Intraindividual comparison of ^{99m}Tc-labelled anti-SSEA-1 antigranulocyte antibody and ^{99m}Tc-HMPAO labelled white blood cells for the imaging of infection

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Abstract. Technetium-99m labelled antigranulocyte antibodies are ready to use and are sensitive and specific in the diagnosis of infectious diseases. ^{99m}Tc-SSEA antigranulocyte antibodies have a very high affinity constant (K_d 10^{-12} *M*) for human neutrophils (PMNs), and excellent imaging qualities with high target/background ratios. The aim of this study was to compare the diagnostic accuracy of the ^{99m}Tc-anti-SSEA-1 monoclonal antibody (Mab) with that of ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO)-labelled white blood cells (WBCs). To this end, 17 patients with 23 proven infectious foci were examined with 555 MBq ^{99m}Tc-anti-SSEA-1 MAb and with 370 MBq ^{99m}Tc-HMPAO labelled autologous leucocytes within a period of 7 days. All the infections were confirmed by culture, biopsy, surgery and follow-up. Whole-body images and planar spot views with the antibody were performed at 1-h, 4-h and 24-h post injection; the biodistribution of the antibody was quantified, absorbed radiation doses were calculated and the diagnostic results were compared with the ^{99m}Tc-HMPAO WBC images. Human anti-mouse antibody (HAMA) evaluation was performed in all patients before and 3 months after antibody injection. Blood was drawn at different times after ^{99m}Tc-anti-SSEA-1 MAb injection to determine the amount of granulocyte-associated radioactivity and to calculate recovery. ^{99m}Tc-anti-SSEA-1 MAb scintigraphy detected all 23 lesions, while 21 were detected with ^{99m}Tc-HMPAO WBC scan. In this small group of patients, the sensitivity and specificity of ^{99m}Tc-anti-SSEA-1 MAb scintigraphy were 95% and 96% respectively, as compared with 91% and 82% respectively for ^{99m}Tc-HMPAO WBC scan. An increasing uptake of the injected activity in the lesion at different time points was indicative of high affinity and of specific PMN binding.There was no HAMA formation. In four of five patients investigated, a transient mild leukopenia was found at 15 min p.i.. There was increased uptake of the antibody in liver and spleen and normal uptake in kidneys and bone marrow.The estimated radiation doses for the whole body and the red bone marrow were 1.1×10^{-2} cGy/37 MBq and 5.3×10^{-2} cGy/37 MBq, respectively. The activity associated to the PMNs in vivo was 33.5%, 30.6%, 21.3% and 9% at 5, 15, 30 and 45 min. post-injection, respectively. It is councluded that use of ^{99m}Tc-anti-SSEA-1 antigranulocyte antibodies demonstrates promising results comparable to those obtained with ^{99m}Tc-labelled autologous WBCs. The ^{99m}Tc-labelled MAb is ready to use, has excellent image qualities and a high target/background ratio.

Key words: Infection imaging – Technetium-99m-labelled anti-SSEA-1 monoclonal antibody – Technetium-99m hexamethylpropylene amine oxime – White blood cells

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Introduction

A variety of radiopharmaceuticals can be used for imaging infection [1].The “gold standard” for localizing infectious diseases is the labelling of isolated granulocytes with indium-111 oxine, first described by Thakur et al. [2]. The use of technetium-99m-labelled white blood cells (WBCs) is comparably effective but offers lower radiation exposure [3, 4].

Monoclonal antibodies (MAbs) specific for neutrophil surface antigens, when labelled with a radionuclide, should serve as ideal agents to selectively label human neutrophils (PMNs) in whole blood vivo by injection into the systemic circulation of the patient. Previously, we have successfully used ^{99m}Tc-labelled monoclonal anti-

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body BW 250/183 [5] and antibody Fab′ fragments [6] for imaging infection or inflammation. The reason for this success was not only the degree of MAb specificity for granulocyte-associated antigens but also the fact that the tissues had leaky blood vessels and enlarged interstitial space that permitted non-specific accumulation of MAb [5]. A disadvantage of the whole-antibody technique is the production of human antimouse antibodies (HAMAs), which seems to be dose dependent. The frequency of HAMA formation ranged from more than 30% in patients receiving repeated injections down to 4.5% in patients with a single dose of 125 μg of the antibody. However, 1.25 mg Fab′ fragments (IMMU-MN3) did not show HAMA formation [6, 7].

Recently, a new antibody has been reported by Thakur et al. [8]. This is an IgM murine monoclonal antibody (anti-SSEA-1) that recognizes the lacto-*N*-fucopentaose or CD-15 antigenic determinants present in human PMNs and eosinophils but not in other circulating blood cells [8].

In light of these results with high target to background ratios and the high PMN affinity constants [8, 9], this study was undertaken to compare the use of this new monoclonal IgM antibody with ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO)-labelled leucocytes in the same group of patients.

