Fever poses a major diagnostic and therapeutic problem in patients with severe granulocytopenia. Approximately 70% of these patients experience one or more febrile episodes. In granulocytopenic patients, clinical signs of infection are often scarce, and in 50% to 70% of the patients, no cause of the fever can be detected. Some authors estimate that 20% to 40% of febrile patients do not have an infection and that the fever is of paraneoplastic origin or caused by drugs. When a focus is found, the identification of the causative microorganism and installation of adequate therapy are facilitated. Recently, the utility of indium-111-labeled human nonspecific polyclonal immunoglobulin G (In-111—IgG) scintigraphy to detect focal infection has been reported. All patients in these studies had either normal or elevated WBC counts. In this study, we addressed the issue of whether In-111—IgG scintigraphy could be helpful in delineating infection in febrile granulocytopenic patients. Since Fe-γ receptors of phagocytic cells could be important for this imaging technique, we decided to perform an animal experiment in granulocytopenic rats to determine if In-111—IgG showed any accumulation in infectious foci while granulocyte counts were low. Stimulated by the positive outcome of the animal experiment, we studied a group of granulocytopenic patients to assess the potential of In-111—IgG scintigraphy in localizing focal infection in such patients.
Animal Study

In four young, randomly bred, male Wistar rats (weighing 200 to 220 g), severe granulocytopenia was induced with intraperitoneally injected cyclophosphamide. On days 1, 3, and 6, a dose of 200, 100, and 100 mg cyclophosphamide per kg of body weight was injected, respectively. The granulocyte count was monitored by daily blood samples obtained by orbital puncture. After severe granulocytopenia had been established, an infection was induced on day 6 by inoculating approximately 10^6 colony-forming units of Staphylococcus aureus in the left calf muscle. One day later, when gross swelling was apparent, 6 mBq of In-111 labeled to 100 μg IgG-DTPA conjugate was injected in the tail vein. Scintigraphic images were recorded at 4, 24, and 48 hours postinjection (PI). Regions of interest with a fixed size were drawn over the infectious focus and the contralateral calf muscle. Ratios of In-111-IgG uptake in infectious foci to that in the contralateral muscle were calculated. All mean values are given ± SEM.

Patient Study

Patients with fever and a granulocyte count below 2.0 × 10^9/L were eligible for the study. The criteria for fever included an oral temperature greater than 38.5°C on one occasion or greater than 38°C on two or more occasions during a 12-hour period, as described by the Consensus Panel of the Immunocompromised Host Society. Antibiotic treatment was not stopped or altered during the study. The experimental nature of the imaging procedure was explained to the patients. Informed consent was obtained. No patient had a history suggestive of IgA- or IgG-deficiency or adverse reaction to previous IgG administration. Pregnant and lactating females were excluded from the study. The study was approved by the institutional research review board.

Patients received an intravenous (IV) dose of approximately 1 mg of IgG labeled with 75 mBq of In-111. One patient, a 15-year-old female, received only 55 mBq of In-111-IgG for dosimetric reasons. The effective dose equivalent after IV administration of 75 mBq In-111-IgG was calculated to be 15 mSv and was similar to that of leukocyte scintigraphy (18 mSv after IV injection of 30 mBq In-111-labeled leukocytes). In-111-IgG images were obtained at 4, 24, and 48 hours PI for a preset time of 5, 7.5, and 10 minutes, respectively. Spot views of the total body were obtained at least 24 hours PI.

Images were interpreted independently by three observers blinded to the results of the verification procedures. An In-111-IgG scan was interpreted as positive if focally increasing accumulation of activity could be noted over time.

In-111-IgG scintigraphy results were verified by clinical, roentgenologic, ultrasonographic, and microbiological methods. All mean values are given ± SEM.

RESULTS

Animal Study

On day 6, when calf muscle infection was induced, all animals had severe granulocytopenia. The mean granulocyte count was 0.1 ± 0.02 × 10^9/L. At all times, infectious foci showed a high degree of In-111-IgG uptake. The ratios of In-111-IgG uptake in the focus relative to that in the noninfected, contralateral muscle steadily increased from 2.6 ± 0.3 (4 hours PI) to 6.0 ± 0.4 (24 hours PI) and finally reached 12.3 ± 1.9 (48 hours PI).

