Cellular Basis for the Elevated Gallium-67 Computed Lung Index in a Rheumatoid Lung Patient

H. David Specht, Antony C. Bakke, Rita Braziel, Abbé Miley, Shahin Rashidi-Nezami, and Linda Germain

Departments of Radiology and Pathology, Oregon Health Sciences University, Portland, Oregon

A patient with high levels of serum rheumatoid factor and an open lung biopsy which showed high-grade interstitial pneumonia with large numbers of lymphocytes and plasmocytes had intense gallium uptake in the lungs. Lymphocytes and/or plasmocytes might be responsible for the gallium uptake even though neutrophils are usually credited with high-level uptake. Differential cell counts demonstrated plasmocyte and lymphocyte preponderance, but neutrophil paucity. In vitro cell cultures of purified neutrophils, monocytes, leukemic plasmocytes, and resting and stimulated lymphocytes with ^{67}Ga showed that plasmocytes take up comparatively low levels of ^{67}Ga, but that activated lymphocytes take up levels that approach neutrophils. It is probable that both rheumatoid lung plasmocytes and activated lymphocytes are responsible for the pulmonary ^{67}Ga concentration in this patient.

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While developing a computer technique (*1*) to improve the utility of ^{67}Ga indices (*2*) in the evaluation of interstitial pulmonary inflammatory disease, we encountered a patient with rheumatoid lung. The patient had a strikingly abnormal index and many plasmocytes in her lung biopsy. This suggested that the two phenomenon might be specifically related.

In an attempt to determine which type of inflammatory cells were responsible for gallium uptake in this rheumatoid lung patient, we evaluated the proportion of different cell types present in the biopsy tissue, using a morphometric method for quantitation. We extended these studies by a series of in vitro culture experiments in which ^{67}Ga uptake of different inflammatory cells was measured. Culture conditions included various transferrin states because transferrin complexes gallium (*3*), and because these complexes bind to transferrin receptors on cells (*4*).

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For reprints contact: H. David Specht, MD, 10805 S.E. Hillside Drive, Portland, OR 97266.

METHODS

The patient underwent computed ^{67}Ga scintigraphy with index generation (*1*). For precise cell differential counts, hematoxylin and eosin-stained lung biopsy slides were re-examined to select paraffin blocks having uniform dispersion of representative inflammatory cells. The selected blocks were re-cut and the resultant slides stained for cytokeratins to assist in differentiating alveolar epithelium from macrophages and other inflammatory cells. After subsequent hematoxylin staining, the identification of inflammatory cell types was performed using nuclear criteria. Two of us independently counted 2000 to 3000 cells from 10 to 12 random fields, using a custom-designed ocular reticle with a full field grid of 2 × 2 mm squares, each of which was subdivided into four 1 × 1 mm squares.

For in vitro correlative studies inflammatory cells were obtained from two sources. Plasmocytes were collected from the peripheral blood of two patients with plasma-cell leukemia. Neutrophils, monocytes and lymphocytes were collected from normal peripheral blood. Leukocytes were separated by 6% dextran gravity sedimentation of erythrocytes, followed by ficoll/hypaque density gradient separation of neutrophils and mononuclear leukocytes. Monocytes and lymphocytes were separated by a 1-hr adherence of monocytes to plastic flasks pre-coated with culture media (RPMI containing 10% fetal calf serum). After incubation, warm media was used to wash away lymphocytes and cold media to remove adherent monocytes. The neutrophil pellet beneath the ficoll/hypaque was resuspended in culture media. Previously isolated lymphocytes underwent two days of stimulation with phytohemaglutinin (PHA) before testing with ^{67}Ga. All cell populations were analyzed with a flow cytometer using monoclonal antibodies. Triplicate or duplicate 1 ml cultures contained 1 × 10^6 cells and 37 kBq of ^{67}Ga with or without either 2 mg/ml of 35% ferric saturated human transferrin (serum concentrations) or 20 μg/ml of human apotransferrin. Forty-two- or 18-hr cell incubations were carried out in 1 ml multiwell plates placed in a 37°C incubator with 5% CO_2. After incubation the cells were transferred to 5-ml conical centrifuge tubes by washing each plate well with 0.5 ml of cold phosphate-buffered saline (PBS) to remove adherent cells. Subsequently the cells were washed twice with PBS, and cell-bound ^{67}Ga assessed using a gamma photon counter.

