Development of a $^{68}$Ge/$^{68}$Ga Generator System Using Polysaccharide Polymers and Its Application in PET Imaging of Tropical Infectious Diseases

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ABSTRACT: Gallium-68 ($^{68}$Ga) is a positron emitter for clinical positron emission tomography (PET) applications that can be produced by a $^{68}$Ge/$^{68}$Ga generator without cyclotron. However, commercially available $^{68}$Ge/$^{68}$Ga generator systems require multiple steps for the preparation of $^{68}$Ga radiopharmaceuticals and are sometimes plagued by metallic impurities in the $^{68}$Ga eluent. We developed a $^{68}$Ge/$^{68}$Ga generator system using polysaccharide-based adsorbents and direct application of the generator-eluted $^{68}$Ga-citrate to PET imaging of tropical infectious diseases. N-Methylglucamine (MG) as a $^{68}$Ge-adsorbing unit (Sepha-MGs) was introduced to a series of Sephadex G-10, G-15, G-25, G-50, and G-75. In the batch method, over 97% of the $^{68}$Ge in the solution was adsorbed onto the Sepha(15)-MG packed columns and 70−80% of the $^{68}$Ga was eluted by 1 mL of 0.1 M trisodium citrate with low $^{68}$Ge contamination (<0.001%). The chemical form of the generator-eluted $^{68}$Ga solution was identified as $^{68}$Ga-citrate. In PET studies, affected regions in mice infected with Leishmania and severe fever with thrombocytopenia syndrome virus were clearly visualized using the $^{68}$Ga-citrate. Sepha-MGs are useful adsorbents for $^{68}$Ge/$^{68}$Ga generator systems with high $^{68}$Ga elution efficiency and minimal $^{68}$Ge breakthrough. These results indicated that eluted $^{68}$Ga-citrate can be directly used for PET imaging of infectious sites in mice. This novel generator system may be useful for straightforward PET imaging of infection in clinical practice.

INTRODUCTION

Positron emission tomography (PET) is a powerful molecular imaging tool for detection of various molecular events in living subjects with high sensitivity. Gallium-68 ($^{68}$Ga) is a positron emitter for clinical PET applications.1,2 Because $^{68}$Ga radio-nuclide can be produced by a $^{68}$Ge/$^{68}$Ga generator, an on-site cyclotron is not necessary, which allows the production of desired $^{68}$Ga radiopharmaceuticals on demand. Its 67.71 min half-life also renders its use practical, allowing it to be bound to low-molecular-weight compounds, peptides, certain fragments of antibodies, and their derivatives.3,4 Several $^{68}$Ge/$^{68}$Ga generators are commercially available and used for clinical and/or research purposes, with matrices based on TiO$_2$ (Eckert-Ziegler GmbH, Berlin, Germany), SnO$_2$ (iThemba, Johannesburg, South Africa), or silica resin (ITG GmbH, Munich, Germany).5 The $^{68}$Ga eluent from these generators can be used for the production of various $^{68}$Ga-labeled radiopharmaceuticals, such as $^{68}$Ga-DOTA-TOC for detection of somatostatin receptor-expressing cancer tissues.1,3−6 However, TiO$_2$- and SnO$_2$-based generators have several drawbacks such as low concentration of $^{68}$Ga in the eluate, contamination with long-half-life parent $^{68}$Ge, and contamination with metallic elements including Zn(II), Ti(IV), and Fe(III). Although these disadvantages can be eliminated by several concentration and purification procedures, complicated apparatuses and operations are needed that may present obstacles to routine clinical use.7−10 Because silica-based resin has a low affinity for the metallic elements, this type of generator has potential as a next-generation system for clinical use.11,12

Received: February 8, 2017
Accepted: March 31, 2017
Published: April 11, 2017
of these generators require HCl solution to elute the $^{68}$Ga, thereby necessitating multiple synthetic and purification steps for the production of $^{68}$Ga radiopharmaceuticals.\(^{13}\) It would be ideal if the generator-eluted $^{68}$Ga solution could directly be used as a radiopharmaceutical similarly to $^{99}$Mo/$^{99m}$Tc.