Patient Study

Twenty patients were studied (13 males, seven females; mean age, 36.9 years; range, 15 to 63 years). Sixteen patients suffered from different types of leukemia, three had malignant lymphoma, one had aplastic anemia. Most patients were granulocytopenic due to remission induction chemotherapy or conditioning regimen before bone marrow transplantation. Patient no. 11 had granulocytopenia of unknown origin. All patients were febrile for approximately 1 to 2 weeks.

Individual hematologic diagnoses, granulocyte cell counts, scintigraphic results, and verification procedures are summarized in Table 1. All patients, except patients no. 2 and 11, received antibiotic treatment. The mean granulocyte count was 0.4 ± 0.1 × 10^9/L; the median granulocyte count, 0.2 × 10^9/L.

Typical Scintigraphic Findings

After remission reinduction therapy for relapse of acute myelogenous leukemia, patient no. 5 developed a molar root infection, resulting in an infiltrate in the left mandibular region (Fig 1A). This focus was believed to be the cause of her fever. Total-body imaging using In-111-IgG revealed a large zone with increased uptake in the area of the middle lobe of the right lung (Fig 1B). The chest x-ray, made a few days before scintigraphy, was normal (not shown). One week later, only mild abnormalities were seen on the chest x-ray (Fig 1C). At that time, initial clinical symptoms of pulmonary infection were noted. A chest x-ray taken 2 weeks after scintigraphy showed a hyperdense area, matching the area of increased uptake on In-111-IgG scintigraphy (Fig 1D). The patient died 1 week later. Postmortem examination revealed a pulmonary infection caused by Aspergillus fumigatus. In this case, In-111-IgG scintigraphy showed a pulmonary infection that preceded clinical symptoms and chest x-ray abnormalities by at least 1 week.

Figure 2 shows the images of patient no. 6, who had aplastic anemia. Despite antibiotic therapy, he developed fever and symptoms of pulmonary infection. Scintigraphy showed multiple coin-shaped lesions in both lungs (Fig 2A) that matched the lesions on the chest.

[Image 0x0 to 2240x3104]
Table 1. Clinical Characteristics, Granulocyte Counts, Results of In-111-lgG Scintigraphy, and Verification Procedures

