Urol Res (1998) 26:23–28

ORIGINAL PAPER

Kiyoshi Koshida · Kunihiko Yokoyama · Tetsuya Imao
Hiroyuki Konaka · Kazuyuki Hirano
Tadao Uchibayashi · Mikio Namiki

Immunolocalization of anti-placental alkaline phosphatase monoclonal antibody in mice with testicular tumors and lymph node metastasis

Received: 31 December 1996 / Accepted: 18 September 1997

Abstract To evaluate the ability of an anti-placental alkaline phosphatase (PLAP) monoclonal antibody (MAb) to localize to PLAP-expressing tumors, we established a model of testicular tumor with metastasis to lymph nodes and liver in severe combined immunodeficient (SCID) mice. ^{131}I-labeled or ^{125}I-labeled MAb was simultaneously administered via the intravenous or lymphatic route, respectively. Preferential accumulation of MAb in PLAP-expressing tumors at primary as well as metastatic sites was demonstrated. The percentage of the injected dose of MAb found in the tumor was generally higher when MAb was administered intravenously. Identical tumor/blood ratios were found with the two routes of administration. These data suggest that intravenous administration of a radiolabeled MAb is superior to lymphatic administration for tumor imaging and radioimmunotherapy.

Key words Immunolocalization · Placental alkaline phosphatase · Testicular tumor · Lymph node metastasis

Introduction

Seminoma, the predominant histologic type of testicular germ cell tumor, lacks a specific tumor marker. However, seminomas express placental alkaline phosphatase (PLAP), mainly on the cell membrane [1]. We have shown that metastatic seminomas in the retroperitoneal and supraclavicular lymph nodes also contain considerable amounts of PLAP, equivalent to that in the primary tumors [8]. We also demonstrated the ability of an anti-PLAP monoclonal antibody to localize PLAP-producing tumors subcutaneously transplanted in nude mice, which present amounts of PLAP similar to that in human seminomas [8]. However, the ability of the monoclonal antibody (MAb) to accumulate in small metastatic foci of seminomas in the retroperitoneal lymph nodes is difficult to assess experimentally, because no suitable animal model has been established. For that reason, we developed a model of a testicular tumor with metastases to the retroperitoneal and intraperitoneal lymph nodes in severe combined immunodeficient (SCID) mice. This model was created by intratesticular injection of HeLa Hep 2 cells that expressed PLAP on the cell membrane.

One of the most critical factors in immunodetection is achieving a high tumor/blood radioactivity ratio. This is achieved by a preferential accumulation of the MAb in the targeted tumors, accompanied by a rapid excretion of MAb from the circulation [4]. Besides reducing background activity from the circulation, an alternative to the intravenous route for administering MAb, i.e., administration through the lymphatic capillaries, is reportedly more efficient in draining regional lymph nodes in the area of the metastases [11]. In this report we describe the biodistribution profiles of a radiolabeled anti-PLAP MAb after its intravenous and subcutaneous (lymphatic) administration.

K. Koshida (✉) · T. Imao · H. Konaka ·
T. Uchibayashi · M. Namiki
Department of Urology, School of Medicine,
Kanazawa University, 13-1, Takara-machi,
Kanazawa, 920 Japan
Tel: +81-076-265-2394, fax: +81-076-222-6726

K. Yokoyama
Department of Nuclear Medicine, School of Medicine,
Kanazawa University, Kanazawa, Japan

K. Hirano
Gifu Pharmaceutical University, Gifu, Japan

Materials and methods

Animal model

Male five-week-old SCID mice were obtained from Clea Japan inc. (Tokyo, Japan), and maintained in a laminar air flow cabinet under specific pathogen-free conditions. The mice used in this study were maintained and sacrificed in accordance with the guidelines of the committee on animal experimentation of Kanazawa University, Takara-machi campus.

One side of the testis of SCID mice was inoculated with approximately 2×10^6 HeLa cells, strain Hep 2, which express surface-bound PLAP [6]. Cell lines were cultured in RPMI 1640 supplemented with 10% (v/v) fetal calf serum. Mice were killed each week up to 3 weeks after implantation of the HeLa Hep 2 cells. Blood and various tissues, including testis, liver, lung, kidney, spleen, omentum, and renal hilar and intraperitoneal lymph nodes, were taken for histologic examination and measurement of PLAP content.