CASE REPORT

A 68-yr-old woman had a 15-yr history of chronic cough accompanied by gradually increasing dyspnea. She also developed

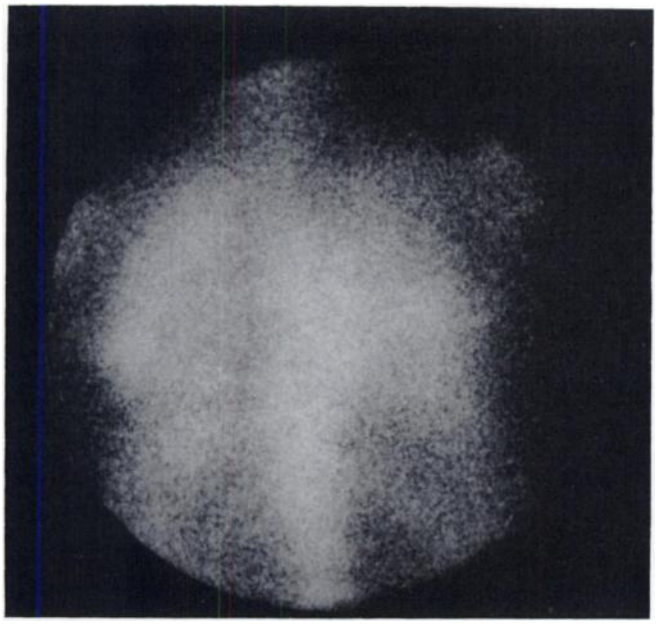

FIGURE 1. Gallium-67 scintigram from the case presented. It is a posterior view of the chest and upper abdomen in which the chest tracer uptake is greater than the liver and abdomen. The index is 308.

migratory polyarthritis and anemia. Her radiographic evaluation showed asymmetric diffuse mixed interstitial and alveolar infiltrates. Her serum rheumatoid factor was 1:2560. Her computed ^{67}Ga lung index was 308, and her lung scintigram is shown in Figure 1. Subsequently, she underwent an open lung biopsy, which showed a marked plasmocytic and lymphocytic interstitial pneumonitis (Figs. 2 and 3).

RESULTS

The lung biopsy differential cell counts demonstrate that plasmocytes occur with the greatest percentage of any

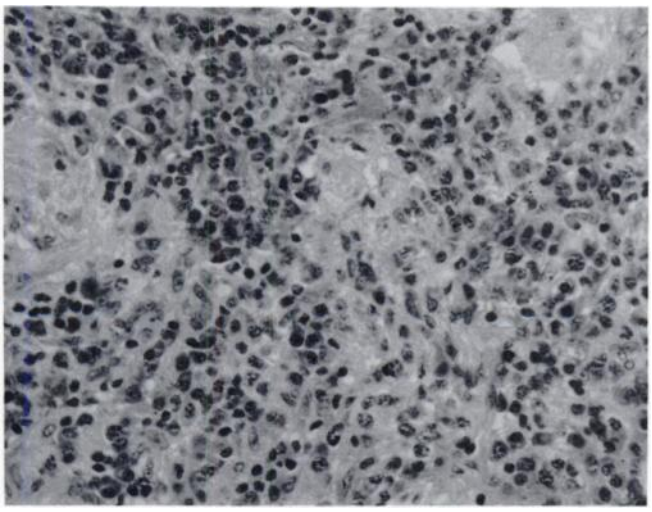

FIGURE 2. Photomicrograph of the open lung biopsy from the case presented. It shows severe effacement of pulmonary architecture with chronic proliferative inflammation dominated by plasmocytes.

