Role of Iron-Binding Proteins and Enhanced Capillary Permeability on the Accumulation of Gallium-67

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We studied the role of the iron-binding proteins transferrin and lactoferrin and of increased capillary permeability on the accumulation of gallium-67 in rabbits.

Intramuscular injection of histamine caused accumulation of gallium-67 (injected i.v. as citrate), and of Tc-99m DTPA, at the i.m. injection site. Normal saline and albumin did not. Intramuscular injection of transferrin or lactoferrin similarly caused Ga-67 uptake. No accumulation of Tc-99m DTPA was observed at the site of transferrin injection but there was a slight accumulation at the site of lactoferrin injection. Prior saturation of transferrin or lactoferrin with ferric ion abolished their effect on Ga-67 accumulation. Gallium-67, pre-bound to transferrin in vitro, did not accumulate at the site of histamine or transferrin injection, but there was a slight accumulation at the lactoferrin site.

Our results suggest that either increased capillary permeability or iron-binding proteins can cause local uptake of Ga-67. Since these factors are present at sites of inflammation, they may contribute to the accumulation of gallium in inflammatory lesions.

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In 1969, Edwards and Hayes (*1*) noted that gallium-67 accumulated in involved lymph nodes in Hodgkin's disease. Since then, Ga-67 has been shown to localize in a variety of tumors as well as inflammatory lesions. Some studies (*2,3*) have suggested that Ga-67 uptake by inflammatory lesions is due mainly to its concentration by polymorphonuclear leukocytes (PMN) at the sites of inflammation. However, in vitro studies of Ga-67 uptake have consistently revealed that PMNs do not significantly accumulate Ga-67 unless the plasma membrane's permeability barrier is disrupted (*4–6*). Gallium-67 is also taken up by a variety of microorganisms (*7*), but analysis of abscess contents reveals that most of the Ga-67 is in the noncellular fraction (2,500 *g* supernate) (*4,8*). Furthermore, Ga-67 accumulates in inflammatory lesions of agranulocytic animals (*8*) and patients (*9*), in whom no PMNs are found in the circulation or at the sites of infection. Thus, neither PMNs nor bacteria are essential for the accumulation of Ga-67 in inflammatory lesions. Noting these observations, Tsan et al. (*10,11*) suggest that Ga-67 uptake in inflammatory lesions is primarily due to leakage of protein-bound Ga-67 out of abnormally permeable capillaries.

Since lactoferrin has a higher binding affinity for Ga-67 than transferrin (*12*) and the secondary granules of PMN are rich in lactoferrin, Hoffer (*13*) has suggested that lactoferrin present at the site of inflammation is responsible for the accumulation of Ga-67. However, there has been no direct evidence to support this hypothesis.

In the present study, we investigated the role of iron-binding proteins, such as transferrin and lactoferrin, and of increased capillary permeability, on the accumulation of Ga-67.

MATERIALS AND METHODS

Materials. Gallium-67 citrate (carrier-free), Tc-99m DTPA, histamine, human albumin, transferrin,* and

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lactoferrin† were obtained from commercial sources. Human transferrin was essentially iron-free, but the lactoferrin preparation contains 5.8 mg of iron per 100 g of lactoferrin.

Nuclear imaging. Female New Zealand white rabbits weighing between 3 and 4 kg were used. A 4-ml solution containing histamine, albumin, transferrin, or lactoferrin in normal saline was injected intramuscularly into the inner aspect of the upper half of one thigh. The concentration of histamine used was 0.5 mg/ml, while that of albumin, transferrin, or lactoferrin was 4 mg/ml. Gallium-67 (500 μCi) or Tc-99m DTPA (diethyltriaminepentaacetic acid, 1.2 mCi) was then injected into a marginal ear vein. When Ga-67 was used, the rabbits were imaged for 20 min with a scintillation camera at 3, 6, 12, 24, and 48 hr after injection. With Tc-99m DTPA the images were made at 3, 6, and 12 hr after injection.