Measurement of enzyme activity

The concentration of PLAP in tissues as well as in the serum of SCID mice was measured by monoclonal immunocatalytic assay, as previously reported [3]. Extraction of PLAP from the tissue homogenate was performed with butanol [9].

Monoclonal antibody

An anti-PLAP MAb (HPMS-1) was obtained from ascites of mice following the intraperitoneal injection of hybridoma cells. The MAb was purified using protein A Sepharose [5]. The affinity constant of HPMS-1 was 4.2×10^9/M.

Radiolabeling

The purified MAb was radiolabeled with ^{125}I or ^{131}I using the chloramine-T method [2]. Free iodine was removed on a Sephadex G 25 column (Pharmacia, Uppsala, Sweden). The labeling efficiency was consistently about 90–94%. Specific activity for each preparation was approximately 1 μCi/μg.

Localization study

The ^{131}I-labeled MAb was injected intravenously, and the ^{125}I-labeled-MAb was simultaneously injected subcutaneously into the hind footpad 3 weeks after testicular implantation of the HeLa Hep 2 cells. The radioactivity injected was 5 μCi for each reagent. Animals were killed at various times after injection. The testis with or without tumor, lymph nodes with metastasis, liver, lung, kidney, spleen, omentum, and blood were weighed. The radioactivity in each organ was measured in a gamma well counter on two occasions. The pharmacokinetics of each tracer can be evaluated independently because of their different half-lives.

Statistical analysis was performed by Mann-Whitney *U*-test.

Results

Results from a preliminary experiment for evaluating the metastatic ability of HeLa Hep 2 cells are listed in Table 1. Tumors developed in all testes inoculated with 2×10^6 HeLa Hep 2 cells, as shown in Fig. 1. Metastases occurred at 2–3 weeks after inoculation in 67% (2/3) of renal hilar lymph nodes, and in 83% (5/6) of the intraperitoneal lymph nodes. An example is shown in Fig. 2. The serum PLAP level was elevated in four of six mice at least 2 weeks later. Mean tissue PLAP levels (±SD) in six testis tumors and seven lymph node metastases were 11.9 ± 5.8 IU/g ($n = 6$) and 41.0 ± 7.8 IU/g ($n = 7$), respectively. Insignificant PLAP levels were seen in other tissues, including normal testis, lung, liver, spleen, kidney and omentum.

Table 1 Metastatic ability of HeLa Hep 2 cells

Tissues/weeks	1	2	3
Testis implanted	2/2[a]	3/3	3/3
Testis untreated	0/2	0/3	0/3
Liver	0/2	0/3	0/3
Lung	0/2	0/3	0/3
Kidney	0/2	0/3	0/3
Spleen	0/2	0/3	0/3
Renal hilar lymph node	0/2	0/3	2/3
Intraperitoneal lymph node	0/2	3/3	2/3

[a] Number with tumor (metastasis)/number investigated

In the localization study all the implanted testes increased in volume due to tumor cell proliferation 3 weeks later. The mean (±SD) weight of the testis with and without tumor was 218 ± 47 mg ($n = 22$) and 91 ± 7 mg ($n = 22$), respectively. Metastasis occurred in 86% (19/22) of the intraperitoneal lymph nodes, and in 36% (8/22) of the renal hilar lymph nodes. Metastasis to the liver was also noted as a small nodule in 18% (4/22) of cases as demonstrated in Fig. 3.

The percentage of the injected dose (%ID) in the blood is shown in Fig. 4. The %ID of ^{131}I-labeled MAb decreased with time. Although a peak was noted 24 h after subcutaneous administration of ^{125}I-labeled MAb, fluctuation of the values was then similar to that of ^{131}I-labeled MAb. The results imply that most of the MAb administered via the lymphatic capillaries entered the bloodstream within 24 h.

