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Natural interferon enhances expression of placental alkaline phosphatase in human seminoma xenograft

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Abstract The purpose of this study was to investigate the effect of natural interferon (IFN) on the expression of placental alkaline phosphatase (PLAP) in a human seminoma xenograft in severe combined immunodeficient mice. Mice were injected intramuscularly with 3×10^5 U/mouse of IFN, twice a day, for five consecutive days. A significant increase in PLAP level of the xenografts followed IFN treatment. A radiolabeled anti-PLAP monoclonal antibody (MAb) was intravenously injected on the first day of IFN administration in order to determine if IFN has the potential to enhance the efficacy of an anti-PLAP MAb in the detection of seminoma. Enhanced retention of the anti-PLAP MAb was observed at 7 and 11 days after MAb administration. Thus, IFN treatment appears to have some effect on the efficacy of the anti-PLAP MAb in the detection of seminoma xenografts.

Key words Interferon · Placental alkaline phosphatase · Seminoma · Immunolocalization

Introduction

Previously we reported that the serum placental alkaline phosphatase (PLAP) level was frequently increased in patients with seminoma [16], and all seminomas were positive for PLAP in immunohistochemical studies [2, 23]. We also reported that tissue PLAP level in testicular seminoma and its lymph node metastasis is approximately 80 times higher than that of normal counterparts [17, 23]. Since PLAP is located chiefly in the cell membrane, it appears to be the most suitable target for immunodetection. Indeed, xenografts of HeLa Hep 2 cells, which produce a comparable level of PLAP to human seminomas, were successfully imaged using an I-125 labeled anti-PLAP monoclonal antibody MAb in nude mice [17]. However, difficulty in imaging for a human seminoma xenograft in SCID mice was found to be due to low blood flow [18]. Although there are some successful reports of tumor imaging with anti-PLAP MAbs for untreated metastatic seminomas [3, 21], radioimmunodetection for residual tumors following radiation/chemotherapy appears to have more potential in clinical application, but it might be more difficult since therapy-induced necrosis/fibrosis would decrease blood flow. An approach to overcome this obstacle to good immunodetection might be enhancement of PLAP expression on the tumor cells, whereby accumulation of an anti-PLAP MAb could be augmented.

Interferons are potent antiviral and antiproliferative compounds [7, 14], and have been shown to have several of the immunoregulatory properties of glycoproteins such as cell surface antigens (1, 19). Treatment of human breast and colon carcinoma cells with recombinant IFNα was reported to enhance the surface expression of tumor-associated antigens recognized by MAbs [5]. This IFN-mediated increase in surface antigens was the result of both the accumulation of more antigens per cell, and of an increase in the percentage of cells expressing antigen. It was shown that administration of recombinant IFN in vivo effectively increased the amounts of tumor antigen expressed by a human colon xenograft and augmented the localization of a radiolabeled MAb to the tumor site [6, 8].

In this study, we investigated the effects of IFN on the expression of PLAP in a human seminoma xenograft in order to determine if IFN has the potential to enhance the efficacy of an anti-PLAP MAb in the detection of seminoma. To confirm the effect of IFN on alkaline phosphatase activity, the tissue level of liver alkaline

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phosphatase (LAP) was measured simultaneously since seminoma also contains much higher amounts of LAP than nonseminoma or normal testis [17, 23].

Materials and methods

Animal model

Male severe combined immunodeficient (SCID) mice, 5–6 weeks old, 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. A seminoma xenograft line had been established from an anaplastic seminoma metastasized to the supraclavicular lymph node from a 50-year-old patient with an increased serum PLAP level [18]. The seminoma xenograft line was maintained by serial subcutaneous transplantation of tumor fragments measuring $3 \times 3 \times 3$ mm, into the back of SCID mice. Tumors of the eighth and ninth generations were used in the experiments.

Interferon treatment

Mice were injected intramuscularly with 3×10^5 U/mouse of IFN (human lymphoblastoid interferon, Sumitomo, Osaka, Japan), twice a day, for five consecutive days. The same schedule was used for untreated mice, which received injections of 0.9% sodium chloride.

Monoclonal antibody

Anti-PLAP MAb (HPMS-1) and anti-LAP MAb (HLMS-1) were obtained from ascites of mice following an intraperitoneal injection of hybridoma cells [11, 12]. The antibody was then purified with protein A sepharose. The affinity constants of HPMS-1 and HLMS-1 were 4.2×10^9/M and 4.1×10^{10}/M, respectively.

