

CASE REPORT

## Demonstration of Lactoferrin in Tumor Tissue from Two Patients with Positive Gallium Scans

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*We have detected lactoferrin in tumor tissue from a patient with Hodgkin's disease and a patient with Burkitt's lymphoma. Both patients had radiogallium scans demonstrating increased uptake in the tumor tissue subsequently found to contain lactoferrin. Tissue assay for lactoferrin was performed by the indirect immunofluorescence method. Control splenic tissue showed either slight lactoferrin content or none.*

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The exact mechanism of gallium-67 localization in tumors is not known. Previous studies indicate an association between Ga-67 and lactoferrin (1-4). In this report we describe an association of Ga-67 uptake and increased lactoferrin content in Hodgkin's disease and Burkitt's lymphoma.

### CASE REPORTS

**Patient A.** A 36-year-old man was admitted to the hospital with a nontender mass in the right side of the neck, first noted 6 wk before admission. A biopsy of the mass, performed 2 wk before admission, revealed nodular sclerosing Hodgkin's disease.

Physical examination on admission revealed six nontender 1- by 1.5-cm palpable nodules in the right cervical region adjacent to the biopsy scar. Chest radiographs, including tomograms, showed superior mediastinal widening and right hilar masses. A Ga-67 citrate scan was performed using 10 mCi of radionuclide. After administration of bowel purgatives, imaging was performed on the fourth day following injection, using the Anger tomographic scanner. The scan (Fig. 1) revealed abnormal activity in the right neck, both supraclavicular fossae, the right axilla, the mediastinum, and behind the left lobe of the liver. Increased splenic uptake was also noted, but no discrete splenic lesions were seen.

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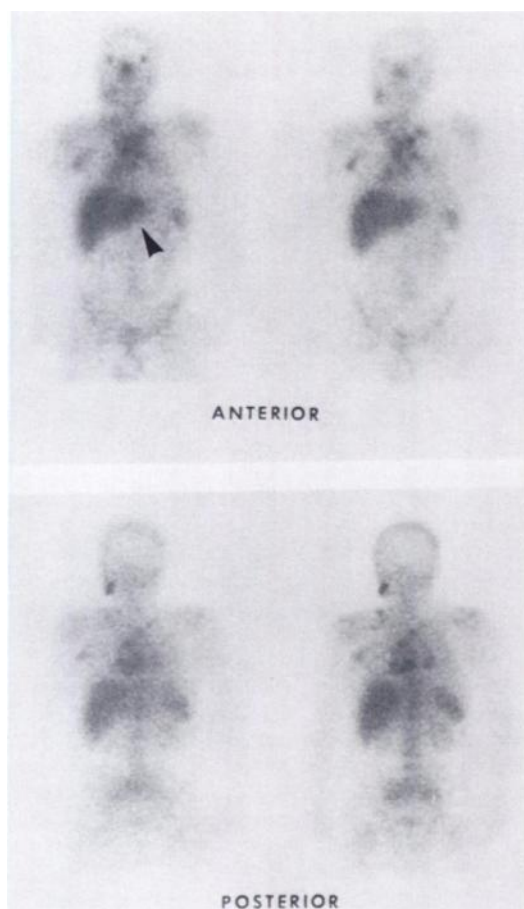
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The day following the scan the patient underwent exploratory laparotomy, with splenectomy and biopsies of liver, bone, and several lymph nodes. Numerous gross nodules were seen in the sectioned spleen; on histologic examination they proved to be nodular sclerosing Hodgkin's disease. Hodgkin's disease was also found in celiac nodes. Other biopsies contained no malignant tissue. Two small pieces of splenic tumor and one piece of normal spleen were quick-frozen in liquid nitrogen for lactoferrin assay.

**Patient B.** A 12-year-old boy was admitted with a 1-mo history of intermittent abdominal pain, fever, a seven-lb weight loss, and a 5-day history of nausea and vomiting. Physical examination revealed abdominal distention and a poorly defined mass involving the entire midabdomen. Scans were made, after appropriate purgation, 24 and 48 hr following injection of 5 mCi of Ga-67 citrate (Fig. 2). A large-field Anger camera with multiple windows and moving table was used for imaging. Increased tracer uptake was seen in the large abdominal mass, as well as in bone lesions in the pelvis and mid-thoracic spine. Two days following the 48-hr scan an exploratory laparotomy was performed. A large tumor mass extending throughout the mesentery was seen. Biopsy sections were obtained. One section was quick-frozen in liquid nitrogen for lactoferrin assay. Histologic examination of the tissues established the diagnosis of Burkitt's lymphoma.