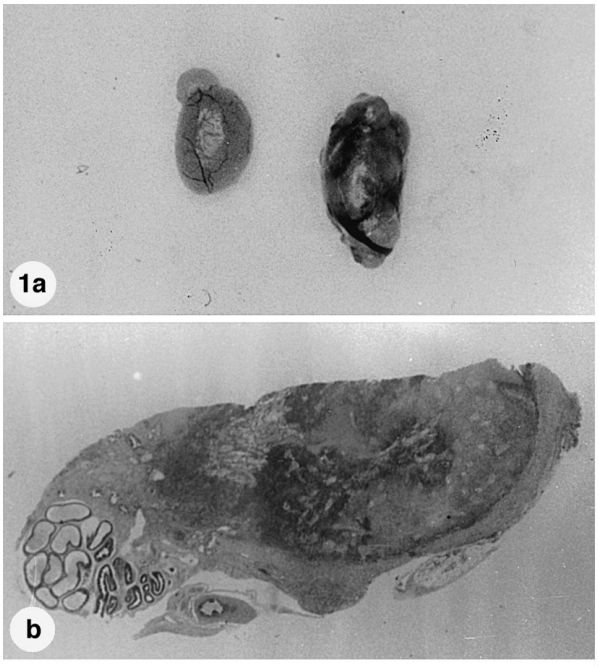
Figure 5 shows the %ID of ^{125}I-labeled or ^{131}I-labeled MAb in the testis tumor. Values of ^{131}I-labeled MAb were higher than those of ^{125}I-labeled MAb. A trend towards an increase in values over time was observed in both cases. Figure 6 indicates the %ID in lymph node metastases, including renal hilar and intraperitoneal lymph nodes. A peak was observed 2 days after administration of each labeled MAb, and fluctuations of the values were parallel.

Figure 7A indicates the ratios of testis tumor to normal testis for accumulation of ^{131}I-labeled and ^{125}I-labeled MAb in 22 mice. The ratio tended to increase with time. The ratios for ^{131}I-labeled or ^{125}I-labeled MAb were identical. The accumulation ratios of intraperitoneal lymph node metastasis to omentum were also equivalent for the two radiolabels Fig. 7B. The accumulation of MAb was significantly increased in renal hilar lymph nodes with metastasis compared with nodes without metastasis ($P < 0.001$). The accumulation of MAb in the metastatic nodules of liver was 4 times higher than that in the normal portion of the liver.

Figure 8 shows the tumor/blood ratio for ^{131}I-labeled and ^{125}I-labeled MAb. No difference was observed between the two routes of administration. Lymph node metastases showed a ratio of more than 3.0 at 2 days after administration, while testis tumors needed more than 7 days to reach this ratio.

Discussion

The model established in this study might resemble the situation in human testicular seminoma. Tumor cells in



