Immunoscintigraphy of Inflammatory Processes with a Technetium-99m-Labeled Monoclonal Antigranulocyte Antibody (MAb BW 250/183)

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Antigranulocyte immunoscintigraphy with a technetium-99m- (^{99m}Tc) labeled monoclonal antigranulocyte antibody (MAb BW 250/183) was performed in 34 in-patients of the departments of accident surgery and internal medicine in order to prove or exclude inflammatory processes. After labeling with ^{99m}Tc, 555 MBq, ^{99m}Tc-MAb (0.5 mg antibody) were slowly injected intravenously over a period of 5 min. A whole-body scan was done 4–6 hr postinjection, and planar or SPECT images were performed 6, 18, and 24 hr postinjection. Leukocyte immunoscintigraphy proved inflammatory suppurating processes in 20 cases (true-positive) and excluded them in 11 cases (true-negative). The findings were false-positive in two patients (hematoma without signs of infection, pseudoarthrosis) and false-negative in one patient (encapsulated lung abscess with pleural fibrosis). Anti-idiotypic human anti-mouse antibodies (HAMA) were found only in one out of 20 patients. According to our experiences, immunoscintigraphy with ^{99m}Tc-MAb BW 250/183 has a sensitivity of 95%, and is, therefore, well suited for the identification of leukocytic inflammations.

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With the introduction of technetium-99m- (^{99m}Tc) labeled antigranulocyte antibodies, leukocyte scintigraphy has become a method that can be performed at any time in any nuclear medicine laboratory. Compared to the time-consuming and elaborate in vitro labeling with indium-111- (^{111}In) oxine or ^{99m}Tc-HM-PAO, in vivo labeling of leukocytes permits easy and quick visualization of inflammatory, suppurating processes (*1–6*).

The monoclonal antibody (MAb BW 250/183, Behring Werke Marburg, FRG) is an intact murine monoclonal antibody, which reacts with an epitope of NCA 95 (nonspecific cross-reacting antigen) on the surface of neutrophil granulocytes (*7*). In spite of its strong binding to the granulocyte membrane, no antibody- or complement-dependent lysis of the cells occurs (*8*). The vitality of granulocytes is not inhibited by the antibody. In contrast to labeling of leukocytes with ^{99m}Tc-HM-PAO, diffusion of free activity into the intestines is prevented due to the high stability of ^{99m}Tc-MAb binding and the high affinity of MAb to the epitope.

The purpose of this study, which included patients hospitalized at the departments of accident surgery and internal medicine, was to investigate the clinical value of antigranulocyte immunoscintigraphy with MAb BW 250/183.

PATIENTS AND METHODS

Antigranulocyte immunoscintigraphy was performed in a total of 34 patients. Twenty-three patients of the department of accident surgery were investigated in order to determine whether inflammatory soft-tissue processes (abscesses, infected hematomas) or osteomyelitis were present; the method was indicated in 11 patients for clarification of inflammatory, suppurating processes of inner organs (lung abscess, colitis ulcerosa, pyonephrosis) or leukocytosis of unknown origin. The antibody (1 mg) was labeled with 1,100 MBq ^{99m}Tc according to the method developed by Schwarz et al. (*9*).

Five to ten minutes after labeling, 555 MBq ^{99m}Tc-MAb BW 250/183 (0.5 mg antibody) were slowly injected intravenously. Antihistaminic or corticoid drugs were not administered prior to injection of the monoclonal antibody; the indwelling cannula was left in place until the first examination 4–6 hr postinjection.

The scintigram was made with an Elscint Apex 409 AG (Haifa, Israel) rotating gamma camera with whole-body option. At first, a whole-body scan was performed 5 hr postinjection followed by SPECT (single-photon emission computed tomography) or planar scans of the regions of interest 6, 18, and 24 hr postinjection. In the meantime, SPECT or planar images of the pathological region 18 hr postinjection have proved to be sufficient, especially for the clarification of specific postoperative events. The SPECT images were taken in

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a 64 × 64 matrix in steps of 6° by continuous 360-degree rotation. The 60 planar projections were reconstructed to transverse slices with the use of a Hanning filter. Using these transverse slices, coronal and sagittal slices were interpolated. The images were interpreted by two independent observers.