Material and methods

Patients. In this study 17 patients (nine men and eight women; mean age 39±6 years) were examined with a ^{99m}Tc-labelled anti-SSEA-1 murine monoclonal IgM antibody (SORIN, Biomedica, Saluggia, Italy and RHOMED, Albuquerque, New Mexico, USA). The patients of this study had to fulfil the following criteria: (a) be 21 years of age or older, (b) not be a pregnant or lactating female, (c) present with a high index of clinical suspicion of having an infection manifested by at least one culturel or scintigraphic or radiological result, and have an elevated WBC count and an elevated sedimentation rate. Exclusion criteria were antibiotic therapy within the past 30 days and a history of renal insufficiency manifested by a BUN level of more than 40 mg/dl or a serum creatinine of ≥2.0 mg/dl. The patients had given their informed, written consent to participation in the study. Of the 17 patients, seven had soft tissue infections and ten osteomyelitis. Six of the patients had more than one infectious lesion, yielding a total of 23 sites of infection. These comprised seven soft tissue infections, ten infections of the knee/foot joints, three infections of the long bones, one infection of the shoulder joint, one infection of the nasal sinus and one case of colitis.

All patients underwent ^{99m}Tc-HMPAO WBC imaging 2–7 days prior to the antibody imaging. All infections were confirmed by culture (n = 9), biopsy (n = 12), surgery (n = 16) or follow up (n = 17).

Methods

Characterization of the antibody. The ^{99m}Tc-labelled monoclonal antigranulocyte antibody is an IgM isotype, with a molecular weight of 900 kDa produced by immunizing mice with murine embryonal carcinoma F9 cells. Although SSEA-1 is selectively expressed on mouse embryonic cells in a stage-specific manner, the epitope is also expressed on the surface of human PMNs, histiocytes, tissue macrophages and reticulo-endothelial cells and presumably on the PMN precursor cells in bone marrow [8–11].

Labelling procedure. The antibody (100 μg) was chemically modified for direct labeling with ^{99m}Tc. For this procedure, the antibody was reduced with dithiothreitol and treated with Sn(II) to protect the exposed sulph hydryl groups and to reduce pertechnetate. Maltose was used to stabilize the formulation. Aliquots of 250 μg of reduced antibody protein were placed in 5-ml serum vials, lyophilized, backfilled with argon gas and sealed. The lyophilized product is estimated to have a shelf-life of 6 months or more when stored at room temperature. However, until stability studies are completed, storage at 4°C was required. Labelling was accomplished in essentially one step: 1110 MBq/1 ml ^{99m}Tc was added, the vial was then swirled to mix the contents and incubated at room temperature for 30 min. The remaining free pertechnetate and reduced ^{99m}Tc were removed by combined gel filtration/anion exchange chromatography on a small DEAE Sephadex A-25 column using 0.9% saline as the eluant [12]. No intermediate purification was required. After reduction of free pertechnetate 555 MBq ^{99m}Tc-anti-SSEA-1 MAb was ready to be injected.

Administration of the antibody. All patients gave their written, informed consent to the study. The administration of the antibody was approved as a clinical trial by the local ethical committee, the local radiation protection authorities and the German federal agency for radioprotection.

Without premedication 555 MBq (15 mCi) of ^{99m}Tc-MAb was slowly infused i.v. over a period of 5 min. In all patients, 100 μg of the MAb combined with 20 mg of human serum albumin as a carrier was used.

The vital signs of all patients were recorded over 4 h p.i. Several blood samples were collected for determination of the radioactivity clearance and of cell blood count. Kinetic studies for the ^{99m}Tc-anti-SSEA-1 MAb were performed.

Data acquisition and image interpretation. In all 17 patients who received the ^{99m}Tc-anti-SSEA-1 antibody and ^{99m}Tc-HMPAO WBCs for diagnostic imaging, a double-headed (Prism 2000, Picker) large field of view gamma camera with an on-line computer system (Odyssey 1500) and equipped with a low-energy high-resolution collimator was used. The windows (20%) were centered over the 140-keV photopeak of ^{99m}Tc. In all cases whole-body images over 15 min were obtained 1 h, 4 h and 24 h post injection in anterior and posterior projections. The pixel matrix applied was always128×128 for planar scintigraphy and 256×256 for whole-body scintigraphy, and the total counts collected were 500 000. In addition, planar imaging of the region of interest was performed at 4 h and 24 h post injection in anterior and posterior views (PCS 2000, Picker) and 300 000 counts were collected. The scintigraphic images were read by a group of three nuclear medicine physicians and the results were correlated with the findings of the ^{99m}Tc-HMPAO WBC scan as well as with the histological and radiological findings.

Peripheral leucocyte count after MAb injection. In all patients blood samples were taken before and at 4 h and 24 h post injection and in five patients at 5, 15, 30 and 45 min after injection for counting of peripheral leucocytes.

Human anti mouse antibody. For the evaluation of HAMA reaction, one blood sample was taken before MAb administration and

in 14 of the 17 patients, 3 months post injection. After anti-SSEA-1 IgM MAb injection for HAMA reaction, IgM HAMA formation has to be expected in the first 2–3 days, whereas IgG HAMA formation is to be anticipated in the subsequent period. Therefore, for the immunoassay for in vitro diagnosis the HAMA ELISA Medac assay was used, this being a one-step enzyme assay. The test was standardized against anti-mouse IgG antibodies and cut-off at 95% specificity was determined with the HAMA titre of 74 ng/ml.