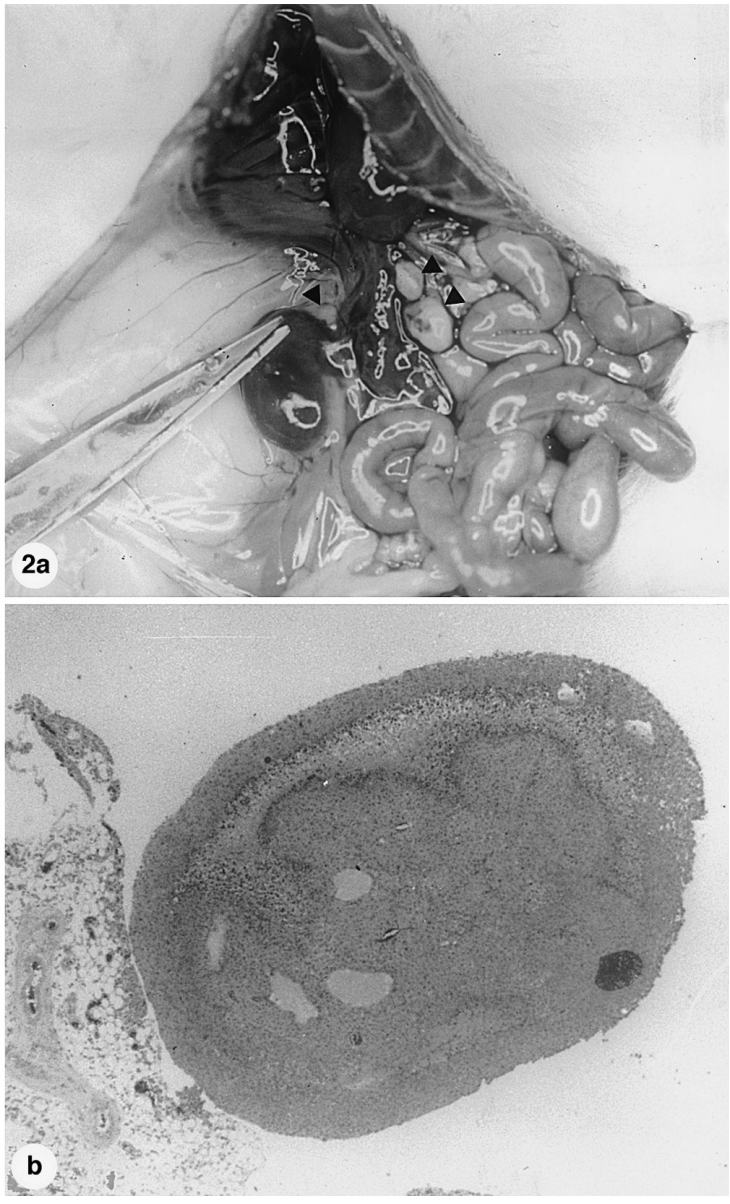


Fig. 1 a Macroscopic appearance of testis tumor and normal testis. **b** Light micrograph of testis tumor. Original magnification ×20

Fig. 2 a Intra-abdominal view of a mouse 3 weeks after intratesticular injection of HeLa Hep 2 cells. *Arrowheads* indicate renal hilar lymph node and intraperitoneal lymph node metastases. **b** Light micrograph of lymph node metastasis. Original magnification ×40

the testis proliferated and frequently metastasized to the retroperitoneal and intraperitoneal lymph nodes, and less frequently to the liver also. The testicular xenograft and lymph node metastasis possessed considerable amounts of PLAP, comparable to the amounts in human seminomas [8]. In addition, PLAP was to some extent secreted into the circulation of the mice, as has been observed in patients with seminoma [7].

We investigated the ability of an anti-PLAP MAb to immunolocalize of the testicular xenograft and its metastases via two different routes of administration: the bloodstream and lymphatic flow. We previously confirmed the ability of the intravenously administered radiolabeled MAb to localize in the xenografts of HeLa Hep 2 cells on the back of nude mice [8]. Good identification of the xenografts was demonstrated, in keeping the %ID of 7–8% up to 216 h after injection. Another group has clearly imaged HeLa xenografts on the back of mice with a %ID of 8–12% using a different MAb against PLAP [10]. In this study, preferential accumulation of the radiolabeled MAb to the testicular xenograft as well as its metastatic lesions in lymph nodes and liver was demonstrated with both routes of administration. Although we could not completely rule out the possibility of non-specific localization of the MAb to the tumors, this appeared unlikely since the %ID and tumor/blood ratio in the testicular xenografts were increased with time and the tumor/blood ratio exceeded 3.0. This was completely different from the results seen using an unrelated antibody, in which the %ID decreased with time and the tumor/blood ratio never exceeded 1.0, as previously reported [8].

The %ID of 30–50% at the testicular xenografts and of over 80% at the metastatic lymph nodes were con-

