In Vivo Radiolocalization of Antiosteogenic Sarcoma Monoclonal Antibodies in Osteogenic Sarcoma Xenografts

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ABSTRACT

Monoclonal antibodies Ost6 and Ost7 (mouse Immunoglobulin G1) to human osteogenic sarcoma were isolated from ascitic fluid and labeled with radioiodine. After injection into athymic nu/nu mice with s.c. xenografts of human osteogenic sarcoma, the uptake of radioactivity in tumors, visceral organs, and blood was determined. Five days after injection, Ost6 and Ost7 showed preferential accumulation in tumors (tumorblood ratio, 4.3). Furthermore, with testicular and bladder tumors, both unreactive with Ost7, there was no localization of radiolabeled Ost7 in xenograft growths.

When Ost7 was labeled with ¹³¹I, its accumulation into human osteogenic sarcoma could be clearly visualized by whole-body γ-scintigraphy without computer-assisted data processing.

INTRODUCTION

We have previously described monoclonal antibodies directed against osteosarcoma-associated antigens (9). These antibodies were obtained by immunizing mice with freshly resected osteosarcoma tissue, and evaluated by histological methods for tumor and organ tissues. Three of these antibodies (Ost6, Ost7, and Ost15) reacted only with osteosarcoma and chondrosarcoma but not with other tumors or normal tissues.

Recent studies have demonstrated that monoclonal antibodies provide a new approach for the in vivo localization of malignant tumors (3, 5, 11) and are potentially useful for targeting antitumor agent (1, 13, 15). Our monoclonal antibodies may contribute to development of reagents with a higher degree of osteosarcoma specificity. The potential value, however, needs to be assessed in model systems before clinical studies are attempted. In this investigation, we report a radiolabeled monoclonal antibody specifically accumulating into osteosarcomas that were implanted s.c. into athymic nude mice.

MATERIALS AND METHODS

Mice and Tumors. BALB/c athymic nu/nu mice were fed a standard diet and housed in steel cages with sawdust bedding under specific-pathogen-free conditions.

SU human osteosarcoma was maintained by serial s.c. transplantation into athymic nude mice (10). KT005 was cloned from SU by one of the authors (S. Toyama) using limiting dilution.

Our monoclonal antibodies were assessed to react in vitro with these human osteosarcomas by the immunohistological method reported previously (9). Monoclonal antibodies, Ost6 and Ost7, are of IgG1 isotype and are highly specific for osteogenic sarcoma and chondrosarcoma.

The antibodies were isolated from ascitic fluids of hybridoma-bearing BALB/c mice by ammonium sulfate fractional salt precipitation and purified by Protein A affinity chromatography (Pharmacia, Uppsala, Sweden).

AFY1 monoclonal antibody used in the control experiments is a monoclonal antibody of IgG1 isotype against human α-protein.

Labeling of Ost6 and Ost7 Monoclonal Antibodies with Radioactive Iodine (¹²⁵I). Iodogen reagent was used for labeling Ost6 and Ost7 monoclonal antibodies with Na¹²⁵I (6). Aliquots of 300 μCi of IODO-GEN (Pierce Chemical Co.), at the concentration of 40 μg of IODO-GEN in 1 ml of chloroform, were evaporated to dryness in a conical glass tube at 37° in an atmosphere of nitrogen. Na¹²⁵I (1 μCi/10 μl) and 0.5 ml of Ost6 or Ost7 monoclonal antibody (2 mg/ml) were added to the tube and incubated for 15 min at room temperature. The reaction was stopped by removing the reaction mixture from the IODO-GEN-coated tube, and free ¹²⁵I was separated by the passage over Sephadex 25 (Pharmacia) in phosphate-buffered saline.

AFY1 was iodinated with ¹²⁵I in the same condition as Ost6 and Ost7, except that 250 μg of AFY1 were iodinated with 4 μCi of NaI.

In Vivo Distribution Test with Radiolabeled Monoclonal Antibodies.

Groups of age- and weight-matched mice with s.c. growth of SU, human osteosarcoma, were injected i.v. via a lateral tail vein under ether anesthesia with ¹²⁵I-Ost6 monoclonal antibody or i.p. with ¹²⁵I-Ost7 monoclonal antibody. Drinking water was supplemented with 0.1% (w/v) NaI from 2 days before injection and throughout experiments.

