Relationship between Gallium 67 Citrate Scanning and Transferrin Receptor Expression in Lung Diseases*

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The uptake of gallium 67 ($^{67}$Ga) into cells is postulated to be through transferrin receptors (TFR) of $^{67}$Ga combined with transferrin. We studied the relationship between gallium 67 citrate scanning ($^{67}$Ga scan) and immunohistochemical TFR expression in lungs of nine patients with lung cancer and eight patients with diffuse interstitial lung diseases. We found that lung cancer tissues of positive $^{67}$Ga scan expressed TFR, but those of a negative scan did not. In all of the five patients with idiopathic pulmonary fibrosis (IPF), TFR were expressed on the membrane of alveolar macrophages that formed clusters. However, TFR were not expressed in lymphocytes, neutrophils, type 2 alveolar epithelial cells, and endothelial cells. In two patients with sarcoidosis and a patient with pneumoconiosis, TFR were expressed positively only on the membrane of foamy alveolar macrophages and epithelioid cells of granuloma. These findings suggest that $^{67}$Ga-citrate initially combines with transferrin in the blood and then the complex is incorporated into cells through TFR. Therefore, $^{67}$Ga scan could be positive when cells have TFR and one should be able to observe cancer cells, clusters of alveolar macrophages, and epithelioid cells through the imaging of $^{67}$Ga scan in lung cancer and diffuse interstitial lung diseases.

(Chest 1992; 102:530-34)

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Gallium 67 citrate scanning ($^{67}$Ga scan) has been used as a tumor scintigram of lung cancer and as an indicator of activity of diffuse interstitial lung diseases such as idiopathic pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis, and pneumoconiosis. As for the uptake mechanism, it has been hypothesized that $^{67}$Ga, instead of iron, combined with transferrin in the blood is taken by transferrin receptors (TFR) on the proliferative cell membrane such as cancer cells. A positive $^{67}$Ga scan reaction has been shown in lung cancers irrespective of histologic types and in active stage of diffuse interstitial lung diseases. However, the cells with $^{67}$Ga uptake remain unclear in these diseases. There have been few reports to date comparing $^{67}$Ga scan and TFR expression using lung immunohistochemistry. Therefore, we investigated the relationship between $^{67}$Ga scan and TFR expression in lung cancers and diffuse interstitial lung diseases.

**Materials and Methods**

**Patients**

Nine patients with lung cancer who underwent $^{67}$Ga scan and operation were studied. Four of them were positive for $^{67}$Ga scan and five were negative. Eight patients with histologically proven diffuse interstitial lung disease (five IPF, two sarcoidosis, and one pneumoconiosis) were also studied with $^{67}$Ga scan (Table 1). Histologically normal tissues obtained from four nonsmoker patients who underwent operation for lung cancer were also investigated.

$^{67}$Ga Citrate Scanning

$^{67}$Ga is a cyclotron-produced radionuclide with a half-life of 78 h.

<table>
<thead>
<tr>
<th>Table 1—Clinical Data of Patients with Lung Cancer and Diffuse Interstitial Lung Disease</th>
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*Grade 0 = no pulmonary activity; grade 1 = uptake less than in the liver; grade 2 = uptake equal to that in the liver; and grade 3 = uptake greater than in the liver.
†IPF = idiopathic pulmonary fibrosis.
‡TBLB = transbronchial lung biopsy.
Patients were scanned 48 h after intravenous injection of 2 mCi of $^{67}$Ga citrate. Imaging was obtained by a large field-of-view gamma camera (Toshiba, GCA-501S). $^{67}$Ga uptake was graded by two observers according to the classification proposed by Gupta et al. Grading was determined in relation to liver uptake activity; no pulmonary activity was graded 0, uptake in the lungs less than in the liver was graded 1, uptake equal to that in the liver was graded 2, and uptake greater than in the liver was graded 3. All patients were scanned within a few days before operation or biopsy.

