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Cellular Bronchoalveolar Lavage Profile Following Induced Bacterial Infection and Rejection of Lung Allografts

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= **Abstract** =

Objective : Experiment was designed to compare cellular profile of bronchoalveolar lavage following induced bacterial infection, acute rejection and acute rejection plus bacterial infection after lung allotranplantation.

Methods : After single lung allotransplantation, dogs were immunosuppressed with standard triple therapy and divided into 4 groups. Group I(n=4) was maintained on immunosuppression as controls. In group II(n=6), infection was induced by bronchoscopic inoculation of *E. coli* at postoperative day 5. In group III(n=6), triple therapy was discontinued to induce acute rejection from postoperative day 5. In group IV(n=8), triple therapy was discontinued and bacterial infection was induced by bronchoscopic inoculation of E coli at postoperative day 5.

At postoperative day 9, bronchoalveolar lavage was obtained in the native and transplanted lung respectively through bronchoscopy. Total cell count and differential cell count of bronchoalveolar lavage were compared in four groups.

Results : In the native lung, there was no significant difference in total cell count and differential cell count in four groups. In the transplanted lung, total cell count of group II (Infection) was increased, compared to group III(Rejection) ($p<0.05$). In the transplanted lung, differential neutrophil count of group II(Infection) and group III(Rejection) were increased, compared to group I(Immunosuppression) ($p<0.05$). In the transplanted lung, differential macrophage count of group II(Infection), III(Rejection) and IV(Rejection plus Infection) were decreased, compared to group I(Immunosuppuression) ($p<0.05$).

Conclusion : Cellular profile of bronchoalveolar lavage reflected the pathological process of infection or acute rejection following lung allotransplantation in the transplanted lung. But conventional total and differential cell counts had limitation to differentiate either process.

KEY WORDS : Lung allograft · Infection · Acute rejection · Bronchoalveolar lavage.

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Introduction

Lung allotransplantation has became a therapeutic option for end-stage lung disease. Graft infection and rejection are the two major postoperative complications. Frequent use of bronchoscopy with transbronchial biopsy and culture is an accepted procedure for diagnosis of infection and rejection, but role of cellular profile of bronchoalveolar lavage is less well defined[1)2)3)].

The purpose of this study is to determine the utility of bronchoalveolar lavage in the diagnosis of infection and rejection following lung allotransplantation.

Material and Methods

Male mongrel dogs of similar weight(20~25%) were used as donors and recipients. In donors, dogs were anesthetized and mehanical ventilation was done. Midsternotomy, thymectomy and anterior pericardiectomy were done in supine position. Azygos vein was ligated and venae cavae, aorta and trachea were encircled with umbilical tape. Heparin and methylprednisolone acetate were given intravenously.

Lungs were flush-perfused with cold(4℃) modified Euro-Collin's solution(60ml/kg) through main pulmonary artery. Topical cooling was obtained by irrigation of cold saline into the thoracic cavity and lungs. Preostaglandin E_1 was infused intravenously before perfusion when flow-perfusion is optimal(lungs were uniformly white). Trachea was clamped with full inflation of lung and heart-lung block was excised.

In recipient dogs, the fifth intercostal space was opened and extrapericardial pneumonectomy was done in lateral position. Transplant of lung was performed with anastomosis of the atria-atria and pulmonary artery by Prolene 5-0 continuously.

Bronchus were anastomosised with interrupted 4-0 Prolene suture using telescoping technique. During procedure, transplanted lung was protected by wrapping with cold soaked sponges and continuous irrigation with cold saline. Before reperfusion, heparin 1000IU and methylprednisolone acetate 125mg were given intravenously.

All dogs received standard triple immunosuppressive therapy(cyclosporine, 10mg/kg/day ; azathio-prine, 2.5mg/kg/day ; methylprednisolone acetate, 1mg/kg/day) and antibiotics(gentamycin sulfate 40mg twice a day ; clindamycin phosphate 300mg twice a day ; cafazolin sodium 250mg twice a day).

Induction of infection and rejection : At postoperative day 5, chest radiography was taken and open lung wedge biopsy was done under general anesthesia. If there was no pathologic evidence, dogs were randomly assigned into one of the following four groups.

