Scintigraphy with J001 Macrophage Targeting Glycolipopeptide: A New Approach for Sarcoidosis Imaging

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Scintigraphy with radiolabeled J001 as a ligand for macrophage targeting is a new approach for sarcoidosis imaging. J001 is a fully characterized acylated peptido-poly (1,3) galactoside isolated from Klebsiella membrane proteoglycans and able to bind electively recruited macrophages. Its physicochemical properties allow rapid absorption by the respiratory tract when this agent, labeled by 99m technetium, is administered as an aerosol. Images are obtained within 3 to 5 h after inhalation. In the present study, we determined the ability of J001 scintigraphy to localize areas of sarcoidosis involvement in 22 patients compared with gallium scanning in ten of them. Nineteen patients underwent bronchoalveolar lavage (BAL) and serum angiotensin-converting enzyme (ACE) assay. J001 scintigraphy was also performed on a control group of six patients with extrathoracic melanoma, in whom J001 scintigraphy was used to evaluate the cutaneous extent of the tumor and the lymph node involvement. In this control group, no fixation appeared in the thoracic area. In the sarcoidosis group, 18 positive results were observed. One stage 0 patient had a mediastinal fixation. Five of the six stage 1 patients had a fixation located in the mediastinum, the lungs, and the wrists. Five of the six stage 2 patients had positive foci located in the mediastinum or the lung areas and in the myocardium in one of them. Six of the nine stage 3 patients had positive J001 scintigraphy occurring in the lungs and/or the mediastinum. One patient had a fixation on the main bronchi. J001 scintigraphy and gallium scanning, performed in ten patients, were positive in seven of them. There were discrepancies between the BAL results and J001 scintigraphy, as well as between the ACE results and J001 scintigraphy. In conclusion, 99mTc-J001 scintigraphy appears to be a sensitive and rapid technique for the imaging of thoracic sarcoidosis at the three stages of the disease.

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ACE = angiotensin-converting enzyme; ROI = region of interest

Sarcoidosis is a granulomatous disease that most commonly affects the lungs. Current concepts of the pathogenesis of the pulmonary involvement suggest that the earliest lesion is an alveolitis characterized by a diffuse infiltration of the lung by mononuclear phagocytes and activated T lymphocytes. Evaluation of this pulmonary cellular involvement appears to be valuable for the follow-up of the disease. With this goal, tests such as the measurement of serum angiotensin-converting enzyme (ACE), quantitation of lung T lymphocytes in the bronchoalveolar lavage (BAL), and gallium 67 scanning are generally performed. Macrophages and neutrophils are known to be involved in the gallium uptake, supporting gallium scanning as a noninvasive technique of possible use for staging alveolitis.

We developed another isotopic approach based on macrophage imaging by J001 scintigraphy. J001 is a fully characterized 34-kDa acylated peptido-poly (1,3) galactoside isolated from Klebsiella membranes. This glycolipopeptide is able to bind electively to recruited macrophages. Due to its amphipathic properties, technetium 99m radiolabeled J001 is absorbed through the respiratory tract after aerosol administration. Images are obtained within 3 to 5 h after inhalation.

In a model of experimental berylliosis, J001 scintigraphy previously revealed both the initial alveolitis and the following stage of inflammatory thoracic lymph nodes.

The aim of the present study was to determine the ability of J001 scintigraphy to localize areas of thoracic sarcoidosis involvement. Results were compared with gallium 67 scanning, serum ACE, and BAL.

