Studies on Gallium Accumulation in Inflammatory Lesions: III. Roles of Polymorphonuclear Leukocytes and Bacteria

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The role of polymorphonuclear leukocytes (PMN) and bacteria in the accumulation of gallium-67 in inflammatory lesions was studied using an animal model. A plastic practice golf ball was implanted in the subcutaneous tissue of the abdominal wall of rabbits, and sterile or bacterial inflammation was induced inside the ball. Gallium accumulated in the inflammatory exudates induced by either sterile casein or* Staphylococcus aureus. *Two rabbits were made agranulocytic by prior treatment with myleran before the injection of* S. aureus *and gallium. These also accumulated gallium, although in one agranulocytic rabbit there were no PMN in the inflammatory exudate. Analysis of the inflammatory exudates showed that most of the gallium was in the noncellular fraction (2,500-g supernatant) in both sterile and bacteria-induced inflammation. The results indicate that gallium accumulates in the inflammatory lesions, even in the absence of either PMN or bacteria.

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Accumulation of gallium in abscesses and inflammatory lesions has been well documented (*1–4*), but its mechanism remains unclear. We have recently demonstrated that gallium is taken up by polymorphonuclear leukocytes (PMN) and a variety of microorganisms (*5,6*). In the present study, we used an animal model to analyze the roles of PMN and bacteria in the accumulation of gallium in inflammatory lesions.

MATERIALS AND METHODS

Female New Zealand white rabbits, weighing between 3 and 4 kg, were used. Under general anesthesia (intramuscular injection of a mixture of ketamine HCl and promazine HCl), a plastic practice golf ball* (a shell containing many holes) was implanted in the subcutaneous tissue of the abdominal wall of each of eight rabbits. Seven to ten days later three of these rabbits were found to be infected, with wound dehiscence and partial extrusion of the ball. Only those rabbits without evidence of wound infection were used for subsequent studies; in them the balls were completely sealed by overlying tissues and no exudate could be obtained from the cavity. Rabbits 1 and 2 were treated with daily intraperitoneal injections of 30 mg/kg myleran (100 mg/ml, in 0.25% methylcellulose), until they developed agranulocytosis. Daily hematocrit values, white blood cell, and differential counts were obtained.

The rabbits were divided into three groups.

1. In each of two rabbits, nos. 5 and 6, a solution containing 30 ml of 1.5% sterile casein in normal saline was injected into the cavity of the implanted ball.

2. In rabbit 8, 25 ml of a *S. aureus* suspension (ATCC 25923, 10^9/ml in normal saline) were injected.

3. Twenty ml of a *S. aureus* suspension containing similar numbers of bacteria was introduced into the ball cavity of the myleran-treated (agranulocytotic)

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FIG. 1. Rectilinear scan of (agranulocytic) rabbit 2, in which inflammation was induced by *S. aureus*.

rabbits 1 and 2 as soon as their neutrophils disappeared from the circulation.

After the introduction of the sterile casein or *S. aureus* suspension into the cavity, 400–500 μCi of carrier-free Ga-67 was injected intravenously through a marginal ear vein. Twenty-four hours later, the rabbits were scanned with a rectilinear scanner. Blood was obtained by cardiac puncture, and inflammatory exudate was obtained from the cavity of the ball with a sterile syringe and needle at desired intervals. The radioactivity of the blood and inflammatory exudates was counted in an automatic gamma well counter with a window setting of 80–320 keV. A standard solution of Ga-67 was prepared at the time of injection and was counted at the same time as the above specimens, and with the same geometry. The blood level was estimated using 4.5% of the body weight as the blood volume. (This has been measured previously in this laboratory using Cr-51-labeled red cells and I-131-labeled albumin). The exudate-to-blood ratio was obtained by counting 1 ml of the exudate and 1 ml of the blood.

A portion of the inflammatory exudates was centrifuged at 2,500 g for 10 min at 4°C. The pellet was washed twice with modified Hanks' solution (*7*). Radiogallium in the 2,500-g supernate and pellet was determined as described above. The rest of the inflammatory exudate was used to determine the number of PMN; also the number of *S. aureus* using a pour-plate method described previously (*8*). The viability of PMN in the inflammatory exudates was determined by the trypan-blue dye exclusion test (*9*).