Group I(Immunosuppression group) was maintained triple therapy.

Group II(Infection group) ; At postoperative day 5, fiberoptic bronchoscopy was done through tracheostomy for assigned dogs. A tip of bronchoscope was wedged into the lower lobe of transplanted lung and 10 milliliters of 10^{10} colony-forming units(CFUs) of *Escherichia coli* with culture media was flushed. In preliminary experiments, three types of bacteria were routinely found from bronchial swabs in dogs ; *E coli, B bronchoseptica and P aeruginosa.* A strain resistant to the antibiotics(gentamycin, clindamycin and cefazolin) was identified, colonized and used to induce pneumonia. Tracheostomy was closed with interrupted 4-0 Prolene suture. Triple immunosuppressive therapy was continued.

Group III(Rejection group) ; At postoperative day 5, assigned dogs were discontinued triple therapy and induced acute rejection.

Group IV(Rejection plus Infection group) ; At postoperative day 5, assigned dogs were discontinued triple therapy and E coli was inoculated in same method.

Fiberoptic bronchoscopic lavage : At postoperative day 9, dogs were anesthetized with intravenous sodium pentobarbital(30mg/kg), intubated and ventilated. Trachestomy was done and a tip of bronchoscope was wedged into the bronchus of lower

lobe of the native lung. 25 – 40ml of saline was flushed and approximately 10 – 15ml of fluid was aspirated through the suction channel of bronchoscope and collected in the suction trap and held at 4℃. Samples were subjected to total cell count using a modified hemocytometer and cytospinned cells were differentially counted after staining with Wright-Giemsa stains.

The procedure was repeated in the same manner in the transplanted lung.

Statistical analysis : All data were expressed as means±standard error and analyzed by statistical software GraphPad Prism. In the native lung, means of total cell, differential neutrophil, differential lymphocyte, differential macrophage and eosinophil count were compared in four groups by one-way ANOVA test. When the one-way ANOVA test had resulted in a significant F test, post hoc comparison were made by Dunnett's test and Tukey's HSD test respectively. In the transplanted lung, one-way ANOVA test and same post hoc tests were repeated. P value was regarded as significant when less than 0.05.

Results

In the native lung, there was no significant difference in total cell count(Table 1 and Fig. 1), differential neutrophil, lymphocyte, macrophage and eosinophil count(Table 2, Fig. 1, 2). In the transplanted lung, total cell count was significantly increased in Group II(infection), compared to group III(Rejection) (4983±1704 vs 740±187/uL ; $p<0.05$*, Table 1 and Fig. 1).

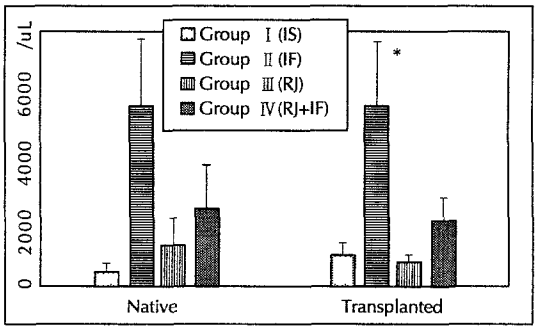

Fig. 1. Total cell count(/uL) in the native and transplanted lung in four groups.
*$p<0.05$ Total cell count of group II (Infection) was increased in the transplanted lung, compared to group III (Rejection).

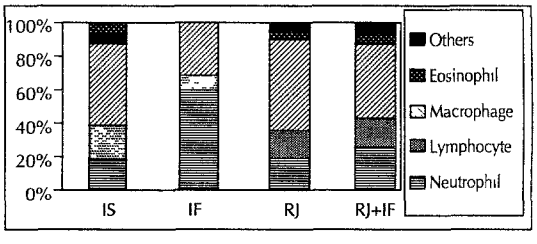

Fig. 2. Differential cell count(%) in the native lung in four groups(IS : Immunosuppression IF ; Infection RJ ; Rejection RJ+IF ; Rejection plus Infection).