Human Anti-Mouse Antibodies

In 20 patients human anti-mouse antibodies (HAMAs) were determined 2–4 mo after anti-granulocyte immunoscintigraphy (Enzygnost HAMA micro, Behring Werke AG, Marburg, FRG). The sera were tested against the specific antibody used for immunoscintigraphy (anti-idiotypic and anti-isotypic response) as well as against unspecific antibodies (anti-isotypic response). As we did not measure HAMAs before the administration of MAb BW 250/183, HAMA response was rated positive if the HAMA factor in the patient serum was twice as high as in the control serum.

RESULTS

Soft-tissue abscesses had been suspected in 13 of the patients referred to us following surgical intervention after accidents and osteomyelitis in 10 of them. Immunoscintigraphy proved suspected inflammatory soft-tissue processes and localized the foci of inflammation, which were confirmed by second-look operations in 10 cases (Table 1, Figs. 1 and 2). A negative immunoscintigram excluded an inflammatory process in two cases (after total prosthetic replacement of the hip). In one patient with slight positive immunoscintigram, merely hematoma fluid was evacuated after surgical intervention; neither the macroscopic finding nor microbial determination furnished evidence of an infection.

In cases of suspected osteomyelitis, positive immunoscintigraphic findings were confirmed by surgery in four patients; the immunoscintigram was true-negative in five cases and false-positive in one patient with

TABLE 1
Suspected Soft-Tissue Abscesses

Patient no.	IS	SO	BSR	LEUKO	MIC	LOC
1	++	+	130/140	11000	+	knee
2	++	+	108/133	12300	+	thigh
3	–	–	42/70	8900	0	hip
4	+++	+	91/128	8300	+	thigh
5	++	+	77/124	7100	–	hip
6	++	+	94/120	8300	–	knee
7	(+)	+	5/15	6600	–	knee
8	++	+	93/121	9300	+	tibia
9	–	–	20/47	6300	0	hip
10	++	+	80/107	7900	–	thigh
11	+++	+	108/130	6400	–	thigh
12	+	0	70/98	9200	0	tarsus
13	+	0	58/92	7630	0	tibia

Antigranulocyte immunoscintigraphy (IS), sonography (SO), blood sedimentation rate (BSR), leukocytes (LEUKO), microbial determination (MIC), and localization (LOC) of postoperative inflammatory suppurating soft-tissue processes.

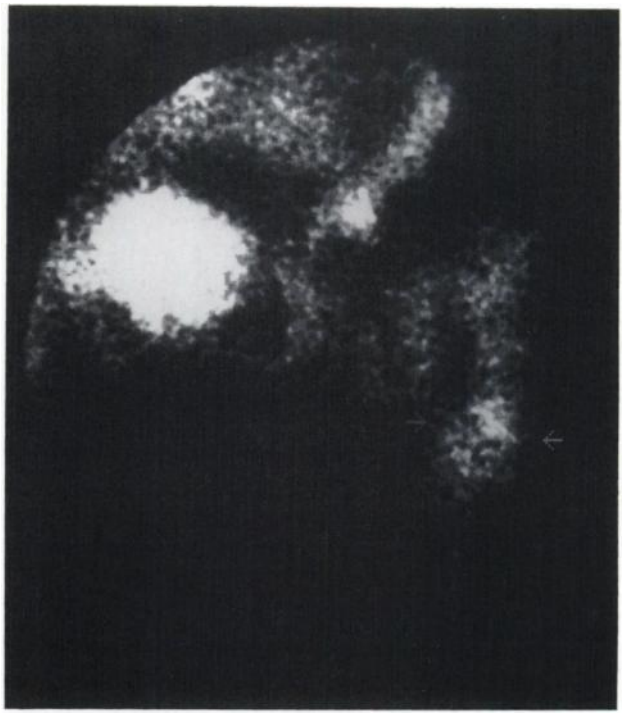

FIGURE 1
A 70-yr-old female patient after left collum femoris fracture; clear uptake of ^{99m}Tc-labeled antigranulocyte antibodies in the soft tissue around the left proximal shaft of the femur (anterior projection); second-look operation: infected hematoma; bacterium: Staph.aureus.