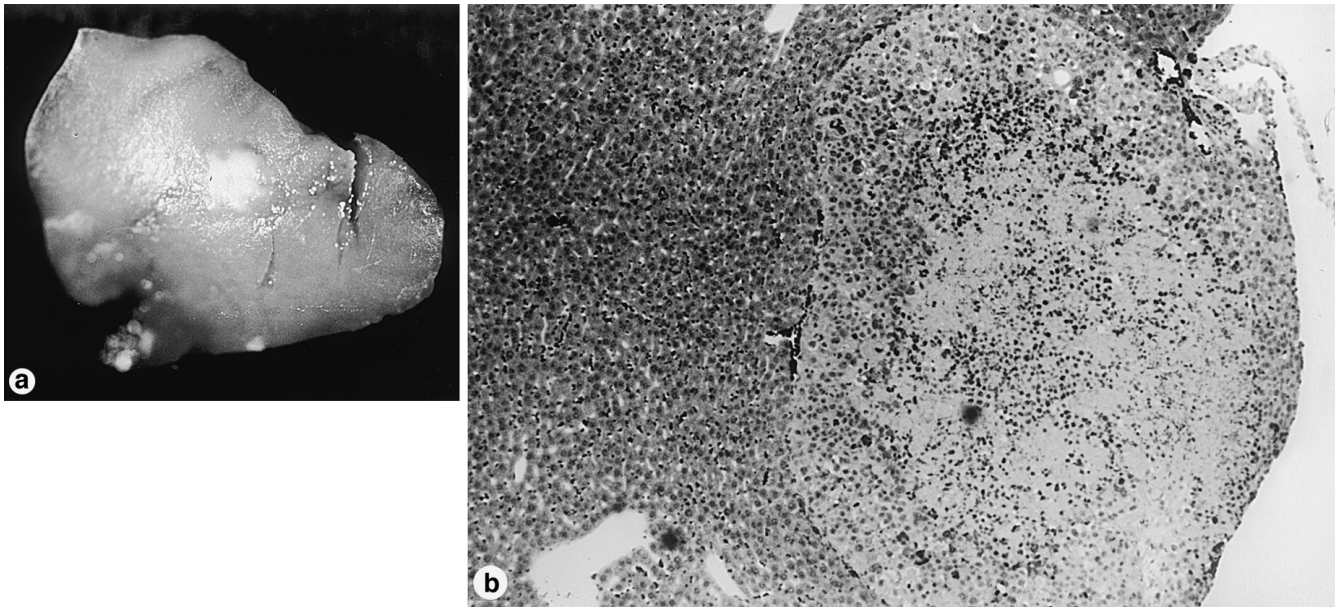


Fig. 3 A Macroscopic appearance of liver metastasis 3 weeks after intratesticular injection of HeLa Hep 2 cells. **B** Histologic section of liver metastasis. Original magnification ×40

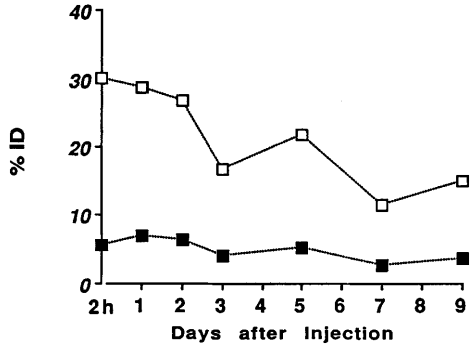


Fig. 4 Biodistribution in blood of ^{131}I-labeled MAb (*open squares*) and ^{125}I-labeled MAb (*filled squares*) as the percentage of injected dose per gram of tissue (%ID/g). Each *point* represents an average of data from two to four mice

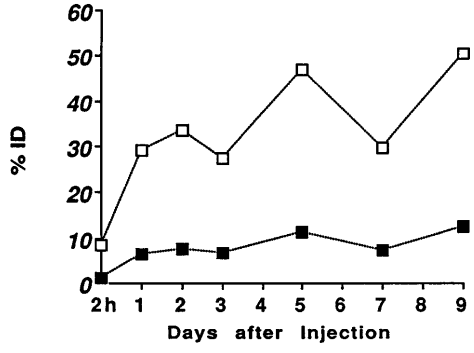


Fig. 5 Biodistribution in testis tumor of ^{131}I-labeled MAb (*open squares*) and ^{125}I-labeled MAb (*filled squares*)

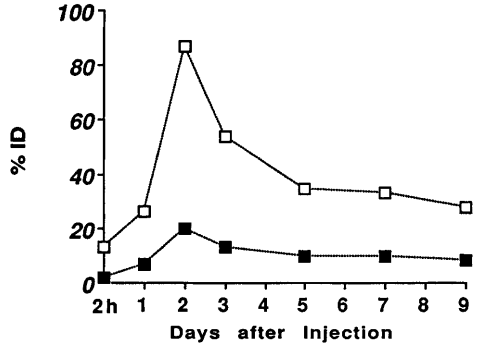


Fig. 6 Biodistribution in renal hilar and intraperitoneal lymph node with metastasis of ^{131}I-labeled MAb (*open squares*) and ^{125}I-labeled MAb (*filled squares*)

siderably higher than the %ID of the xenografts on the back. The higher %ID in the tumors with ^{131}I-labeled MAb implies that delivery of the MAb to the lesions by intravenous administration is more efficient. According to the %ID and tumor/blood ratio, localization of the MAb in lymph node metastases was more efficient than localization in the testicular xenografts. This might be due to differences in the size of the lesion and the amount of the antigen expressed. Identical results for the tumor/blood ratio with the two different routes of administration imply that specific binding of the MAb to the lymph node metastasis via lymphatic flow was unlikely to occur in this experimental model.