Radiolabeling

The purified MAb was radiolabeled with iodine-125 using the chloramine-T method [4]. Free iodine was removed on a Sephadex G 50 medium column (Pharmacia, Uppsala, Sweden). The labeling efficiency was consistently between 85% and 90%.

Localization study

The radiolabeled antibodies were intravenously injected on the first day of IFN administration with a total radioactivity of 10 μCi for the distribution study and 150 μCi for the imaging procedure. In the distribution study, the animals were killed at various times after injection, and their tumor, blood, liver, kidney, spleen, muscle and heart weighed. The amount of radioactivity in each organ was measured in a gamma well counter. For imaging, radioactivity distribution in the animals was recorded at various times with a Gamma camera (Technicare, Sigma 414, Solon, Ohio).

Measurement of enzyme activity

Concentrations of PLAP and LAP in the tissues as well as sera of SCID mice were measured by monoclonal immunocatalytic assays (MICAs) as previously reported [9]. Briefly, the microtiter plates were coated with 100 μl of purified MAb (7 μg/ml) dissolved in phosphate buffered saline containing 0.02% sodium azide at 4°C overnight. After removal of the unbound MAb, the plates were coated with bovine serum albumin, then kept at 4°C until use. One hundred μl of aliquots of the samples were added to each well and were allowed to react at 4°C for at least 12 h. After washing, 100 μl of substrate solution, prepared according to the International Federation of Clinical Chemistry (IFCC) method [13], were added to each well and the plate was incubated at 37°C for the appropriate length of time. The resulting yellow color, corresponding to the intensity of activity was tested at 405 nm using an automatic immunoplate reader and the activity of the isozyme was calculated making use of the standards of alkaline phosphatase isozyme