Two or 5 days after injection, the mice were anesthetized with ether, and blood samples were withdrawn from the heart. The animals were then killed and placed on X-ray film (HS-film; Fuji Ltd., Tokyo, Japan) for a few days at −80° to obtain ¹²⁵I γ-ray photograms. To assess the orientation of imaging of ¹²⁵I, the mice were radiated with soft X-ray (18 to 35 kV; 5 mA) for 10 sec before the film was developed. Then, tumors and visceral organs were removed, weighed, and the ¹²⁵I counts were assessed using ¹²⁵I-γ-counter.

Gamma-scintigraphy of KT005 with ¹²⁵I-labeled Ost7. Ost7 antibody (200 μg) was labeled with 2 to 4 μCi of ¹²⁵I using IODO-GEN. Gamma-scintigraphy was carried out from 1 to 7 days after i.v. injection of ¹²⁵I-labeled Ost7 (100 μCi) into KT005 xenografted mice. Images were obtained using a γ-camera with a pinhole collimator. To avoid artifacts caused by movement, the mice were anesthetized with Nembutal. Views were directly taken without data processing. After 7 days, the mice were killed, and the tumors as well as visceral organs were weighed and assayed for radioactivity using a γ-counter.

¹²⁵I-AFY1 was injected into KT005-bearing mice. The animals were killed 7 days after injection, and radioactivities of tumor or visceral organs were assessed using a γ-counter.

In a control experiment, ¹³¹I-Ost7 was injected into mice inoculated with human testicular tumor or human bladder tumor that failed to react with Ost7. Then, their scintigrams were obtained, and radioactivities of tumor and visceral organs were detected in the same way as described in KT005.

RESULTS

Purification of Monoclonal Antibodies. The purity of Ost6 and Ost7 was analyzed by electrophoreses on 12.5% sodium...
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...dodecyl sulfate-polyacrylamide slab gel (16) with and without disruption by β-mercaptoethanol (Fig. 1). The molecular weight of the monoclonal antibodies was approximately 150,000. After disruption with β-mercaptoethanol, the proteins migrated, showing 2 clear bands consistent with the size of the heavy and light chains. Ost6 and Ost7 were labeled with 0.8 to 1.0 μCi of 125I per μg of protein, and AFY1 was labeled with 14 μCi of 131I per μg of protein. Ost7 was labeled with 7 to 14 μCi of 131I per μg of protein.

γ-Ray and Soft X-Ray Photogram. Ost6 was injected i.v. via the tail tendon vein, and Ost7 was injected i.p. (10 to 50 μCi). The mice receiving Ost6 were sacrificed 2 or 5 days after injections, and those receiving Ost7 were sacrificed 5 days after injections. No tumors were detected on γ-ray photograms after 2 days but, after 5 days, tumors were clearly visible in photograms from both the Ost6 and Ost7 groups (Figs. 2 and 3). The tumors appeared as black shadows of exposure.

Ost6 and Ost7 Distribution in Tumors and Visceral Organs. The 125I distribution is summarized in Tables 1 and 2 showing the cpm value (per 1 mg of tissue) of each tumor and organ. Chart 1 shows their ratios to the blood value. The tumors did not show specific accumulation of Ost6 monoclonal antibody 2 days after the injection compared with the blood cpm value, although they showed higher accumulations than other visceral organs. Five days after injection, both the Ost6 and Ost7 groups exhibited very high accumulations of antibodies, although the cpm values varied. The tumor: blood cpm ratios were between 2.5 and 9.1.

<table>
<thead>
<tr>
<th>Radioactivity (cpm)</th>
<th>Sacrificed 2 days after injection</th>
<th>Sacrificed 5 days after injection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>Tumor</td>
<td>523.5</td>
<td>633.2</td>
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<tr>
<td>Lung</td>
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<td>Liver</td>
<td>165.6</td>
<td>196.0</td>
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<td>Kidney</td>
<td>380.6</td>
<td>478.7</td>
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<tr>
<td>Muscle</td>
<td>82.5</td>
<td>135.7</td>
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<tr>
<td>Brain</td>
<td>15.5</td>
<td>15.0</td>
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<tr>
<td>Spleen</td>
<td>ND</td>
<td>84.7</td>
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<tr>
<td>Blood</td>
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<td>469.3</td>
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ND, not determined.

