BRONCHIAL SMEAR IN LUNG TRANSPLANTATION

— Early detection of rejection in canine lung allotransplantation —

MOTOAKI ITO, KIMIYOSHI TSUJI, HIROSHI MIYAMOTO,
and HISAO YAMASHITA

*Division of Transplantation and Histocompatibility, Department of Radiology,
School of Medicine, Keio University, Tokyo, Japan*

(Received for publication March 22, 1972)

INTRODUCTION

It is certain that how to detect the rejection reaction in lung allotransplantation as fast as possible is important in the stand point of immunosuppression therapy under transplantation surgery. The authors have studied lung allotransplantation experimentally and clinically^{5,6,9} over the past several years, and developed a method for the early detection of rejection reaction of the transplanted lung at cell level.

MATERIALS AND METHODS

This report is based on 30 successive unselected operations in mongrel dogs of which 20 represent pulmonary left lower lobe allografts and 10 represent pulmonary lower lobe autografts as a control.

The recipient dogs were sacrificed one by one after the transplantation, and the grafted lungs were examined according to the following steps. The cytologic study of the grafted lungs by bronchial brushing and patho-histological examination were carried out using H-E staining.

The bronchial brushing smear from the pre- and post-operative donor dog were prepared as control for cytologic study. Furthermore, daily examination of the bronchial smear and patho-histological examination were carried out in the

two experimental groups of dogs with auto and allo-lobe transplantation.

RESULTS

According to a differential cell count of the bronchial brushing smear from the donor lung^{2,7}, the ciliated cells originating supposedly from the bronchus were markedly observed in number and were followed by the basal, cylinder, goblet cells in order. A differential cell count was 68% by calculating a total of these cells as a bronchial cell type. The alveolar cell type; alveolar epithelial, endothelial cell and the sum of the degenerated alveolar cells were about 21% in a differential cell count.

With respect to other cells related to the alveolar region, differential cell counts of free dust cell and the migratory cell (polymorphonuclear neutrophil, lymphocyte) were about 5% and about 6% respectively (Figs. 1 and 2).

The bronchial cells were much more than the alveolar cells in a differential cell count of the bronchial brushing smear from the autografted lungs at the first postoperative day, and there was marked appearance of the polymorphonuclear neutrophils and it was about 30% of the migratory cell. On the 2nd and 3rd postoperative day, the number of the bronchial cell was almost the same as that of the alveolar cell and polymorphonuclear neutrophils gradually increased. The alveolar cells were more frequent than the bronchial cells in the bronchial brushing smear of allotransplanted lung on 1st and 2nd postoperative day, and the

Fig. 1 Percentage of bronchial brushing smear from donor lung.

Fig. 2 Bronchial brushing smear in donor lung as a control. $\times 400$

Fig. 3 Branchial brushing smear with allograft at 2 days. Degenerated alveolar cells were observed more than bronchial cells. $\times 400$

coexistence of dust cells and polymorphonuclear neutrophiles was observed (Fig. 3).

The histological findings revealed the polymorphonuclear neutrophiles infiltration (Fig. 4). The bronchial cells were greater in number to the alveolar cells on 3rd and 4th postoperative day and a few lymphocytes were observed in Fig. 5. The process of cytological findings in allografted lung as shown in Table 1. Histological findings indicated a mild infiltration of the small round cells around

the small vessels and a slight thickening of the alveolar wall. On the 5th and 6th postoperative day, there were changes (swelling of the nuclei, reduction of chromatin, the ratio of cytoplasm to nucleus) in the alveolar cells and the number of the alveolar cells was almost equal to that of the bronchial cells, and polymorphonuclear neutrophils further increased with a few lymphocytes. Histolog-

Fig. 4 Same allograft as in Figure 3. The round cell infiltrate is present in perivascular location and in alveolar spaces in association with focal disruption of the alveolar septum. $\times 100$

Fig. 5 Bronchial brushing smear with allograft at 4 days. $\times 400$

The early peribronchiolar infiltration is seen in association with focal intrabronchiolar destruction in same allograft.

Table 1

The process of cytological findings of bronchial brushing smear in allografted lung

	1—2	4	5—6	7—8th day
Alveolar cells	++	+	+	+
Dust cells	±	+	++	++
Granulocytes	++	+	++	++
Lymphocytes	—	+	+	++
Bronchiolar cells	+	++	(+)-(++)	±

ical findings at this time indicated a survival of the ciliated cell without cilia and bronchial epithelial cells falling from the bronchial wall. Polymorphonuclear neutrophils, plasma cells and lymphocytes were scattered in the alveolar cavity and so-called macrophage was also observed. In addition, there was an eruption of secretion faintly stained with Eosin as an inflammatory product in the bronchial space. The infiltration of the round cells around the small vessels, the thickening of the alveolar walls, granuloma-like formation around the bronchiole were observed at the 7th postoperative day, and the typical rejection pattern was accomplished in the histological level.

DISCUSSION AND SUMMARY

The authors also examined the changes in rejection reaction using R.I., RISA and ^{133}Xe from the standpoint of the function of the transplanted lung,^{3,5} but it was very difficult to know accurately the technical factors on transplantation and the finer development of rejection reaction. Especially with respect to early detection of rejection reaction, the above-mentioned methods are still unestablished in practice. Moreover, there is no established theory on the mechanism of rejection reaction in lung transplantation, and the typical pattern^{1,8} in the tissue level always relied on. From these viewpoints, the cytologic study of the bronchial brushing smear was noted in order to carry out early detection of the rejection in the transplanted lung.