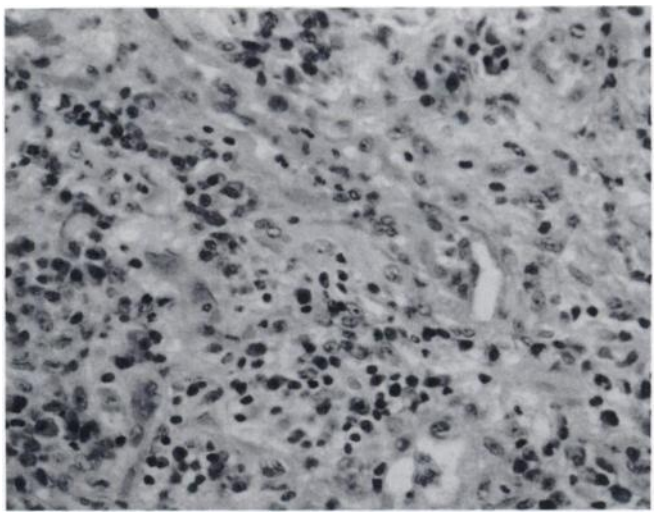

FIGURE 3. Additional photomicrograph of the same open lung biopsy. It shows an area where more lymphocytes are visible, but many plasmocytes are also present.

of the inflammatory cell types (Table 1). Neutrophils have a very low percentage occurrence. Lymphocytes and histiocytes occur 9% and 21% less frequently than plasmocytes.

In vitro culture experiments (Table 2) at 42 hr show that monocytes have the highest ^{67}Ga uptake followed by neutrophils, resting lymphocytes and leukemic plasmocytes in decreasing order. Culturing with human serum transferrin levels does not change this order. This transferrin concentration suppresses ^{67}Ga uptake of every inflammatory cell type. At 18 hr neutrophils have the highest ^{67}Ga uptake followed in decreasing order by PHA-stimulated lymphocytes, monocytes, and resting lymphocytes. When analyzed by flow cytometry using anti-CD3 and CD20 monoclonal antibodies, 90% of the PHA-stimulated lymphocytes were T cells and 3% were B cells.

Cultures show that low apotransferrin concentrations cause stimulation of neutrophil and macrophage ^{67}Ga uptake, but suppression of both activated and resting lymphocytic ^{67}Ga uptake.

DISCUSSION

Morphometric observations indicate that both plasmocytes and lymphocytes are probably responsible for ^{67}Ga uptake in rheumatoid lung. Plasmocytes are the dominant inflammatory cell in the patient's open lung biopsy. Lymphocytes are only slightly less prevalent than plasmocytes. This suggests that either or both plasmocytes and lymphocytes could cause the high ^{67}Ga index observed. Neutrophils have an extremely low occurrence. Although histocytes occur in moderate numbers, they have a significantly lower occurrence than plasmocytes or lymphocytes.

In vitro culture data support the conclusion that both plasmocytes and lymphocytes are responsible for the high ^{67}Ga index seen in rheumatoid lung. Although plasmocytes

TABLE 1
Differential Inflammatory Cell Count Percentages of Open Lung Biopsy from the Rheumatoid Lung Patient Obtained by Two Different Observers

	Plasmocytes	Lymphocytes	Histocytes	Neutrophils	Eosinophils
Observer 1	42.1	35.2	20.0	2.1	0.6
Observer 2	42.8	31.2	22.5	2.8	0.7

have the lowest ^{67}Ga uptake comparatively, the large plasmocyte burden in rheumatoid lungs could still make this low-level ^{67}Ga uptake significant. Plasmocyte ^{67}Ga uptake is selective because it is suppressed by the human transferrin concentrations used in our cultures and was augmented by the trace transferrin concentrations used by Sephton and Harris when culturing mouse myeloma cells (*5,6*). The comparatively high ^{67}Ga uptake exhibited by our PHA-generated lymphoblasts suggests that the lymphocyte population in rheumatoid lung is also responsible for the patient's high ^{67}Ga index. This is likely because PHA-generated lymphoblasts are analogous to the probable activated immunologic status of lymphocytes seen in rheumatoid lungs. The fact that our PHA-generated lymphoblasts were T- rather than B-derived is not likely to be significant. Parallel behavior in T and B lymphocytes has been demonstrated by Chitambar (*7*).

Our observation that traces of apotransferrin suppress lymphoid cell ^{67}Ga uptake, but stimulate neutrophil and monocyte ^{67}Ga uptake, suggests that ^{67}Ga uptake by lymphoid cells has a different mechanism from the transferrin-lactoferrin gradient previously shown to account for neutrophil ^{67}Ga uptake (*8*). Gallium-67 lymphocytic uptake has previously been demonstrated to be a membrane phenomenon that is not closely related to cell proliferation (*9*). It may not be due solely to transferrin receptors either, because an apparent non-transferrin receptor pathway has been demonstrated (*4*).