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex, Age</th>
<th>Medical History</th>
<th>Granulocytes (×10^9/L)</th>
<th>IgG Scan</th>
<th>Verification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 39</td>
<td>ALL, BMT, GVHD</td>
<td>0.4</td>
<td>Negative</td>
<td>Thoracic wall infiltrate after Hickman catheter caused by SA.</td>
</tr>
<tr>
<td>2</td>
<td>M, 45</td>
<td>Hairy cell leukemia</td>
<td>1.0</td>
<td>Negative</td>
<td>No infection proven.</td>
</tr>
<tr>
<td>3</td>
<td>M, 58</td>
<td>B-CLL</td>
<td>0.1</td>
<td>Lungs</td>
<td>Pulmonary infection [BAL: SV, SA, HI].</td>
</tr>
<tr>
<td>4</td>
<td>M, 54</td>
<td>NHL stage IV-B</td>
<td>1.3</td>
<td>Negative</td>
<td>No infection (autopsy).</td>
</tr>
<tr>
<td>5</td>
<td>F, 43</td>
<td>AML, BMT, relapse AML</td>
<td>0.05</td>
<td>Mandible</td>
<td>Extraction of infected molar tooth.</td>
</tr>
<tr>
<td>6</td>
<td>M, 22</td>
<td>Aplastic anemia</td>
<td>0.1</td>
<td>Lungs</td>
<td>AF infection of the lung, proven at autopsy.</td>
</tr>
<tr>
<td>7</td>
<td>F, 41</td>
<td>AML</td>
<td>0.3</td>
<td>Lungs</td>
<td>Clinical and roentgenologic diagnosis: pulmonary Aspergillus infection.</td>
</tr>
<tr>
<td>8</td>
<td>F, 15</td>
<td>Ileocecal NHL</td>
<td>1.7</td>
<td>Lungs</td>
<td>Pulmonary infection [BAL: CF, HSV].</td>
</tr>
<tr>
<td>9</td>
<td>F, 44</td>
<td>AML, BMT, GVHD</td>
<td>0.5</td>
<td>Lungs</td>
<td>Pulmonary infection [BAL: AF].</td>
</tr>
<tr>
<td>10</td>
<td>M, 31</td>
<td>Mediastinal NHL, stage I-A</td>
<td>0.6</td>
<td>Lungs</td>
<td>Generalized toxoplasmosis infection (autopsy).</td>
</tr>
<tr>
<td>11</td>
<td>M, 63</td>
<td>CLL</td>
<td>1.0</td>
<td>Right side of lower abdomen</td>
<td>Thoracic wall ulcer after diagnostic parasternal mediastinotomy. Pleural effusion: photopenic on IgG; culture: no infection.</td>
</tr>
<tr>
<td>12</td>
<td>M, 44</td>
<td>ALL, BMT</td>
<td>0.2</td>
<td>Wrist</td>
<td>Surgery: perforated appendix.</td>
</tr>
<tr>
<td>13</td>
<td>M, 43</td>
<td>AML</td>
<td>0.2</td>
<td>Colon</td>
<td>Septic arthritis with prompt recovery after vancomycin.</td>
</tr>
<tr>
<td>14</td>
<td>F, 18</td>
<td>Relapse ALL</td>
<td>0.6</td>
<td>Negative</td>
<td>No infection; skin lesions (IgG negative): allergic vasculitis.</td>
</tr>
<tr>
<td>15</td>
<td>F, 17</td>
<td>ALL, BMT, GVHD</td>
<td>0.1</td>
<td>Negative</td>
<td>No infection proven.</td>
</tr>
<tr>
<td>16</td>
<td>F, 17</td>
<td>AML</td>
<td>0.06</td>
<td>Right side of lower abdomen</td>
<td>Pain and tenderness in the right side of the abdomen, disappeared in a few days, no microbiologic confirmation.</td>
</tr>
<tr>
<td>17</td>
<td>M, 20</td>
<td>ALL</td>
<td>0.1</td>
<td>Negative</td>
<td>No infection proven.</td>
</tr>
<tr>
<td>18</td>
<td>M, 50</td>
<td>AML</td>
<td>0.01</td>
<td>Distal colon</td>
<td>No infection proven.</td>
</tr>
<tr>
<td>19</td>
<td>M, 34</td>
<td>CML</td>
<td>0.03</td>
<td>Lower left side of abdomen</td>
<td>Unverified.*</td>
</tr>
<tr>
<td>20</td>
<td>M, 40</td>
<td>CML</td>
<td>0.05</td>
<td>Oral cavity</td>
<td>Fulminant oral mucositis.</td>
</tr>
</tbody>
</table>

Abbreviations: BAL, bronchoalveolar lavage; BMT, bone marrow transplant; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin’s lymphoma; AF, Aspergillus fumigatus; CF, Citrobacter freundii; HI, Haemophilus influenzae; GVHD, graft-versus-host disease; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; HSV, herpes simplex virus; SA, Staphylococcus aureus; SV, Streptococcus viridans.

*This patient died 1 week after scintigraphy. Permission for autopsy was not given.

Overall Results

Proven Inflammatory and Infectious Foci Showing Increased In-111–lgG Uptake (True-Positive Studies)

Pulmonary lesions. Two A. fumigatus infections were proven by culture (patients no. 5 and 8). In one patient (no. 6) the clinical and roentgenologic course was compatible with aspergillosis. Two patients (no. 3 and 7) had pulmonary infection caused by bacteria. Patient no. 9 had a toxoplasmosis infection.

Bone and joint lesions. Septic arthritis showed increasing In-111–lgG accumulation (patient no. 12). An aseptic, prednisone-induced, femoral head necrosis was also positive on In-111–lgG scintigraphy (patient no. 9).
Abdominal lesions. An appendicular infiltrate that was proven at surgery (patient no. 11) and an infection in the right side of the lower abdomen that was suspected on clinical grounds (patient no. 16) showed increased In-111-IgG uptake.

Soft tissue lesions. A mandibular infiltrate due to a molar root infection (patient no. 5), a postmediastinotomy thoracic wall ulcer (patient no. 10), and a fulminant oral mucositis (patient no. 19) were clearly visualized.