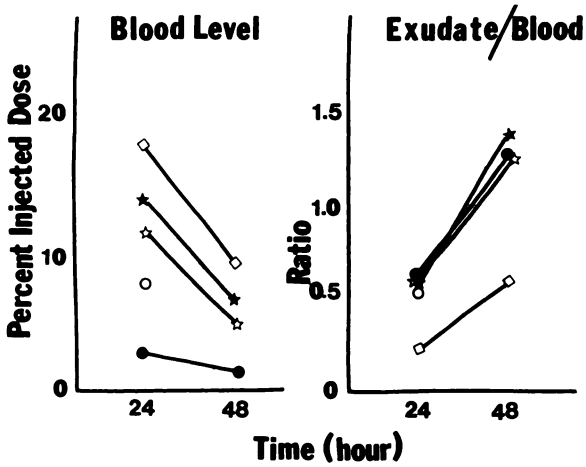

FIG. 2. Radiogallium blood levels and exudate/blood ratios in experimentally induced inflammatory lesions. Casein-induced: ★ rabbit 5; ☆ rabbit 6. *S. aureus*-induced: □ rabbit 8; ○ rabbit 1; ● rabbit 2. (Rabbits 1 and 2 were without circulating neutrophils, due to prior treatment with myleran.)

RESULTS

Figure 1 shows a total-body rectilinear scan of rabbit 2, 24 hr after the injection of *S. aureus* into the cavity of the implanted ball and i.v. injection of Ga-67. This rabbit had been treated previously with daily intraperitoneal injections of myleran, and at the time of this study its total white blood cell count was 1,050/mm³, with no circulating neutrophils (agranulocytosis). As the scan shows, most of the radioactivity was localized at the area corresponding to the implanted ball. Some activity also was noted in the area of liver and pelvis. Similar scan patterns were observed in all five rabbits studied, whether they were injected with sterile casein or bacteria, and whether they were treated with myleran or not.

The blood levels of Ga-67, and the exudate-to-blood ratios, are shown in Fig. 2. There was a marked variation in the blood level among different rabbits, but the blood level at 48 hr after injection was about half that at 24 hr. (Rabbit 1 died after 24 hr.) In contrast, the exudate-to-blood ratio more than doubled during this interval.

Table 1 shows the distribution of Ga-67 in the casein-induced inflammatory exudates of rabbits 5 and 6. There were 9–30 million PMN per milliliter of exudate, of which more than 98% were viable, and there was no bacteria on culture. More than 80% (84–92%) of the radioactivity was in the 2,500-g supernate, which did not contain cellular elements.

Table 2 summarizes the results of the *S. aureus*-induced exudates. Rabbit 8 was not treated with myleran, and *S. aureus* induced an intense inflammatory reaction. Twenty-four hours after injection,

TABLE 1. DISTRIBUTION OF Ga-67 IN CASEIN-INDUCED INFLAMMATORY EXUDATES*

Rabbit No.	Time† (hr)	Radioactivity (%) supernate (2,500 g)	Radioactivity (%) pellet	PMN in exudate
5	24	92	8	20×10^6/ml
	48	84	16	10×10^6/ml
6	24	85	15	16×10^6/ml
	48	88	12	9×10^6/ml

* All exudates were sterile upon culture.
† Time indicates hours after injections of casein and Ga-67.

there were 60 million PMN per milliliter of exudate, of which more than 98% were viable, and there were 5.2 million *S. aureus* per milliliter of exudate. Ninety percent of the radioactivity was in the 2,500-g supernate. At 48 hr, there were 120 million PMN per milliliter exudate, of which 20% were nonviable, and there were 300,000 *S. aureus* per milliliter exudate. Only 53% of the radioactivity was in the 2,500-g supernate. Rabbits 1 and 2 were pretreated with myleran, and at the time of this study there were no neutrophils in the circulation. Rabbit 1 had no PMN in the exudate and died 24 hr after injection. There were 1.7 million *S. aureus* per milliliter of exudate. Rabbit 2 was able to mobilize 0.9–5 million PMN per milliliter of exudate, of which more than 98% were viable, and there were 0.23–4.9 million *S. aureus* per milliliter exudate. In these two rabbits, more than 80% (80–90%) of the radioactivity was in the 2,500-g supernate.

DISCUSSION

The purpose of this study was to determine the role of PMN and bacteria in the accumulation of Ga-67 in inflammatory lesions. The animal model used is particularly suited to this purpose. The plastic practice golf ball has multiple holes allowing easy access to its cavity. Seven to ten days after implantation in the subcutaneous tissue, the holes are sealed by the overlying tissue. This creates a closed space that has direct contact with surrounding tissue. The model has the advantage of allowing us to introduce various substances into the cavity and to sample repeatedly from the cavity for analysis.