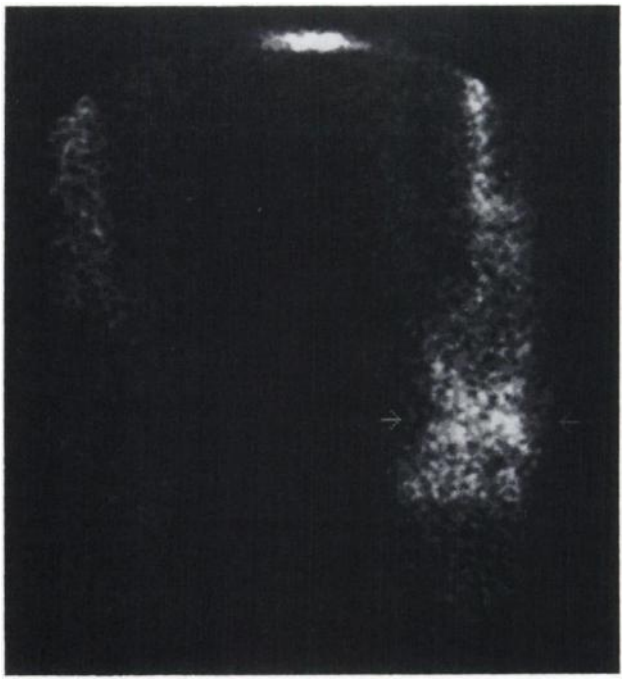

FIGURE 2
A 60-yr-old female patient after open supra- and diacondylar fracture of the left femur; clear uptake of ^{99m}Tc-labeled antigranulocyte antibodies in the soft tissue around the left knee joint (anterior projection); second-look operation; infected hematoma; bacterium: Vibrio fluvialis.

pseudoarthrosis (Table 2 and Fig. 3). In the search for inflammatory abscesses of inner organs or of inflammations associated with internal diseases, the immunoscintigram was true-positive in eight cases, true-negative in two cases, and false-negative in one case of lung abscess with pleural fibrosis (Table 3, Figs. 4A–B, 5A–B, 6A–C). The investigation of 34 patients produced 20 true-positive findings, 11 true-negative, 2 false-positive, and 1 false-negative. Thus, the sensitivity of antigranulocyte immunoscintigraphy with ^{99m}Tc-MAb BW 250/183 was 95% in the patients studied by us; its specificity was 85%. Side effects, such as allergic reactions, were not observed in any of our patients.

HAMA Results

In 20 patients, the antibody response to the first administration of the murine monoclonal antibody (MAb BW 250/183) was measured 2–4 mo after in vivo application. Only one patient developed HAMAs (IgG type), which were able to bind to MAb BW 250/183 at its variable region (anti-idiotypic antibodies). In two patients, the response was anti-isotypic (IgG and/or IgM). However, in all three patients the calculated HAMA factor (patient serum − background/negative serum − background) was very low (Fig. 7).

DISCUSSION

Visualization of inflammatory suppurating processes via nuclear medicine techniques has become an acknowledged method since the introduction of leukocyte scintigraphy with ^{111}In-oxine (*10–13*). Since in vitro labeling is rather elaborate and cells are sometimes damaged during leukocytes separation, possibilities of in vivo labeling have been investigated intensively in the past few years (*14*).