In the transplanted lung, differential neutrophil count was significantly increased in group II(Infection) and group III(Rejection), compared to group I (Immunosuppression) (83±1.9 and 73.5±2.4 vs 35.8±13.1% ; $p<0.05$**, Table 3 and Fig. 3). In the

Table 1. Total cell count(/uL) in the native and transplanted lung in four groups

	Group I (IS)	Group II (IF)	Group III (RJ)	Group IV (RJ+IF)
Native lung	391±231	4933±1798	1130±723	2144±1223
Transplanted Lung	900±333	4983±1704*	740±187	1850± 612

*$P<0.05$ Total cell count of group II(Infection) was increased in the transplanted lung, compared to group III(Rejection).
(IS : Immunosuppression, IF : Infection, RJ : Rejection, RJ+IF : Rejection plus Infection)

Table 2. Differential cell count(%) in the native lung in four groups

	Neutrophil	Lymphocyte	Macrophage	Eosinophil	Others
Group I (IS)	17.3± 8.8	22.5±9	47.8± 5.9	12.5±9	0
Group II (IF)	56.8±14	9±5.5	27.3±10	0.2±0.2	0
Group III (RJ)	17.2± 6.1	13.5±3.5	62.7± 7.7	6.5±3.1	0.2±0.2
Group IV (RJ+IF)	28.2±12.9	17.2±8	48.3±12.2	3.3±1.9	3.1±3

Table 3. Differential cell count(%) in the transplanted lung in four groups

	Neutrophil	Lymphocyte	Macrophage	Eosinophil	Others
Group Ⅰ(IS)	35.8±13.1	12.8±2.8	48.5±14.6	2±2	1±1
Group Ⅱ(IF)	83± 1.9**	2.8±1.5	14.2± 1.4***	0	0
Group Ⅲ(RJ)	73.5± 2.4**	8±2.1	16.8± 3.6***	1.7±1	0
Group Ⅳ(RJ+IF)	59.8± 9.1	18.3±9.5	16.9± 6.1***	4±2.6	1.1±1

**P<0.05 Differential neutrophil count of group Ⅱ(IF) and Ⅲ(RJ) were increased in the transplanted lung, compared to group Ⅰ(IS)

***P<0.05 Differential macrophage count of group Ⅱ(IF) and Ⅲ(RJ) and Ⅳ(RJ+IF) were decreased in transplanted lung, compared to group Ⅰ(IS)

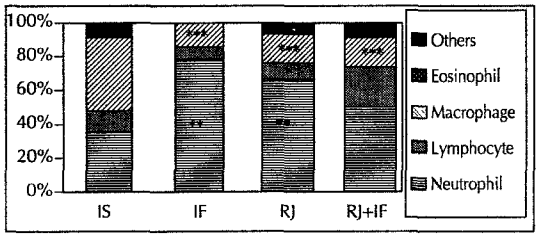

Fig. 3. Differential cell count(%) in the transplanted lung in four groups.
**p<0.05 Neutrophil count of group Ⅱ and Ⅲ were increased in transplanted lung.
***p<0.05 Macrophage count of group Ⅱ, Ⅲ and Ⅳ were decreased in transplanted lung.

transplanted lung, differential macrophage count was significantly decreased in group II(Infection), III (Rejection) and IV(Rejection plus Infection), compared to group I(Immunosuppression) (14.2±1.4, 16.8±3.6 and 16.9±6.1 vs 48.5±14.6% ; p<0.05***, Table 3 and Fig. 3). Differential lymphocyte and eosinophil count were comparable in the transplanted lung.

Discussion

During the acute rejection after lung allotransplantation, patients experience a low-grade fever, leukocytosis, an increased alveolar-arterial oxygen gradient, a feeling of lethargy and dyspnea and a radiological perihilar flare or infiltrate. However these couldn't differentiate rejection from pneumonia, atelectasis, or pulmonary edema. Transbronchial biopsy is now considered to be the standard for determining the presence of rejection or infection, but possible complications and clinical limitations need bronchoalveolar lavage evaluation. Bronchoalveolar lavage through bronchoscopy can provide direct access to cellular and fluid components within the lung safely.

Typical composition of human bronchoalveolar lavage contained about 95% macrophages, 4% to 5% lymphocytes, and 0% to 1% polymorphonuclear leukocytes. Normal canine have 65% to 85% macrophages, 10% to 20% lymphocytes, and 4% to 10% polymorphonuclear leukocytes.