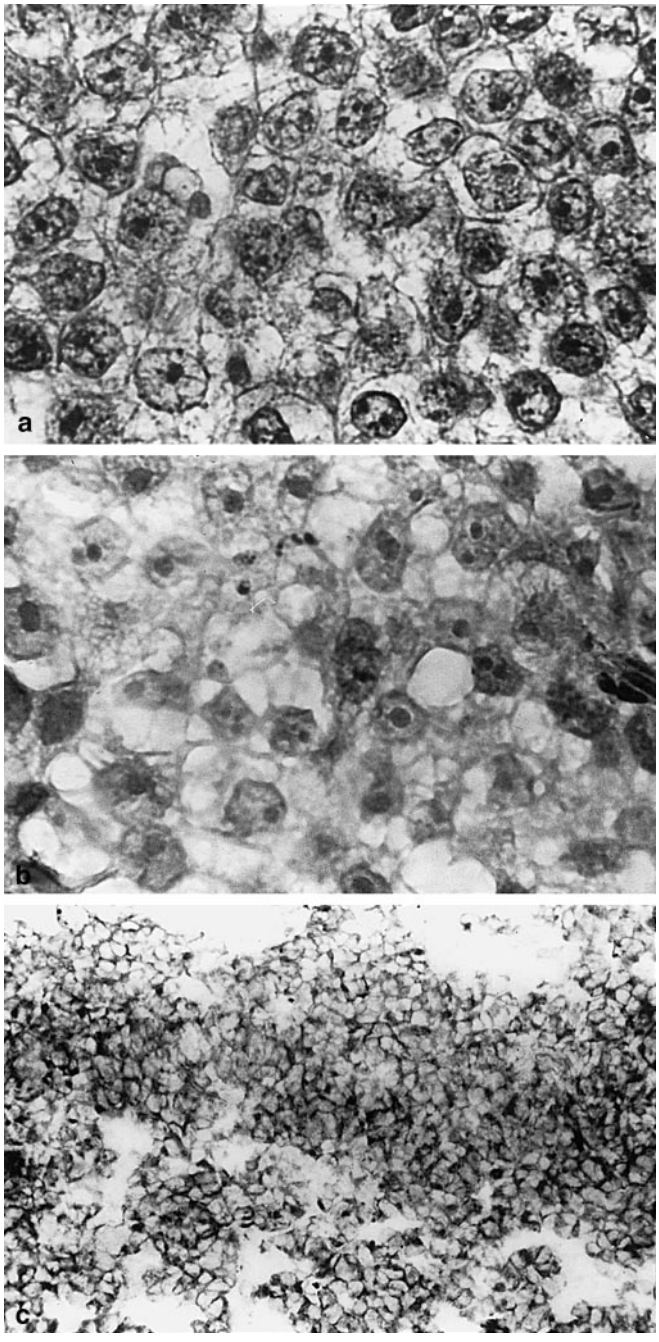

Fig. 1a, c Microscopic images of the original tumor of the anaplastic seminoma metastasized to the supraclavicular lymph node (**a**) and seminoma xenograft in severe combined immunodeficient (SCID) mice (**b**). Immunoperoxidase staining of seminoma xenograft for placental alkaline phosphatase monoclonal antibody (PLAP MAb) (**c**). (Original magnification ×400 for **a** and **b**, and ×100 for **C**)

activities determined simultaneously. Extraction of PLAP and LAP in tissue homogenate was performed with butanol [20].

Statistical analysis was performed with the Mann-Whitney U test.

Results

Histologic findings of the original tumor and of the xenograft, as well as immunohistochemical staining of the xenograft for PLAP are shown in Fig. 1. The xenograft clearly preserved the histologic characteristics of the seminoma and presented PLAP mainly on the cell membrane. Significant correlations between tumor weight and serum alkaline phosphatase levels in both PLAP and LAP are shown in Fig. 2, indicating that these antigens are released from the xenografts into the circulation and that the extent of elevation depends on the tumor burden. Tumor alkaline phosphatase levels in mice with and without IFN administration are shown in Fig. 3. A significant increase in antigen expression was demonstrated in the xenografts 24 h after the last administration of IFN. The extent of increase was almost the same for the two antigens (146% for PLAP, 169% for LAP). Alkaline phosphatase levels in other tissues such as liver, lung, spleen, heart, and muscle were less than 0.06 IU/g. However, the levels of PLAP and LAP in the kidney were somewhat higher than in other tissues, probably reflecting excretion of the circulating antigens into the urine. Biodistribution of an anti-PLAP MAb was studied in mice with and without IFN administration. No significant difference in biodistribution was found between the two groups in blood and other tissues including liver, lung, kidney, spleen, heart, and muscle (data not shown). The exceptions were the seminoma xenografts at 7 and 11 days after injection of iodine-125-labeled MAb, corresponding to 2 and 6 days after the last administration of IFN, as shown in Fig. 4A. Figure 4B indicates that the tumor/blood ratio increased with time in mice with IFN administration but not in mice without IFN. Figure 5 represents posterior images of mice with xenografted human seminoma. The images were obtained at 7 and 11 days after injection of iodine-125-labeled anti-PLAP MAb. Increased retention of the MAb was seen in the mouse treated with IFN when compared with the untreated mouse. Tumors removed 11 days after injection of anti-PLAP MAb were nearly equal in volume and showed grossly homogeneous tissues without necrosis. Increased retention of anti-PLAP MAb in the tumor from the mouse treated with IFN is shown in Fig. 6. Biodistribution results from the two mice are presented in Fig. 7. All areas of the tumor from the mouse treated with IFN showed greater accumulation of anti-PLAP MAb than that from the

