## Cellular Basis for Elevated Gallium-67 Uptake in Rheumatoid Lung Patient

**TO THE EDITOR:** Specht et al. (*1*) recently suggested that on the basis of cell culture experiments that plasmocytes and lymphocytes are responsible for $^{67}$Ga uptake in a patient with rheumatoid lung disease. While we agree with some of the conclusions, we were surprised that no statistical analysis accompanied the data to determine if the difference in means observed were actually significant. Even the standard deviations were not available. Some of the differences in values appeared significant.

For example, $^{67}$Ga uptake for monocytes cultured for 42 hr in the presence of transferrin (TF) was reduced about eightfold. The $^{67}$Ga uptake in other cell types was also decreased four- to sevenfold. It would be expected that the addition of TF would suppress (at high concentrations, 700 μg/ml) $^{67}$Ga uptake in all cell types except the cultured monocytes. Normally neutrophils, monocytes and lymphocytes do not have high concentrations of TF receptors and thus the TF would act as a chelator and prevent the $^{67}$Ga from being incorporated into the cells. However, culturing monocytes for at least 4 days appears to cause these cells to undergo terminal maturation to macrophages and express TF receptors (*3*).

Andressen and coworkers (*3*) demonstrated that TF stimulated $^{67}$Ga uptake of these monocytes by 3–15-fold, but monocytes cultured for 1 day or uncultured cells did not incorporate measurable amounts of $^{67}$Ga. The shorter time in culture (1.8 days) is a partial explanation for lack of TF-stimulated $^{67}$Ga uptake in monocytes observed by Specht and coworkers (*1*). In addition, these investigators used bovine serum not human serum as did Andressen et al. (*3*) in their culture milieu. Andressen and coworkers argue that only human serum causes true terminal maturation and this results in a 10–100-fold increase in TF receptor number.

When the different types of leukocytes were cultured for 18 hr, neutrophil uptake was the greatest and resting lymphocytes the least. It was stated that TF had an effect on all cell types. However, if the standard deviation of the mean was estimated to be approximately ±10%, using a simple t-test, all the differences observed in the presence of TF were not statistically significant ($p > 0.1$). It would be expected that the addition of TF would have minimal effect (at a low concentration, 20 μg/ml) on $^{67}$Ga uptake in all cell types except activated lymphocytes. Mitogen stimulation of lymphocytes causes a > tenfold increase in TF receptor concentration (*2*). Therefore, it was surprising that TF had no stimulatory effect on the activated lymphocytes and may be related to the concentration of TF used for stimulation or the length of time in culture.

If the inhibitory effect of TF on lymphoid cells was indeed significant this does not preclude lactoferrin (LF) from being involved in $^{67}$Ga uptake in this patient or in lung inflammations. Specht et al. (*1*) have misinterpreted our previous work with regard to the role of LF and $^{67}$Ga uptake in neutrophils (*4*). We originally suggested that LF was a repository for $^{67}$Ga in the neutrophil and not involved in enhancing uptake. Neutrophils have very few LF receptors (*5*) and other data suggest that $^{67}$Ga uptake in this leukocyte is not a receptor-based process (*6*).

In addition, LF and TF are distinct proteins and have distinct receptors. TF, a serum protein, is used for iron transport. LF is found in secretions that bathe the epithelium and in the secondary granules of neutrophils. Due to LF's bacteriostatic and bactericidal activity, it is likely that this protein participates in host defense mechanisms (*7,8*).

In regard to lung inflammations, LF is present in bronchial mucus and synthesized by the glandular acini in the bronchial tree (*7*). Inflammations can cause increased LF concentration at the site due to both synthesis and the presence of neutrophils (*9, 10*). For example, in mice, LF concentration increased fourfold in lung lavage fluid upon bacterial challenge with aerosolized *E. coli* (*11*). Even though there are few (2%) neutrophils in this patient, LF could be present at the site by both increased synthesis and secretion and neutrophil exocytosis, providing the site with this $^{67}$Ga avid molecule. LF also could stimulate $^{67}$Ga uptake in alveolar macrophages, activated lymphocytes and T lymphocytes by a receptor mediated process. These leukocytes do have a large number of LF receptors (*5*).

We believe that the data provided by Specht and coworkers could be useful. While there have been a number of studies on $^{67}$Ga uptake by human neutrophils and lymphocytes (*6*), there have been few reported studies on monocytes or macrophages (*3*). We are unaware of any previous reports on $^{67}$Ga incorporation by plasmocytes. Since uptake studies on these cell types were performed simultaneously, a correlation can readily be made. However, to adequately compare this data, it is essential that at least the standard deviations of the data be presented so that the significance of these results can be determined. We hope that Specht and coworkers will share this data with the nuclear medicine community.