Calculation of recovery. The radioactivity counting of other blood samples permitted us to determine the clearance of blood activity as well as the percentage of activity bound to plasma and neutrophils. PMNs were separated using the Percoll/plasma gradient.

Recovery of granulocyte-bound ^{99m}Tc activity in the patient was calculated according to the formula: recovery = activity/ml×blood volume×100/injected activity.

Blood collection and counting. Blood samples (3 ml) were taken from a cubital vein in MAb-receiving patients at 5 min, 15 min, 30 min, 45 min and 60 min post injection for the determination of plasma clearance (supernatant, pellet). After centrifugation, the activity was measured separately in the supernatant and in the cell pellet.

Percoll/plasma gradients. In accordance with to the previously published procedure [13], in five patients 40 ml peripheral venous blood was drawn once for a discontinuous Percoll/plasma gradient after antibody injection. In this way one sample at 5, 15, 30, 45 and 60 min was taken from different patients. The activity distribution in the Percoll/plasma gradient was measured using a small field of view gamma camera from Picker, and 100 000 counts were collected. The activity distribution was roughly estimated by a region of interest technique over the different gradient layers.

Biodistribution and radiation dosimetry. In all patients the distribution of radiolabelled monoclonal antibody was quantified in the whole body and in various organs. The kinetic considerations included whole body imaging at 1 h, 4 h and 24 h post injection. The estimated absorbed radiation dose to each organ was calculated using the MIRD scheme [14].

Affinity determination of SSEA-1. Leucocytes were isolated from 50 ml of pooled blood from two healthy donors. Granulocytes were isolated by Percoll/plasma gradient centrifugation. ^{99m}Tc-labelled anti-SSEA-1 MAbs were diluted and incubated with approximately one million granulocytes in PBS pH 7.4 for approximately 1 h. The samples were centrifuged, and the activity in the supernatant and in the pellet were measured in the scintillation counter. Scatchard plot analysis of these data was performed (bound/free activity versus total protein; affinity = slope of the regression line) [15].

Results

In 17 patients, the total number of infections detected was 23. All 23 infectious lesions were correctly identified with the ^{99m}Tc-anti-SSEA-1 MAb. The ^{99m}Tc-HMPAO WBC scans were positive in 21/23. Seven patients suffered from soft tissue infections, ten patients from infections of the knee or the foot joint and three patients from infections of the tibial or femoral bone. One infection of the shoulder joint was found, as well as one infection of the nasal sinus and one case of colitis.

The 2/23 foci of infection that were detectable with the ^{99m}Tc-anti-SSEA-1 MAb comprised one foucus in the nasal sinus in a 45-year-old female patient with post-