Previously, we found that the commercially available organic copolymer Diaion CRB-02 (R-MGlu, Mitsubishi Chemical Company, Tokyo, Japan) has high affinity for $^{68}$Ge and low affinity for $^{68}$Ga. CRB-02 is a macroporous styrene-divinylbenzene copolymer containing 1-deoxy-1-(methylamino) sorbitol (N-methylglucamine, MG). It has been demonstrated that the MG groups in CRB-02 are responsible for the strong $^{68}$Ge adsorption and high $^{68}$Ga separation efficiency.\(^{14}\) In contrast to the other generator systems, CRB-02 shows high $^{68}$Ga elution efficiency at nearly neutral pH, which makes it possible to use the eluate directly as a $^{68}$Ga radiopharmaceutical. We have found that trisodium citrate can elute a solution of $^{68}$Ga-citrate with high efficiency.\(^{14,15}\) $^{68}$Ga-citrate planar and single-photon emission computed tomography (SPECT) imaging have been widely used for diagnosis of cancers,\(^{16–18}\) sarcoidosis,\(^{19}\) and inflammatory and/or infectious diseases\(^{20,21}\) in clinical practice. $^{68}$Ga-citrate has shown promise in PET imaging of prostate cancer,\(^{22}\) Staphylococcus aureus infection,\(^{23}\) and osteomyelitis.\(^{24}\) The $^{68}$Ga-citrate used in these studies was prepared from a strong acid solution of $^{68}$GaCl$_3$ produced from the currently available $^{68}$Ge/$^{68}$Ga generators. A generator system that can produce $^{68}$Ga-citrate by elution with trisodium citrate would be more convenient. One disadvantage of the CRB-02-based generator system is that a large amount of trisodium citrate (10 mL) is required to elute the $^{80}$% yield of the $^{68}$Ga solution.\(^{14,15}\) Considering that approximately 1.5–2.0 mL of $^{68}$Ga-citrate has generally been used for one patient in clinical practice,\(^{25,26}\) a more concentrated $^{68}$Ga-citrate solution is desirable. CRB-02 is mainly composed of the styrene-divinylbenzene copolymer and is highly hydrophobic. As $^{68}$Ga-citrate is a hydrophilic molecule, hydrophilic environments physically close to MGs may boost the elution efficiency of $^{68}$Ga. Sephadex (GE Healthcare Life Sciences, Pittsburgh, PA) series, chromatographic carriers for gel filtration, are composed of dextran. These polymers have many surface hydroxyl groups that can be covalently linked with functional MG groups. Several types of Sephadex with different degrees of cross-linkage are commercially available. The rank order of the degree of cross-linking is as follows: G-10 > G-15 > G-25 > G-50 > G-75, whereas the order in increasing degree of expansion is as follows: G-75 > G-50 > G-25 > G-15 > G-10.\(^{27}\) Differences in their properties allow the systematic evaluation of synthesized generators. Hence, we sought to develop a new $^{68}$Ge/$^{68}$Ga generator system based on these polysaccharides. In this study, we developed MG-containing Sephadex (Sepha-MGs) that showed strong $^{68}$Ge adsorption power and minimal $^{68}$Ge breakthrough. $^{68}$Ga-citrate could be effectively eluted by the Sepha-MG packed columns using a much smaller amount of trisodium citrate than that of Diaion CRB-02. Furthermore, we have demonstrated that PET imaging with generator-eluted $^{68}$Ga-citrate successfully visualized mice models of tropical infectious diseases. Our results demonstrate the application of Sepha-MGs for the convenient $^{68}$Ge/$^{68}$Ga system of which the $^{68}$Ga eluent can be directly used for the diagnosis of various infectious diseases.