The fact that the number of the alveolar cells and polymorphonuclear neutrophils (PMN Leukocyte) was greater to that of the bronchial cells in the bronchial brushing smear of the resected lung on the 1st and 2nd day after autotransplantation was considered as follows: the transplanted antigen flowed from the allografted lung might be carried to the host lymph nodes and humoral antibody which recognized the antigen and sensitized cell return to the allografted

Fig. 6 Rejection mechanism of lung allograft (Hypothesis).

A: alveolus	lymph: lymphocyte
ADC: alveolar dust cell	PMN: polymorphonuclear leukocyte
Alv. Duct: alveolar duct	PSE: pulmonary surface epithelium
bbm: bronchiolar epithelium	sbm: superficial basement membrane
C: capillary	cbm: capillary basement membrane
ctm: capillary basement membrane	CT: connective tissue
CT: connective tissue	T. Br.: terminal bronchiole

lung, and it attacks the endothelium of the capillary around the alveoli and a part of it attacks the alveolar cells through the alveolar basal cell, so that it is thought that the alveolar cells are fallen off and flown out. At any rate, in the pulmonary tissue, if antigen-antibody reaction occurs more rapidly at the alveoli, the appearance of the alveolar cells, migratory cells, especially polymorphonuclear neutrophils in the bronchial brushing smear is understandable. Hume⁴ reported that there were three types of rejection as rejection mechanism: acute, chronic and hyperacute rejection and he explained each mechanism. The rejection reaction which was observed in our experiment is acute or hyperacute type of rejection. But with respect to its mechanism, the specificity involving the lung should be noted different from kidney transplantation. If we assume that "the lung is immunologically competent tissue" as advocated by Tsuji,^{10,11} we might be able to consider the specific interaction in the pulmonary tissue, especially in the alveoli as shown in Fig. 6, and polymorphonuclear neutrophils may be possible to get ahead of lymphocytes as non-specific reaction.

Summarizing our results, the alveolar cells were greater in number to the bronchial cells in the brushing smear with a peak on the 2nd postoperative day, but the latter increased remarkably with a peak on the 4th day. In other words, in the early stage of lung allotransplantation the alveolar cells might be fallen off, and polymorphonuclear neutrophils appeared on 1st and 2nd day after surgery and increased day after day. In addition, lymphocytes were observed at about the 4th postoperative day. Histologically, the infiltration of mainly polymorphonuclear neutrophils, lymphocytes, reticulum-like cells and round cells composed of plasma cells were observed from about on the 4th postoperative day. Polymorphonuclear neutrophils flowed out in early stage in the alveolar cavity and continued to appear until the accomplishment of the typical rejection pattern in the tissue level.

CONCLUSION

We studied the bronchial brushing smear from the transplanted lung in order to use cytologic study of the sputum for the detection of the rejection reaction when clinical lung transplantation is carried out routinely. This preliminary report deals with one of methods for early detection of the reaction of the host recognizing the antigenicity of the allografted lung before the occurrence of the typical rejection reaction now defined at the tissue level.

REFERENCES

1. Barnes, B. A., Flax, M. H., Burke, J. F., and Barr, G. (1963) Experimental Pulmonary homografts in the dog. I: Morphological studies. *Transplantation* 1: 351-364.
2. Bertalanffy, F. D. (1964) Respiratory tissue—Part II: Structure, Histophysiology, Cytodynamics. *International Review of Cytology* 17: 213-297.
3. Hardy, J. D. (1969) Lung transplantation, Organ transplantation today. Excerpta Medica Foundation, Amsterdam.
4. Hume, D. M. (1969) Prospects of kidney transplantation, organ transplantation today. Excerpta Medica Foundation, Amsterdam.
5. Ito, M. (1969) Experimental and clinical studies of lung transplantation, especially denervation of the grafted lung. *J. Japanese Associ. Thoracic Surgery* 17: 1167-1185 (in Japanese).
6. Ito, M., Tsuji, K., Miyamoto, H. *et al* (1969) The morphological studies of denervation in the grafted lung. *Transplantation J.* 4: 166-172 (in Japanese).
7. Ito, M., Tsuji, K., Miyamoto, H., and Yamashita, H. (1970) The specific findings of rejection in lung transplantation; Especially the early detection of pulmonary allograft rejection. *IGAKU no AYUMI* 72: 370-371 (in Japanese).
8. Ito, M., Tsuji, K., and Miyamoto, H. (1970) The specific findings of rejection in lung transplantation, so called "allogeneic lung syndrome." *IGAKU no AYUMI* 73: 20-22 (in Japanese).

9. Shinoi, K., Ito, M. *et al* (1966) Pulmonary lobe homotransplantation in human subjects. *Amer. J. Surg.* 111: 617-628.
10. Tsuji, K., Ito, M., and Miyamoto, H. (1970) PMN-L and transplantation immunology. *IGAKU no AYUMI* 2: 68 (in Japanese).
11. Tsuji, K., Ito, M., and Miyamoto, H. (1971) The lung in transplantation immunology. *The Japanese J. Chest Diseases* 30: 548-554 (in Japanese).