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<tr>
<th>Radioactivity (cpm)</th>
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<th>B*</th>
<th>C*</th>
<th>D*</th>
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<tr>
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<td>2762.3</td>
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<td>Lung</td>
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<tr>
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<tr>
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<td>Blood</td>
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<td>120.7</td>
<td>943.2</td>
<td>1086.7</td>
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* A and B were given injections of 10 μCi of Ost7 monoclonal antibody. * C and D were given injections of 50 μCi of Ost7 monoclonal antibody.

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5%) of purified Ost7 monoclonal antibody. Right, sample containing marker proteins (LMW Pharmacia Electrophoresis Calibration Kit); middle, sample prepared in the presence of 1% mercaptoethanol; left, sample prepared under nonreducing conditions.

Chart 1. Distribution of 131I-labeled Ost6 and Ost7 injected into groups of 3 athymic mice with SU xenografts. Values are expressed as ratios to the blood values. Tu, tumor; Lu, lung; Li, liver; Ki, kidney; Br, brain; bars, S.D.

Chart 2 shows 131I-labeled Ost7 distribution in mice with xenografts of KT005 and mice with xenografts of human testicular tumor and bladder tumor. The tumor: blood ratios in the Ost7 group were much lower in the testicular tumor and bladder tumor than those in KT005, although the individual organ: blood ratios did not differ between 2 groups of mice. Radioactivity in testicular and bladder tumors was lower than that in blood.

Chart 2 also showed the distribution of 131I-labeled AFY1 in
KT005-bearing mice. The ratios were similar to those of Ost7 in mice with the xenograft of testicular tumor and bladder tumor. The tumor: blood ratio was lower than 1.0. These results showed that uptake of Ost7 by KT005 was not a nonspecific accumulation of Ost7.

**Scintigram of $^{131}$I-labeled Ost7.** One to 3 days after injection, $^{131}$I-Ost7 were observed as spots not only in tumors but also in blood pool (Fig. 4); however, the blood spots generally decreased and, at 7 days, the tumor was clearly imaged by scintigram (Fig. 4). In contrast with KT005, testicular and bladder tumors that failed to react with Ost7 showed a high accumulation in the blood pool, but no specific accumulation was seen in the tumor 7 days after injection (Fig. 5).

**DISCUSSION**

Murine monoclonal antibodies produced against osteogenic sarcoma bind at much higher levels to the tumor than to normal tissues. Consequently, these monoclonal antibodies may be useful for targeting antitumor agents to tumor or for tumor detection by external body scanning following injection of radioisotopically labeled preparations. However, in order to develop these approaches, it is necessary to establish that the monoclonal antibody preparations localize preferentially in tumors following specific interactions compared to nonspecific uptake into normal tissues.

Ost6 and Ost7 were selected on the basis of immunohistological studies on frozen tissue sections. Immunofluorescent techniques demonstrated no reaction with cultured cells of human osteogenic sarcoma. However, we found that some human osteogenic sarcomas, which were maintained in athymic nude mice, reacted to Ost6 and Ost7 using immunohistological methods; SU is one of such osteogenic sarcomas. After more than 70 passages, SU now grows stably in athymic nude mice. Therefore, SU and KT005 are suitable tumors for investigating the distribution of Ost6 and Ost7 in vivo.

Radiolabeled Ost6 and Ost7 monoclonal antibodies showed preferential localization in SU and KT005 compared with muscle or several other visceral organs except for a high accumulation of Ost6 in the kidney. One reason for this exception may be that antigens from tumor tissue are in blood and immune complex precipitates in kidney tissue. At present, there is no direct evidence as to whether antigens against Ost6 in blood are released from a tumor. However, considering the fact that no accumulation was observed in the kidney with either $^{125}$I-labeled or $^{131}$I-labeled Ost7, which were centrifuged before injections, the Ost6 accumulation in a kidney may have resulted from aggregation of Ost6 antibodies that were not centrifuged before injections.