TABLE 2
Gallium-67 Uptake by Different Types of Leukocytes

	42-hr cultures*		18-hr cultures†	
	^{67}Ga alone	^{67}Ga + transferrin‡	^{67}Ga alone	^{67}Ga + transferrin§
Neutrophils	6601	1253	26707	35402
Monocytes	13452	1637	10142	12740
Resting lymphocytes	5023	725	5932	4526
Activated lymphoyctes	n.d.	n.d.	18386	13797
Plasmocytes #1¶	677	168	n.d.	n.d.
Plasmocytes #2	900	143	n.d.	n.d.

* Mean of triplicate cpm.

† Mean of duplicate cpm.

‡ Transferrin concentrations of 2 mg/ml with 35% iron saturation is similar to human serum concentrations.

§ 20 μg/ml ferric desaturated transferrin (apotransferrin) as per Harris and Sephton (*6*).

¶ Plasmocytes are from two patients with plasmocyte leukemia.

n.d. = not done.

Dose-dependent transferrin effects on ^{67}Ga uptake have been reported before, but not related to human serum concentrations. Sephton et al. (*5*) used trace quantities of transferrin when compared with our first culture experiment, and obtained uptake stimulation. His later paper (*6*) did show a plateau in ^{67}Ga uptake at higher transferrin concentrations, which were still far below human serum levels.

Comparison of 42- and 18-hr data shows that monocytes have the highest ^{67}Ga uptake at 42 hr, but neutrophils have the highest at 18 hr. Although one explanation for this is decreased neutrophil survival with longer culturing, another augmentative explanation is that longer culturing increases monocyte ^{67}Ga uptake. Culture-time dependent ^{67}Ga uptake of monocytes has been previously reported and correlated with increased cell size and numbers of transferrin receptors (*10*).

In conclusion, it is probable that both rheumatoid lung plasmocytes and lymphocytes account for the elevated computed pulmonary ^{67}Ga uptake index.

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REFERENCES

1. Specht HD, Brown PH, Haines JE, McNeill M. Gallium-67 lung index computerization in interstitial pneumonitis. *J Nucl Med* 1987;28:1826–1830.
2. Line BR, Fulmer JD, Reynolds HY, et al. Gallium-67 citrate scanning in the staging of idiopathic pulmonary fibrosis: correlation with physiologic and morphologic features and bronchoalveolar lavage. *Am Rev Resp Dis* 1978;118:355–65.
3. Clausen J, Edeling CJ, Fogh J. Gallium-67 binding to human serum proteins and tumor components. *Cancer Res* 1974;34:1931–1937.
4. Chitamber CR, Zivkovir Z. Uptake of gallium-67 by human leukemic cells: demonstration of transferrin receptor-dependent and transferrin-independent mechanisms. *Cancer Res* 1987;47:3929–3934.
5. Sephton RG, Harris AW. Gallium-67 citrate uptake by cultured tumor cells, stimulated by serum transferrin. *J Natl Cancer Inst* 1974;54:1263–1266.
6. Harris AW, Sephton RG. Transferrin promotion of ^{67}Ga and ^{59}Fe uptake by cultured mouse myeloma cells. *Cancer Res* 1977;37:3634–3638.
7. Chitamber CR, Seigneuret MC, Matthaeus WG, Lum LG. Modulation of lymphocyte proliferation and immunoglobulin production by transferrin-gallium. *Cancer Res* 1989;49:1125–1129.
8. Weiner R, Hoffer PB, Thakur ML. Lactoferrin: its role as a Ga-67 binding protein in polymorphonuclear leukocytes. *J Nucl Med* 1981;22:32–37.
9. Merz T, Malmud L, McKusick K, Wagner HN. The mechanism of ^{67}Ga association with lymphocytes. *Cancer Res* 1974;34:2495–2499.
10. Andreesen R, Sephton RG, Gadd S, Atkins RC, DeAbrew S. Human macrophage maturation in vitro: expression of functional transferrin binding sites of high affinity. *Blut* 1988;57:77–83.