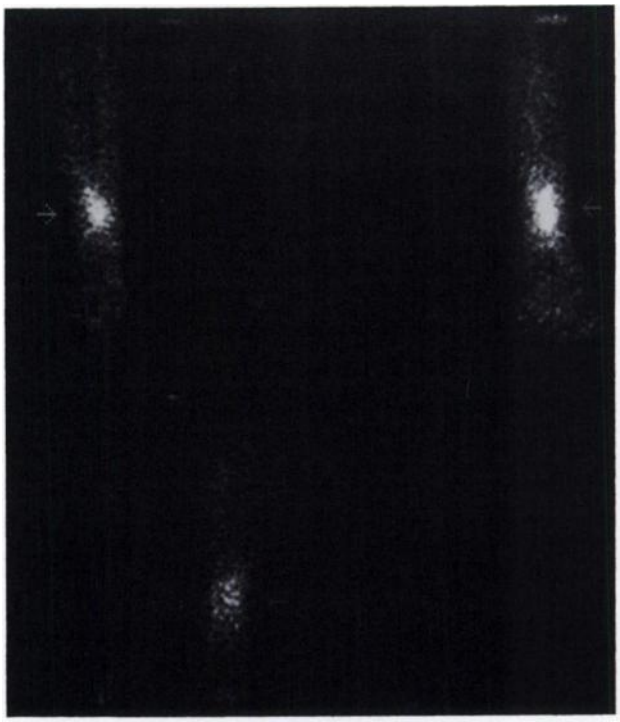

FIGURE 3
A 26-yr-old male patient after right tibia fracture; suspected osteomyelitis; circumscribed uptake of ^{99m}Tc-labeled antigranulocyte antibodies in the area of the right distal tibia (anterior projection—left upper image; right lateral projection—right upper image; posterior projection—left lower image); second-look operation: removal of the osteomyelitic focus; no bacteria detected.

In 1984 Buchegger and Mach defined a monoclonal antibody, which is directed against the nonspecific cross-reacting antigen (NCA 95), an epitope of the carcinoembryonic antigen (CEA). This antigen is also present on the surface of neutrophil granulocytes (*15*). In vivo experiments and pharmacokinetic data have

TABLE 2
Suspected Osteomyelitis

Patient no.	IS	X-RAY	BSR	LEUKO	LOC	SURG
1	−	+	11/35	13900	tibia	0
2	++	+	87/123	7800	tibia	+
3	++	(+)	46/81	7800	tibia	−
4	++	+	50/93	12300	tibia	+
5	+++	+	60/91	9300	tibia	+
6	−	(+)	49/97	5700	hip	0
7	−	−	8/21	4600	tibia	0
8	++	+	57/81	5600	tibia	+
9	−	−	107/120	4900	thigh	0
10	−	(+)	70/108	7800	tibia	0

Antigranulocyte immunoscintigraphy (IS), x-ray findings (X-RAY), blood sedimentation rate (BSR), leukocytes (LEUKO), localization (LOC), and results of second-look operation (SURG) for questionable osteomyelitic events.

TABLE 3
Suspected Abscesses of Inner Organs

Patient no.	IS	Diagnosis	BSR	LEUKO
1	++	Lung abscess	121/126	12500
2	+++	Colitis ulcerosa	118/131	6400
3	++	Colitis ulcerosa	88/107	11100
4	+	Pylonephrosis	93/123	16400
5	+++	Infarction pneumonia	112/126	5000
6	−	Lung abscess	146/150	8500
7	−	Suspected abscess	54/102	18500
8	−	Suspected discitis	43/75	8100
9	+	Appendicitis	120/142	12700
10	++	Diabetes gangrene	70/102	9300
11	++	Diabetes gangrene	69/98	10400

Antigranulocyte immunoscintigraphy (IS) and blood sedimentation rate (BSR), leukocytes (LEUKO) for suspected inflammatory suppurating processes of inner organs.