Data processing and quantification. A computer was used to analyze the images. Quantification was performed with joystick to choose the regions of interest. The noninjected thigh was used as a control. The average background counts per pixel were calculated for both the injected side (BKG-i) and the contralateral (BKG-c). The background regions were chosen as close to the thighs as possible. The region of interest was then drawn at the site of injection, with area fixed at 36 pixels. Average counts per pixel at the injection site (CPU-i) were obtained; then a control region of interest (also 36 pixels) was drawn at a corresponding area on the contralateral thigh, and the average counts/pixel (CPU-c) obtained there. The injected/control ratio was calculated by the following formula:

$$\text{Injected/control ratio} = \frac{(\text{CPU-i}) - (\text{BKG-i})}{(\text{CPU-c}) - (\text{BKG-c})}$$

Measurement of unbound iron-binding capacity (UIBC). This was performed using the Res-O-Mat Fe-59 Diagnostic kit.

Saturation of transferrin or lactoferrin with iron. This was performed as follows. Aliquots of 0.1 ml of 0.01 *N* HCl containing 0.55 mg of $FeCl_3$ (2.5 μM) were added to 5 ml of normal saline containing 20 mg of transferrin or lactoferrin (about 0.23 μM). After mixing for 30 min at room temperature, the pH of the solution was adjusted to 7.4.

Preparation of transferrin-bound gallium-67. Gallium-67 (4 mCi) was added to 4 ml of normal saline containing 120 mg of transferrin and 0.4 m*M* of $NaHCO_3$. After incubation for 8 hr at room temperature with constant agitation, the mixture was dialyzed against 2.5 liters of normal saline for 12 hr. At the end of incubation, about 500–700 μCi of Ga-67 remained with transferrin. Five hundred microcuries of transferrin-bound Ga-67 were used in each experiment.

RESULTS

Effect of intramuscular injection of histamine, transferrin, or lactoferrin on the accumulation of gallium-67. To determine the role of increased capillary permeability on the accumulation of Ga-67, histamine was injected intramuscularly followed by i.v. Ga-67. The injection caused local edema due to capillary leakage of plasma

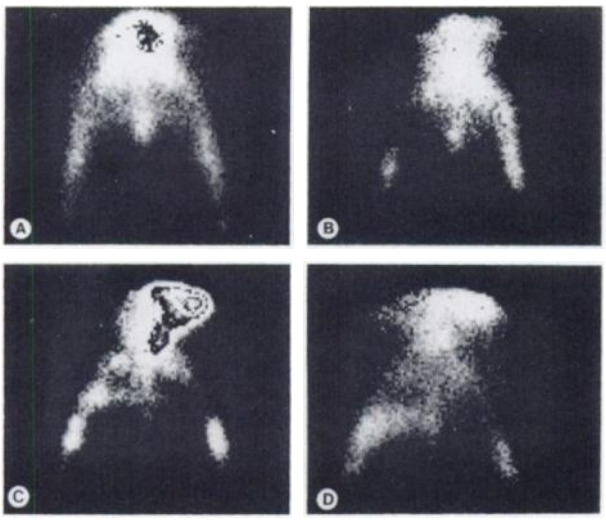

FIG. 1. Representative images, of caudal half of rabbits, showing effects of i.m. injection of normal saline, histamine, transferrin, or lactoferrin on accumulation of Ga-67. Images were made 6 hr after i.v. injection of Ga-67. (A) Normal saline, (B) histamine; (C) transferrin; (D) lactoferrin. With the exception of histamine, all agents were injected into right thigh (viewer's right).