## RESULTS

### Characterization of Sepha-MGs.

The nucleophilic addition of the epoxy groups in the polymers with MG gave the desired Sepha-MG (Figure 1). The MG-modified Sephadex beads were used as polymer matrices for immobilizing the $^{68}$Ge. Figure 2 shows the $^{68}$Ge adsorption rates of the Sepha-MGs by the batch operation method. Diaion CRB-02 was used for the comparison of basic performance with Sepha-MGs. All Sepha-MGs showed >97% of the adsorption rate of the $^{68}$Ge in the solution after 15 min of shaking. In contrast, only 78% of $^{68}$Ge was adsorbed by CRB-02 at 15 min. Table 1 shows the characteristics of the different Sepha-MGs. Because Sephadex has no nitrogen atoms, the nitrogen content indicates the amount of introduced MGs. The numbers following “G-” of their names such as 25, 50, and 100 indicate their swelling property and the number of hydroxyl groups; the larger the

![Figure 1. Chemical derivatization of Sephadex beads to Sepha-MGs.](image-url)
number, the higher the degree of swelling and the larger the number of hydroxyl groups. The degree of swelling and nitrogen content of Sepha-MGs increased with increasing the numbers, indicating that the basic properties of Sephadex did not appear to change by the modification of MGs. The Ge adsorption capacities in the wet state of Sepha(10)-MG, Sepha(15)-MG, and Sepha(25)-MG were higher than those of Sepha(50)-MG and Sepha(75)-MG. Accordingly, Sepha(10)-MG, Sepha(15)-MG, and Sepha(25)-MG were used for further evaluation.

Sepha(10)-MG, Sepha(15)-MG, and Sepha(25)-MG were separately packed into a teflon-made column, and then the adsorption, Ge breakthrough, and elution efficiency of Ge were evaluated. When 3.7 MBq of Ge was loaded onto the Sepha-MG packed columns, >97% of Ge was absorbed in each generator and over 60% of the Ge could be eluted by 1 mL of 0.1 M trisodium citrate. The three Sepha-MGs showed much higher Ge elution efficiencies than that of CRB-02 (Figure 3). Breakthrough of Ge was less than 0.01% for the three Sepha-MG packed columns (data not shown).

Sepha(10)-MG, Sepha(25)-MG, and Sepha(10)-MG showed equivalent elution profiles. Sephadex G-15 has a higher degree of cross-linkage than that of Sephadex G-25, which indicates that the physical durability of Sepha(15)-MG is greater in comparison to that of Sepha(25)-MG. Accordingly, further evaluation of Sepha(15)-MG as an adsorbent for Ge/Ge generator was performed using higher amounts of radioactivity. Figure 4 shows the evaluation of 74 MBq in a generator composed of Sepha(15)-MG. This generator showed a similar performance, with high Ge elution efficiency and quite low Ge breakthrough (<0.001%), as shown in Figure 4A. Furthermore, elution of Ge was used after 244 days to check the reproducibility of the generator system and resulted in identical elution efficiency (Figure 4B). Paper chromatography analysis revealed that >95% of the Ge solution was in the form of Ge-citrate (Figure 5).

PET/CT Imaging of Infected Mice with Ge-Citrate. Characterization of Sepha-MGs indicated that the generator-eluted Ge solution can be directly used as Ge-citrate. Therefore, we performed small animal PET/CT studies with Ge-citrate to visualize inflammatory foci in mice infected with Leishmania and severe fever with thrombocytopenia syndrome virus (SFTSV). Leishmaniasis is one of the neglected tropical diseases caused by infection with Leishmania parasites, with symptoms ranging from self-healing cutaneous lesions to a fatal visceral disorder. SFTSV is a recently identified emerging disease in East Asia that shows various symptoms, including fever, gastrointestinal symptoms, myalgia, and regional lymphadenopathy caused by the SFTSV. Figure 6 shows representative PET/CT images acquired 120–180 min after intravenous injection of Ge-citrate in mice infected with Leishmania major in the left hind footpad. There was significant Ge-citrate accumulation in the infected left hind footpad, whereas no significant signals were observed in the phosphate-buffered saline (PBS)-injected right hind footpad. Figure 7A shows representative PET/CT images of SFTSV-infected or mock-infected mice at 120–180 min after intravenous injection of Ge-citrate in mice infected with Leishmania major in the left hind footpad. Significant accumulation of Ge-citrate is clearly visible in the gastrointestinal tract, a target site of SFTSV, in the SFTSV-infected mice. On the other hand, there is quite low activity in the gastrointestinal tract of mock-infected mice (Figure 7A). Next, we performed similar PET studies using FDG, which can be used for the detection of infectious inflammatory regions. As shown in Figure 7B, the gastrointestinal tract was clearly visualized in the SFTSV-infected mice, whereas there were no significant signals in the mock-infected mice. The accumulation pattern of FDG is similar to that of Ge-citrate, indicating that Ge-citrate could also visualize the inflammation induced by SFTSV infection.