In this investigation, only $^{125}$I-Ost7 was injected i.p. to compare the results with $^{131}$I-Ost7 injected i.v., while other antibodies were injected i.v. Hedin et al. (7) reported no obvious difference in the distribution of labeled antibody between animals given i.v. or i.p. injections. In our study, the distribution of Ost7 injected i.p. was similar to that of Ost7 injected i.v., although the tumors were different.

The highest ratio (95:1) of Ost7 tumor to normal tissue radioactivity uptake was seen into the brain and its lowest (6:1), into the lung. Levine et al. (11) noted a very high tumor: blood ratio (15:1) with a monoclonal antibody to mouse teratoma, but this tumor was syngeneic, and the antibody was IgM. In addition, the tumor was implanted into a muscle rather than s.c. According to Epenot (4), i.m. implanted tumors showed higher uptake than those implanted s.c. In other studies on the tissue: blood ratios of xenografted human cancers, the ratios were between 1.5 and 5 (2, 4, 12, 14). Compared with these values, Ost6 and Ost7 yielded fairly high ratios.

In the present investigation, the high uptake of Ost7 was seen in SU and KT005 with no elevated radioactivity level being found in the testicular or bladder tumor that failed to react with Ost7. In addition, KT005 did not absorb nonspecific immunoglobulin, because no specific accumulation of AFY1 was detected in KT005. These results suggested that uptake of Ost7 by KT005 was specific.

We could not obtain scintigrams using $^{125}$I, because our $\gamma$-camera did not respond to $^{125}$I $\gamma$-rays, although small animals can be imaged by $^{131}$I-labeled antibody (2). Accordingly, we tried to take $\gamma$-ray photos for preliminary localization of $^{125}$I-labeled antibody as described in "Materials and Methods." This simple method permitted the $^{125}$I-labeled antibody to be localized with a low dose of isotope and, as the photos were taken after blood was withdrawn from heart, tumor specificity was accelerated.

The usefulness of Ost7 for imaging was demonstrated by the fact that $^{131}$I scintigram showed clear tumor image in 7 days without data processing. However, it may not be suitable for clinical use, since more than 5 days are needed to obtain clear images. The subtraction method assisted by a computer or using F(ab')2 instead of whole IgG may overcome this limitation (2, 14).

The present study suggests that Ost7 can be of clinical value for the detection or treatment of osteogenic sarcoma. In our preliminary experiment, this antibody had no cytotoxic effects against osteogenic sarcoma xenografted s.c. Herlyn et al. (8) reported that the in vivo cytotoxic effects of monoclonal antibodies differ according to their subclass, and only monoclonal antibodies of IgG2a isotype specifically inhibited growth of human tumors in nude mice. Ost6 and Ost7 are of IgG1 isotype and, by themselves, have very little effect on osteogenic sarcoma. However, at least Ost7 appears to be useful for the specific delivery to osteogenic sarcoma of antitumor agent which are conjugated to Ost7.
ACKNOWLEDGMENTS

We thank Professor Seiichi Ishii and Professor Shinzo Nishi of Hokkaido University for their generosity in providing human osteosarcoma, SU, and a monoclonal antibody, AFY1, respectively.

REFERENCES


Fig. 3. \( \gamma \)-Ray photograms of mice with SU xenografts in the backs injected with \(^{141}\text{Ce}\)-labeled Ost7. Top, soft X-ray photos taken after maintaining the mice on the films for 4 days in a deep freezer. Bottom, soft X-ray photos taken of the same mice without maintaining them on the films. Soft X-ray photos were taken at 18 kV for 10 sec.
Fig. 4. Serial scintigrams of a mouse with KT005 xenografts injected with \(^{131}I\)-labeled Ost7. With time up-take of radioactivity in a tumor increased and at 7 days after injection, tumor was clearly localized by \(^{131}I\)-labeled Ost7.

Fig. 5. Whole-body scan of a mouse with KT005 xenograft (left) and of a mouse with a testicular tumor xenograft (right) 7 days after injection of Ost7. KT005 was reactive to Ost7; testicular tumor was unreactive to Ost7.
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