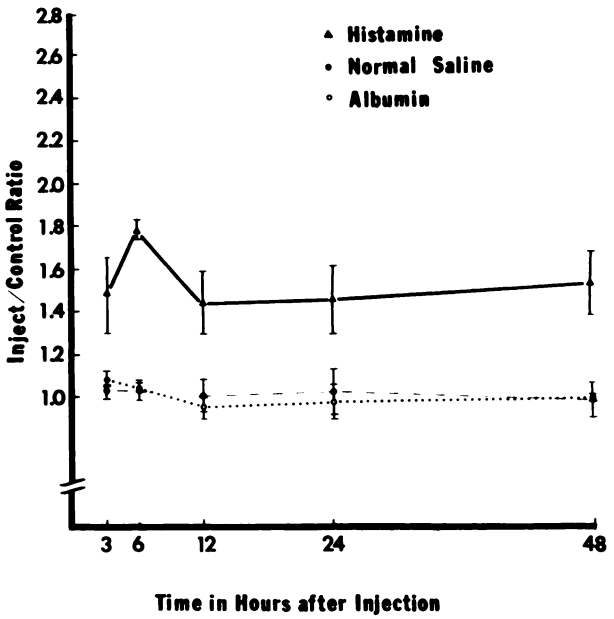

FIG. 2. Effect of i.m. injection of histamine, albumin, and normal saline on accumulation of Ga-67 injected i.v. as citrate. Histamine (2 mg/4 ml), normal saline (4 ml), or albumin (16 mg/4 ml) was injected into thigh of one leg. Contralateral thigh was used as control. Each point gives mean and range of three experiments.

components. Transferrin and lactoferrin bind Ga-67 (*12,14*); their effects were also studied. As shown in Fig. 1, intramuscular injection of histamine, transferrin, or lactoferrin (but not normal saline) caused an accumulation of intravenously injected Ga-67. These representative images were taken 6 hr after the injections. Except with saline (A), the Ga-67 uptake at each injection site is obvious. Quantitative versions of the results are shown in Figs. 2 and 3. The Ga-67 trapped at the sites of histamine, transferrin, or lactoferrin injection, but not at those of normal saline or albumin, persisted throughout the 48-hr study period.

Effect of intramuscular injection of histamine, transferrin, or lactoferrin on the accumulation of Tc-99m DTPA. This agent does not penetrate the plasma membrane and is widely used as an extracellular agent. In order to determine whether the above observed Ga-67 uptake was due to increased capillary permeability and expanded extracellular space, we studied the effect of i.m. injection of histamine, transferrin, or lactoferrin on the accumulation of Tc-99m DTPA. As shown in Fig. 4, i.m. injection of histamine—but not of normal saline or transferrin—caused a marked accumulation of i.v. injected Tc-99m DTPA, whereas lactoferrin caused a more modest accumulation. Our lactoferrin thus caused a slight increase of the extracellular space, and whether this is due to lactoferrin or contaminants in the preparation is currently not clear.

Gallium uptake after prior saturation of the iron-binding capacities of transferrin and lactoferrin. There is ample evidence that transferrin and lactoferrin bind

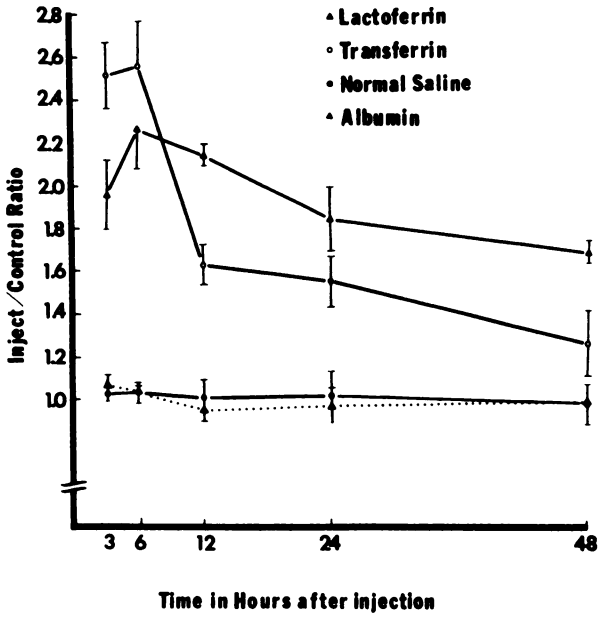

FIG. 3. Effect of i.m. injections of transferrin and lactoferrin on accumulation of i.v. Ga-67 citrate. Concentrations: transferrin 16 mg/4 ml, lactoferrin 16 mg/4 ml. Each point gives mean and range of three experiments.