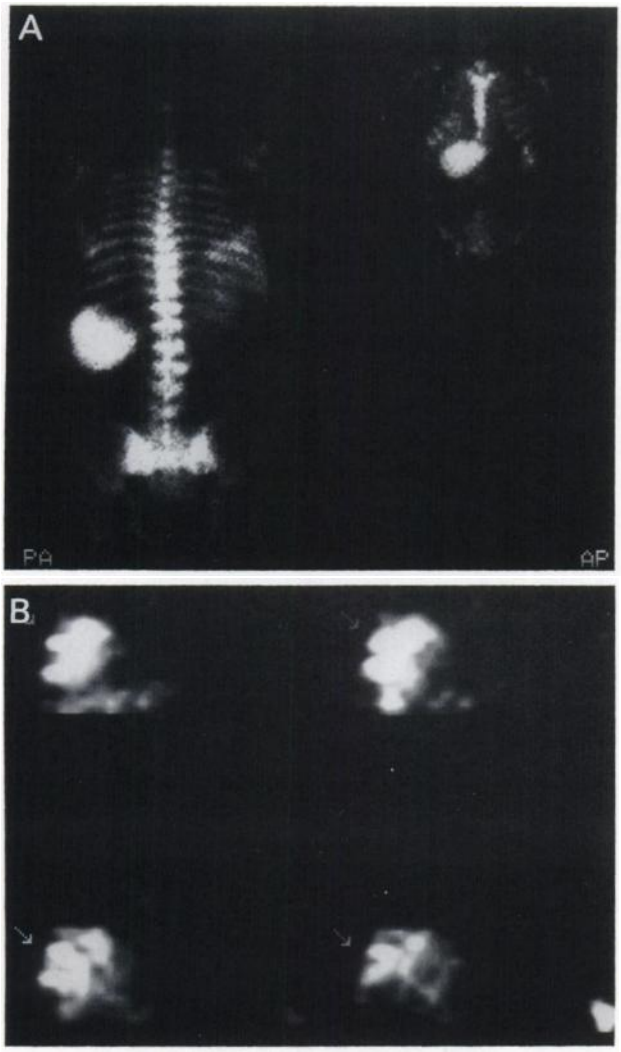

FIGURE 4
(A) An 80-yr-old male patient with suspected infarction pneumonia on the right side; circumscribed uptake of ^{99m}Tc-labeled antigranulocyte antibodies in the right lower area of the lung. (Whole-body scan 5.5 hr p.i. in posterior and anterior projection). (B) SPECT 6 hr postinjection (coronal slices) shows massive antibody uptake in the right lower pulmonary area.

shown that the function of granulocytes is not influenced by in vivo labeling with monoclonal antibodies (*16,17*).

First clinical results with iodine-123-labeled monoclonal antibodies (^{123}I-MAb47) were reported by Locher in 1986 and Seybold in 1988 (*14,18*). They confirmed the high sensitivity of leukocyte immunoscintigraphy and its clinical relevance. However, the disadvantages of immunoscintigraphy with ^{123}I are the high costs of ^{123}I and the fact that the radionuclide is not always available in routine nuclear medicine laboratories. In 1987, Schwarz et al. succeeded in achieving stable labeling of monoclonal antibodies with ^{99m}Tc; thus, the utilization of leukocyte immunoscintigraphy has become possible in routine nuclear medicine (*9*).

The ^{99m}Tc-labeled MAb BW 250/183 defined by Bosslet is also directed against NCA 95, an epitope of CEA and does not lead to antibody-dependent or complement-dependent lysis of granulocytes (*8*). Schorlemmer showed that MAb BW 250/183 does not inhibit cell-specific functions such as endocytosis, lysosomal enzyme secretion, and superoxide anion production (*19*). Bosslet et al. could demonstrate that no changes in physiologic granulocyte function, such as lysosomal enzyme secretion or oxidative bursts, occur (*20*).

The specificity on human tissue was reported by Bosslet et al. (*7*). MAb BW 250/183 binds to the membrane of neutrophil granulocytes by more than 90%. When this ^{99m}Tc-labeled monoclonal antibody is used, radiation exposure amounts to only ~15% of the dose of ^{67}Ga or ^{111}In. Other than with ^{99m}Tc-HM-PAO, no radioactivity passes into the intestines so that inflammatory processes such as colitis ulcerosa or Crohn's disease can be visualized with ^{99m}Tc-MAb BW 250/183 without the uncertainty of a false-positive unspecific uptake. Kroiss et al. have demonstrated in patients with inflammatory bowel disease that the sensitivity of leukocyte immunoscintigraphy with ^{99m}Tc-labeled monoclonal antibodies to be 77% for planar imaging and 91% for SPECT (*21*).

HAMA response to murine monoclonal antibodies depends on the route of application and the amount and type of the antibody (*22*). In our investigations (0.5 mg antibody), only one out of twenty patients showed an anti-idiotypic, two an anti-isotypic response. However, no side effects occurred in our patients. Second administration of the antibody in two cases with ulcerative colitis neither lead to a diminished image quality nor to allergic reactions.

