Different biodistribution of $^{99m}$Tc-labelled chimeric mouse–human monoclonal antibody between athymic mice model and human

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Summary Biodistribution of chimeric mouse/human monoclonal antibody against non-specific cross-reacting antigen (chNCA Ab) was studied in athymic mice and patients with metastatic bone disease. $^{99m}$Tc-chNCA Ab showed a high labelling efficiency, stability and also a high binding ratio to human granulocytes. Since NCA showed cross-reactivity with carcinoembryonic antigen (CEA), animal experiments showed that $^{99m}$Tc-chNCA Ab was accumulated in the xenografted tumour which expressed CEA, suggesting the preserved immunoreactivity of labelled materials. In the clinical study, injected $^{99m}$Tc-chNCA Ab formed a high molecular weight complex immediately after intravenous administration and was trapped mainly in liver. The first-phase blood half-life was 6.4 ± 1.1 min. None of the patients showed adverse reaction or human anti-murine or anti-chimeric antibody in their serum. $^{99m}$Tc-chNCA Ab demonstrated remarkably different biodistribution between patients and the animal model and showed different pharmacokinetics from other murine and chimeric Abs reported previously. For safety HPLC analysis should be performed before clinical radioimmunodetection or radioimmunotherapy by incubating radiolabelled MAb with human serum under strict conditions.

Keywords: technetium-99m; chimeric antibody; non-specific cross-reacting antigen; immune complex; animal model; immunoscintigraphy

Materials and methods

Murine and chimeric mouse – human anti-NCA antibodies

Murine anti-NCA Ab (mNCA Ab), IgG, isotype, was purified from ascites of Balb/c mouse inoculated intraperitoneally with hybridoma cells that were produced by the fusion of mouse myeloma cells and anti-NCA Ab-producing B-cells derived from Balb/c mouse immunised with CEA. Derived mNCA Ab reacted to NCA 50 and NCA 90. chNCA Ab was prepared by the method previously reported (Koga et al., 1990). Vθ(Vκ) gene, isolated from hybridoma 2.7.1G.10., was linked to Cγ(Cκ) gene from ARH77 human myeloma cell line. Recombinant DNA was sequentially transfected into p3x63-Ag8.653 mouse myeloma cell line by electroporation (Potter et al., 1984). The transfectants were adapted to be grown in Isecove's modified Dulbecco's medium supplemented with bovine insulin (5 μg ml$^{-1}$), human transferrin (10 μg ml$^{-1}$) and ethanolamine (1.53 μg ml$^{-1}$) and then cultured in a hollow fibre cell culture system (Cellmax 100, Cellox Advanced Bioreactors, Kensington, MD, USA). chNCA Ab was purified from the supernatant on SP-TOP OPEAL (Tosoh, Tokyo, Japan), QAE-TOP OPEAL (Tosoh), protein A – Sepharose (Pharmacia, Uppsala, Sweden) and Sephacryl S-300 (Pharmacia) column. MAb against human chorionic gonadotropin, IgG, was used as an irrelevant control Ab. Serum samples obtained from five patients who received $^{99m}$Tc-labelled murine MAb against CEA, designated BW431/126 (Baum et al., 1989) were used as control in the measurement of serum levels of HAMA and human anti-chimera antibody (HACA).

Radiolabelling procedure

$^{99m}$Tc labelling $^{99m}$Tc labelling was performed according to the method reported previously (Mather and Ellison, 1990). Purified mNCA Ab and chNCA Ab, 1 mg ml$^{-1}$ of 0.05 m
phosphate-buffered saline (PBS), pH 7.5, were reduced by 4.8 μl of 97%, 2-mercaptoethanol (2-ME, Wako, Osaka, Japan) with 2-ME/MAb molar ratio of 10000:1 at 25°C for 30 min. The reduced antibodies were then purified by gel chromatography using G-25M Sephadex column (Phar-macia). The protein fraction was separated into 0.5 mg aliquots. For \( \text{Tc}^{99m} \) labelling, 50 μl of hydroxymethylene diphosphonate (HMDP, Nihon Meddi-physics, Nishinomiya, Japan) solution reconstituted with 5 ml of 0.9% sodium chloride was added to 0.5 mg of reduced antibody. Antibody–HMDP mixture was then incubated with 740 MBq of \( \text{Tc}^{99m} \) pertechnetate eluted from a \( \text{Mo}^{99m}/\text{Tc}^{99m} \) generator (Dainabo, Tokyo, Japan) for 10 min. The labelling efficiency was determined by the cellulose acetate electrophoresis and quantitative measurement as reported previously (Watanabe et al., 1994).