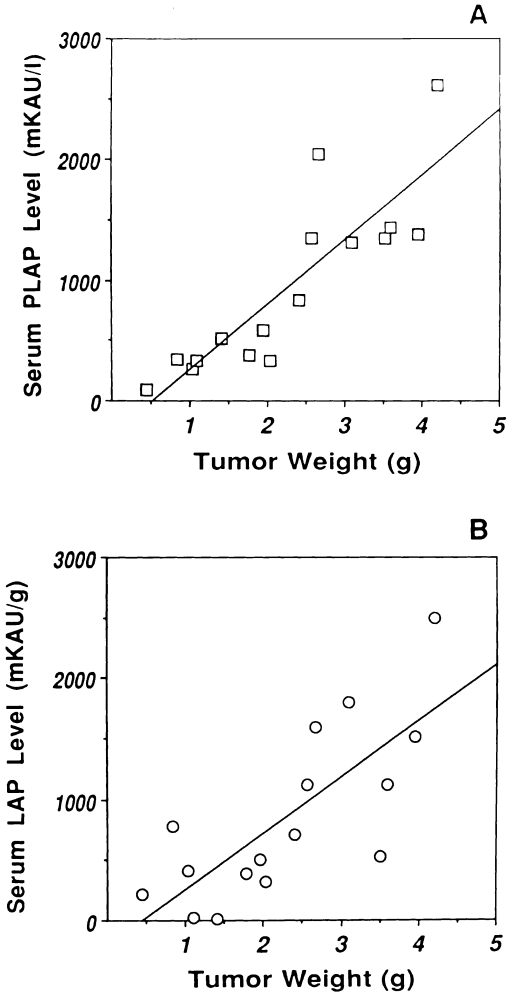

Fig. 2A, B Correlation between tumor weight and serum PLAP (**A**) and liver alkaline phosphatase (LAP) (**B**) levels in 16 untreated mice. Correlation coefficients are $r = 0.861$ for PLAP and $r = 0.761$ for LAP

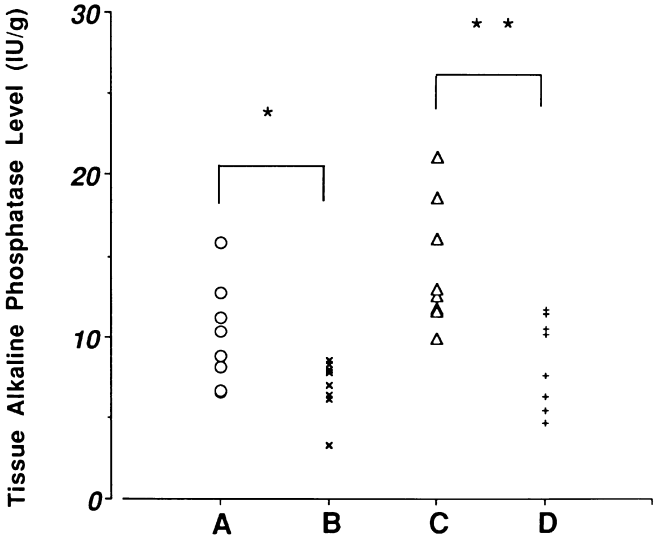

Fig. 3 Tumor alkaline phosphatase levels in mice with and without interferon (IFN) administration. Each group consisted of eight mice. PLAP in mice with IFN (**A**) and without IFN (**B**); LAP in mice with IFN (**C**) and without IFN (**D**). $*P < 0.05$, $**P < 0.01$, Mann-Whitney U test

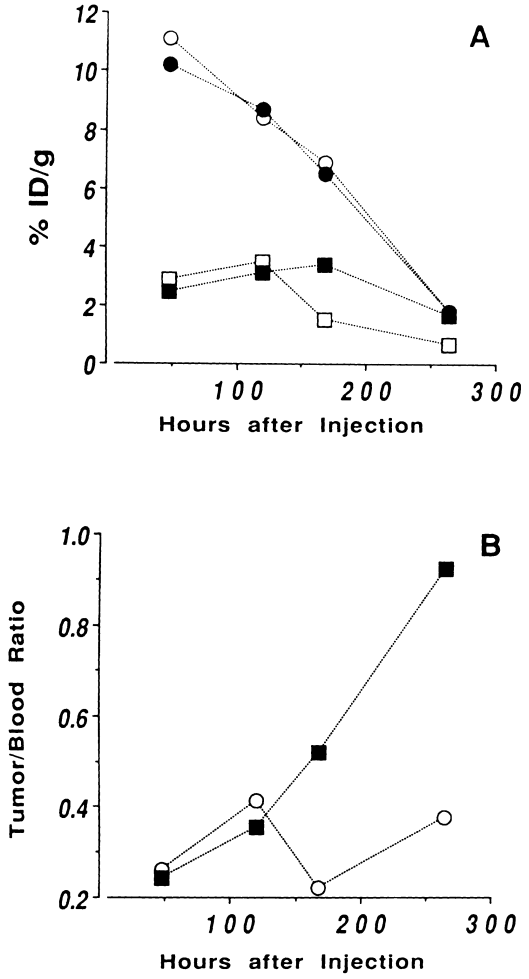

Fig. 4 **A** Biodistribution of anti-PLAP MAb in blood and tumor. Percentage injected dose per gram (%ID) of blood in mice treated with IFN (*filled circles*), and in untreated mice (*open circles*), and %ID of tumor in mice treated with IFN (*filled squares*), and in untreated mice (*open squares*). Each point represents average data from five mice. For the tumor, the mean of the values for four different portions was employed for calculation. **B** Tumor/blood ratio of anti-PLAP MAb in mice with IFN (*filled squares*) and without IFN administration (*open circles*)