Apart from its merits in the search for abscesses of inner organs, leukocyte immunoscintigraphy can be very helpful in the decision for or against second-look operations of patients with inflammatory soft-tissue and bone processes following orthopedic surgery. However, it also must be borne in mind that all noninflammatory processes accompanied by leukocytosis, such as hematomas or peripheral bone marrow expansion (e.g. pseudoarthrosis) will produce positive results. The sensitivity of leukocyte immunoscintigraphy was very high in our study (95%). On the other hand, precisely these leukocytes not associated with inflammatory events produced false-positive results in the clarification of inflammatory suppurating processes, thus reducing the specificity to 85%. Therefore, a differentiation between infected and noninfected postoperative hematomas by leukocyte scintigraphy does not seem to be possible. Yet this qualification applies to the visualization of leukocytes by nuclear medicine techniques in general and is not limited to leukocyte immunoscintigraphy. Perhaps a differentiation between infected and noninfected hematoma is possible by a recently described new method

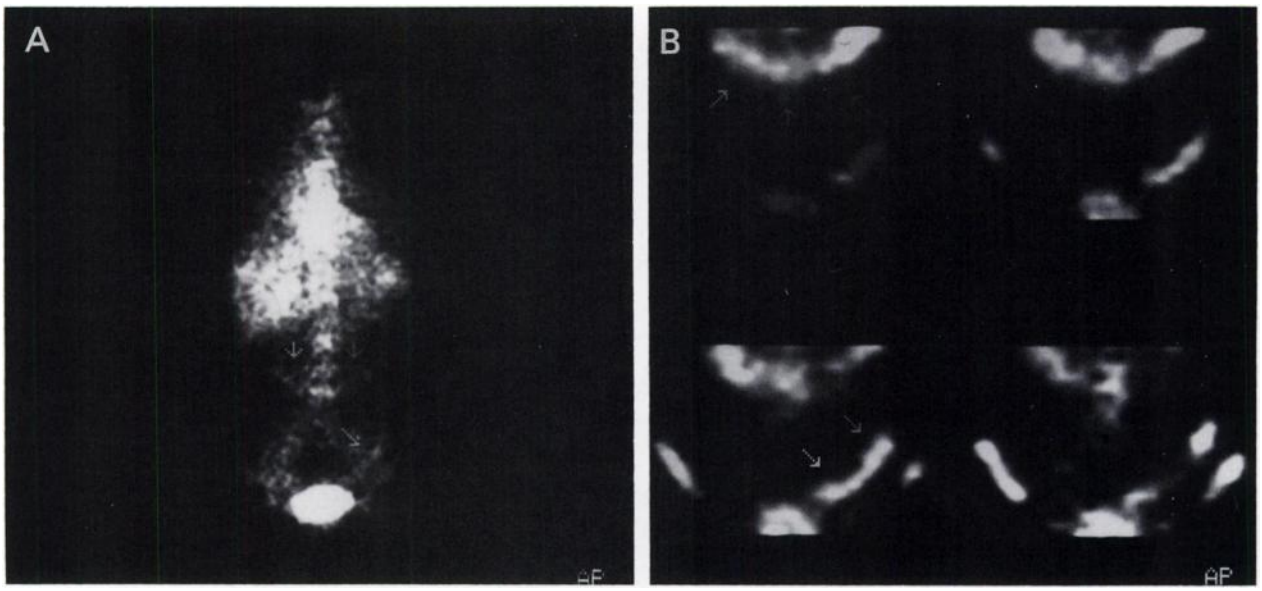

FIGURE 5
(A) A 31-yr-old male patient with hyperacute ulcerative colitis. The whole-body scan shows a positive uptake in the transverse, descending, and pelvic colon. (B) SPECT imaging 6 hr postinjection (coronal slices) circumscribed antibody uptake in transverse, descending, and pelvic colon.