METHODS

Patients

Twenty-two patients with pulmonary sarcoidosis proved by biopsy specimen were included in this study from 1988 to 1990. All patients were fully informed and gave written consent. The study was approved by the ethics committee of our institute. Ages ranged from 22 to 61 years, with a mean age of 38 years. Twelve patients were women and ten were men. Eighteen subjects were nonsmokers and four were smokers (five to ten pack-year) but did not smoke during the four days preceding gallium scan and J001 scintigraphy. Eight patients had clinical signs of extrathoracic involvement such
as erythema nodosum, lacrimal glands, uvea, liver, spleen, myocardium, or cutaneous nodules. Thoracic assessment of sarcoidosis included chest roentgenogram and computed tomographic (CT) scan. One patient was in roentgenographic stage 0; six patients were in stage 1 (hilar adenopathy only); six were in stage 2 (hilar adenopathy plus pulmonary infiltrates); and nine were in stage 3 (pulmonary infiltrates only). Sixteen patients were untreated at the time of entry in the study. Five patients were still taking prednisolone (10 to 30 mg/day) after 3 to 42 months' treatment and one patient continued taking inhaled beclomethasone (1,000 μg/day) after 6 months' treatment.

Control Population

Due to ethical considerations, J001 scintigraphy was not applied to healthy subjects. J001 scintigraphy was applied to a control population consisting of six patients with extrathoracic melanoma who had no evidence of metastatic pulmonary neoplasia, prior chest irradiation, or chemotherapy. Macroagglutination is of value to evaluate the cutaneous extent of the tumor and the lymph node involvement. Four control subjects were nonsmokers, and two were smokers (15 to 25 pack-years) but did not smoke during the four days before gallium scan and J001 scintigraphy.

Methodology

Bronchoalveolar Lavage: Nineteen patients underwent fiberoptic bronchoscopy with BAL within ten days after gallium scan and J001 scintigraphy. BAL was performed as previously described. Briefly, 150 ml of sterile saline solution was infused in divided portions in the lingula or the middle lobe and was immediately aspirated. After centrifugation, lavaged fluid cells were analyzed for total numbers and differential cell counts. After staining with modified Wright-Giemsa, a minimum of 300 cells were counted to obtain a differential cell count.

ACE Assay: This test was performed by the method of Lieberman et al. Values of 400 U/ml or higher were considered as abnormal.

Gallium 67 Scanning: Ten patients with sarcoidosis underwent gallium 67 scintigraphy. Each patient received citrate gallium intravenously, 1.11 MBq/kg of body weight. Twenty-four and 48 h later, anterior and posterior scans were recorded from the neck to the pelvis using a gamma camera (Sopha, France). Activity in the lungs was considered to be increased when it exceeded the activity of the adjacent chest wall soft tissues. Abnormal accumulation was considered as diffuse when localized in the lungs and focal when it corresponded to regional or perihilar lymph nodes.

An uptake index was calculated from the activity in regions of interest (ROI) corresponding to the abnormal scintigraphic areas. The activity was counted in anterior and posterior views and the mean square was calculated to take into account the photon attenuation. The index was referred to the nonspecific activity in a

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Stage and Clinical Signs</th>
<th>Lavage % Lymphocytes</th>
<th>Corticosteroid Treatment</th>
<th>Serum ACE, U/ml</th>
<th>Gallium Scanning Fixations</th>
<th>J001 Scintigraphy Fixations</th>
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<tr>
<td>1</td>
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*ND = not done; ACE = angiotensin-converting enzyme.
J001 scintigraphy: One milligram of freeze-dried J001 (Laboratoire Pierre Fabre, France) was reduced with 125 μg stannous fluoride (Hepatek II, Amersham England) and labeled with 740 MBq pertechnetate. Patients inhaled the 4 ml radiolabeled J001 preparation as an aerosol in an especially devised aspiration hood (ESI, France) using an ultrasound TV 6000 inhaler (Siemens, West Germany) operating at 110 kHz. Aerosolization was always limited to 15 min to prevent any degradation of the radiolabeling. To avoid false-positive results resulting from skin contamination, patients wore gloves and clothes that were changed after inhalation and before each scintigraphy. Three and 5 h after inhalation, anterior and posterior images were recorded, using a gamma-camera with a high-resolution parallel collimator and connected to a computer for image processing (Sopha Medical, S 2000, France). Large-scale contamination was generally observed due to the deposition of the aerosol in the pharynx, esophagus, and stomach. Consequently, patients were given a meal just after the aerosol inhalation to reduce esophageal activity.