Table 1. Physicochemical Properties of the Sepha-MGs

| resin     | wet volume (mL/g-dry resin) | nitrogen content (mmol/g-dry resin) | Ge binding capacity |
|-----------|----------------------------|------------------------------------|--------------------|
|           |                            | (mmol/g-dry bead)                  | (mmol/mL-wet bead) |
| Sepha (10)-MG | 2.6                         | 0.23                               | 0.21               | 0.081               |
| Sepha (15)-MG | 3.2                         | 0.37                               | 0.25               | 0.078               |
| Sepha (25)-MG | 4.7                         | 0.63                               | 0.34               | 0.072               |
| Sepha (50)-MG | 12.4                        | 0.72                               | 0.46               | 0.038               |
| Sepha (75)-MG | 18.8                        | 0.73                               | 0.61               | 0.032               |
DISCUSSION

Commercially available $^{68}$Ge/$^{68}$Ga generator systems composed of TiO$_2$ and SnO$_2$ are subject to several major problems. First, considerable metallic impurities can be detected in the generator-eluted solution. Second, $^{68}$Ga$^{3+}$ needs to be eluted with HCl in association with the necessity of multiple steps for preparing $^{68}$Ga radiopharmaceuticals, which makes $^{68}$Ga labeling complicated and inconvenient for PET imaging in clinical practice. In view of these concerns, we developed a new $^{68}$Ge/$^{68}$Ga generator system to overcome such problems. We have previously reported that organic polymer CRB-02 exhibited selective adsorption of $^{68}$Ge and had a higher elution efficiency using trisodium citrate without significant $^{68}$Ge breakthrough. The chemical form of the eluate was $^{68}$Ga-citrate, which can be directly applied to PET imaging without further modification. However, the elution volume needed reduction for clinical applications, which requires significant improvement of elution efficiency. This may be due to poor accessibility of trisodium citrate to the MG groups on the hydrophobic backbone polymer of CRB-02. We developed MG-group-containing Sephadex (Sephaga) that showed strong $^{68}$Ge adsorption ability and low $^{68}$Ge leakage and allowed $^{68}$Ga-citrate to be directly eluted with much smaller volumes of trisodium citrate than that in CRB-02. The parameters of commercially available and newly developed generators, including gallium elution capabilities, metallic impurity, and

![Figure 4](image-url) (A) Elution of $^{68}$Ga from 74 MBq Sepha(15)-MG columns eluted by 0.1 M trisodium citrate within 1 week after the preparation of the $^{68}$Ge/$^{68}$Ga generator. (B) Long-term evaluation of the $^{68}$Ga elution efficiency of the Sepha(15)-MG columns. The data indicate the percentage of elution efficiency of 1 mL of $^{68}$Ga-citrate solution eluted from the columns.

![Figure 5](image-url) (A) Cellulose acetate electrophoresis of the $^{68}$Ga solutions eluted from Sepha(15)-MG columns and commercially available $^{67}$Ga-citrate (Medi-physics). (B) Quantitative profile of the radioactivity of the $^{68}$Ga solution (green) and $^{67}$Ga-citrate (blue).