## REFERENCES

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**REPLY:** We are happy to share additional data with the nuclear medicine community from our study on the cellular basis for the elevated $^{67}$Ga computed lung index in a rheumatoid patient. A two-tailed t-test was performed using the three variables: cell type, incubation time and transferrin (TF) presence or absence in 20 combinations. The pertinent data combinations are seen in the accompanying tables of P values. The 42-hr incubation culture data are shown in Table 1, where different TF states of either the same cell type or different cell types are compared. The p values for 22 of the 25 possible combinations range from 0.001 to 0.053. This indicates that most of these data are significant. When considering only TF+ cells incubated for 42 hr, comparisons between neutrophils, monocytes, resting lymphocytes, plasmocytes #1 and plasmocytes #2 gave p values ranging from 0.001 to 0.030 (not illustrated). The only exception was when plasmocytes #1 and #2 were compared, which gave a p value of 0.622 as expected.

The 18-hr culture data are shown in Tables 2 and 3. When considering only TF+ cells of different cell types, five of six possible combinations gave significant P values (Table 2). When considering different cell types that had different TF states, 9 of 12 possible combinations gave significant P values (Table 3). When considering different TF states for the same cell type, the p values ranged from 0.146 to 0.206 (Table 3). Technical and kinetic differences are the reasons for this occurrence. Statistical analysis of our 18-hr incubation culture data shows that some of the observations published in our case report on the effects of apotransferrin may need to be reconsidered. In summary, statistical analysis of the culture data supports the conclusion that both plasmocytes and activated lymphocytes are responsible for the radiogallium uptake in rheumatoid lung.

With regard to the use of human TF, our Materials and Methods section did specify that we added human TF to the culture milieu in addition to fetal calf serum. Fetal calf serum has been used before as a relatively nontransferrin-stimulatory general growth additive to culture media when transferrin-effect experiments are performed (*1,2*). From the work of Harris et al. (*2*), it would *not* be "expected that the addition of transferrin would have minimal effect (at low concentration 20 μg/ml) on $^{67}$Ga uptake in all cell types except activated lymphocytes." They showed that this low concentration promoted optimal uptake of radioiron in mouse myeloma cells after which increasing concentrations of transferrin led to a decline in radioiron uptake and a plateau in radiogallium uptake. They also showed that apotransferrin had the maximal effect by demonstrating a declining radiogallium uptake with increasing iron loading of the apotransferrin (*2*).

With regard to the role of lactoferrin (LF), we should state that the experimental data in most publications, including Dr. Weiner's later paper (*3*), deal primarily with cellular uptake of radiogallium, not extracellular-based LF uptake of radiogallium. The exception is the work of Tsan (*4*). In contrast to his work with bacterially and chemically induced rabbit thigh abscesses, significant extracellular LF is unlikely to be present in our case of rheumatoid lung because this disease is a chronic proliferative inflammation, not an exudative inflammation. It appears that only sequestered lactoferrin (contained in a walled-off abscess) would resist removal by tissue vasculature, especially in the lung with its rich vasculature.

**TABLE 1**
Statistical Comparisons of the Influence of Human Transferrin Absence and Presence at 2 mg/ml with 35% Iron Saturation on $^{67}$Ga Uptake in Cultures of Different Types of Leukocytes at 42-Hr Incubation

| | Tf+ | | | | |
|---|---|---|---|---|---|
| Tf− | Polys | Monos | Rest lymph | Plasm #1 | Plasm #2 |
| Polys | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Monos | 0.005 | 0.006 | 0.004 | 0.004 | 0.004 |
| Rest lymph | 0.005 | 0.006 | 0.002 | 0.002 | 0.002 |
| Plasm #1 | 0.015 | 0.006 | 0.374 | 0.001 | 0.004 |
| Plasm #2 | 0.137 | 0.042 | 0.511 | 0.082 | 0.053 |

**TABLE 2**
Statistical Comparisons of $^{67}$Ga Uptake in Different Types of Leukocytes Cultured for 18 Hours in the Presence of Human Transferrin at 20 μg/ml with 0% Iron Saturation

| | 18-hr incubation | | | |
|---|---|---|---|---|
| 18-hr incubation | Polys | Monos | Resting lymph | Activated lymph |
| Polys | — | 0.020 | 0.002 | 0.008 |
| Monos | 0.020 | — | 0.061 | 0.252 |
| Resting lymph | 0.002 | 0.061 | — | 0.024 |
| Activated lymph | 0.008 | 0.252 | 0.024 | — |

**TABLE 3**
Statistical Comparisons of the Influence of Human Transferrin Absence and Presence at 20 μg/ml with 0% Iron Saturation on $^{67}$Ga Uptake in Cultures of Different Types of Leukocytes at 18-Hr Incubation

| | Tf+ | | | |
|---|---|---|---|---|
| Tf− | Polys | Monos | Resting lymph | Activated lymph |
| Polys | 0.150 | 0.062 | 0.062 | 0.089 |
| Monos | 0.003 | 0.146 | 0.021 | 0.029 |
| Resting lymph | 0.006 | 0.041 | 0.154 | 0.000 |
| Activated lymph | 0.069 | 0.124 | 0.087 | 0.206 |