### MATERIALS AND METHODS

The examination of tissue for human lactoferrin (HLF) content was performed in the following manner (5). Rabbit anti-serum



**FIG. 1.** Patient A, age 31, Hodgkin's disease. Ga-67 citrate: 10 mCi + 96 hrs. Four tomographic planes, two ant., two post. Increased tumor uptake seen in involved areas, including right neck, both supraclavicular fossae, mediastinum, right axilla, behind left lobe of liver (arrow) and spleen. Splenic uptake is diffuse, with no focal accumulation.

to human lactoferrin\* (anti-HLF) and goat anti-serum to rabbit IgG (anti-RIgG) labeled with fluorescein<sup>†</sup> were obtained commercially. Phosphate-buffered saline was prepared using analytical-grade reagents: 1.38 g  $\text{NaH}_2\text{PO}_4$ , 1.42 g  $\text{Na}_2\text{HPO}_4$ , 8.78 g NaCl, and one liter distilled water. A HLF-rich whey sample was prepared from mature human milk by centrifugation at 8000 rpm at 4°C for 30 min, followed by skimming off the fat and precipitation of the casein by titration to pH 4.6. The supernatant constituted the HLF-rich whey sample (6).

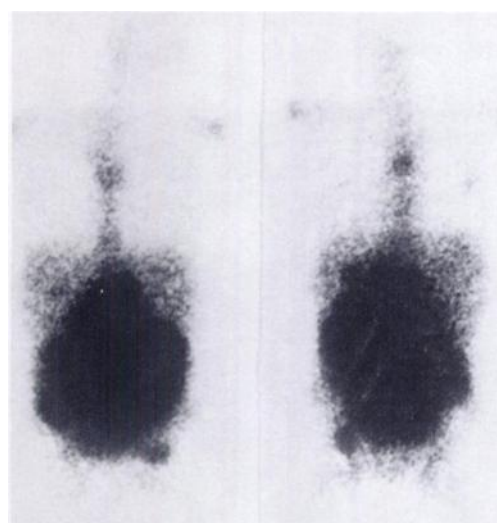
Tissues were prepared by rapid freezing of one- to two-mm tissue cubes to  $-150^\circ\text{C}$ . Sections were cut at 6 microns, kept frozen, and dried overnight in vacuo at  $-30^\circ\text{C}$ . The dry sections were then placed in sealed desiccators and brought to room temperature. Sections were fixed to slides using a thin coat of egg albumin. Sections were treated with one of the following reagents for 30 min: a) rabbit anti-HLF, b) human serum, or c) rabbit anti-HLF plus the HLF-rich whey sample. Each sample was then washed with three changes of phosphate-buffered saline (pH 7.4). The fluorescein-conjugated goat anti-RIgG, used in 1:5 dilution, was then added and incubated for 5 min with the sections treated with rabbit anti-HLF (a), human serum (b), or rabbit anti-HLF plus the HLF-rich whey sample (c). A control was also run with the sample tissue and fluorescein-conjugated anti-RIgG alone. The slides were again washed in three changes

of phosphate-buffered saline. In addition, control fluorescence was determined using smears of whey protein reacted with rabbit anti-HLF and fluorescein-conjugated goat anti-RIgG. The slides were examined with a fluorescent microscope<sup>‡</sup> employing a 200 w/2 mercury lamp and a BG 38 FITC excitation filter combined with a K 510 barrier filter. Sections were also stained with hematoxylin and eosin for routine histologic examination.

The following specimens were examined: from the patient with Hodgkin's disease, (Patient A), pieces of tissue from the splenic tumor nodules and one piece of normal splenic tissue; from the child with Burkitt's lymphoma (Patient B), a single piece of tumor tissue; and as a normal control, a single piece of splenic tissue obtained at autopsy of a 73-year-old man who died of nephrosclerosis.

## RESULTS

Marked fluorescence was observed in the tumor tissues from Patients A and B. The fluorescence occurred primarily in the perivascular regions (Figs. 3 and 4). No fluorescence was seen in the specimen of normal spleen from Patient A, and only slight fluorescence in the splenic tissue from the patient who died of nephrosclerosis (Fig. 5). In the technical controls, no fluorescence was observed in the specimens treated with fluorescein-conjugated RIG alone, or with rabbit anti-HLF and HLF-rich whey followed by fluorescein-conjugated goat anti-RIG (c). Some diffuse nonspecific fluorescence was observed in specimens from Patient A treated with human serum followed by fluorescein goat anti-RIG (b). This fluorescence did not occur when the serum was diluted 1:5 with saline, and was most probably due to the presence of small amounts of anti-human-serum antibodies in the goat anti-RIG (7). The suppression of this cross reactivity by slight dilution, the diffuse nature of the slight fluorescence observed, and the absence of fluorescence in the control splenic tissue from Patient A, indicate that this slight cross-reactivity does not interfere with the interpretation of the assay.



**FIG. 2.** Patient B, age 12, Burkitt's lymphoma, Ga-67: 5 mCi + 48 hr. Ant. (L) and post. (R) views. Diffuse uptake in abdomen corresponds to tumor distribution in mesentery seen at surgery. Bone lesions also seen in pelvis and thoracic spine.