![Figure 6](image-url) Representative PET/CT images of footpad regions in BALB/c mice infected with L. major in the left hind footpad 6 days after infection. PET/CT images were acquired 120–180 min after intravenous injection of $^{68}$Ga-citrate (4.2 MBq). The significant $^{68}$Ga-citrate signal was observed at the site of leg edema (yellow arrows) but not in the uninfected contralateral leg (blue arrows).
68Ge breakthrough, are summarized in Table 2. It is reported that over 3 mL of HCl solution was required to elute about 75% of 68Ga from commercially available generators composed of TiO2, SnO2, or SiO2 (Table 2) and recently developed 68Ge/68Ga generators using the CeO2-polyacrylonitrile sorbent.13 We demonstrated that 75% of 68Ga solution was eluted by 1 mL of trisodium citrate from a Sepha(MG)-15-based generator (Figure 4A), indicating that the elution efficiency of Sepha(MG)-15 is superior to that of the previously reported 68Ge adsorbents. Several metallic impurities have been found in the commercially available generators.13 We have not examined the metal impurity of Sepha-MGs, but the organic polymers might not contain any intrinsic metal. The 68Ge breakthrough from the Sepha(MG)-15-based generator was less than 0.001%, which is comparable to that from the clinically available generators (Table 2).13 The elution efficiency of Sepha(MG)-15 has high stability over time. Accordingly, Sepha(MG)-15 may be an optimal adsorbent for 68Ge/68Ga generator systems. Indeed, PET imaging with 68Ga-citrate eluted with this system successfully visualized the site of infection with L. major and SFTSV in mice. BALB/c mice inoculated in the footpad with L. major develop inflammation at the injection site, which is a consequence of the parasite growth and the host immune responses.29 As shown in Figure 6, PET imaging with 68Ga-citrate can recognize the inflammatory foci caused by L. major infection. To our knowledge, this is the first report of Leishmania-infected regions visualized by small animal molecular imaging techniques. Previously, we reported that PET imaging with 18F-FDG could visualize the intestinal tract as a pathological site of SFTSV-infected mice.30 We also demonstrated that 68Ga-citrate showed significant signals in the gastrointestinal tract of SFTSV-infected mice. In contrast to that of 18F-FDG-PET, physiological accumulation of 68Ga-citrate was absent in the muscle, as shown in Figure 7. Accordingly, our data suggested that 68Ga-citrate PET can be a useful imaging tool for the detection of SFTS-infected regions. It is suggested that 68Ga-citrate PET can be useful for the detection of infectious sites, investigation of disease progression, and monitoring of therapeutic effects of various infectious disorders, such as the neglected tropical diseases. 68Ga-labeled radiopharmaceuticals can be produced without an expensive cyclotron, and our generator may be cost-effective compared to commercially available generators in terms of the 68Ga elution efficiency and cost of matrices. This economic advantage would be especially important for the developing countries spreading the neglected tropical diseases. In our preliminary experiments for clinical application, 68GeCl4 of higher radioactivity (337 MBq) was loaded onto Sepha-MG(15), resulting in 78% elution of 68Ga-citrate from the generator by 4 mL of eluent but 68Ge breakthrough was increased to over 0.02%. However, most of the 68Ge impurity can be removed by the pre-column packed with a small amount of Sepha-MG(15) (unpublished data). We have previously demonstrated that 68Ga-citrate can be reacted with ethylenediaminetetraacetate and deferoxamine to label polypeptides.14,15 Therefore, this generator system can also be used for the production of various 68Ga

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Representative axial (left panels), coronal (middle panels), and sagittal (right panels) the PET/CT images of SFTSV-infected and mock-infected A129 mice 3 days after infection. PET/CT images were acquired 120–180 min after intravenous injection of 68Ga-citrate (A) or 30–90 min after intravenous injection of 18F-FDG (B). The yellow arrows indicate the gastrointestinal tract.