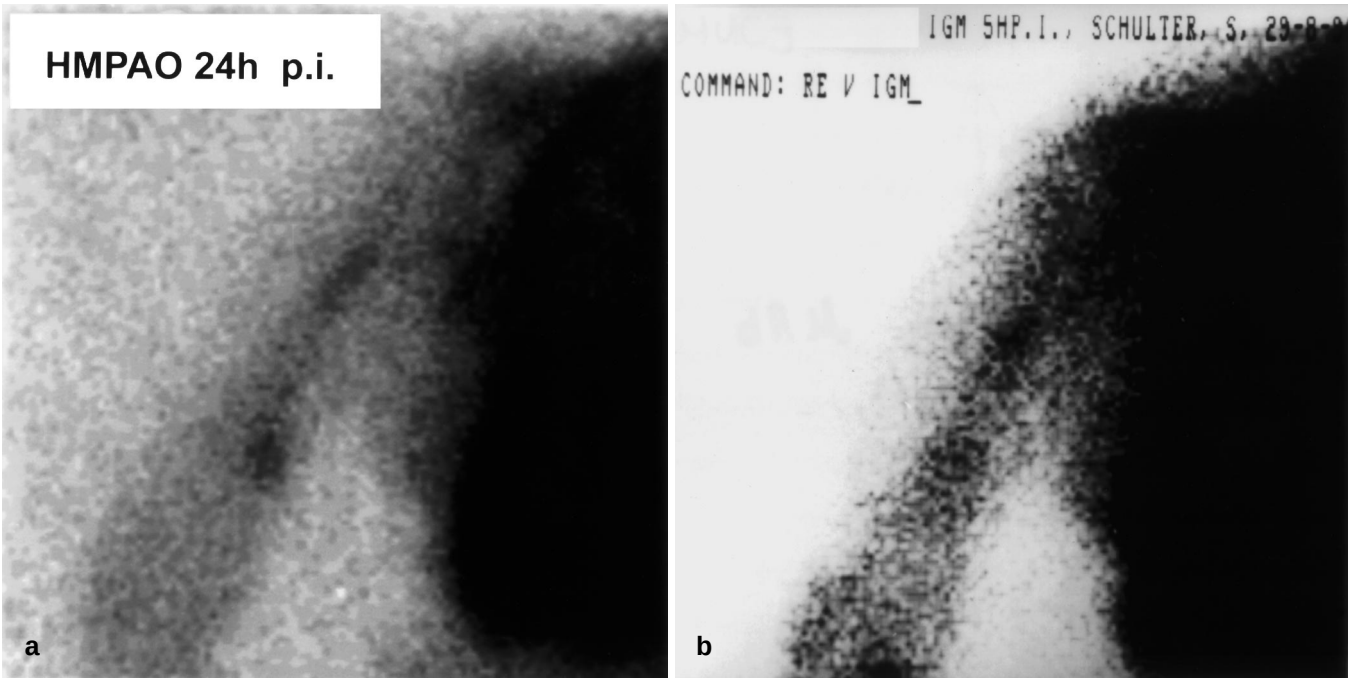

Fig. 1a, b. Seventy-five year old female patient with an infected prosthesis of the right elbow. ^{99m}Tc-HMPAO WBC scan (**a**) shows a larger area of uptake than the SSEA-1 scan (**b**), on which uptake is mostly focally limited to the upper end of the prosthesis shaft. Radiological findings were negative

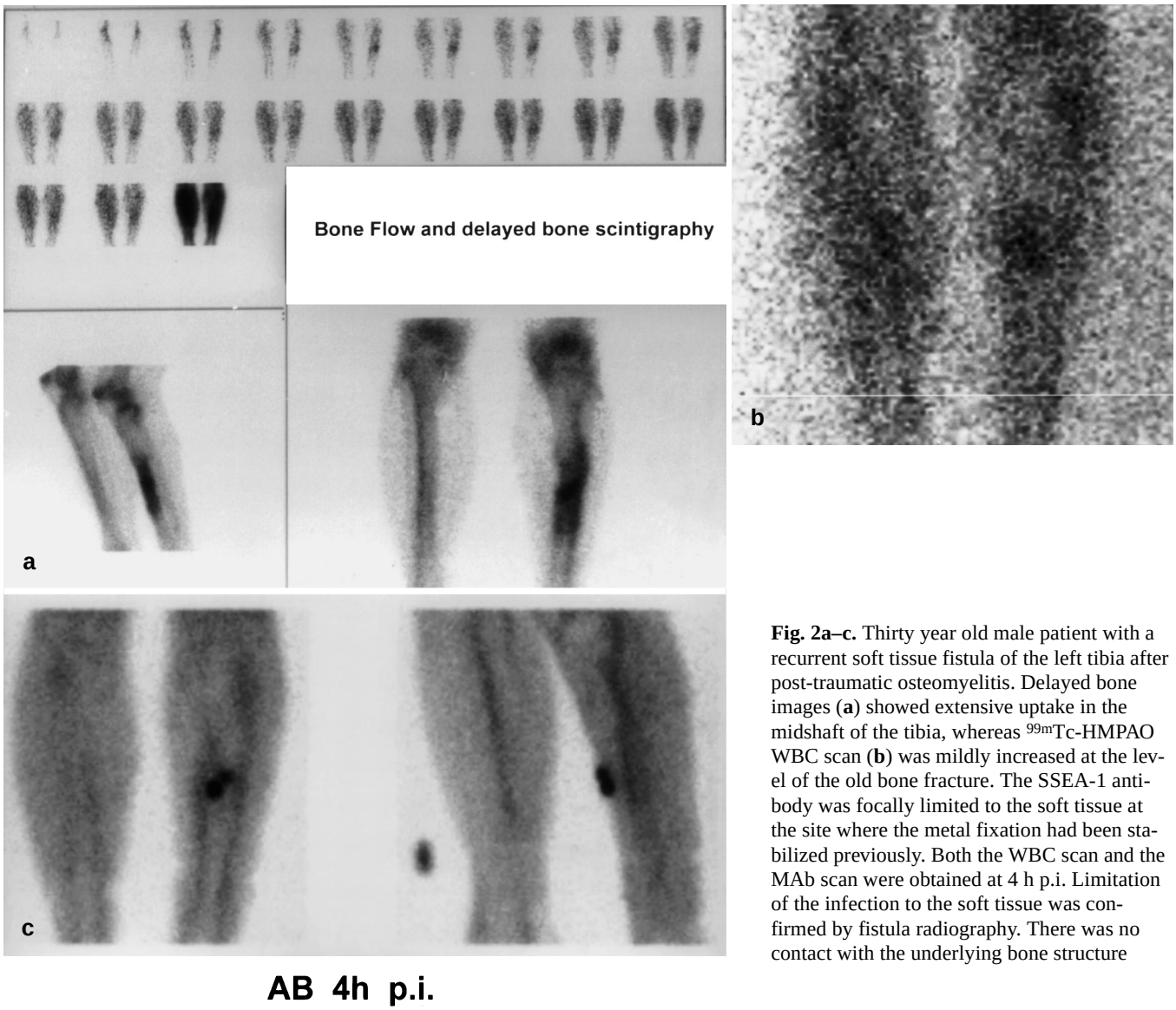

Fig. 2a–c. Thirty year old male patient with a recurrent soft tissue fistula of the left tibia after post-traumatic osteomyelitis. Delayed bone images (**a**) showed extensive uptake in the midshaft of the tibia, whereas ^{99m}Tc-HMPAO WBC scan (**b**) was mildly increased at the level of the old bone fracture. The SSEA-1 antibody was focally limited to the soft tissue at the site where the metal fixation had been stabilized previously. Both the WBC scan and the MAb scan were obtained at 4 h p.i. Limitation of the infection to the soft tissue was confirmed by fistula radiography. There was no contact with the underlying bone structure

traumatic osteomyelitis of the right tibia and one case of ascending colitis in a 68-year-old male patient with a diabetic forefoot. The infection of the nasal sinus was verified with radiographically.

