Monoclonal Antibody and Nuclear Medicine; Radioimmunoimaging of Tumor with Radiolabeled Monoclonal Antibody

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Introduction

Gallium(Ga)-67 citrate has been widely used for the diagnosis of malignant diseases, but it is not an ideal radionuclide, since it is accumulated in inflammatory lesions as well as in malignant tumors. Furthermore Ga-67 scans are useful only for such tumors as malignant lymphoma, malignant melanoma and lung cancer[1].

In contrast, the antigen-antibody reaction can show a high level of specificity, because the binding sites of an antibody directed against determinants on one antigen are not complementary to determinants of another antigen. The technique using hybridomas, first established by Kohler and Milstein in 1975 is now widely used for the production of anti-tumor monoclonal antibodies.

Monoclonal Antibodies

Recently various monoclonal antibodies directed against tumor-associated antigens have been developed (Table 1) and clinically used as a transport of radionuclides and anti-cancer drugs for the diagnosis and treatment of various cancers.

Monoclonal antibodies are obtained from hybridomas, derived from fusions between myeloma cells and sensitized B cells of mice immunized with tumor cells. Hybridomas produce monoclonal antibodies which recognize only one antigenic determinant, which make them valuable for the localization and diagnosis of tumor and for target cytotoxic therapy. From the ascitic fluid of mice, we could obtain gram quantities of homogenous antibodies, which is a distinct advantage over the conventional polyclonal antibodies.

Anti-tumor monclonal antibodies are practically applied in many fields of the clinical oncology due to their purity, specificity and homogeneity[2]. Radiolabeled monoclonal antibodies are useful in the radioimmunoassay of cancer markers, such as CA 19-9, CA 15-3 and CA 125, employed in the ma-

Table 1. Monoclonal Antibodies Against Tumor-Associated Antigens

Carcinoembryonic antigen (CEA)	Malignant Melanoma
	Stomach
Alpha-fetoprotein (AFP)	Colon
	(Pancreas: CA 19-9)
	Lung
	Breast(CA 15-3)
	Prostate
	Bladder
	Ovarium(CA 125)
	Osteosarcoma

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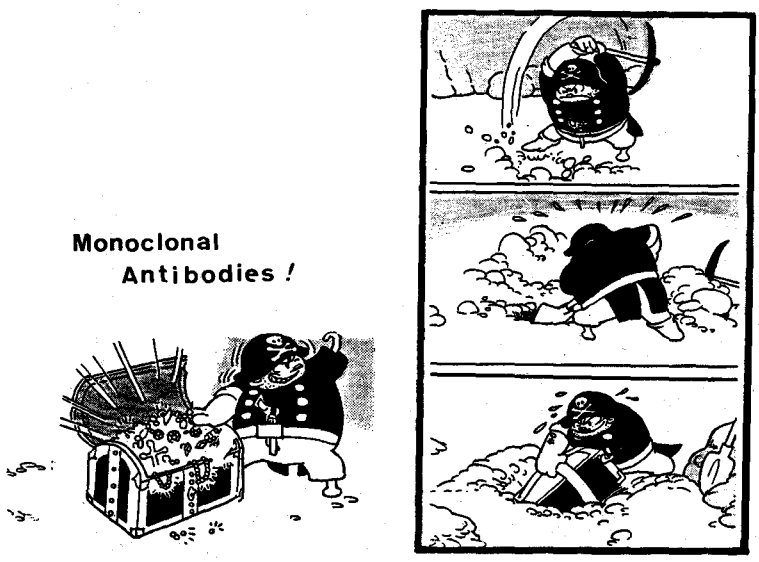

Fig. 1. Production of monoclonal antibodies.

nagement of patients with pancreatic, breast and ovarian cancers, respectively. These cancer markers were developed through the practical application of monoclonal antibody techniques. Furthermore, anti-tumor monoclonal antibodies are also used for the radioimmunoimaging and radioimmunotherapy of various cancers. In the present study, we examined the use of labeled monoclonal antibodies for the diagnosis of malignant melanoma. Some of this work, especially that on the preparation and property of labeled antibodies with metallic radionuclides has been reported in the previous issue of Korean Journal of Nuclear Medicine[3].

Malignant Melanoma

Although malignant melanoma is relatively rare in the Asian countries, it accounts for 1.4% of all cancers in the United States. Vast surgical excision is the treatment of choice, but its prognosis is still poor in advanced cases.