The scintigraphic images were interpreted without background subtraction and after digitized contrast enhancement by setting the maximum of intensity to a lower value than the maximum count. The scintigraphy was considered normal when no activity was detectable in the lung, in the hilar regions, or in the mediastinum 5 h after inhalation. When activity was present in the lung, the percentage of change between 3 and 5 h after inhalation was calculated. A 21 percent decrease corresponded to the physical 99mTc decay and indicated stable uptake of J001. This data processing was performed to assess the contribution of a potent impaction or a lower clearance in the constitution of the scintigraphic picture. The radioactive J001 concentration in abnormal fixing areas was evaluated by an uptake index referring the mean count in these areas to the nonspecific vascular mean count in the shoulder area.

Investigations performed in the 22 patients with sarcoidosis are summarized in Table 1. The control group had only J001 scintigraphy.

RESULTS

Control Population

The lung clearance was always complete 5 h after J001 inhalation and neither focalized nor diffuse activity was observed in the thoracic area of the six control patients with extrathoracic melanoma.

Patients

J001 scintigraphy showed regions of uptake in 18 of the 22 patients with sarcoidosis (Table 1).

Patient 1, who was at stage 0, had a J001 mediastinal region of uptake. Uptake index was 1.4 at 3 h and 1.2 at 5 h. No fixation was observed in the areas of cutaneous nodules localized in the forearms and the neck.

Four of the six stage 1 patients (patients 2, 3, 4, and 5) had a region of uptake in the mediastinum area corresponding to mediastinal lymph nodes. Uptake index at 3 h was, respectively, 1.2, 1.6, 1.3, and 1.5. At 5 h, uptake indices were 1.1, 1.3, 1.3, and 1.1. In two patients lung fixation was observed: patient 5 with lung J001 fixation had the highest BAL lymphocytosis (60 percent). Patient 2 had a gallium scan that showed mediastinal and lacrimal gland regions of uptake (Fig 1). When this patient was studied with J001 scintigraphy, we did not perform scintigraphy on the lacrimal gland areas. Patient 7 had Löfgren syndrome with normal BAL and ACE: no J001 region of uptake was observed in the mediastinum but only in the wrists. In patient 6 with normal BAL and increased serum ACE, gallium region of uptake was observed in the mediastinum but no J001 fixation.

Five of the six stage 2 patients showed J001 regions of uptake. Patients 9, 10, and 12 had J001 uptake both in the lungs and the mediastinum and patient 13 had a region of uptake localized to the mediastinum. Uptake indices at 3 and 5 h were calculated in the mediastinal uptake area. They were 1.8, 1.5, 1.4, and

![Figure 1A (left): Patient 2: Stage 1 sarcoidosis. Anterior J001 scintigraphy with regions of interest on bilateral mediastinal regions of uptake and in shoulder area 3 h after inhalation (scintigraphic index: 1.2).](image1a.jpg)

![Figure 1B (right): Patient 2: Stage 1 sarcoidosis. Anterior thoracic gallium scan 48 h after injection. There is a bilateral mediastinal fixation and a physiologic gallium uptake in the liver.](image1b.jpg)
1.0, and 1.8, 1.7, 1.6, and 1.6, respectively. Patient 8 had a region of uptake localized throughout the lungs with an uptake index of 1.6 at 3 h and of 1.3 at 5 h. This patient had glandular (parotid and lacrimal glands) and ocular (uveitis) involvement of sarcoidosis: J001 uptake was observed in the eye areas. No region of uptake appeared in the eyes with the gallium scintigraphy in contrast with intensive activity focused on the parotids and the lacrimal glands. Patient 11 had a stage 2 sarcoidosis with 35 percent of lymphocytes in BAL. J001 scintigraphy was performed three weeks after BAL: no J001 uptake was observed. Two months later, chest roentgenogram was quite normal in this patient 11. Patient 13 had severe myocarditis related to sarcoidosis: both J001 and gallium scintigraphy demonstrated an intense myocardial fixation (J001 uptake index of 1.5 at 3 h and of 1.4 at 5 h).