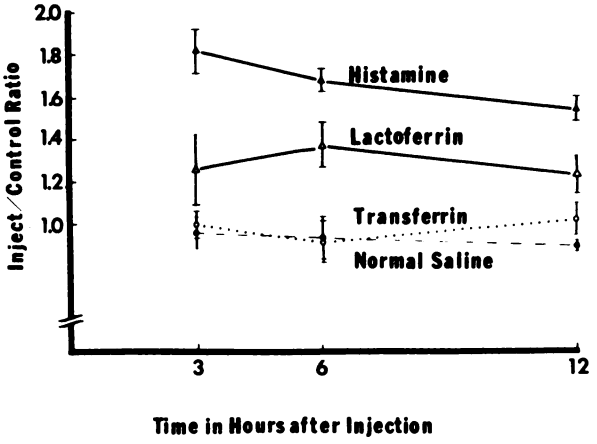

FIG. 4. Effect of i.m. injection of histamine, lactoferrin, and transferrin on accumulation of i.v. injected Tc-99m DTPA. Each point gives mean and range of three experiments.

iron and gallium at the same binding sites (*12,14*). In order to determine whether our observed Ga-67 uptake associated with transferrin or lactoferrin was due to the iron-binding capacities of these two proteins, they were saturated with iron before their intramuscular injection. The unbound iron-binding capacities (UIBC) of transferrin and lactoferrin at the concentration used (4 mg/ml) were 490 μg/dl and 190 μg/dl, respectively. As shown in Fig. 5, saturation of the UIBC of transferrin and lactoferrin completely abolished their effect on the accumulation of Ga-67. However, there were quantitative differences. At an UIBC of 96 μg/dl, transferrin failed to accumulate Ga-67, whereas at an UIBC of 75 μg/dl, lactoferrin still caused a slight gallium uptake. When the UIBC of lactoferrin was reduced to 42 μg/dl, however, it failed to cause Ga-67 uptake.

Effect of intramuscular injection of histamine, transferrin, or lactoferrin on the accumulation of transferrin-bound gallium-67. Lactoferrin is present in the secondary granules of neutrophils, and since it binds Ga-67 more strongly than does transferrin, it has been suggested that transferrin-bound gallium in the plasma might transfer to the lactoferrin in an inflammatory lesion, thus causing the uptake (*13*). In order to test this possibility, Ga-67 was bound to transferrin before injection, and the effect of intramuscular histamine, transferrin, or lactoferrin on the uptake of the bound Ga-67 was studied. The results are shown in Fig. 6, with the behavior of free Ga-67 included for comparison. The already transferrin-bound gallium fails to concentrate near intramuscular histamine or transferrin, although i.m. lactoferrin causes mild Ga-67 uptake. It is clear, however, that the lactoferrin will cause a much stronger uptake if the gallium is injected unbound.

DISCUSSION

Our present results suggest that either increased

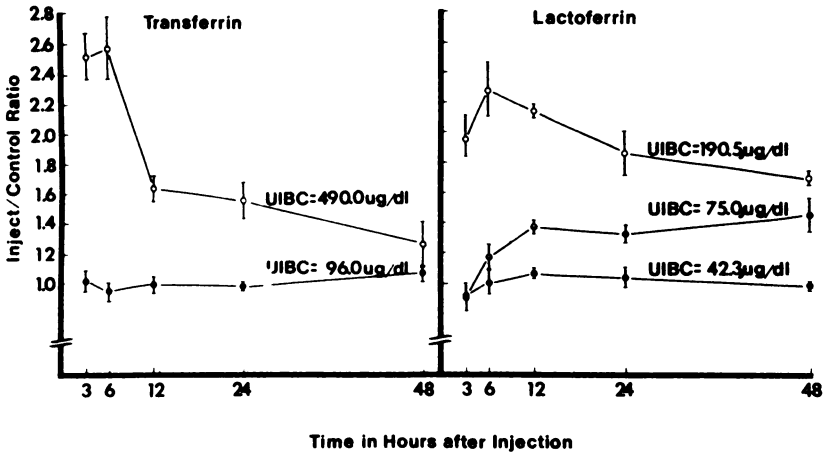

FIG. 5. Effect of the saturation of iron-binding capacities of transferrin and lactoferrin on accumulation of Ga-67. Each point gives mean and range of three experiments.