Weinstein et al. [11] reported that in a guinea pig model of chemically induced hepatocarcinoma, a clear image of the regional lymph node with metastasis was obtained 2.5 h after subcutaneous injection of a radiolabeled MAb. Accumulation of the MAb 24 h later was approximately 4 times higher than the accumulation after intravenous administration. In our study, however, there was no significant accumulation of the MAb on

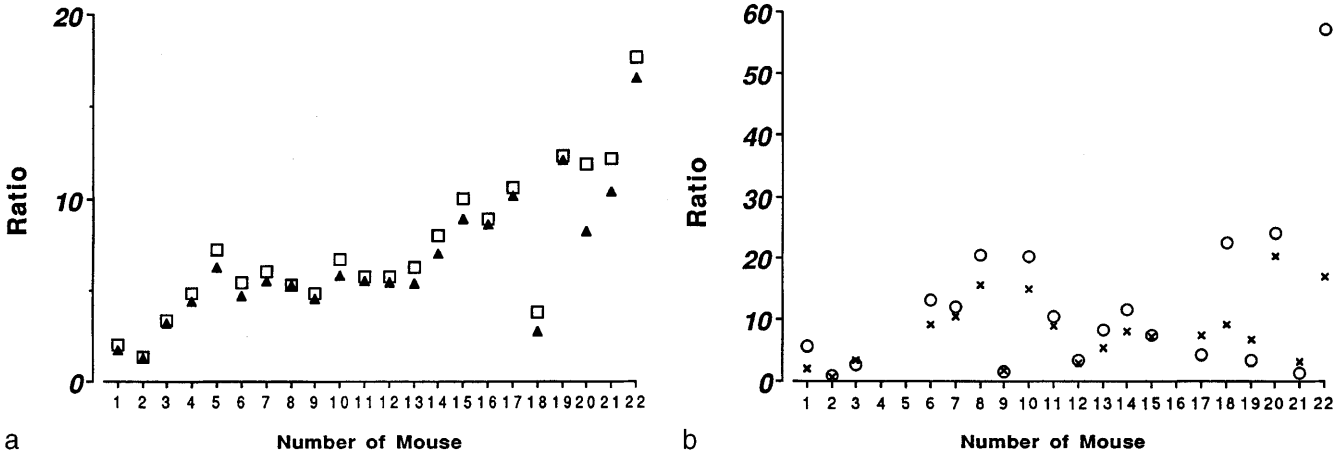


Fig. 7 a Accumulation ratio for testis tumor to normal testis of ^{131}I-labeled MAb (*open squares*) and of ^{125}I-labeled MAb (*filled triangles*). Mice nos. 1 and 2 are from day 0 (2 h), nos. 3–5 from day 1, nos. 6–9 from day 2, nos. 10–13 from day 3, nos. 14–17 from day 5, nos. 18–20 from day 7, and nos. 21 and 22 from day 9. **b** Accumulation ratio for intraperitoneal lymph node to omentum of ^{131}I-labeled MAb (*open circles*) and of ^{125}I-labeled MAb (*crosses*). Data from mice nos. 4, 5 and 16 are not presented due to lack of metastasis

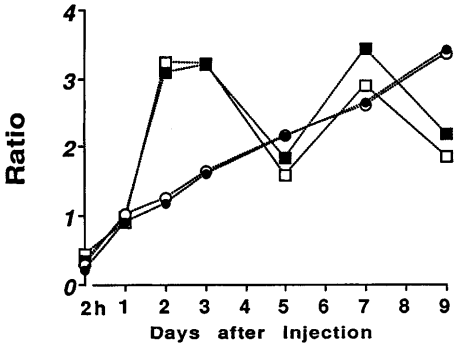


Fig. 8 Tumor/blood ratio of ^{131}I-labeled MAb (*open symbols*) and ^{125}I-labeled MAb (*filled symbols*). Testis tumor/blood ratio is indicated as *circles*, lymph node metastasis/blood ratio as *squares*

either renal hilar or intraperitoneal lymph nodes with metastasis 2 h after subcutaneous administration, when approximately 70% of the radioactivity had been drained from the hindpaw where the MAb was injected. Even 24 h later, the %ID in lymph node metastases after subcutaneous administration of the MAb was lower than that after intravenous administration.