| generator                  | eluent        | required volume of eluent (mL) for over 75% elution | metal impurity                                                                 | 68Ge breakthrough          |
|----------------------------|---------------|------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------|
| TiO2-based (Eikert & Zeigler, Germany) | 0.1 M HCl    | 4.515                                                | Ti, Fe, Cu, Mn, Al (1–10 ppm)13                                               | 0.001–0.01% (1–12 month)13  |
| SnO2-based (iThemba Labs, South Africa) | 0.6 M HCl    | 5.015                                                | Sn, Fe, Cu, Mn, Al (1–20 ppm)13                                               | 0.001–0.01% (1–12 month)13  |
| SiO2-based (ITG Germany)       | 0.05 M HCl   | 2.515                                                | Si, Fe, Cu, Mn, Al (<0.1 ppm)13                                               | <0.001% (0–12 month)13      |
| Sephadex-based (this study)    | 0.1 M trisodium citrate | 1.0                                                   | n.d.                                                                          | <0.001% (0–3 month)         |
radiopharmaceuticals. However, $^{68}$Ga-citrate, eluted using Sepha-MG, had a relatively lower reaction efficiency with chelating agents such as 1,4,7-triazacyclononanetricarboxylic acid (NOTA) than GaCl$_3$ did. Approximately 27 $\mu$m precursor NOTA-arginylglycylaspartic acid (NOTA-RGD) was reacted with $^{68}$GaCl$_3$ in aqueous HCl produced by a commercially available generator for 10 min at room temperature to synthesize $^{68}$Ga-NOTA-RGD in 89% yield. 35 In contrast, 500 $\mu$m precursor NOTA-RGD and heating were required to synthesize $^{68}$Ga-NOTA-RGD in 90% yield when $^{68}$Ga-citrate was used as the $^{68}$Ga source (data not shown). Regarding the production of other $^{68}$Ga-labeled radiopharmaceuticals using chelating groups, further studies are required to develop more useful eluents with high reaction efficiency. Nevertheless, our study suggests that Sepha(15)-MG is a promising adsorbent for the effective $^{68}$Ge/$^{68}$Ga generator and may be useful in clinical practice.

## CONCLUSIONS

Sephadex-based $^{68}$Ge adsorbents were prepared from the Sephadex series without complicated procedures. Seventy to eighty percent of the $^{68}$Ge in Sepha(15)-MG columns was eluted in 1 mL of the eluate, and the $^{68}$Ge breakthrough was $<$0.001%. No remarkable changes in the elution efficiency of $^{68}$Ge were observed at 244 days after absorption of $^{68}$Ge, suggesting that our generator could provide a long shelf life. We demonstrated that generator-eluted $^{68}$Ga-citrate can be directly used for PET imaging of infectious mouse models. Our polysaccharide-based $^{68}$Ge/$^{68}$Ga generators are prospectively cost-effective production systems for $^{68}$Ga radiopharmaceuticals.

## EXPERIMENTAL SECTION

### Materials and Reagents

All reagents were commercial products and used without further purification unless otherwise indicated. Sephadex beads (G-10 (particle size distribution, 40–120 $\mu$m), G-15 (40–120 $\mu$m), G-25 (20–80 $\mu$m), G-50 (20–80 $\mu$m), G-50 (40–120 $\mu$m)) were purchased from GE Healthcare (Piscataway, NJ). $^{68}$GeCl$_4$ was obtained from Isotope Products Lab, (Valencia, CA). $^{68}$Ge-citrate and $^{18}$F-FDG were purchased from Nihon Medi-Physics Co., Ltd. (Chiba, Japan). An automated $\gamma$ counter with a Nal(Tl) detector (2470 WIZARD$^2$, PerkinElmer, Norwalk, CT) was used to measure the radioactivity of $^{68}$Ga samples. The solutions in the $^{68}$Ge/$^{68}$Ga generator were moved with a MP-3 peristaltic pump (EYELA, Tokyo Rikakai Co., Tokyo, Japan).