In four patients, the results of the ^{99m}Tc-MAb and ^{99m}Tc-HMPAO WBC scans were not concordant. The *first* such patient (no. 9 in Table 1)was a 75-year-old female with an infected prosthesis of the right elbow joint (Fig. 1). ^{99m}Tc-HMPAO WBC scan showed a much larger area of uptake than the anti-SSEA-1 MAb scan, on which uptake was limited to the upper end of the prosthesis shaft. Radiological findings were negative. On the basis of the anti-SSEA-1 MAb scan the prosthesis was not removed. Instead, the patient was set on antibiotics and the infection had resolved 2 weeks later. The *second* patient (no. 4 in Table 2) was a 30-year-old male with a recurrent soft tissue fistula of the left tibia after post-traumatic osteomyelitis (Fig. 2). Delayed bone images showed extensive uptake in the midshaft of the tibia, whereas ^{99m}Tc-HMPAO WBC scan showed a mild increase in uptake at the level of the old bone fracture. The anti-SSEA-1 MAb scan demonstrated infection focally limited to the soft tissue; this was confirmed by fistula radiography, which showed no contact with the underlying bone structure. The third patient (no. 17 in Table 1) was a 68-year-old male with the clinical signs of a diabetic forefoot (Fig. 3). ^{99m}Tc-HMPAO WBC scan showed normal finding at the forefoot, whereas the anti-SSEA-1 MAb scan showed a focally increased uptake in the fourth and fifth toes of the right foot. Superinfected gangrene was confirmed sugically. The *fourth* patient (no. 15 in Table 1) was a 56-year-old woman with post-traumatic osteomyelitis of the upper and lower right tibia (Fig. 4). The findings with both methods at this site were consistent. In addition, however, the anti-SSEA-1 MAb showed focally increased uptake at the right nasal sinus, which was not recognized on the ^{99m}Tc-HMPAO scans. A swelling of the mucosa of the nasal sinus was than

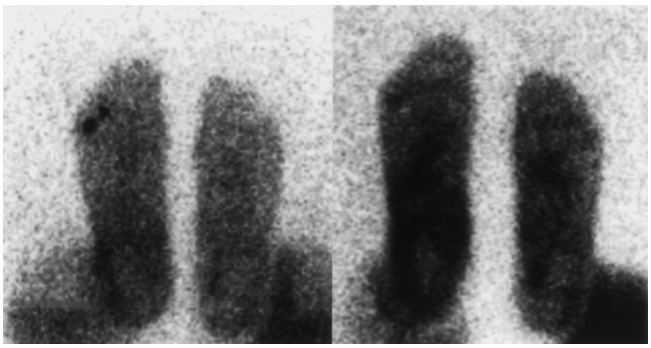

Fig. 3. Sixty-eight year old male patient with suspected diabetic forefoot. ^{99m}Tc-HMPAO WBC scan (*right*) was normal or mildly positive in the forefoot, whereas the SSEA-1 scan (*left*) showed focally increased uptake in the fourth and fifth toes of the right foot. Superinfected gangrene was confirmed surgically

confirmed by normal radiography. A summary of the case details is provided in Table 1.

Regarding the *biodistribution* of the anti-SSEA-1 MAb, the quantitative mean uptake as % injected dose at 1 h, 4 h and 24 h post injection was, respectively 37.4%, 36% and 27.9% in the liver, 22%, 21.9% and 11% in the spleen, 2.5%, 2.7% and 3.9% in one kidney and 8.4%, 9.9% and 12.5% in a clearly defined bone marrow area. In one lesion we also observed increasing uptake of 0.3%, 0.7% and 2.2% at 1 h, 4 h and 24 h post injection, respectively.

The *dosimetric* radiation dose delivered to the organs was calculated and is shown in Table 2.

With the *HAMA* ELISA medac assay, no HAMA formation was detectable at three months after injection in 14/17 patients. One patient had a high HAMA level before injection of the anti-SSEA-1 MAb and in the remaining two patients 3-month blood samples were not available.

In four of the five patients in whom blood samples were taken at regular intervals during the first hour following MAb injection the *peripheral leucocyte count* showed a transient decrease at about 15 min post injection. In two patients, this decrease was by about 48% whereas in the other two it was by about 21% (Fig. 5).

The *PMN association affinity constant* of the anti-SSEA-1 IgM MAb was determined to be 1.6×10^{-12} *M*.