**FIG. 3.** Splenic tumor nodule from Hodgkin's patient examined by indirect fluorescence method using anti-HLF antibody. Marked fluorescence indicates high lactoferrin content. (400 $\times$  magnification).



**FIG. 4.** Tumor tissue from child with Burkitt's lymphoma, examined by indirect fluorescence method using anti-HLF antibody. Lactoferrin content is high. (540 $\times$  magnification).



**FIG. 5.** Normal splenic tissue (autopsy) from nephrosclerotic patient. Tissue prepared exactly as for Figs. 3 and 4 shows only minimal fluorescence; i.e., low lactoferrin content. Uninvolved splenic tissue from Hodgkin's patient (not shown) similarly showed no fluorescence.

#### DISCUSSION

Following i.v. injection, Ga-67 in the blood is found primarily in association with transferrin (8-10). Subsequent tissue localization of Ga-67 is believed to occur as a result of migration of gallium from transferrin to other metal-binding molecules. Lactoferrin is similar to transferrin in molecular weight ( $\sim 80,000$  daltons), but its tissue distribution is significantly different. Tissues and secretions with known high lactoferrin content are observed to concentrate Ga-67—e.g. lacrimal glands and tear fluid, breast (under lactational stimulation) and breast secretions, and polymorphonuclear leukocytes (PMN). Gallium-67 in association with lactoferrin has been demonstrated in breast secretions (1) and tears (3) of patients receiving radiogallium. Gallium-67 is also bound to lactoferrin in PMN (4). Furthermore, transfer of Ga-67 from transferrin to lactoferrin has been demonstrated in vitro (2). The affinity of Ga-67 for lactoferrin is greater than for transferrin, especially at low pH (2).

The purpose of this study was to determine whether tumors known to localize Ga-67 also contained significant quantities of lactoferrin. Increased lactoferrin content in breast tumors has previously been observed, based on tissue absorption of anti-lactoferrin antibody (11), and de Sousa has recently observed increased lactoferrin content in the spleens of patients with Hodgkin's disease (12). The latter study involved no controls.

In this report we demonstrate increased lactoferrin content in tumor tissue from two patients corresponding to areas of increased Ga-67 uptake seen on scan. In view of the previously demonstrated association of lactoferrin and Ga-67, it is highly probable that at least some of the uptake in these tumors is related to their lactoferrin content. However, these observations raise two additional important questions. Why is lactoferrin present in tumor tissue and is it the only gallium-binding molecule in such tissue?

In normal individuals significant amounts of lactoferrin are found primarily in organs associated with secretory activity, the secretions from these organs, and in PMN (13-15). The primary function of lactoferrin relates to its binding affinity for ferric ion. Its presence in secretions acts to diminish the amount of free iron present and thereby inhibit bacterial growth (16). Bacteria and other microorganisms are also capable of producing ferric-ion-binding molecules, called siderophores (17), which facilitate trapping and incorporation of iron. Lactoferrin and siderophores appear to compete for available ferric ion. Both lactoferrin and siderophores (18) also avidly bind gallium, presumably because of its similarity to ferric ion in atomic radius and charge.

In inflammatory lesions, lactoferrin is deposited by PMN and binds to the surface of monocytes and macrophages (19 and personal communication from R. M. Bennett and T. Kokocinski 1977). Its function appears to be to inhibit bacterial growth by competing with siderophores for available ferric ion.

It is likely, therefore, that lactoferrin in tumor tissue is also deposited as a reactive response, rather than by the tumor itself. (A less likely alternative is that the tumor may produce lactoferrin and use it to capture ferric ion.) Recently, Fernandez-Pol (20) has isolated a siderophore-like substance in mammalian tumor tissues grown in iron-deficient culture media. The presence of such an iron-binding molecule in tumor, if confirmed, would help explain the presence of lactoferrin in tumor tissue. The siderophore-like substance would probably bind gallium also and serve to augment Ga-67 uptake in the tumor.

The exact pathophysiologic role of lactoferrin in tumor tissue is still speculative. However, our current studies demonstrate an association between Ga-67 uptake and increased lactoferrin content in tumors of two patients, one with Hodgkin's disease and the other with Burkitt's lymphoma. In view of previous evidence of Ga-67 binding to lactoferrin, this association could account for tumor uptake of the radionuclide. These findings do

not exclude the possibility that a competitive Ga-67 binding molecule may also be present in tumors and contribute to uptake.

## FOOTNOTES

\* The rabbit anti-serum to human lactoferrin (Behring) has been demonstrated by the manufacturer to be monospecific for purified human lactoferrin and nonreactive with the secretory component of IgA, colostrum IgA, and serum IgA by double immunodiffusion technique and to be monospecific for lactoferrin and nonreactive with human serum and plasma. This product was not further purified before use. (Behring Diagnostics, Somerville, NJ, lot 2573 AA)

† Heyland Labs, Travenol Lab, Costa Mesa, CA

‡ Leitz Metrilax

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