capillary permeability, or iron-binding proteins such as transferrin or lactoferrin alone, can cause accumulation of intravenously injected Ga-67. Its accumulation at the site of histamine injection was primarily due to the enhanced capillary permeability and resultant expanded extracellular space, since histamine also caused accumulation of Tc-99m DTPA, an extracellular agent. Failure of histamine to cause accumulation of transferrin-bound Ga-67 suggests that the transferrin-gallium complex does not diffuse across the capillary membrane very well, even at a site of enhanced capillary permeability. This finding is consistent with that of Sheldon et al. (*15*), who observed that the transferrin concentration in inflammatory exudates was much lower than that in serum.

The accumulation of Ga-67 at the site of transferrin or lactoferrin injection was largely due to the iron-binding sites on these two proteins, since their prior saturation with ferric iron abolished their effects. The initial accumulation of Ga-67 at the site of transferrin injection was higher than that of lactoferrin (Fig. 3). This is probably due to the higher iron-binding capacity of the transferrin preparation used (Fig. 5). The accumulation of Ga-67 at the site of transferrin injection declined 12 hr after injection, whereas that of lactoferrin remains high throughout the study period of 48 hr. This may be because gallium binds to lactoferrin more strongly than to transferrin. The results presented here suggest that transfer of Ga-67 from transferrin to lactoferrin can occur in vivo, since lactoferrin caused Ga-67 uptake even if the gallium were injected transferrin-bound. However, one should be cautious in extrapolating this observation to the accumulation of Ga-67 in inflammatory lesions and tumors. The secondary granules of neutrophils are rich in lactoferrin (*16,18*), but analysis of radioactivity in abscesses reveals that only a minor fraction of Ga-67 is associated with intact neutrophils (*4,8*). Thus the mere presence of lactoferrin in cells does not ensure that the cells will accumulate Ga-67.

In the current study, lactoferrin was injected intramuscularly and was primarily in the interstitial fluid rather than inside the cells. At the site of inflammation, lactoferrin is probably present in exudate, being discharged from the granules of nonviable PMNs during their lysis. Thus the trapping of Ga-67 by this free lactoferrin may play a role in the localization of Ga-67 in inflammatory lesions. Transferrin is also present in small quantity in inflammatory exudates (*15*). However, we do not know the iron-binding capacity of the transferrin or lactoferrin present in inflammatory exudates. It remains to be shown that Ga-67 in inflammatory exudates is transferrin- or lactoferrin-bound.

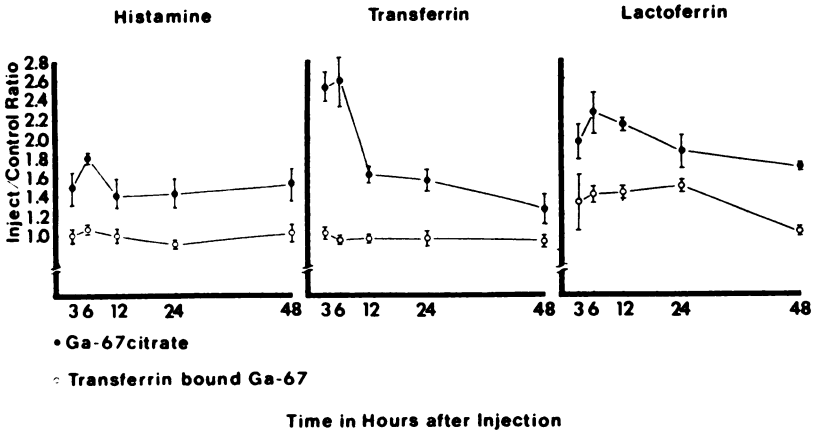

FIG. 6. Effect of i.m. injection of histamine, transferrin, and lactoferrin on accumulation of i.v. injected transferrin-bound Ga-67. Each point gives mean and range of three experiments.