\[ \text{In vitro immuneactivity and animal experiments} \]

Immunoreactivity of radiolabelled MAb was determined by the in vitro cell binding assay and in vivo tumour accumulation studies using LS-180, human colorectal carcinoma cells that expressed CEA molecule on their surfaces. The cell binding assay was performed by incubating radiolabelled MAb (30 ng in 100 μl of 0.05 M PBS) with increasing numbers of LS-180 (1 × 10^3–1 × 10^7) in microcentrifuge tubes for 1 h at 25°C. After centrifugation at 10 000 r.p.m. for 10 min, supernatant was aspirated. The upper portion of tubes were cut and removed. The radioactivity of cells located on the bottom of tubes was counted by a gammacounter. The percentage of radioactivity bound to cells was calculated.

Athyric nude mice bearing LS-180 human colorectal cancer cells were used for studying in vivo immunoreactivity and biodistribution of radiolabelled MAb. Average weight of tumours was 0.46 g at 2 weeks after inoculation of LS-180 (1 × 10^5 cells) subcutaneously into a rear flank. The mice were injected intravenously with approximately 400 ng of both \( \text{Tc}^{99m} \) labelled and \( \text{I}^{123} \) labelled MAb. At 3 and 18 h post injection, the mice were anaesthetised and sacrificed, and the weight and radioactivity of major organs were measured. Biodistribution was presented as a percentage of the injected dose per gram of organ corrected for 20 g of body weight. Four to five mice with tumours of a defined size were examined in each group. Statistical analysis was made by Student’s t-test. Scintigrams of tumour xenograft were obtained at 18 h after injection of high-dose \( \text{Tc}^{99m} \)-mNAAb or \( \text{I}^{123} \)-chNCA Ab into mice with large tumours.

**Binding to human granulocytes**

Venous blood was collected from a normal volunteer in a 50 ml syringe containing 0.4 ml of heparin and 7 ml of hydroxy ethyl starch, and sedimented at 1 × g for 60 min. Supernatant was collected and centrifuged at 1000 r.p.m. for 5 min to separate leucocytes. Leucocytes were resuspended in autologous plasma. A 10-fold dilution was repeated three times and the number of granulocytes were measured by flow cytometry and cytochemical analysis using Technicon (Technicon Instruments, Tarrytown, NY, USA). Radiolabelled MAb (30 ng in 0.05 M PBS) were incubated with increasing numbers of granulocytes (7.4 × 10^3–7.4 × 10^9) in microcentrifuge tubes for 1 h at 25°C. The percentage of radioactivity bound to granulocytes was calculated in the same manner with the cell binding assay.

**Clinical study**

Four patients (63 to 74 years of age) with metastatic bone tumour from prostate cancer were studied. All patients gave informed consent to participate in the study which was approved by the ethical committee of our university. \( \text{Tc}^{99m}/\text{chNCA Ab} (1110 MBq, 1 mg) \) was mixed with 100 ml physiological saline followed by the intravenous injection for 10 min. Temperature, blood pressure, heart rate, respiratory rate and subjective symptoms were monitored before, during and until 30 min after the infusion.

**Pharmacokinetics and immune response**

Blood samples were collected sequentially at 5, 10, 15, 20, 25, 30 and 60 min, and 24 h after injection of \( \text{Tc}^{99m}/\text{chNCA Ab} \). Radioactivity of whole-blood samples and plasma samples were counted with a gamma counter to determine the blood clearance of \( \text{Tc}^{99m}/\text{chNCA Ab} \). High-pressure liquid chromatography (HPLC) analysis was performed using 5 min and 30 min plasma samples with a G3000SW column (Tosoh). Blood samples were also obtained at intervals from 2 to at least 19 weeks to investigate the serological immune response to the injected chNCA Ab.