Melanoma has a strong affinity for Ga-67[1], but the detection rate of Ga-67 scans is between 39% and 82% for grossly involved or pathologically proven sites. The antigenicity of melanoma is considered to be high, and autoantibodies against melanoma cells have been reported in some cases. The hybridoma methodology has been most successfully applied to develop monoclonal antibodies to melanoma-associated antigens. Tumor imaging using radiolabeled monoclonal antibodies has been carried out mostly in the United States[4~7]. Several institutes reported that anti-melanoma monoclonal antibodies labeled with indium (In)-111 were useful for the diagnosis of metastatic melanoma with a detection rate of 50 to 70%. Furthermore they helped detect some lesions that were not suspected by other tests. In these studies subtraction techniques were not employed. None of the patients experienced any serious side effects due to the administration of murine monoclonal antibodies.

Since the clinical human trial of In-111 labeled monoclonal antibodies was approved by the Public Health and Welfare of Japan in April, 1985. Collaboratory study among Kitazato University Hospital (Kanagawa), National Cancer Institute (Tokyo) and Kyoto University Hospital (Kyoto) has been in progress to evaluate the clinical usefulness of two radiolabeled anti-melanoma antibodies, a monoclonal antibody (96.5), which reacts with an Mr 97,000 antigen[8], and an antibody (225.28S) reactive with a high molecular weight melanoma-associated antigen[9].

Animal studies were performed using nude mice

Table 2. Distribution of Radioiodinated Monoclonal Antibodies in Melanoma Xenograft

Melanoma	Monoclonal Antibody	%Dose/g tissue		Tumor-to-Blood Ratio
		Tumor	Blood	
M1	96.5	7.23~ 7.35	5.03~ 5.40	1.34~1.46
	225.28S	8.33~10.80	6.23~ 6.70	1.24~1.73
M2	96.5	6.63~ 9.52	9.79~14.53	0.66~0.68
	225.28S	7.88~14.66	7.86~14.16	1.00~1.04

Monoclonal antibodies; 96.5 and 225.28S was radiolabeled with I-125 and injected intravenously into two nude mice carrying human melanoma cells (M1 and M2). Data show the range of percent injected dose per gram tissue and tumor-to-blood ratios obtained 4 days after administration.

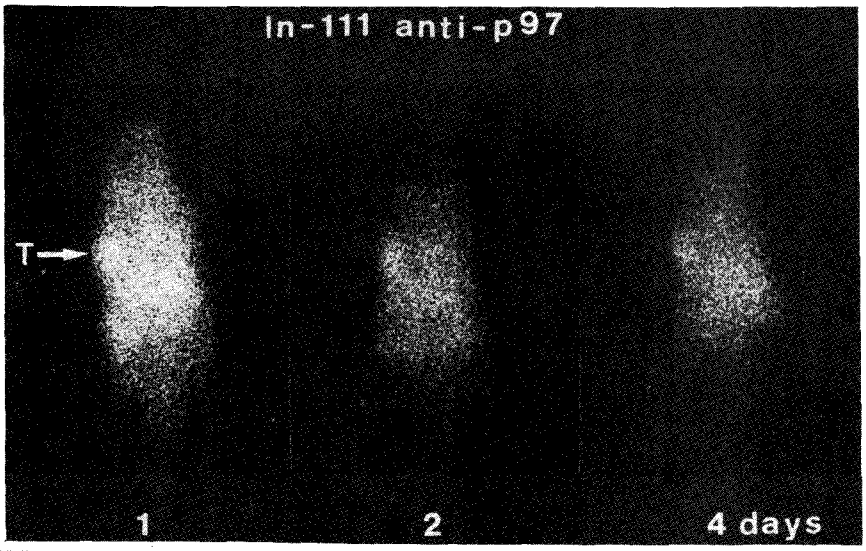

Fig. 2. Scintigram of a nude mouse injected with the In-111 labeled anti-p97 monoclonal antibody (96.5.). The nude mouse transplanted with human melanoma cell line M1 showed tumor localization(arrow) with tumor-to-blood ratio of 3.7, in spite of small tumor size.

transplanted with human melanomas; M1 (melanotic) and M2 (amelanotic) cells. Localization of the radioiodinated antibody was observed in both transplanted melanoma tissues (Table 2). In-111 labeled antibody (96.5) also demontrated a significant uptake of the radioactivity at 1, 2 and 4 days after the intravenous injection, as shown in Figure 2.

The In-111 labeled antibody is prepared easily and rapidly using DTPA as a bifunctional chelating agent.