**Immunohistochemistry**

Each lung specimen was immediately fixed in periodate-lysine 4 percent paraformaldehyde for 6 h, and washed in phosphate-buffered saline solution (PBS) containing increasing concentrations of sucrose. The fixed specimens were embedded in Tissue-Tec OCT compound (Miles Pharmaceutical, Naperville, Ill), frozen in dry ice ethanol, and sectioned at 6 μm on a cryostat microtome. The sections were placed on egg-albumin coated slides and dried in air. Monoclonal antibody OKT9, which recognizes TFR, was purchased from Ortho Pharmaceutical Co (Ortho Japan, Tokyo, Japan). Monoclonal antibody NU-Tfr2, which differs from OKT9 in the recognition of antigen epitope of TFR, was purchased from Nichirei Co, (Seikagaku Kogyo, Tokyo, Japan). They were used at a dilution of 1:10. Goat anti-mouse F(ab')2 fragments of IgG labeled with horseradish peroxidase (HRP) (Cosmo Bio Co, Tokyo, Japan) was the second antibody. It was used at a dilution of 1:100. Cryostat sections to be observed by light microscopy were treated with 100 percent methanol containing 0.03 percent hydrogen peroxidase to inactivate endogenous peroxidase. Then, indirect HRP-labeled antibody method was applied for the immunologic reaction as previously described. Briefly, the procedure involves successive incubations with the first antibodies for 12 h at 4°C, and the second antibodies for 6 h at 4°C. They were then reacted with 0.25 percent dianinobenzidine (DAB) solution containing 0.01 mol/L sodium azide and 0.01 mol/L hydrogen peroxide, and counterstained with methyl green. The specificity of histochemical staining was confirmed by use of either nonimmune rabbit serum or ascitic fluid from mice injected with nonsecreting hybridoma cells instead of primary antiserum.

**RESULTS**

In normal lung tissues, bronchoalveolar epithelial cells, bronchial glands, vascular endothelial cells, and other interstitial cells were negative for TFR. Only alveolar macrophages observed scarcely in alveolar spaces of normal lung tissues showed mildly positive TFR staining on its membrane, but no clusters of alveolar macrophages were observed. All lung cancer tissue specimens from four patients with lung cancer who were positive for $^{67}$Ga scan showed strong positive TFR staining on the membrane of cancer cells. Patient 1 was presented as the most typical case (Fig 1). However, all of the lung cancer tissue specimens from five patients with lung cancer who were negative for $^{67}$Ga scan showed negative TFR staining (data not shown).

As for diffuse interstitial lung diseases, lung tissue specimens of IPF showed strong positive TFR staining of the membrane of alveolar macrophages that formed clusters in alveolar spaces surrounded by slightly thickened alveolar septa. We could not recognize the expression of TFR in other cells such as proliferative type 2 alveolar epithelial cells, bronchiolar epithelial cells, and inflammatory cells such as lymphocytes and neutrophils (Fig 2). However, alveolar macrophages that were located in severely fibrotic lung tissues showed a small number of such cells. In lung tissue specimens of sarcoidosis, the membrane of foamy alveolar macrophages in alveolar spaces and epitheli-
oid cells in granuloma showed positive TFR staining, but lymphocytes did not show positive as those in IPF did not (data not shown). In lung tissue specimens of pneumoconiosis, the same results as those in the patient with sarcoidosis were obtained (data not shown). We confirmed the same results of TFR staining of two monoclonal antibodies throughout the study.

**Discussion**

$^{67}$Ga scan has been used as a tumor scintigram to detect lung cancer and as an indicator of active diffuse interstitial lung diseases. First, the present study demonstrated that TFR were strongly expressed on lung cancer tissues of all $^{67}$Ga scan-positive patients, but TFR were not expressed on those of $^{67}$Ga scan-negative patients. These results suggest uptake of $^{67}$Ga combined with transferrin by cancer cells whose membranes express TFR.