At 5, 15, 30 and 45 min post injection the activity found in the single blood samples was 93%, 85%, 59% and 25% of the injected dose respectively. This was 40% of the injected activity. Over 90% of this cell-bound activity was associated with PMNs. This means that at 5, 15, 30 and 45 min, the recovery of the PMN-bound activity in vivo was 33.5%, 30.6%, 21.3% and 9% of the total injected dose.

Discussion

Radioiodinated (^{131}I, ^{123}I) [16, 17] or ^{99m}Tc-labelled MAbs or fragments [18, 19] have been used for the scintigraphic detection of tumours and infections in humans for many years. The initial clinical experience with in vivo markers for PMNs for the imaging of infection was excellent [19, 20]. Images of diagnostic quality were obtained earlier and the image quality was better compared with corresponding images obtained with ^{111}In-WBCs [20–23]. The better image quality could be attributed to suitable gamma energy and to the large quantity of ^{99m}Tc

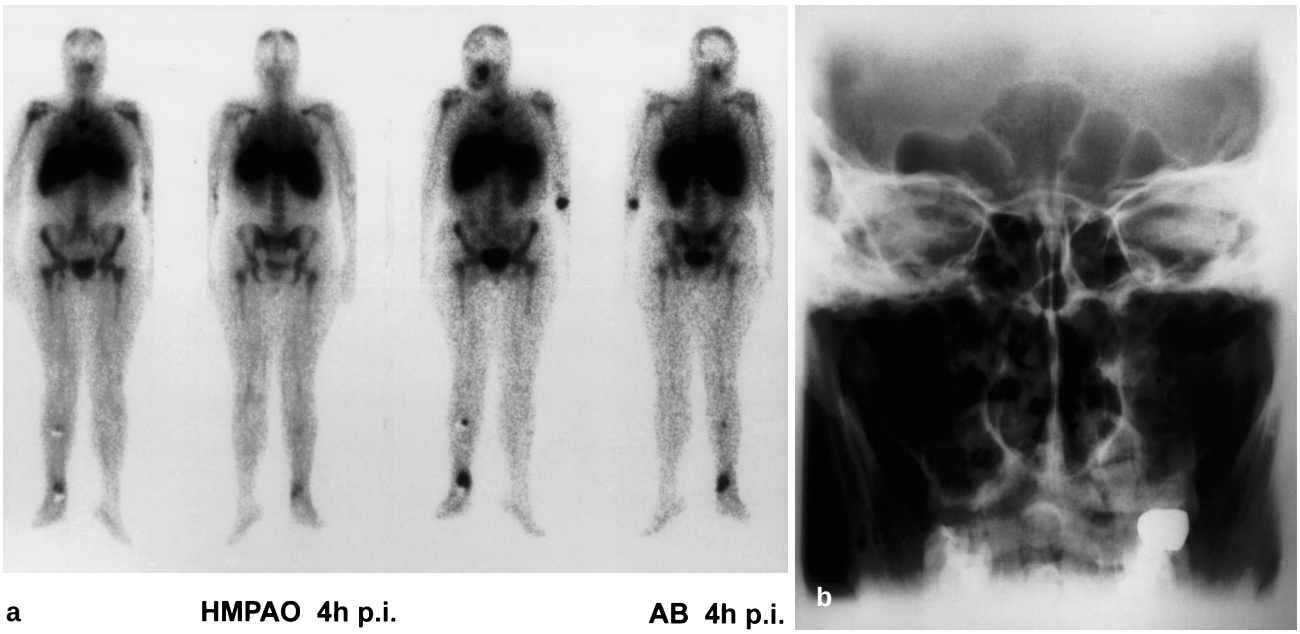

Fig. 4a, b. Fifty-six year old woman with post-traumatic osteomyelitis of the upper and lower right tibia. Imaging was performed with both methods 4 h. p.i., and the findings at this site were consistent (**a**). In addition, the SSEA-1 antibody scan showed focally increased uptake at the right nasal sinus, which was not recognized on the ^{99m}Tc-HMPAO scan. Sinusitis was confirmed by normal radiography (**b**)

Table 1. Patient details and diagnoses based on scintigraphic results with ^{99m}Tc-MAb and ^{99m}Tc-HMPAO WBCs

Patient	Age (yrs)	Sex	Diagnosis	Confirmation by
1	35	m	Osteomyelitis of the tibia	Culture, bone scan
2	28	f	Osteomyelitis of the tibia and foot joint	Culture
3	31	f	Infected external metall fixation at the femur	Surgery
4	30	m	Soft tissue infection	Fistula radiography
5	33	f	Post-traumatic infection of the foot joint and soft tissue of the upper leg	Biopsy, culture
6	35	m	Osteomyelitis of the knee joint and infected scar soft tissue	MRI and culture
7	37	m	Perforating soft tissue fistula 3 phase bone scan	
8	33	m	Multiple sites of osteomyelitis in the upper right ankle/foot and soft tissue	Culture
9	75	f	Infected prosthesis of the elbow	Follow up
10	34	f	Infected knee joint	Biopsy and culture
11	44	m	Infected shoulder joint and colitis	biopsy, endoscopy
12	22	m	Post-traumatic osteomyelitis of the knee and foot	Culture
13	45	m	Infected prosthesis of the knee	Bone scan, surgery
14	30	f	Soft tissue infection of the femoral muscle and osteomyelitis of the knee and foot joint	Culture
15	45	f	Osteomyelitis of the right tibia and sinusitis	Radiography
16	34	f	Post-traumatic midfoot infection of the plantar soft tissue	Radiography, biopsy, culture
17	68	m	Diabetic superinfected forefoot gangrene and ascending colitis	Surgery