### Preparation of Sepha-MGs

Sephadex beads (G-10, 3.9 g; G-15, 3.2 g; G-25, 2.2 g; G-50, 0.8 g; and G-75, 0.5 g) were added to 10 mL of distilled water and degassed. After the supernatants had been removed, 10 mL of 1 M NaOH was added to the residue, and the mixture was stirred overnight at room temperature. Epichlorohydrin (1 mL) was added, and the mixture was stirred at 120 rpm for 24 h. After the supernatant had been removed by decantation, the resin was washed with distilled water three times. MG (2 g) was added in 20 mL of 0.5 M carbonate–bicarbonate buffer (pH 10), and the mixture was stirred at 120 rpm for 24 h. The resulting mixtures were washed with distilled water three times and stored in 0.01 M phosphate buffer until use.

### Measurement of Nonradioactive Ge Adsorption on the Sepha-MGs

A solution of nonradioactive Ge(IV) was prepared by dissolving germanium dioxide in a small volume of 0.1 M NaOH. The concentration and the initial pH were, respectively, adjusted to 0.01 M and 7 with 0.01 M HCl and 0.01 M phosphate buffer. The binding capacity of Sepha-MG for Ge(IV) was determined by the batch operation method. Fifty milligrams of Sepha-MGs was shaken for 24 h at 30 °C with 10 mL of 0.01 M Ge(IV). The contents of Ge(IV) in the supernatant were colorimetrically measured using phenylfluorone. 28 The nitrogen content of the Sepha-MGs was determined by elemental analysis.

### Adsorption of $^{68}$Ge onto the Sepha-MGs by the Batch Method

Each stock Sepha-MG in 0.01 M phosphate buffer was washed with H$_2$O five times and then freeze-dried. Sepha-MGs or Diaion CRB-02 (5 mg; Mitsubishi Chemical, Yokohama, Japan) were shaken in a glass vial with 2 mL of 0.01 M phosphate buffer (pH 7) containing 1.5 kBq of $^{68}$GeCl$_3$ at room temperature for 1 h. Then, 100 $\mu$L of supernatant was collected, and 48 h after standing, the radioactivity was compared to that of a standard solution taken from the equilibrium mixture before incubation with each adsorbent.

### Separation of $^{68}$Ga from $^{68}$Ge

Half milliliter of Sepha-MGs or CRB-02 presoaked in 0.01 M phosphate buffer (pH 7) was packed in a teflon-made column (5 cm length, 0.8 cm internal diameter; Flon Chemical, Inc., Osaka, Japan). Then, 5 mL of $^{68}$Ge (3.7 MBq for preliminary assessment and 74 MBq for optimizing Sepha-MG items) in 0.01 M phosphate buffer (pH 7) was passed through the column at a flow rate of 0.5 mL/min at room temperature. After being washed with 0.01 M phosphate buffer, the $^{68}$Ga in the column was eluted with the trisodium citrate solution at a flow rate of 0.5 mL/min. The elution profiles of $^{68}$Ga and the total yield of $^{68}$Ga were characterized by measuring the $^{68}$Ga activity in each 1 mL aliquot of eluate and normalizing counts to the end of the elution procedure. $^{68}$Ga elution efficiency was expressed as a percentage of the amount of $^{68}$Ge contained in the resin at the time of elution. The leakage of $^{68}$Ge from the columns was determined by re-counting the eluates at 48 h after collection.

### Analysis of the Generator-Eluted $^{68}$Ga Solution by Cellulose Acetate Electrophoresis

The $^{68}$Ga solution eluted with 0.1 M trisodium citrate from the Sepha(15)-MG generator was electrophoresed on a Separax SP (Joko, Tokyo, Japan) cellulose acetate membrane at a constant current of 4.8 mA/cm$^2$ with 0.1 M trisodium citrate from the Sepha(15)-MG generator (pH 7) was electrophoresed on a Separax SP (Joko, Tokyo, Japan) and the mixture was electrophoresed on a Separax SP (Joko, Tokyo, Japan) for 30 min in 72 mM veronal buffer (pH 8.6). The cellulose acetate membrane was dried under a stream of cold air and placed in contact with imaging plates (BAS-MS 2040; Fuji Film, Tokyo, Japan) for 10 min. The distribution of the radioactivity on the plates was analyzed by a FLAS100 fluor image analyzer (Fuji Film).