It has been reported that 85 to 95 percent of lung cancers are $^{67}$Ga scan positive. Most squamous cell carcinomas show positive reaction on $^{67}$Ga scan; however, that of adenocarcinoma is somewhat low. As for TFR, 82 percent of all lung cancers are positive, and according to the histologic type, squamous cell carcinomas are all positive and positive rate of adenocarcinoma is somewhat low. Thus, the abovementioned reports suggest the correlation between $^{67}$Ga scan positivity and TFR expressing cells, and we could confirm the relationship with immunohistochemical study.

TFR expression shows a strong significant correlation with proliferating activity in any kind of cancer, and it also has a significant correlation with Ki67 antigen, which preferentially reacts with a human nuclear antigen expressed on all proliferating cells during late G1, S, M, and G2 phases of cell cycle. From these facts, we considered that $^{67}$Ga scan-positive lung cancer has a strong proliferating activity that demands much iron. If the $^{67}$Ga scan were positive in patients with lung cancer, there would be marked TFR expression on membranes of cancerous tissues, and reduction of the iron supply as well as use of cisplatin-transferrin complex would presumably be effective therapy. Given the TFR expression of $^{67}$Ga scan-positive cancer, we investigated whether TFR expression would reflect the positive $^{67}$Ga scan in diffuse interstitial lung disease. It has been reported that $^{67}$Ga uptake would be by macrophages, neutrophils, and T lymphocytes. We found that TFR were expressed on the membrane of alveolar macrophages that formed clusters in IPF and epithelioid cells of granuloma that are considered to be differentiated from activated alveolar macrophages in sarcoidosis and pneumoconiosis.

However, inflammatory cells, including lymphocytes and neutrophils, proliferative type 2 alveolar epithelial cells, fibroblasts, and vascular endothelial cells did not show TFR expression. Therefore, we could observe imaging of $^{67}$Ga scan through $^{67}$Ga uptake of these TFR-positive cells in diffuse interstitial lung disease.

The uptake mechanism would be one in which $^{67}$Ga-transferrin complex in blood passes through the cap-

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**Figure 2.** A: $^{67}$Ga scan showing uptake by lung parenchyma in patient 10 with idiopathic pulmonary fibrosis (IPF). B: OKT9 is reactive with the membrane of alveolar macrophages (arrows) that formed clusters in alveolar spaces. Type 2 alveolar epithelial cells (arrowheads) are not stained with OKT9 (original magnification x100). C: OKT9 is reactive with the membrane of alveolar macrophages that formed clusters, but not with neutrophil (arrow) and lymphocyte (arrowheads) membranes (original magnification x1,000).
illary wall with accelerated permeability and is taken up by activated alveolar macrophages or epithelioid cells through TFR that are expressed intensively. Although TFR are expressed on lymphocytes stimulated by mitogen, TFR were not expressed on lymphocytes in the present study. Tsuda et al also reported that TFR were positive on epithelioid cells, but negative on lymphocytes in sarcoidosis. Angiotensin-converting enzyme (ACE) is produced from activated alveolar macrophages and epithelioid cells in sarcoidosis, and $^{67}$Ga scan and serum ACE level have a strong correlation with each other. The findings agree with those of the present study. In sarcoidosis, positive $^{67}$Ga uptake by fibrotic parts is weak, and the present result that alveolar macrophages located in severely fibrotic lung tissues of IPF showed a small number may agree with this finding. The results of the present study lend support to the hypothesis of $^{67}$Ga-transferrin complex uptake by cells through TFR on their membranes. Thus, cancer cells, clusters of macrophages, and epithelioid cells may be observed on $^{67}$Ga scans of lung cancer and diffuse interstitial lung diseases.

ACKNOWLEDGMENTS: The writers thank Professor Hidehiko Saito, First Department of Internal Medicine, Nagoya University School of Medicine, and Dr. Tatsunari Satake, Pathology section, Nagoya Ekisaiai Hospital, for their valuable suggestions and comments.

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