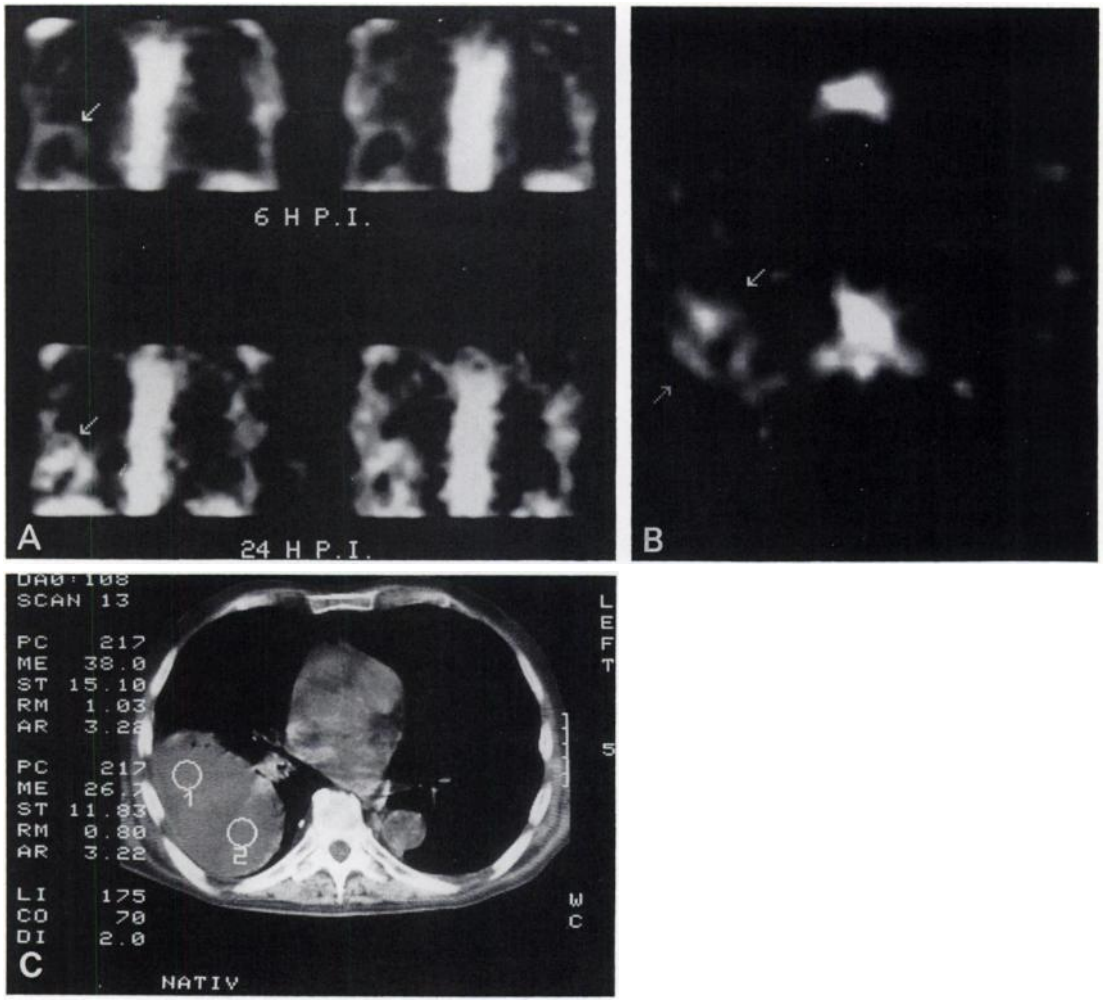

FIGURE 6
(A) A 75-yr-old female patient with suspected right pulmonary abscess. SPECT 6 hr postinjection (upper row) and 24 hr postinjection (lower row): circumscribed, primarily marginal antibody uptake in the right lower dorsal area. (B) SPECT imaging 24 hr postinjection (transaxial slice) demonstrates uptake of the ^{99m}Tc-labeled MAb in the area of the pulmonary abscess. (C) TCT (transaxial slice) shows hyperdense lesion in the right lower dorsal pulmonary area.