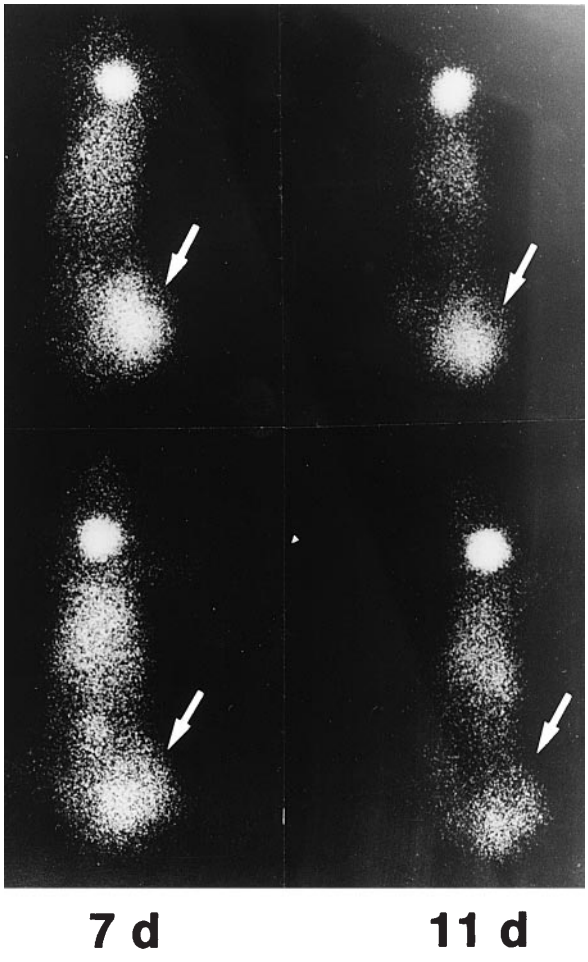

Fig. 5 Posterior images of SCID mice with xenografted human seminoma, obtained 7 days (*d*) and 11 days after injection with 150 μCi iodine-125-labeled anti-PLAP MAb. *Upper row* represents a mouse treated with IFN and the *lower row* an untreated mouse. *White arrows* indicate xenografts inoculated

untreated mouse. In addition, anti-PLAP MAb tended to accumulate more in the central region (facing the muscle of the back) of the tumor than the peripheral region (facing the skin), although tissue levels of PLAP in these regions were equivalent.

Discussion

We previously demonstrated the ability of the anti-PLAP MAb (HPMS-1) to localize to xenografts of HeLa Hep 2 cells, which contained a considerable amount of PLAP equivalent to the level of human testicular seminomas [17]. The PLAP level of the seminoma xenografts used in this study was higher than that of the HeLa xenografts or human testicular seminomas. Although good immunolocalization of the anti-PLAP MAb to the seminoma xenografts was expected, visualization of the xenografts was unexpectedly poor, partly due to low blood flow [18]. Since the seminoma xenografts might represent characteristics of human seminoma more than do the HaLa xenografts, difficulty in imaging for human metastatic seminoma is suspected. In order to develop a system for imaging of metastatic seminoma, an experiment was conducted to determine whether IFN was capable of enhancing PLAP expression and subsequent MAb binding in vivo. The injected dose of IFN was rather high compared with the doses previously reported, since the extent of enhancement would depend on the plasma level of circulating IFN, which in turn seemed to be related to the initial amounts of IFN administered [5, 6]. As a result, significant increases in tissue levels of alkaline phosphatases, PLAP and LAP, were observed in mice treated with IFN.

A prerequisite for good imaging was reported to be a tumor/blood ratio of more than two to three [10]. The