A possible explanation for the discrepancy might be the difference in the tumor burden of the lymph node. The metastatic lymph node in the model of Weinstein et al. [11] contained relatively few tumor cells, corresponding to a tumor weight of 2.3–6.7 mg. By contrast, the entire lymph node was replaced by tumor cells in our model, resulting in an increase in weight ranging from 11.1 to 164.8 mg (mean 33.0 mg). Replacement of the entire lymph node by tumor cells might interrupt the lymphatic flow and impede access of the MAb to the tumor cells. Another possible explanation is the difference in the anatomical relationship between the lymph nodes with metastasis and the injection site of the MAb. The ipsilateral superficial distal axillary node of the guinea pig might collect almost all the lymphatic flow from the front foot, while the situation in the retroperitoneal or intraperitoneal lymph node might be different when the MAb is administered in the hindpaw. In addition, the difference in affinity of the MAb to the targeting antigen should be taken into consideration.

The results from this study suggest that the lymphatic approach should be considered at an earlier stage of lymph node metastasis, when lymphatic flow is well preserved. From the therapeutic viewpoint intravenous administration of a radiolabeled MAb appears to be superior, since the %ID in tumorous lesions was always higher than with subcutaneous administration. The potential of the radiolabeled MAb for radioimmunotherapy against tumors is under investigation.

Acknowledgements The HeLa cell line was kindly provided by Dr. T. Stigbrand, Department of Medical Biochemistry and Biophysics, University of Umea, Sweden. This work was supported by a Grant-in-Aid for general scientific research from the Ministry of Education and Culture, Japan.

References

1. Brehmer-Andersson E, Ljungdahl-Stahle E, Koshida K, Yamamoto H, Stigbrand T, Wahren B (1990) Isoenzymes of alkaline phosphatases in seminomas: an immunohistochemical and biological study. APMIS 98:977
2. Greenwood FC, Hunter WM, Glover JS (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem J 89:114
3. Hayashi Y, Mitani T, Kurono M, Hirano K, Hayashi K, Iino S, Domar U, Stigbrand T (1991) Improved monoclonal immunocatalytic assays (MICAs) for human alkaline phosphatase isozymes. Jpn J Clin Chem 20:125
4. Hendrix PG, Dauwe SE, Van De Voorde A, Nouwen EJ, Hoylaerts MF, De Broe ME (1991) Radiolocalisation and imaging of stably HPLAP-transfected MO4 tumours with monoclonal antibodies and fragments. Br J Cancer 64:1060
5. Hirano K, Iiizumi Y, Hayashi Y, Tanaka T, Sugiura M, Hayashi K, Lu ZD, Iino S (1986) A highly sensitive assay

method for human placental alkaline phosphatase involving a monoclonal antibody bound to a paper disk. Anal Biochem 154:624
6. Jeppsson A, Wahren B, Millan JL, Stigbrand T (1984) Tumour and cellular localization by use of monoclonal and polyclonal antibodies to placental alkaline phosphatase. Br J Cancer 49: 123
7. Koshida K, Uchibayashi T, Yamamoto H, Hirano K (1996) Significance of placental alkaline phosphatase (PLAP) in the monitoring of patients with seminoma. Br J Urol 77:138
8. Koshida K, Uchibayashi T, Yamamoto H, Yokoyama K, Hirano K (1996) A potential use of a monoclonal antibody to placental alkaline phosphatase (PLAP) to detect lymph node metastases of seminoma. J Urol 155:337
9. Morton RK (1954) The purification of alkaline phosphatase of animal tissues. Biochem J 57:595
10. Stigbrand T, Hietala SO, Johansson B, Makiya R, Riklund K, Ekelund L (1989) Tumor radioimmunolocalization in nude mice by use of antiplacental alkaline phosphatase monoclonal antibodies. Tumor Biol 10:243
11. Weinstein JN, Steller MA, Keenan AM, Covell DG, Key ME, Sieber SM, Oldham RK, Hwang KM, Parker RJ (1983) Monoclonal antibodies in the lymphatics: selective delivery to lymph node metastases of a solid tumor. Science 222:423