Casein is a phosphoprotein occurring in milk, beans, and nuts. It is widely used in experimental animals to induce sterile peritonitis for the procurement of PMN. In this study, casein induced a sterile exudate in the cavity of the implanted balls with 9–20 million PMN per milliliter of exudate. These exudates accumulated Ga-67, suggesting that bacteria are not necessary for the localization of Ga-67 in inflammatory lesions. On the other hand, in two rabbits, in whom agranulocytosis had been induced previously, injection of *S. aureus* induced accumulation of Ga-67 in the cavity. In one of these rabbits (rabbit 1) there were no PMN present in the exudate, suggesting that PMN also are not necessary for the accumulation of Ga-67. One could argue that there might be some PMN accumulated in the cavity after the injection of *S. aureus*, but at the time of our analysis (24 hr after injection) all of them were lysed after engulfing enormous numbers of bacteria. Although we are unable to rule out this possibility, this observation at least suggests that overwhelming accumulation of PMN at the site of inflammation is not required for Ga-67 accumulation. In rabbit 2, there were some PMN in the exudate, suggesting that this rabbit still had some bone-marrow reserve, although during the course of this study no neutrophils could be seen in the circulation.

The numbers of *S. aureus* decreased dramatically 24 hr after injection into these two agranulocytic rabbits. This reduction in the number of bacteria could not be due to dilution alone, secondary to outpouring of exudate into the cavity. It has been reported that among several laboratory animals, the rabbit is most resistant to the development of staphylococcal abscess (*10*). Moreover, rabbit serum kills

TABLE 2. DISTRIBUTION OF Ga-67 IN *S.-AUREUS*-INDUCED INFLAMMATORY EXUDATES*

Rabbit No.	Time† (hr)	Radioactivity (%) supernate (2,500 g)	Radioactivity (%) pellet	PMN in exudate	*S. aureus*
8	24	90	10	60×10^6/ml	5.2×10^6/ml
	48	53	47	120×10^6/ml	5.2×10^6/ml
1	24	90	10	0	1.7×10^6/ml
2	24	80	20	5×10^6/ml	4.9×10^6/ml
	48	85	15	0.9×10^6/ml	0.23×10^6/ml

* Rabbits 1 and 2 lacked circulating neutrophils due to prior treatment with myleran.
† Time indicates hours after injection of *S. aureus* and Ga-67.

S. aureus (*10*). Thus, some humoral mechanism may be responsible for the destruction of *S. aureus* in these two rabbits.

Gelrud and coworkers (*4*) have studied the kinetics of Ga-67 accumulation in experimentally induced inflammatory lesions in monkeys. They reported that in two neutropenic monkeys, there was either no localization or delayed accumulation of Ga-67 at inflammatory sites, and concluded that localization of Ga-67 within areas of inflammation was chiefly mediated by binding of the radionuclide within granulocytic inflammatory cells (*4*). However, they scanned their subjects only up to 2 hr after i.v. injection of Ga-67. No analyses of the abscesses were carried out to determine the actual localization of the radioactivity. In addition, their neutropenic monkeys had absolute neutrophil counts of 1,125/mm^3 and 1,500/mm^3, respectively, both of which are adequate for mobilization of PMN into sites of inflammation.

The majority of the radioactivity present in our exudates was in the 2,500-g supernate, whether the inflammation was induced by casein or *S. aureus*. This confirms our previous observations of turpentine- and *E. coli*-induced muscle abscesses (*5*). In rabbit 8, more Ga-67 was associated with the cellular fraction in the exudate 48 hr after injection. Although this is partly due to the more numerous PMN in the exudate, the most likely explanation is that 20% of these PMN were nonviable. We have previously demonstrated that nonviable PMN take up much more Ga-67 than intact, viable cells (*5*).

The exact mechanism of Ga-67 accumulation in inflammatory lesions remains unclear. Since most of the Ga-67 present in the exudate is in the noncellular fraction, it is possible that some components of the exudate bind Ga-67 preferentially. Recently, Hoffer and coworkers (*11*) demonstrated that lactoferrin has higher gallium-binding ability than transferrin. Whether lactoferrin plays a role in Ga-67 accumulation in inflammatory lesions needs further investigation.

FOOTNOTE

* Whiffle Ball Inc., Shelton, Conn.

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