Cellular profile in the bronchoalveolar lavage during an uncomplicated post-transplant course is characterized by slightly to moderately increased total cell count with increases in lymphocytes, neutrophils, and macrophages. The differential cell count displays neutrophilia, the proportion of polymorphonuclear leukocytes rising to up to 30% to 40%. All these elevations fall with time, persisting for weeks to months.

The reason for the neutrophilic reaction remained obscure. It has been attributed to several factors : inflammations of the bronchial epithelium, reimplantation response, disturbed clearance mechanisms, initiation of incipient acute rejection, tissue injury and latent infection. All lung transplant patients have cellular abnormalities in their early period. Change in heart-lung transplant recipients are possibly more pronounced when compared with those in double-lung or single-lung allograft patients. In uncomplicated cases the cellular profiles can approach normal by 3 months.

Diverse results were reported during rejection and infection ; in the absence of immunosuppression, rejecting allografts manifested a trend toward polymorphonuclear leukocytosis 3 to 7 days after allograft.

This was usually accompanied by a proportional decreased in the macrophage and lymphocyte populations[4] ; acute rejection was significantly different from bacterial pneumonia by a decrease in total cell count resulting from a lower proportion and number of neutrophils but by an increased proportion and number of lymphocytes[1] ; total cells were reduced and macrophages were also reduced and lymphocytes were increased[5]. But progressive fall in the number of alveolar macrophage was consistent findings in rejection[6].

Many studies failed to distinguish infection from rejection with bronchoalveolar lavage cells[7]. More sophisticated methods have been recently considered to potentiate the role of bronchoalveolar lavage including flow cytometric phenotypic analysis of mononuclear cell[8], functional analysis of T lymphocyte, concentration of thromboxane B_2, endothelin and nitric oxide[9][10].

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폐 이식후에 합병된 감염증과 거부반응시의 기관지폐포 세척액의 양상

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박 영 식

= 국 문 초 록 =

본 실험은 폐 이식후에 감염증이나 거부반응이 합병되었을 때, 기관지폐포 세척액내의 양상이 서로 다른가를 비교하기 위해 계획되었다.

황견에서 동종 폐이식을 시행한 뒤에, 네집단으로 분류하였다. 제1군(대조군 ; n=4)은 수술 후 면역억제제의 투여를 정상적으로 지속받았다. 제2군(감염군 ; n=6)에서는 수술후 5일에 기관지경을 통하여 대장균을 이식된 폐에 주입하여 감염을 유발시켰다. 제3군(거부반응군 ; n=6)에서는 수술 후 5일부터 면역억제제의 투여를 중단하여, 거부반응을 유발시켰다. 제4군(거부반응 및 감염군 ; n=8)에서는 수술 후 5일에 대장균을 주입하고, 면역억제제의 투여를 중단하여, 감염과 거부반응을 동시에 유발하였다.

수술 후 9일에 기관지경을 통하여 기관지폐포 세척액을 취하여, 총세포수, 감별 세포수를 조사하여 각 군에서 서로 비교하였다. 검사는 수술받지 않은 정상 폐와 이식 수술받은 폐에서 각각 따로 시행하였다.

정상 폐에서는 총세포수나 감별 세포수에서 의의있는 변화는 없었다. 이식된 폐에서의 총세포수는, 제2군(감염군)에서 제3군(거부반응군)에 비하여 증가하였다(4983±1704 vs 740±187/uL ; $p<0.05$). 이식된 폐에서, 감별 호중구수가 제2군(감염군)과 제3군(거부반응군)에서 제1군(대조군)에 비하여 증가되었다(83±1.9 and 73.5±2.4 vs 35.8±13.1% ; $p<0.05$). 이식된 폐에서의 감별 대식세포수가, 제2군(감염군), 제3군(거부반응군)과 제4군(거부반응 및 감염군)에서 제1군(대조군)에 비하여 감소하였다(14.2±1.4, 16.8±3.6 and 16.9±6.1 vs 48.5±14.6% ; $p<0.05$).

감염증과 거부반응의 병리학적인 변화는 이식된 폐의 기관지폐포 세척액 검사에 잘 반영되어있지만, 두 변화를 감별진단하기에는 미흡하였다.