FOOTNOTES

* Human transferrin, Sigma Chemical Company, St. Louis, MO.

† Human lactoferrin, Calbiochem-Schering Corporation, CA.

‡ Mallinckrodt Nuclear, St. Louis, MO.

ACKNOWLEDGMENT

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REFERENCES

1. EDWARDS CL, HAYES RL: Tumor scanning with ^{67}Ga citrate. *J Nucl Med* 10:103-105, 1969
2. BURLESON RL, JOHNSON MC, HEAD H: Scintigraphic demonstration of experimental abscesses with intravenous ^{67}Ga citrate and ^{67}Ga labeled blood leukocytes. *Ann Surg* 178:446-452, 1973
3. GELRUD LG, ARSENEAU JC, MILDER MS, et al: The kinetics of ^{67}gallium incorporation into inflammatory lesions: experimental and clinical studies. *J Lab Clin Med* 83:489-495, 1974
4. TSAN MF, CHEN WY, SCHEFFEL U, et al: Studies on gallium accumulation in inflammatory lesions: I. Gallium uptake by human polymorphonuclear leukocytes. *J. Nucl Med* 19:36-43, 1978
5. MCAFEE JG, THAKAR ML: Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. I. Soluble agents. *J Nucl Med* 17:480-487, 1976
6. ENGLISH D, CLAIROUX-MOREAU S, MCPHERSON TA: Uptake of ^{67}Ga citrate by physiologically impaired cells in vitro. *Clin Res* 25:693A, 1977
7. MENON S, WAGNER HN, TSAN MF: Studies on gallium accumulation in inflammatory lesions: II. Uptake by *Staphylococcus aureus:* Concise communication. *J Nucl Med* 19:44-47, 1978
8. TSAN MF: Studies on gallium accumulation in inflammatory lesions: III. Roles of polymorphonuclear leukocytes and bacteria. *J Nucl Med* 19:492-495, 1978
9. DHAWAN VM, SZIKLAS JJ, SPENCER RP: Localization of Ga-67 in inflammations in the absence of circulating polymorphonuclear leukocytes. *J Nucl Med* 19:292-294, 1978
10. TSAN MF, CARMARGO EE, WAGNER HN JR. Gallium accumulation in inflammatory lesions. Second International Congress of the World Federation of Nuclear Medicine and Biology. Washington, DC, 1978, p 83 (abst)
11. TSAN MF, SCHEFFEL U: Gallium-67 accumulation in inflammatory lesions. *J Nucl Med* 20:173, 1979
12. HOFFER PB, HUBERTY J, KHAYAM-BASHI H: The association of Ga-67 and lactoferrin. *J Nucl Med* 18:713-717, 1977
13. HOFFER PB: The utility of gallium-67 in tumor imaging: A comment on the final reports of the cooperative study group. *J Nucl Med* 19:1082-1084, 1978
14. GUNASEKERA SW, KING LJ, LAVENDER PJ: The behavior of tracer-gallium-67 towards serum proteins. *Clin Chim Acta* 39: 401-406, 1972
15. SHELDON WH, MILDVAN D, ALLEN JC: Some serum protein and cellular constituents of inflammatory lesions. Collection of exudates in a chamber adhered over skin wounds of rabbits, *Johns Hopkins Med J* 121:113-133, 1967
16. MASSON PL, HEREMANS JF, SCHONNE E: Lactoferrin, an iron-binding protein in neutrophilic leukocytes. *J Exp Med* 130:643-656, 1969
17. SPITZNAGEL JK, DALLDORF FG, LEFFEL MS, et al: Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. *Lab Invest* 30:774-785, 1974
18. RAUSCH PG, PRYZWANSKY KB, SPITZNAGEL JK: Immunocytochemical identification of azurophilic and specific granule markers in the giant granules of Chediak-Higashi neutrophils. *N Engl J Med* 298:693-698, 1978