Detection of HAMA in the serum samples after injection of \( \text{Tc}^{99m}/\text{chNCA Ab} \) was performed by the radioimmunoassay as reported previously (LoBuglio et al., 1986; Khazaéli et al., 1991). Serum samples (100 μl) were incubated with mNAAb-coated beads for 90 min at 25°C. After washing with purified water, \( \text{Tc}^{99m}/\text{mNAAb} \) was added and incubated for 30 min at 25°C. Purified water was then added and unbound radioactivity was washed away. Radioactivity bound to beads was counted with a gamma counter. HAMA was determined as positive when bound radioactivity to beads was over the mean values plus three standard deviations of 20 normal individuals. For the assay of HACA, the same procedure was applied using chNCA Ab instead of mNAAb. The same criteria were applied to catecholine as a positive HACA response. Sera obtained from 20 healthy individuals and five patients receiving \( \text{Tc}^{99m}/\text{BW431/26} \) (Oriuchi et al., 1995) were also examined, and their HAMA and HACA titres were determined. Three of five patients receiving \( \text{Tc}^{99m}/\text{BW431/26} \) developed HAMA detectable with this method.

**HPLC analysis of \( \text{Tc}^{99m}/\text{chNCA Ab} \) incubated with human serum**

In order to assess the interaction of \( \text{Tc}^{99m}/\text{chNCA Ab} \) with the human serum, \( \text{Tc}^{99m}/\text{chNCA Ab} \) was incubated with human serum in vitro for 1 h at 37°C and HPLC analysis was performed with a G3000SW column (Tosoh) eluted at a flow rate of 1 ml min^-1 and 0.5 ml fractionation. Doses of \( \text{Tc}^{99m}/\text{chNCA Ab} \) used for the incubation were: (1) 5 μg of \( \text{Tc}^{99m}/\text{chNCA Ab} \) with 20 μl of human serum, which was performed as one of the preclinical tests before the clinical trial; and (2) 5 ng of \( \text{Tc}^{99m}/\text{chNCA Ab} \) with 20 μl of human serum, which was performed to confirm the presence of high molecular weight complex after the clinical trial. The latter dosage (2) was calculated as equivalent to that of the clinical situation.
Results

The cellulose acetate electrophoresis of $^{99m}$Tc-chNCA Ab showed that labelling efficiency was more than 96% and there was no evidence of colloid formation. Specific activities of $^{99m}$Tc-labelled mNCA Ab and chNCA Ab were calculated as 1.5 GBq mg$^{-1}$. The cell binding assay showed that the percentage binding of $^{99m}$Tc-chNCA Ab to LS-180 cells increased as the cell number increased (Figure 1, top) and it was almost comparable with $^{125}$I-chNCA Ab and $^{99m}$Tc-mNCA Ab. Percentage binding of $^{99m}$Tc- and $^{125}$I-labelled chNCA Ab to human granulocytes also increased in proportion to granulocyte numbers added, and 73.4% and 66.7% of bound radioactivity were obtained at granulocyte numbers of $7.4 \times 10^6$ per tube (Figure 1, bottom). $^{99m}$Tc-labelled chNCA Ab and mNCA Ab showed a slightly different biodistribution in athymic mice bearing LS-180 as shown in Table 1. Clearance of $^{99m}$Tc-chNCA Ab from the circulation was faster than that of $^{99m}$Tc-mNCA Ab resulting in a lower radioactivity of $^{99m}$Tc-chNCA Ab (% injected dose per gram tissue) in all organs except kidney. Tumour-normal organ ratio was high with $^{99m}$Tc-chNCA Ab except tumour–kidney ratio. At 18 h after injection of $^{99m}$Tc-chNCA Ab, tumour–blood ratio was 1.74; tumour–liver, 2.73; tumour–muscle, 13.7; whereas the ratios for $^{99m}$Tc-mNCA Ab were 0.74, 1.62 and 6.89 respectively. Tumour–kidney ratios for $^{99m}$Tc-labelled chNCA Ab and mNCA Ab were 0.33 and 0.87 respectively. Scintigrams of athymic mouse confirmed the results of biodistribution studies, showing a higher radioactivity in the tumour and kidney of mice administered with $^{99m}$Tc-chNCA Ab (Figure 2).