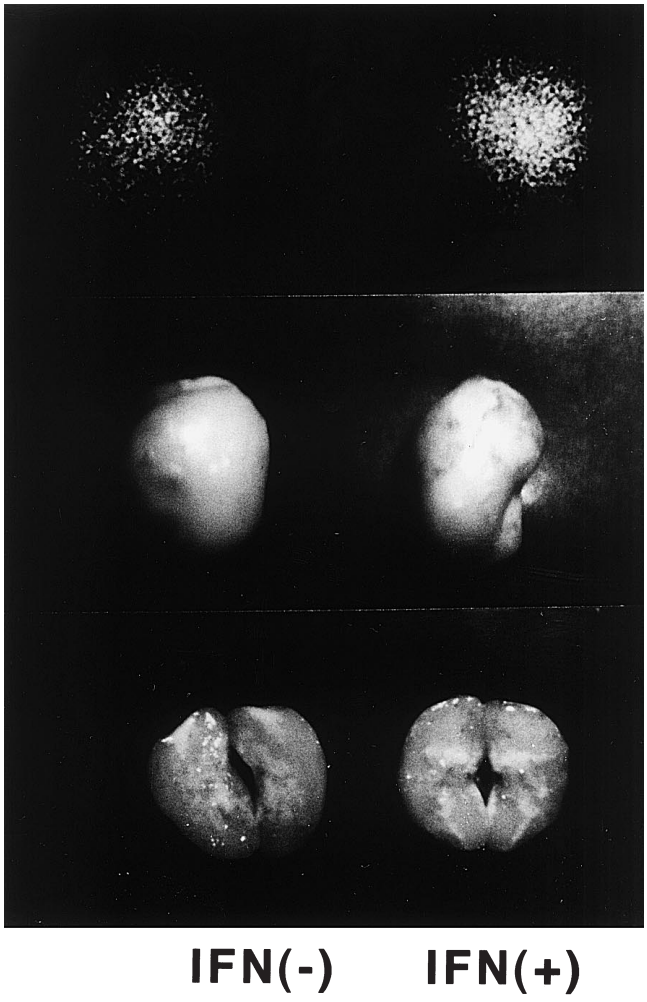

Fig. 6 Macroscopic appearance of tumors removed from a mouse with (*right*) and without (*left*) IFN administration. Accumulation of iodine-125-labeled anti-PLAP MAb to the tumors is also shown

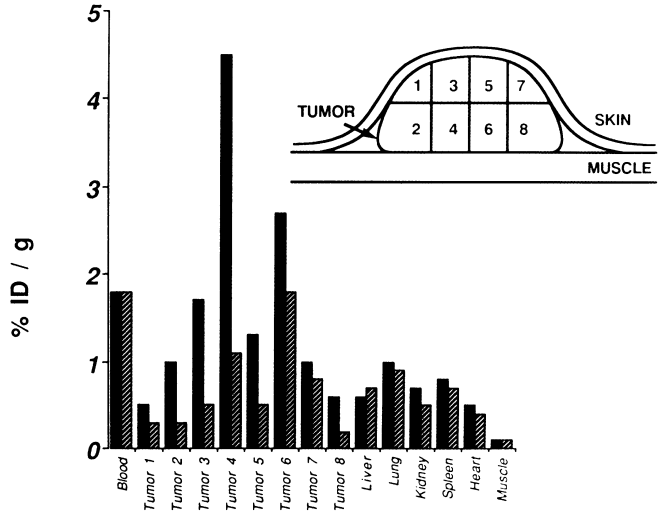

Fig. 7 Biodistribution results of the tumors presented in Fig 5. Numbers in the tumor correspond to the area of the tumor illustrated. *Solid bars* indicate data from the mouse treated with IFN and *shaded bars* from the untreated mouse

results presented here imply that this approach for enhancement of MAb localization has a limited effect, since the tumor/blood ratio did not exceed 1.0. However, we previously observed that tumor imaging for xenografts in mice was possible in the late phase when the tumor/blood ratio was around 1.0 [18]. This is also the case for mice treated with IFN showing enhancement of tumor localization of MAb with the tumor/blood ratio close to 1.0. The reason for increasing MAb localization appears to be, in part, the augmentation of antigen expression, as demonstrated for tissue PLAP level. However, the extent of the increase was much less than that seen in a previous study [6] (146% versus 380%).

To obtain good visualization of the seminoma xenograft, we previously showed that fragmentation of the MAb was effective [18]. This approach, however, did not increase MAb accumulation, but facilitated excretion of MAb from the circulation, resulting in a higher tumor/blood ratio. To increase MAb distribution in the target tumor, elevation of blood pressure with angiotensin II appears to be effective due to increase of blood flow in the tumor [15]. Increase of vascular permeability might be another aspect to increase penetration of MAb into the target tumor. For that purpose, pretreatment with interleukin-2 (IL-2) was reported to facilitate the localization of tumor-specific MAb at the site of tumor through a mechanism of increased capillary permeability (22). Thus coadministration of angiotensin II or IL2 with IFN appears to be of interest.