No Infection, Normal In-111-IgG Scintigraphy (True-Negative Studies)

A pleural effusion was photopenic on In-111-IgG scintigraphy. Repeated cultures remained sterile (patient no. 10). Skin lesions showed no increased uptake and were diagnosed as allergic vasculitis rather than septic emboli (patient no. 14). An infectious origin of a right shoulder swelling in patient no. 20 could not be proven by cultures or roentgenologic examination. At a
later date, chloroma was proven by biopsy, histologic examination, and subsequent response to irradiation. In five other patients with normal In-111-IgG scintigraphies, infection could not be proven.

Noninfected Areas With Increased In-111-IgG Uptake (False-Positive Studies)

In two cases (patients no. 13 and 18) infection could not be established. Intense activity was seen in the large bowel. In both patients, no clinical or microbiologic signs of abdominal infection were present. Patient no. 13 remained febrile for approximately 2 weeks. During this
period he was treated with cefazidime and co-trimoxazole. Normalization of temperature coincided with an increase of the blood granulocyte count. After treatment with ciprofloxacin and co-trimoxazole, patient no. 18 became afebrile within 2 weeks after scintigraphy.

Proven Focal Infections With No Increased In-111-IgG Uptake (False-Negative Studies)

In patient no. 1, a thoracic wall infiltrate caused by S aureus showed no increased In-111-IgG uptake. During scintigraphy, however, the size of the infiltrate and the signs of inflammation had diminished. In the following weeks, an extensive P aeruginosa infection of the chest wall developed.

For patient no. 9 who had toxoplasmosis, proven infection of the heart, kidneys, and liver showed no abnormal uptake.

Unassessable Study

In patient no. 19 a focus in the left side of the lower abdomen could not be verified. The patient died 1 week after scintigraphy. Permission for autopsy was not given.

Calculations for In-111-IgG Scintigraphy

Using the number of evaluated lesions (n = 26) mentioned above, we calculated a sensitivity of 76%, a specificity of 78%, and an accuracy of 77% for In-111-IgG scintigraphy, with a positive predictive value of 87% and negative predictive value of 64%.

DISCUSSION

Results from the animal study showed high In-111-IgG uptake in the infected muscle. Although the number of animals was small and only S aureus infection was studied, the experiment showed that In-111-IgG accumulates in infectious foci, despite low granulocyte counts. In rats without granulocytopenia, the ratio of activity uptake appeared to be lower (between 2.3 and 3.4). Higher uptake in granulocytopenic rats may be explained by greater outgrowth of bacteria in the absence of granulocytes. We chose S aureus infections for these experiments because it is one of the few not rapidly fatal infections in granulocytopenic rats. Data in the literature suggest that the causative microorganism is not a determining factor for accumulation of radiolabeled IgG in infectious foci, even in immunocompromised animals.

Our results in humans indicate that In-111-IgG scintigraphy shows performance characteristics in granulocytopenic patients similar to those in patients with normal or elevated WBC counts. Pulmonary, abdominal, joint, and soft tissue infections were detected. In patients no. 3 and 7 with positive In-111-IgG images of the lungs and clinical and roentgenologic evidence of pulmonary infection, the possibility must be considered that bronchoalveolar lavage might have identified resident oral contamination when the bronchoscope was passed through the nasopharynx rather than recovering the actual pathogenic microbial flora. With In-111-IgG, not only bacterial, but also fungal infections were localized adequately and sometimes in early stages, as demonstrated by patients no. 5 and 11. The diagnosis of infections (especially those caused by fungi) in granulocytopenic patients is notoriously difficult, and the ability to detect such infections holds great promise as it may lead to early microbiologic diagnosis and specific treatment. Even when microbiologic confirmation is not achieved, localization of a focus may give an indication of the causative pathogen.

With regard to our false-positive studies, no infection could be proven in two patients showing marked In-111-IgG uptake in the large bowel. One reason for this accumulation might be significant mucosal barrier damage by cytarabine, given 2 to 3 weeks before imaging. This drug is known to cause extensive desquamation of the intestinal mucosa with exudation and bleeding. Therefore, increased In-111-IgG uptake in the large bowel might have been due to increased protein leakage. Another possible explanation is that local bowel uptake occurred because of a chemotherapy-induced break in the mucosal integrity with local bacterial invasion but negative blood cultures, and the patient’s temperature normalized due to empiric antibiotic treatment.