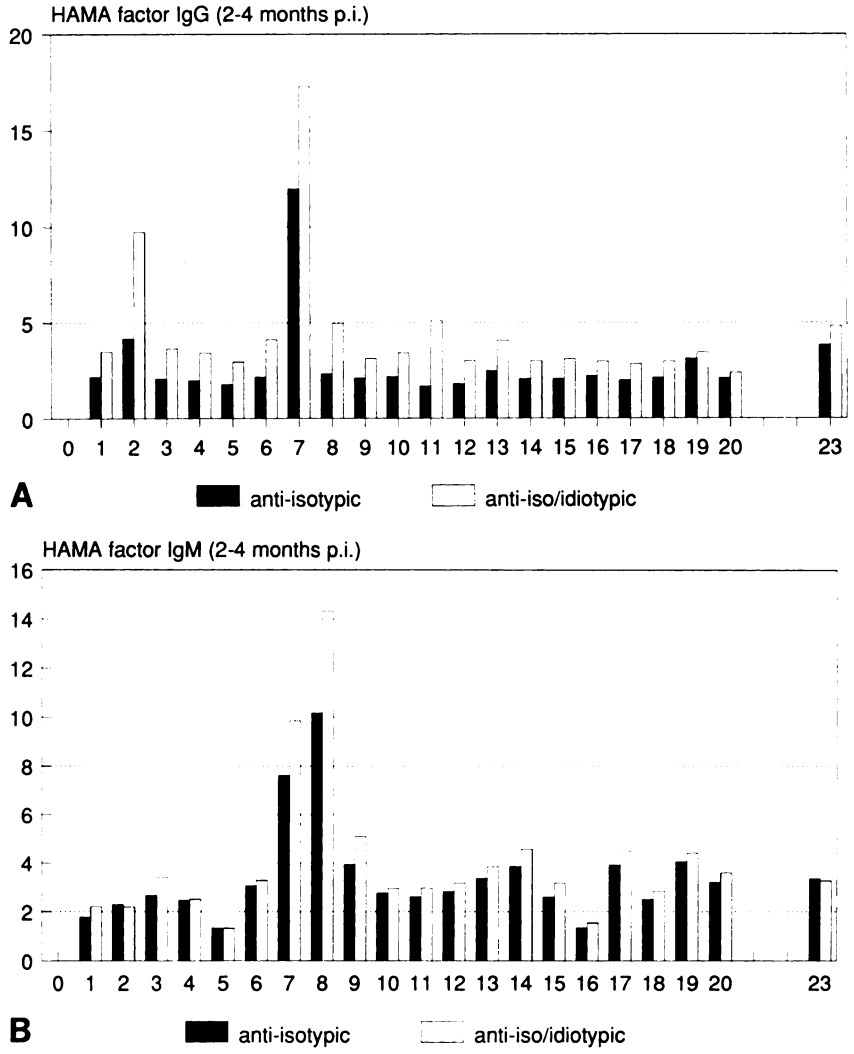

FIGURE 7
(A) Anti-isotypic and/or anti-idiotypic HAMA response (IgG) in twenty patients (1–20 patients, 23 control serum) after antigranulocyte immunoscintigraphy with 0.5 mg MAb BW 250/183. (B) Anti-isotypic and/or anti-idiotypic HAMA response (IgM) in twenty patients (1–20 patients, 23 control serum) after antigranulocyte immunoscintigraphy with 0.5 mg MAb BW 250/183.

with an ^{111}In-labeled nonspecific immunoglobulin (*23*). False-negative findings, on the other hand, must be expected in the demonstration of older encapsulated abscesses, in which exchange or migration of neutrophil granulocytes have been reduced.

A consecutive scan (24 hr postinjection) is indispensible in these cases, since quite frequently, at least marginal leukocytosis is proved in the delayed image even though the 6-hr scan has been negative. The sensitivity in cranial, thoracic, abdominal, and hip regions can be increased significantly by use of the SPECT technique (*24,25*), while planar imaging (especially when the x-ray findings are known) is sufficient for the identification of inflammatory processes in the extremities.

The introduction of ^{99m}Tc-labeled antigranulocyte antibodies allows a further clinically relevant in vivo application of monoclonal antibodies in addition to anti-CEA immunoscintigraphy for the diagnosis of colorectal tumor recurrences (*26*). Easy labeling, the optimum gamma energy of ^{99m}Tc and the resulting high-quality SPECT images offer considerable advantages compared to in vitro labeling of leukocytes with ^{111}In oxine.

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