Nine patients had stage 3 sarcoidosis. A J001 region of uptake was observed in the lung or mediastinal areas of six patients (patients 14, 16, 17, 19, 20, and 22). Uptake index at 3 and 5 h were 1.9, 1.3, 1.6, 1.5, 2.1, and 1.2, and 1.9, 1.9, 1.5, 1.6, 1.7, and 1.2, respectively. In two patients (patients 17 and 19), J001 uptake occurred in the lungs (Fig 2). These two patients had gallium scintigraphy, with a positive result only in patient 17 who had the highest count of lymphocytes in BAL (33 percent) among the stage 3 patients.

Patients 13, 14, and 16 with splenomegaly had a gallium uptake in the spleen area whereas no J001 uptake was observed.

Patient 21 had diffuse fibrosis diagnosed ten years ago with an inflammation of the bronchial tree: J001 uptake was localized in the main bronchial areas with a high uptake index (3.6 and 2.9 at 3 and 5 h, respectively).

In patients with lung activity, the mean decay between 3 and 5 h after inhalation was −21 percent (ranges: +5 percent and −40 percent). This result corresponded to the physical decay of 99m technetium during the 2-h interval. The lung decay was lower than −21 percent (range, −21 percent to +5 percent) in two stage 1 patients, two stage 2 patients, and three stage 3 patients: this lung activity was considered to correspond to an active uptake. The lung decay was higher than −21 percent (range, −21 percent to −40 percent) in four stage 3 patients, corresponding to a light fixation at 5 h referring to the absence of any activity at that time in the control population.

A comparison of J001 scintigraphy and gallium scanning was possible for ten patients. Thoracic regions of uptake obtained in J001 scintigraphy corresponded to gallium uptake in seven of these cases (patients 2, 8, 10, 13, 14, 16 and 17) (Fig 3). Patient 6, who was at stage 1 of sarcoidosis, had a gallium region of uptake in the mediastinum, whereas J001 scintigraphy was negative. This patient had only 5 percent of lymphocytes in the BAL. Patient 15, who also was at stage 3, had a very mild gallium uptake. J001 scintigraphy was negative. An opposite result was observed in patient 19 whose J001 result was mildly positive in the lungs whereas gallium scan was negative. At the three stages of sarcoidosis, discrepancies were observed between J001 scintigraphy and BAL lymphocyte count (Fig 4).

Among the 19 patients whose serum ACE was measured, seven had a high level: three stage 1 patients, two stage 2 patients, and two stage 3 patients. There was no relation between J001 fixations and ACE results.
lymph nodes, salivary glands, lacrimal glands, eyes, or spleen. However, gallium imaging is relatively expensive and its low tissue clearance requires a 48- or 72-h delay after intravenous administration to obtain images. For these reasons, gallium scan is difficult to perform as a routine index for evaluating the clinical course of sarcoidosis.

Previous studies comparing evaluation of the alveolitis by BAL and gallium scan have demonstrated that the two cellular components of alveolitis, ie, macrophages and lymphocytes, can be activated either together or separately. Thus, a functional evaluation of macrophage alveolitis appears to be of interest.

Recruited macrophages are able to interact in vitro with bacterial proteoglycans. This property was applied to develop a new scintigraphic strategy using J001 labeled with 99m technetium for imaging sarcoidosis and inflammatory foci characterized by macrophage infiltration. J001 is a fully characterized acylated peptido-poly (1,3) galactoside, isolated from Klebsiella membrane proteoglycans that is able to recognize macrophages selectively both in vitro and in vivo. Due to its low molecular weight (34 kDa) and its amphiphatic structure, J001 is absorbed by the respiratory tract after aerosol administration. In those conditions, J001 rapidly diffuses in the blood, lymph, and extracellular space. Kidney clearance of unbound J001 molecules occurs within 4 h after administration.

