, group 3 grafts significantly increased to 19.1±5.7% (Fig. 2, range 15.7 to 27.6%, $P < 0.05$ vs group 1), which showed mitotic figures indicating a proliferative immune response.

In the perivascular cellular infiltrates (Fig. 3), the PCNA index in the proup 3 grafts increased to 23.9±3.7%

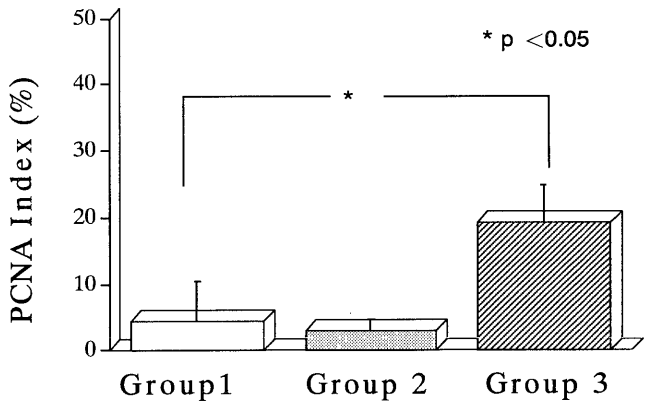

Fig. 1. The PCNA index (% of nuclei immunostaining for PCNA) of the BALT cells in the group 1 and 2 grafts was 4.2±6.2% (range 0 to 11.3%) and 2.95±1.72% (range 1.25 to 4.69%). Conversely, group 3 grafts significantly increased to 19.1±5.7% (ranged 15.7 to 27.6%, $p < 0.05$ vs group 1).

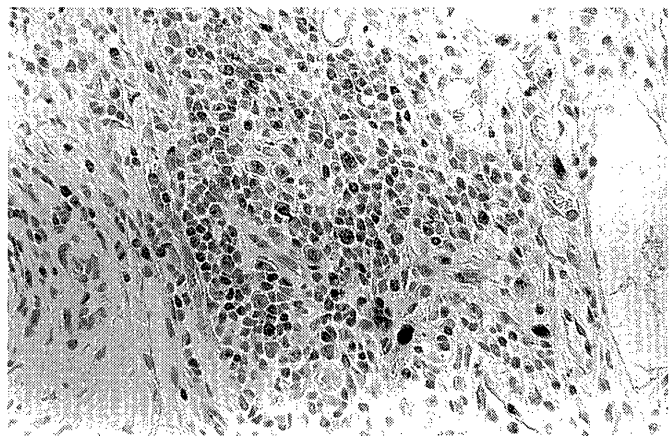

Fig. 2. PCNA staining of the BALT cells (balck dots) were present in the group 3 graft.(x 600)

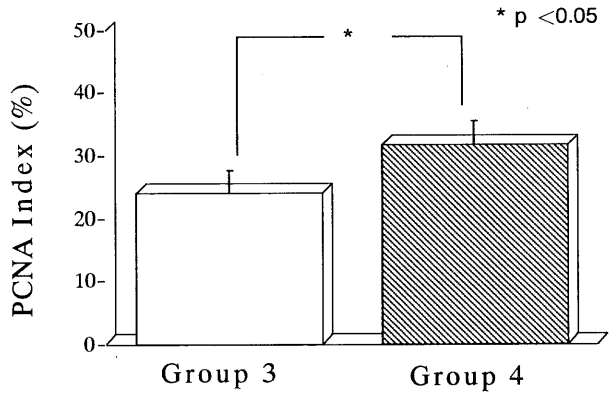

Fig. 3. In the perivascular cellular infiltrates, the PCNA index in the group 3 grafts increased to 23.9±3.7% (range 19.1 to 27.9%), and reached 31.6±4.1% (range 27.0 to 36.4%, $p < 0.05$ vs group 3) in the group 4 grafts.

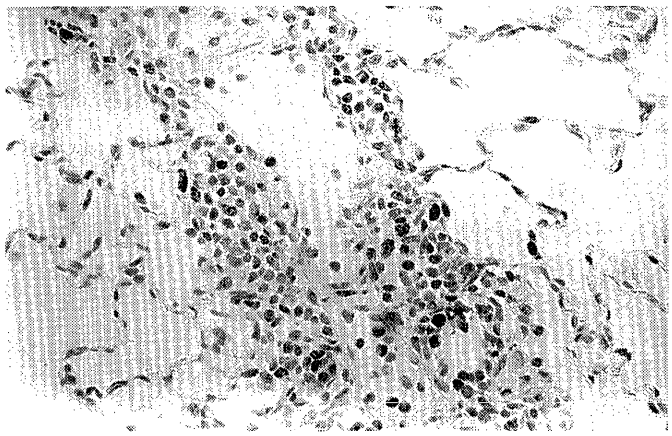

Fig. 4. PCNA staining in the perivascular cellular infiltrates (black dots) were present in the group 3 graft.(x 600)

(Fig 4, range 19.1 to 27.9%), and reached 31.6 ± 4.1% (range 27.0 to 36.4%, $p < 0.05$ vs group 3) in the group 4 grafts. In the group 1 and 2 grafts, there were few cellular infiltrates in the perivascular area, and PCNA-positive cells were rare in the alveolar space and septum.

Discussion

In this sutdy, we evaluated PCNA expression of the perivascular infiltrates and BALT cells in rat lung allograft models. PCNA is an acidic polypeptide with a molecular weight of about 36 kDa and can be detected in several species, indicating that this is a highly conserved cellular protein. Expression of PCNA is increased in late G1 and S phases and is reduced in G2-M phases[4,7]. Therefore, the presence of this antigen identifies cells that are preparing for mitosis. The advantage of PCNA immunostaining over other standard methods for assessing cell proliferation is that they can be used in routinely processed paraffin-embedded tissues[4].

Lung contains a considerable amount of lymphoid tissue in contrast to most other organs. Prop et al. noted that BALT magnifies the interaction between lung graft and recipient host that is likely to accelerate the induction of the rejection response both locally in the graft and systemically in the recipient's lymphoid organs[3]. In this study, the PCNA-positive BALT cells dramatically increased in the vascular phase of allograft rejection compared with syngeneic graft. In the perivascular infiltrating cells, the high PCNA index at 3 days following transplantation was similar to BALT cells in the same specimen. Sequentially, the index was increasing at 5 days following transplantation. These results indicated that the cells stimulated by rejection reaction could increase the expression of PCNA, and the increasing severity of rejection was paralleled by an increase in the number of PCNA-positive cells. In a recent study, it was also reported that PCNA staining may be of use in the differential diagnosis of rejection in routinely processed biopsies of renal allografts where there are only focal inflammatory infiltrates[8].

In conclusion, the detection of increased expression of PCNA in the lung allografts may be helpful in the diagnosis of acute cellular rejection. Further studies are needed to evaluate the effect of immunosuppressive drugs and viral infection.

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