The number of false-negative studies was low. One focal lesion (a thoracic wall infiltrate) was missed. Temporary improvement of the infiltrate in the period of scintigraphic imaging may have accounted for the absence of In-111-IgG accumulation. In a patient with generalized toxoplasmosis, the lungs showed increased In-111-IgG uptake, whereas the heart, kidneys, and liver, containing multiple small lesions, appeared normal on scintigraphy. This is not surprising, as these organs show a relatively high physiologic In-111-IgG uptake in all patients, thereby obscuring possible small lesions in these organs.

If we assume that In-111-IgG scintigraphy is not appropriate for the detection of lesions in organs such as the liver, kidneys, and heart because of high background activity, it is probably justified to delete the missed toxoplasmosis lesions from the calculations. This would
lead to a sensitivity of 93%, a specificity of 78%, an accuracy of 87%, a positive predictive value of 87%, and a negative predictive value of 88%.

Until now, all investigations in febrile granulocytopenic patients showed low yield. Clinical signs of inflammation are often lacking. Chest x-rays may show a delay between the onset of infection and the appearance of abnormalities, as demonstrated by patient no. 5. The same holds true for ultrasonography and x-ray computed tomographic scanning. Surveillance cultures are time-consuming and costly and have a low predictive value. Bronchoalveolar lavage also has a low yield, especially in pulmonary aspergillosis.

For scintigraphic delineation of infectious foci, several techniques are available. However, scarce data are reported regarding scintigraphic imaging in granulocytopenic patients. The use of gallium-67 (Ga-67) scintigraphy in granulocytopenic patients is questionable because lactoferrin from granulocytes is necessary for Ga-67 accumulation, resulting in frequent false-negative studies. Autologous leukocyte scintigraphy is not possible, and antihuman granulocyte monoclonal antibody scintigraphy seems inappropriate in patients with low leukocyte counts. Moreover, the development of human antimouse antibodies poses a serious problem using the latter technique. Several reports document the utility of donor leukocyte scintigraphy. However, preparation of this radiopharmaceutical is complicated, time consuming, costly, and it is not free of side effects, such as transfusion reactions, HLA-antibodies, and disease, and the possibility of viral infection. On the other hand, In-111-IgG is pathogen-free and ready available as a kit. No side effects have been seen after the administration of 1 mg dosages of radiolabeled IgG in more than 350 patients (W.J.G.O., unpublished data, February 1991). In addition, the safety of intravenous IgG preparations has been established by long-term therapeutic administration in gram doses.

In-111-IgG, like all scintigraphic imaging techniques used for detection of focal infection, does not discriminate between sterile inflammation and focal infection. In the present study, this is exemplified by In-111-IgG accumulation in a sterile femoral head necrosis.

In one patient, an artifact was observed in the subcutaneously tunneled part of the Hickman catheter due to injection of In-111-IgG through the catheter. Since Hickman catheters are known to be the site and source of infection, an optimal scintigraphic image of the tissue around the catheter should be obtained. Therefore, we recommend administering In-111-IgG through direct venipuncture in the contralateral arm.

Despite the use of antibiotics in 18 patients, we obtained high-quality images. It seems unnecessary to stop or alter antibiotic medication for imaging purposes.

Both the animal and patient studies indicate that neutrophils are not a major factor in the uptake of In-111-IgG in infectious foci, making Fcγ receptor-mediated accumulation an unlikely mechanism. This is in agreement with the hypothesis by Morrel et al., who suggest that In-111-IgG accumulation is caused by enhanced vascular permeability.

Patients with clinically proven infectious foci represent a population different from those with no clinically identifiable focus (eg, with regard to the recommended antibiotic treatment, time course for treatment, and the outcome of the febrile episode). It appears that In-111-IgG scintigraphy will become a valuable additional diagnostic tool for those febrile patients in whom no focus can be identified by conventional techniques or in whom an inflammatory component of known abnormalities is to be evaluated. Further studies are needed to establish whether In-111-IgG scintigraphy can contribute to the improvement of therapy in febrile granulocytopenic patients due to early localization of focal infection, especially in patients with fever of unknown origin who do not respond to antibiotic treatment. Furthermore, the use of follow-up scans during or after adequate treatment of infection warrants further studies.

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