### Animals

BALB/c mice were purchased from SLC (Shizuoka, Japan). The 129 strain background $\alpha/\beta$ interferon receptor knockout (IFNAR-KO) mice (A129 mice) were purchased from B & K Universal Limited. All mice were maintained in the Laboratory Animal Center for Animal Research at Nagasaki University. The experiments with animals were conducted in accordance with our institutional guidelines and were approved by the Nagasaki University Animal Care Committee (approval number: 1003240836-4). The infectious animal experiments were conducted under biosafety level 3 containment in accordance with institutional guidelines.

### Preparation of Leishmania-Infected Mice

BALB/c mice at the age of 8–14 weeks were infected by subcutaneous injection of stationary state promastigotes ($5 \times 10^5$) of L. major (MHOM/SU/73-5-ASKH strain) in the left hind footpad as described previously. 29 The right hind footpad was injected with sterile PBS. The Leishmania-infected mice underwent PET studies 6 days after infection.
Preparation of SFTS-Infected Mice. Adult A129 mice were subcutaneously inoculated with 10⁶ focus-forming units of the SFTSV diluted in Eagle’s minimal essential medium containing 2% fetal bovine serum.³⁰

PET/CT Imaging of Infected Mice with ⁶⁸Ga-Citr and ¹⁸F-FDG. PET/CT images of Leishmania-infected mice (BALB/c, 8 week old, male, 17.5−20.8 g, n = 4), SFTSV-infected mice (IFNAR-KO, 8 week old, male, 16.3−26.5 g, n = 4), and control mice injected with sterile medium (IFNAR-KO, 8 week old, male, 22.6−29.4 g, n = 4) were acquired with a Triumphant combined PET/SPECT/CT system (TriFoil Imaging Inc., CA). Generator-eluted ⁶⁸Ga-citrate was diluted with 1.3× volume of H₂O to adjust the osmotic pressure. Each mouse was administered ⁶⁸Ga-citrate (4.5−6.0 MBq) or ¹⁸F-FDG (10.5−12.5 MBq) in the tail vein. The mice were anesthetized with 1.5% isoflurane, and the CT acquisitions were performed for anatomical reference. Subsequently, 1 h ⁶⁸Ga PET acquisitions commenced 2 h after injection of ⁶⁸Ga-citrate or 30 min ¹⁸F-FDG acquisitions commenced 30 min after injection of ¹⁸F-FDG. The PET data were reconstructed using a three-dimensional maximum-likelihood expectation maximization algorithm (Iterative3D). Acquired PET and CT data were processed, and PET images were quantified using OsiriX MD software (Pixmeo, Geneva, Switzerland). Tracer uptake was expressed as the standardized uptake value (SUV), which was calculated as follows

\[
\text{SUV} = \frac{\text{radioactivity (MBq/mL)} \times \text{body weight (g)}}{\text{ injected radioactivity (MBq)}} \times 100
\]

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Prof. Matsuda (Nagasaki University) for his help with the PET/CT experiments. Financial support was provided by a Grant-in-Aid for Scientific Research (B) (Grant Nos. 18390015 and 16H05392), the Grants-in-Aid for the Development of Systems and Technology for Advanced Measurement and Analysis Program from Japan Science and Technology Agency, JST, and JSPS Research Fellows Program, the Joint Usage/Research Center on Tropical Disease, Institute of Tropical Medicine, Nagasaki University (2017-Ippan-17) and the Program of the network-type joint Usage/Research Center for Radiation Disaster Medical Science of Hiroshima University, Nagasaki University, and Fukushima Medical University.

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