Eighteen of the 22 patients included in this study had a positive J001 scintigraphy result. J001 scintigraphy appears to be sensitive at the three stages of thoracic sarcoidosis. In stage 1, the regions of uptake concerned mediastinum in the four positive cases. In addition, an uptake was observed in the lungs in two patients, which might correspond to a subradiologic parenchymal involvement. Moreover, a stage 1 patient with lung J001 uptake had the highest BAL lymphocytosis (60 percent). In stage 2, five positive results...
were observed among the six patients studied. In three patients, J001 fixations were located both in the mediastinum and lungs. Six of the nine stage 3 patients had positive J001 scintigraphy occurring both in the lungs and/or the mediastinum, demonstrating the sensitivity of J001 scintigraphy in interstitial lung sarcoidosis. One patient with diffuse lung fibrosis had a fixation on the main bronchi. This patient had an inflammation of the bronchi despite corticotherapy administration.

The absence of J001 fixation in a stage 2 patient, despite lymphocyte alveolitis three weeks before, was of particular interest. Two months later, the chest roentgenogram was normal. These data could be compared with other studies that have shown the value of gallium lung scan for the follow-up of patients with sarcoidosis. A negative gallium scan is associated with stability of pulmonary function whereas a positive scan has no specificity in terms of capacity to evolve. Further studies are necessary to define whether J001 scintigraphy might have the same potential as gallium scan to follow up patients and discuss the indications for treatment. Therefore, J001 scintigraphy would appear very valuable because it is easier to perform than gallium scintigraphy and requires only 5 h to obtain images.

Due to the aerosol administration of J001, some extrathoracic localizations of sarcoidosis are difficult to visualize. Among the three patients who had cutaneous nodules or erythema nodosum, only one had a positive J001 scintigraphy involving the wrists. Due to the facial and digestive tract contamination after J001 administration, it is not possible to study the parotid region with J001 scintigraphy. In contrast, one patient with uveitis demonstrated an eye fixation with J001 scintigraphy. In gallium scintigraphy, activities focusing on the parotid and the lacrimal gland areas were noted in three patients. Similarly, J001 scintigraphy failed to reveal the splenic involvement in three patients whereas it was obvious on gallium scintigraphy. We have no hypothesis as yet to explain this lack of sensitivity of J001 scintigraphy for imaging the spleen. Nevertheless, J001 scintigraphy does not appear to be a promising method to image the abdominal region due to the digestive contamination that could hinder active fixation in many patients. On the other hand, the high fixation in the myocardium in a patient with myocarditis may be an indication for further studies.

As already demonstrated in previous studies comparing BAL results and gallium scan, it seems that there are also discrepancies between BAL results and J001 scintigraphy. Only three patients had BAL lymphocytosis higher than 30 percent: in two of these patients, a high pulmonary fixation was observed with J001 scintigraphy. Some patients with low BAL lymphocytosis had a mediastinal J001 fixation but rarely a pulmonary one. Likewise, there is no relation between ACE results and J001 scintigraphy. In fact, this is not surprising bearing in mind the close specificity of the macrophage-J001 interaction. J001 scintigraphy and gallium scanning, performed in ten patients, were both positive in seven of them. In two patients with positive gallium scan and negative J001 scintigraphy, BAL lymphocytosis was low.

In conclusion, J001 scintigraphy could be a convenient and rapid test of potential value to characterize the severity and anatomic location of parenchymal inflammation in sarcoidosis. The use of 99m technetium for the labeling of J001 results in a much lower cost of J001 scintigraphy when compared with gallium scintigraphy.

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REFERENCES

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**Practical Spirometry Courses**

This two-day NIOSH approved course for training in pulmonary function testing will be presented September 18-19 in Chicago and November 6-6 in Orlando. The courses are presented by Mayo Pulmonary Services. For information, write Mayo Pulmonary Services, 432 Plummer Bldg, Rochester, MN 55905 or call 800:533-1653.

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**Annual Meeting, National Society for Cardiovascular Technology/National Society for Pulmonary Technology**

This meeting has been scheduled for September 16-20 at the Miami Airport Hilton and Marina, Miami, Florida. For information, contact the NSCT/NSPT, 10703 Courthouse Road, Fredericksburg, Virginia 22401 (703:891-0079).