Four patients with bone metastases received $^{99m}$Tc-chNCA Ab and no adverse reaction was noted after the administration in these patients. Dynamic images obtained immediately after injection demonstrated intense radioactivity in the liver

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Table 1: Biodistribution of $^{99m}$Tc- and $^{125}$I-labelled chNCA Ab and mNCA Ab in athymic mice carrying human colorectal cancer cell xenografts at 3 and 18 h after intravenous administration.

| Organ      | $^{99m}$Tc-chNCA (n = 4) | $^{125}$I-chNCA (n = 4) | $^{99m}$Tc-mNCA (n = 4) | $^{125}$I-mNCA (n = 4) | $^{99m}$Tc-chNCA (n = 5) | $^{125}$I-chNCA (n = 5) | $^{99m}$Tc-mNCA (n = 4) | $^{125}$I-mNCA (n = 4) |
|------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Blood      | 16.7 ± 2.0              | 17.7 ± 0.9              | 20.8 ± 1.4              | 17.5 ± 0.7              | 2.6 ± 0.5                | 5.4 ± 1.5                | 6.7 ± 0.8               | 5.5 ± 0.9               |
| Liver      | 4.8 ± 0.5               | 6.0 ± 1.2               | 9.1 ± 1.2               | 6.6 ± 1.1               | 2.3 ± 1.0                | 1.8 ± 0.4                | 6.5 ± 0.9               | 2.4 ± 0.7               |
| Kidney     | 19.3 ± 3.0              | 8.0 ± 1.3              *     | 12.5 ± 1.1              | 7.4 ± 1.1              *     | 17.6 ± 4.3               | 2.5 ± 0.7              *     | 13.9 ± 1.2              | 2.4 ± 0.2              *     |
| Intestine  | 3.2 ± 0.5               | 2.3 ± 0.7               | 2.3 ± 0.2               | 2.5 ± 0.5               | 2.1 ± 0.6                | 1.4 ± 0.9                | 2.1 ± 0.4               | 1.1 ± 0.3               |
| Stomach    | 1.2 ± 0.6               | 4.4 ± 2.0               | 1.2 ± 0.1               | 3.8 ± 1.9               | 1.4 ± 0.8                | 2.8 ± 1.3                | 1.3 ± 0.3               | 2.5 ± 1.2               |
| Spleen     | 3.9 ± 1.3               | 6.2 ± 3.0               | 7.2 ± 1.4               | 6.4 ± 1.4               | 1.5 ± 0.4                | 2.1 ± 0.5                | 3.5 ± 1.6               | 2.1 ± 0.8               |
| Lung       | 7.5 ± 3.2               | 8.7 ± 4.1               | 9.0 ± 5.1               | 8.2 ± 4.3               | 1.8 ± 0.8                | 3.0 ± 1.0                | 2.6 ± 0.5               | 2.2 ± 0.5               |
| Muscle     | 0.7 ± 0.2               | 1.0 ± 0.3               | 1.1 ± 0.3               | 0.8 ± 0.3               | 0.2 ± 0.02               | 0.4 ± 0.07              *     | 0.6 ± 0.2             *     | 0.5 ± 0.1              *     |
| Bone       | 1.8 ± 0.2               | 2.6 ± 0.8               | 3.0 ± 0.6               | 2.8 ± 0.7               | 0.6 ± 0.1                | 0.9 ± 0.2                | 1.5 ± 0.4              *     | 1.0 ± 0.2              *     |
| Tumour     | 7.7 ± 1.3               | 8.7 ± 1.0               | 10.0 ± 0.8              | 7.6 ± 0.9               | 5.4 ± 2.7                | 6.8 ± 3.8                | 9.8 ± 1.3               | 6.7 ± 0.8               |

*P < 0.001 compared with $^{99m}$Tc-chNCA. **P < 0.01 compared with $^{99m}$Tc-chNCA.

Data are shown as % injected dose per g of organs (mean ± s.e.).

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Figure 1. Immunoreactivity of $^{99m}$Tc-chNCA Ab ( — ), $^{125}$I-chNCA Ab ( — — ), $^{99m}$Tc-mNCA Ab ( — ), $^{99m}$Tc-labelled control murine Ab ( — ), and $^{125}$I-labelled control murine Ab ( — — ) evaluated by in vitro binding to human colorectal cancer cells (top) and to human granulocytes (bottom).