Table 2. Absorbed radiation dose in cGy/37 MBq

Organ	^{99m}Tc SSEA-1
Whole body	1.1×10–2
Liver	6.5×10–2
Spleen	2.4×10–1
Kidneys	5.9×10–2
Lung	1.2×10–2
Ovaries	9.4×10–3
Testes	5.9×10–3
Red marrow	5.3×10–2
Thyroid	7.9×10–3
Eff. dose equiv.	1×10–2
Eff. dose	1.9×10–2

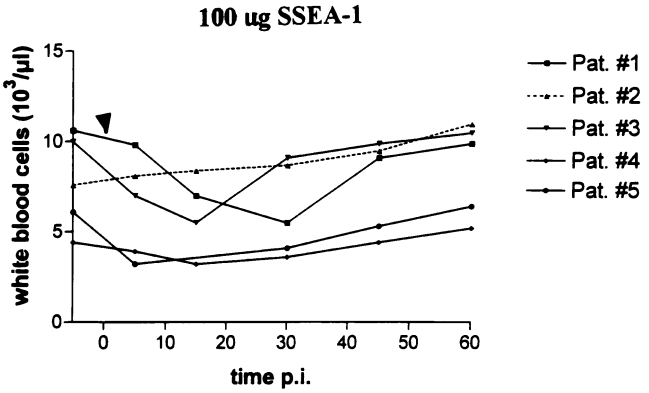

Fig. 5. Peripheral leucocyte count after MAb injection in five patients. A temporary decrease of about 21%–48% was observed in the first 15 min without any clinical symptoms or changes in the vital signs

administered. This direct method is easy to use and practicable even in very ill or septic patients. Furthermore, no allergic reactions and only slight leukopenia have been observed.

The aim of this study was to compare the efficacy of ^{99m}Tc-anti-SSEA-1 IgM MAb scintigraphy with that fo with ^{99m}Tc-HMPAO-labelled autologous WBCs the technique widely accepted in nuclear medicine for imaging of infectious diseases. In the small group of patients investigated the ^{99m}Tc-MAb detected infection unequivocally and showed high sensitivity and specificity. With the ^{99m}Tc-anti-SSEA-1 MAb, acute infective lesions were found to be positive within 1 h post injection; this is consistent with the findings of Thakur et al [8]. More clinically useful information was obtained with this new antibody than with ^{99m}Tc-HMPAO WBCs in four patients with simultaneous chronic and acute sites of infection (Figs. 1–4). In one patient an additional inflammatory focus located in the nasal sinus was seen, which was not detectable with ^{99m}Tc-HMPAO WBCs, but was subsequently verified by planar radiography. Thus, the sensitivity and specificity of ^{99m}Tc-anti-SSEA-1 MAb were 95% and 96%, respectively, compared with firgures of 91% and 82%, respectively, for ^{99m}Tc-HMPAO WBC scans.

Consistent with previous findings [8], the anti-SSEA-1 IgM MAb showed excellent imaging qualities with high target to background ratios despite its high molecular weight of 900 kDa and the relatively high liver uptake of radioactivity. More than 90% of the cell-bound activity was associated with PMNs. This high in vivo association of this anti-SSEA-1 MAb with PMNs, which is consistent with its high PMN association constants (10^{-12} M) in vitro, together with the fast blood pool clearance, may be the primary reason for the excellent imaging quality. With another PMN-specific MAb, BW

250/183, the PMN-bound activity is reported to be only 10%–20% [6]. ^{99m}Tc-HMPAO-WBCs, are reported to be unstable, with an elution rate of 7% per hour [24–26]. As a consequence of this instability of ^{99m}Tc, the secondary hydrophilic complexes of HMPAO are excreted via kidney and bladder within a few minutes after reinjection of the labelled cells. Intestinal uptake also appears rapidly. which frequently interferes with image quality. No such gastrointestinal uptake was noticeable with the anti-SSEA-1 MAb.

Even though we did find a significant 21–48% decrease of the circulating granulocytes over the first 15 min in four of the five patients studied, there were no clinical symptoms or any changes in the vital signs at this time. To our knowledge, there are two possible explanations for this transient decrease in the circulating granulocytes. The first is increased expression of an adhesion-promoting, surface glycoprotein which may lead to sequestration of the granulocytes in the spleen as a reservoir of vital cells and afterwards in the lung [27]. The second possibility is cell opsonization with the MAb, which leads to the removal of cells by the reticulo-endothelial system as described by Dillman et al. [28]. However, in their study all the patients suffered from nausea, emesis, chills and temperature >37.6°C, whereas such symptoms and sign were not seen in our study. Previous studies have shown that exposure of this MAb in human PMNs at much higher concentrations than were used here does not alter the chemotactic ability or directional migration of human PMNs [29].