In-111……DTPA——monoclonal antibody

DTPA coupled antibody is stable at 4°C for one year and radiolabeling is efficiently performed by mixing with In-111 chloride and DTPA-coupled antibody, just before use. The monoclonal antibody melanoma diagnostic imaging kit consists of In-111 chloride, DTPA-coupled antibody (1 mg), unlabeled antibody (19 mg), labeling buffer (0.26 M citric acid) and neutralizing buffer (0.1 mM DTPA in 0.13 M sodium citrate). The "cold" antibody was added to increase the amount of injected antibody to 20 mg, following the findings that the number of sites imaged increased as the dose of antibody increased, despite an overall decrease in specific activity of In-111[4,5]. Then the obtained In-111 labeled antibody (3 mCi, 20 mg antibody/100 ml) was administered as a 60 min i.v. infusion. Images were taken at multiple interval times of up to 6 days. The radiolabeling of In-111 was

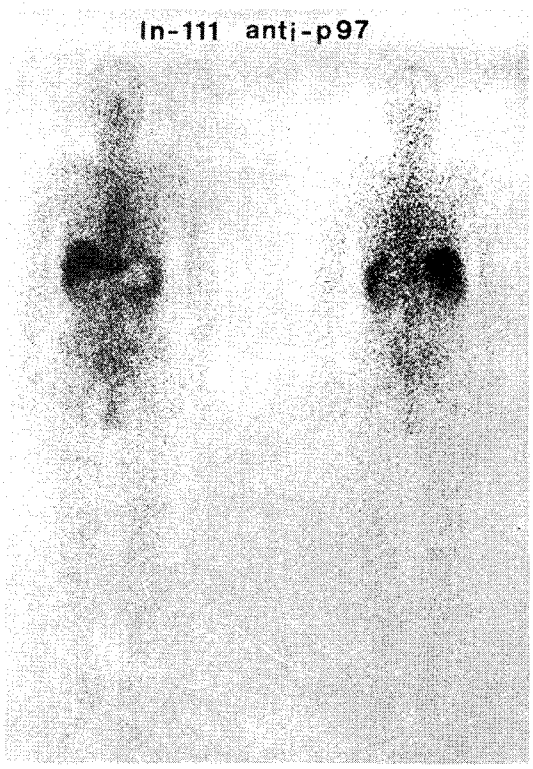

Fig. 3. Normal study of the In-111 labeled antibody in a melanoma patient (case 1) who had involvement to left inguinal lymph node. Scans were taken 3 days after administration and shows a typical normal distribution.

Fig. 4. Localization of In-111 labeled monoclonal antibody in both lower extremities (case 2). Multiple deposits of In-111 was seen throughout the subcutaneous metastases from malignant melanoma with the size of 1 to 2 cm in diameter. The scintigram was taken 3 days after the injection of 20 mg and 2 mCi of In-111 labeled anti-p97 monoclonal antibody(96.5).

efficiently performed through the chelation with DTPA. The labeling efficiency was 80 to 95% without a further purification step and protein unbound In-111 was quickly excreted into the urine in the form of In-111 DTPA chelates.

As a preliminary study we scanned patients with the In-111 labeled antibody, who showed a normal distribution of radioactivity or multiple localization in subcutaneous melanomas. A 75 year-old male was well until 7 years earlier when an operation was performed to remove a malignant melanoma of the left lower extremity. Recently he noticed a tumor of about 3 cm in diameter in his left inguinal region. There was no uptake in his left inguinal tumor either with Ga-67 or with In-111 labeled anti-p97 monoclonal antibodies (Figure 3). A normal distribution of the In-111 labeled antibodies 3 days after the injection was seen in the nasopharyngeal regions, heart, liver, gastrointestinal tract, bones and bladder. The liver contained the highest concentration of the radioactivity, as expected from the animal studies[10].

Figure 4 is the In-111 labeled antibody scan of a 54-year-old man presented with malignant melanoma, widely metastatic to subcutaneous tissue. Multiple subcutaneous deposits of the radiolabled antibody was seen throughout subcutaneous tissues, in masses that varied from 1~2 cm in diameter. All palpable lesions of both lower extremities were clearly visualized. No side effects, nor toxicity due to the infusion of murine monoclonal antibodies was observed.

Human melanoma cells, like other types of tumor cells in men and in other animals are heterogeneous for a variety of phenotypic traits, including morphology, growth properties, drug resistance, metastatic potential, oncogene expression, and antigenic profile[11]. Antigenic heterogeneity revealed by the use of many anti-melanoma monoclonal antibodies indicated that immunodiagnostic and immunotherapeutic approaches to melanoma should rely on the use of a combination of monoclonal antibodies. Furthermore, the combined use of Ga-67 and In-111 labeled monoclonal antibodies was found to improve the detection of metastic melanoma, for which sensitivity improved from about 70% to 91%[12]. These findings suggest that a combination of imaging techniques as well as of monoclonal antibodies may help detect the tumor more accurately. The most important finding we obtained was that the In-111 labeled anti-tumor monoclonal antibody revealed multiple tumors in humans as well as in animals with no side effects.