Figure 2. Scintigrams of athymic mice carrying human colorectal cancer cell xenograft (arrow) at 18 h after administration of $^{99m}$Tc-mNCA Ab (left) and $^{99m}$Tc-chNCA Ab (right). Arrowheads: kidneys.
(data not shown). Static images of a patient demonstrated remarkable accumulation of the tracer in liver accompanied by moderate accumulation in spleen, lung and kidney (Figure 3). Metastatic bone lesion could be seen as a photopenic area on 1 and 4 h posterior images (Figure 4). Blood clearance of the radioactivity in four patients demonstrated that the biphasic curve was found to be best fitted with the first-phase half-life of 6.4 ± 1.1 min (mean ± s.d.) and the second-phase half-life of 70.8 ± 41.6 h (Figure 5). Figure 6 represents the HPLC elution profile of plasma samples of a patient obtained at 5 and 30 min after injection of 99mTc-chNCA Ab. More than 60% of the plasma radioactivity was found at the first peak at around 29 min of the retention time corresponding to a high molecular weight material in the circulation.

HPLC analysis showed that there was the first peak before the second peak of 99mTc-chNCA Ab under incubation condition similar to those pertaining to the clinical situation (5 ng of 99mTc-chNCA Ab with 20 µl serum), which indicated the evidence of a high molecular weight complex formation. However, there was apparently no complex formation when 1 × 10^-5-fold excess of 99mTc-chNCA Ab was incubated with serum (5 µg of 99mTc-chNCA Ab with 20 µl serum) as shown in Figure 7.

No patient showed positive HACA or HAMA response in their sera within 19 weeks after injection of 99mTc-chNCA Ab, whereas 60% of patients produced HAMA in their sera after injection of the same dose of 99mTc-BW431 26 (Figure 8).

![Figure 3](image-url) Anterior static images obtained 1, 4 and 24h after administration of 99mTc-chNCA Ab in a 64-year-old patient with metastatic bone tumour from prostate cancer. Intense radioactivity was seen in the liver.

![Figure 4](image-url) Posterior static images obtained 1 and 4h after administration of 99mTc-chNCA Ab and 99mTc-HMDP bone scintigraphy in the same patient as Figure 3. Metastatic bone lesion can be seen as a vague photopenic area on 99mTc-chNCA Ab images (arrows), and a hotspot on bone scintigraphy (arrowhead).

![Figure 5](image-url) Blood clearance of 99mTc-chNCA Ab in four patients with metastatic bone tumour. A biphasic curve fitting was demonstrated with mean first-phase half-life of 6.4 min and second-phase half-life of 70.8 h.

![Figure 6](image-url) HPLC elution profile of plasma drawn at 5 min (---) and 30 min (- - - - - - -) after the administration of 99mTc-chNCA Ab.
Figure 7 HPLC elution profiles of $^{99m}$Tc-chNCA Ab after incubation with normal human serum for 60 min. $^{99m}$Tc-chNCA Ab and normal human serum were incubated under two different chNCA Ab to serum ratios: 5 µg of $^{99m}$Tc-chNCA Ab with 20 µl serum (○○); 5 ng of $^{99m}$Tc-chNCA Ab with 20 µl serum (●●●)....

Discussion

Chimeric anti-NCA Ab was successfully labelled with $^{99m}$Tc with high specific activity of 1.5 GBq mg$^{-1}$. $^{111}$I and $^{125}$I have been conjugated with MAb and used for radioimmunodetection. $^{99m}$Tc is relatively new for conjugating MAb, especially for chimeric MAb. However, $^{99m}$Tc has several advantages for imaging. $^{99m}$Tc is easily available since it is produced by a generator system and photon energy is suitable for gamma camera imaging. The relatively short half-life of $^{99m}$Tc enables sufficient amount of injection dose to provide tomography with a high resolution and low statistical error.

Animal experiments showed that $^{99m}$Tc-chNCA Ab was accumulated in the xenografted tumour which expressed CEA. In contrast to animal studies, clinical examination revealed that most radioactivity was present in the liver immediately after intravenous injection of $^{99m}$Tc-chNCA Ab. Blood clearance of $^{99m}$Tc-chNCA Ab showed a biphasic clearance with the first-phase half-life of 6.4±1.1 min, although reported mean half-lives of chimeric MAb s in humans were approximately 18 h (first phase) and 100 h (second phase), six times longer than those of murine MAb s (LoBuglio et al., 1989; Meredith et al., 1991). HPLC analysis of plasma from the patient who received $^{99m}$Tc-chNCA Ab revealed the formation of a high molecular weight complex in the circulation and there was no increment in free $^{99m}$Tc pertechnetate in the plasma detected by either whole body imaging or HPLC analysis. NCA has been found in various human tissues such as lung, spleen, granulocytes and also in the serum with a variety of molecular weights ranging from 50 to 160 kDa (von Kleist et al., 1972; Bosslet et al., 1985). In vitro instability of reduction-mediated $^{99m}$Tc-labelled Ab has been reported (Sakahara et al., 1993) but no evidence of aggregation or complex formation was noted when $^{99m}$Tc-chNCA Ab was incubated with PBS (data not shown).