In conclusion, IFN treatment appears to have some effect on the efficacy of the anti-PLAP MAb in the detection of seminoma xenografts through a mechanism of enhanced antigen expression. These data may have implications for clinical application of this procedure in detecting micrometastasis in seminoma.

References

1. Attallah AM, Needy CF, Noguchi PD, Elisberg BL (1979) Enhancement of carcinoembryonic antigen expression by interferon. Int J Cancer 24: 49
2. Brehmer-Andersson E, Ljungdahl-Stahle E, Koshida K, Yamamoto H, Stigbrand T, Wahren B (1990) Isoenzymes of alkaline phosphatases in seminomas. An immunohistochemical and biochemical study. APMIS 98: 977
3. Epenetos AA, Carr D, Johnson PM, Bodmer WF, Lavender JP (1986) Antibody-guided radiolocalisation of tumours in patients with testicular or ovarian cancer using two radioiodinated monoclonal antibodies to placental alkaline phosphatase. Br J Radiol 59: 117
4. Greenwood FC, Hunter WM, Glover JS (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem J 89: 114
5. Greiner JW, Hand PH, Noguchi P, Fisher PB, Pestka S, Schlom J (1984) Enhanced expression of surface tumor-associated antigen on human breast and colon tumor cells after recombinant human leukocyte α-interferon treatment. Cancer Res 44: 3208
6. Greiner JW, Guadagni F, Noguchi P, Pestka S, Colcher D, Schlom J (1987) Recombinant interferon enhances monoclonal targeting of carcinoma lesion in vivo. Science 235: 895

7. Gresser I, Toney MG (1978) Antitumor effects of interferon. Biochim Biophys Acta 516: 231
8. Guadagni F, Schlom J, Pothen S, Pestka S, Greiner JW (1988) Parameters involved in the enhancement of monoclonal antibody targeting in vivo with recombinant interferon. Cancer Immunol Immunother 26: 222
9. 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
10. 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
11. 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
12. Hirano K, Matsumoto H, Tanaka T, Hayashi Y, Iino S, Domar U, Stigbrand T (1987) Specific assay for human alkaline phosphatase isozymes. Clin Chim Acta 166: 265
13. International Federation of Clinical Chemistry. Scientific committee, expert panel on enzymes (1983) IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase. Clin Chim Acta 183: 339F
14. Isaac A, Lindenmann J (1957) Virus interference. I. The interferon. Proc R Soc Lond 147: 258
15. Kinuya S, Yokoyama K, Konishi S, Tonami N, Hisada K (1996) Effect of induced hypertension with angiotensin II infusion on biodistribution of ^{111}I-labeled monoclonal antibody. Nuc Med Biol 23: 137
16. 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: 128
17. 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
18. Koshida K, Yokoyama K, Uchibayashi T, Yamamoto H, Hirano K, Namiki M (1997) Factors contributing to imaging of xenografts using anti-placental alkaline phosphatase monoclonal antibody. J Urol 157: 1941
19. Liao S-K, Kwong PC, Khosravi M, Dent PB (1982) Enhanced expression of melanoma-associated antigens and B2-microglobulin on cultured human melanoma cells by interferon. J Natl Cancer Inst 68: 19
20. Morton RK (1954) The purification of alkaline phosphatase of animal tissues. Biochem J 57: 595
21. Pectasides D, Vonorta P, Tsialta-Salihou A, Pateniotis K, Barbounis V, Kayianni H, Arapantoni P, Taylor-Papadimitriou J, Epenetos A, Koutsiouba P, Athanassiou A (1990) Immunoscintigraphy with ^{131}I-labelled H17E2 monoclonal antibody compared with conventional lymphangiography and computed tomography in the detection of metastases in patients with testicular germ cell tumours. Br J Cancer 62 (Suppl X): 74
22. Shultz KR, Badger CC, Dombi GW, Greenberg PD, Bernstein ID (1992) Effect of interleukin-2 on biodistribution of monoclonal antibody in tumor and normal tissues in mice bearing SL-2 thymoma. J Nat Cancer Inst 84: 109
23. Yamamoto H, Uchibayashi T, Koshida K, Hirano K, Hisazumi H (1993) Immunopathology of alkaline phosphatase isozymes in seminoma. Urol Int 50: 33