Future of Radiolabeled Monoclonal Antibody

The selection of radioisotopes for radioimmunoimaging is based on the same criteria used in choosing nuclides for other imaging studies[3]. In-111 has been most extensively investigated and widely used for the diagnosis of cancers.

Tc-99m is nearly the most ideal radionuclide not only judging from its nuclear properties, but from its ready availability from a generator. However, little has been reported on the labeling of monoclonal antibodies with Tc-99m[13]. As reported before, dithiosemicarbazone (DTS) is a very useful chelating agent for the labeling of biologically active molecules with Tc-99m[3].

The Tc-99m labeled monoclonal antibody clearly revealed a transplanted tumor at 6 to 24 hours after administration which matches well with the half-life of Tc-99m (6 hours) (Figure 5). Thus, Tc-99m-labeled products with desirable nuclear properties will be clinically used in the community hospital as well as in research centers within several years.

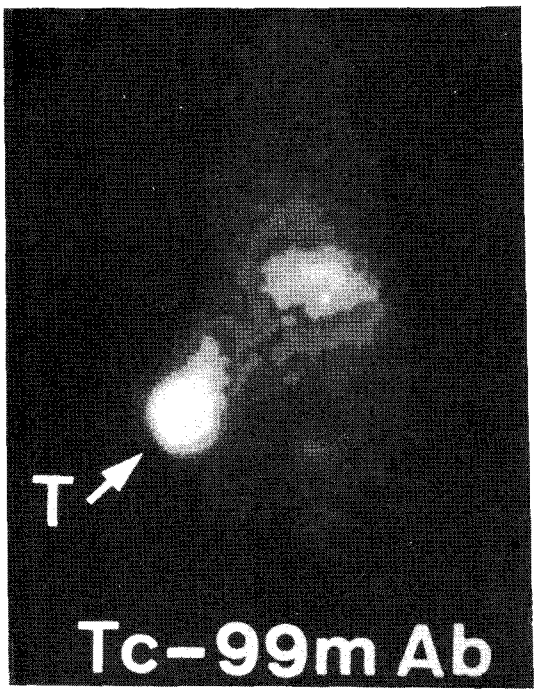

Fig. 5. Scintigram of a nude mouse injected with the Tc-99m labeled monoclonal antibody. Picture was taken at 24 hours after administration. The transplanted tumor (arrow) was clearly visualized, indicating that Tc-99m could be successfully used for the labeling of monoclonal antibodies.

Anti-tumor monoclonal antibodies are expected to transport large quantities of radioisotope and to kill tumor tissue without giving toxic effects to normal tissue, as performed in the I-131 treatment of lurg and bone metastasis from thyroid cancer.

Preliminary studies of radioimmunotherapy have been performed by usirg I-131 and yttrium (Y)-90 labeled antibodies. Sufficient radiation could be delivered to the tumor to treat it[7,14,15]. Y-90 has been described as one of the best radionuclides for tumor therapy when tagged to anti-tumor monoclonal antibodies[15]. Furthermore, Y-90 forms chelates with DTPA. Therefore techniques similar to those used in In-111 labeling could be applied to Y-90.

Y-90……DTPA——monoclonal antibody

The in vitro stability and biodistribution of In-111 and Y-90-labeled antibodies have been reported to be similar. Attemps are being made on the specific treatment of malignant neoplasms with monoclonal antibodies coupled with anti-cancer drugs or toxins. The coupling of monoclonal antibodies with anti-neoplastic drugs and radionuclides suitable for the therapy of cancer share common problems with the diagnosis of neoplasmas. Scintigraphic studies are very useful to know the biodistribution of the radionuclides and drugs attached to antibodies.

Monoclonal antibodies have opened a new era in nuclear medicine and it is unevitable that radiolabeled monoclonal antibodies will have a significant impact on the practice of nuclear medicine as cancer markers in radioimmunoassay. However there are numerous factors as to whether radioimmunoimgaing will be ultimately successful.

We have just begun clinical work with In-111 labeled monoclonal antibodies directed toward human melanoma. As is snown in Figure 3 and 4, the targeting has limitations. Much more work is needed to improve the imaging techniques to create a stronger distinction between tumors and surrounding normal tissues. Monoclonal antibodies specific for cancer alone, hopefully derived from human origin, has to be developed. Experience to date teaches us that the outcome appears to depend largely on the property of monoclonal antibody being used.

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