HPLC analysis of the diluted $^{99m}$Tc-chNCA Ab incubated with normal human serum in vitro demonstrated the same radioactivity peak corresponding to a high molecular weight complex (Figure 7). Serum samples, which were absorbed by anti-NCA Ab and then incubated with $^{99m}$Tc-chNCA Ab, showed only one peak which corresponded to $^{99m}$Tc-chNCA Ab (data not shown). From these results, increased radioactivity in the liver is supposed to be caused by the complex formation of injected $^{99m}$Tc-chNCA Ab with circulating NCA resulting in subsequent clearance by the reticuloendothelial system. Of note was the fact that a high molecular weight complex was only detected by HPLC under a certain incubation ratio of radiolabelled MAb to human serum. According to the recommendation of the Food and Drug Administration in USA (Center for Biologies Evaluation and Research, FDA 1993), $^{99m}$Tc-chNCA Ab was examined for binding to serum protein using HPLC by incubating $^{99m}$Tc-chNCA Ab with human serum as a preclinical safety testing, though the high molecular weight complex was hardly demonstrable probably because of the saturation of antigen-binding capacity present in the serum. On the contrary, when $^{99m}$Tc-chNCA Ab and human serum were incubated under the strict dilution ratio (1 × 10$^{-5}$-fold), which was the equivalent ratio of $^{99m}$Tc-chNCA Ab to serum in the circulation, then a high molecular weight complex was clearly demonstrated by HPLC.

The biodistribution profile observed in this study was different from other studies. The disparity between the results of the biodistribution of $^{111}$I-labelled MAb in man and mice has been reported (Ledermann et al., 1993). In the report, most of the radioactivity was located in the bone marrow and spleen, since the radiolabelled MAb was bound to circulating granulocytes. Lack of immunodetection and complications associated with MAb cross-reactive with circulating cells had been reported (Dillman et al., 1984).

Several authors have suggested that the presence of antigen in the blood does not significantly affect the results of imaging, though it would form a circulating immune complex (Goldenberg et al. 1978; Mach et al. 1980; Primus et al., 1980). Pharmacokinetics and biodistribution of $^{111}$In-
labelled MAb which formed a circulating immune complex have been reported (Hnatowich et al., 1987; Davidson et al., 1991). In the present study, increased liver uptake of radiolabelled MAb was noted as in the previous studies, although the pharmacokinetics were different and imaging was not successful. Blood clearance of 99mTc-chNCA Ab was much faster than in other studies and the mechanism of fast clearance and the liver accumulation of 99mTc-chNCA Ab remains to be clarified.

None of the patients produced HAMA or HACA within 19 weeks after 99mTc-chNCA Ab injection, although three of five patients who received the same 1 mg of 99mTc-labelled murine Ab produced HAMA in their serum. There have been a few reports describing the immunogenicity of chimeric Abs. Chimeric Abs designated 17-1A, L6 and NR-LU-13 had low immunogenicity, whereas 131I-labelled chimeric Ab B72.3, has considerable immunogenicity (Khazaeli et al., 1991; LoBuglio et al., 1989; Meredith et al., 1991). In the latter paper, seven of 12 patients with metastatic colon cancer had an antibody response after intravenous injection of 3.4 to 6.9 mg of chimeric B72.3, and a small portion of the antibody response was directed to epitopes requiring the presence of both murine V-region and human CH-1/κ constant regions. These results may indicate that the immunogenicity of chimeric Abs may depend on the amino acid sequences of murine V-region.

In conclusion, chNCA Ab was stably labelled with 99mTc pertechnetate and immunoreactivity was completely reserved with high-binding capacity to human granulocytes and CEA-expressing colon cancer cells. 99mTc-chNCA Ab was safely administered to patients without generating HAMA or HACA response. In animal studies, however, radiolabeling and circulating antigenic molecules reactive with chNCA Ab formed a high molecular weight complex immediately after the administration of 99mTc-chNCA Ab and were taken up in the liver. For safety HPLC analysis should be performed before clinical radioimmunodetection or radioimmunotherapy by incubating radiolabelled MAb with human serum under strict conditions.

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