As determined by Scatchard plot analysis, the affinity constant of this new IgM monoclonal antigranulocyte antibody is about 1.6×10^{-12} *M*, similar to that reported by Thakur and colleagues [8]. This high affinity assures low circulating free antibody levels and less non-specific uptake in infections. These qualities, together with the rapid liver and bone marrow uptake, result in a rapid clearance of the MAb associated with a high endogenous background subtraction [8]. The affinity constant of the ^{99m}Tc-antigranulocyte antibody (IgG1) BW 250/183 was calculated to be 2×10^{-9} *M* [6] and of the ^{99m}Tc Fab′ fragments of IMMU-MN3, $0.5\pm0.2\times10^{-8}$ *M* [7]. About 10% of the injected ^{99m}Tc-antigranulocyte antibody (IgG1) BW 250/183 circulates as free immunoglobulin. The good image quality with high target/background ratios results from the specific binding of high amounts of injected antibody to epitopes in bone marrow and spleen and the resulting low background activity [6]. The uptake mechanism of radiolabelled monoclonal antigranulocyte whole antibody has been described elsewere [6] as consisting in the migration of the antibody-labelled circulating granulocytes to the focus because of their chemotactic behaviour and the non-specific, non-antigen-related uptake of free antibody. In routine clinical practice this often precludes reliable differentiation between non-specific uptake in inflammatory lesions (observed due to their increased capillary permeability) and granulocyte-associated specific uptake in infectious lesions. Thus, the above described characteristics of this IgM antibody lead to a nearly complete in vivo labelling of human PMNs.

As mentioned above, at 1 h, 4 h and 24 h post injection the ^{99m}Tc-MAb uptake was 37.4%, 36% and 27.9%, respectively, in the liver, 22%, 21.9% and 11% in the spleen, 2.5%, 2.7% and 3.9% in one kidney and 8.4%, 9.9% and 12.5% in a clearly defined bone marrow area. In dynamic studies after the injection of ^{99m}Tc-BW 250/183 we have previously shown [7, 26, 27] an ascending antibody uptake curve over the bone marrow at the rate of 1.1%/min, with a much higher value at prolonged times. Since the anti-SSEA-1 MAb detects reticulo-endothelial cells, its liver uptake is higher than that of ^{99m}Tc-HMPAO WBCs. In this respect our data are similar to those reported by Thakur et al. [8]; in addition, while they show higher uptake in the spleen than was observed by Thakur et al., results in respect of the kidneys and bone marrow are similar. The reasons for the discrepancy in the spleen uptake are not clearly understood.

The absorbed radiation dose in cGy/37 MBq to the whole body was 1.1×10^{-2} and the effective dose, 1.9×10^{-2}. These data show nicely that the absorbed radiation dose is comparable to that of ^{99m}Tc-HMPAO, i.e. $1.5\times10^{-2}/3.1\times10^{-2}$, and lower than that of ^{111}In-oxine WBCs, i.e. $1.7\times10^{-1}/5.9\times10^{-1}$ [30, 31].

For the determination of HAMA we used the HAMA ELISA Medac assay. This assay detects human anti-mouse IgG antibodies. Human anti-IgM antibodies are to be anticipated only during the first 2–3 days after anti-SSEA-1 MAb injection in a mature individual, whereas in the following period human anti-IgG antibody formation is to be expected. In 14 patients no IgG HAMA was determined with the HAMA ELISA Medac assay. This was also true in one patient who was positive for HAMA before and who showed no further increase in HAMA production over the 3 months following the administration of the anti-SSEA-1 MAb. Only Fab′ fragments (IMMU-MN3) [7] are known to show comparable results. Further studies are being carried out.

We observed that as a function of time after injection of anti-SSEA-1 MAb activity in one infection site increased from 0.3% to 0.7% and 2.2% of the injected dose at 1 h, 4 h and 24 h post injection, respectively. The ^{99m}Tc-anti-SSEA-1 antibody correctly detected two more lesions that were not detectable by ^{99m}Tc WBC scans. In four patients the results of ^{99m}Tc- anti-SSEA-1 MAb and the ^{99m}Tc-HMPAO WBC scans were not concordant. All the other findings of ^{99m}Tc-antigranulocyte scans were identical to the ^{99m}Tc-HMPAO WBC scans 4 h and 24 h post injection.

In conclusion, this new ^{99m}Tc-labelled anti-SSEA-1 murine IgM MAb provides several advantages. It can be injected directly, it labels PMNs in vivo efficiently and it does not cause any allergic reactions. In four out of five patients investigated, a transient leukopenia was seen. ^{99m}Tc-anti-SSEA-1 is ready to use and has excellent image qualities with high target/background ratios; in the

small group of patients considered in this study it detected more lesions than died ^{99m}Tc-labelled autologous WBC scans.

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