Radioimmunodetection of human pancreatic tumor xenografts
using DU-PAN II monoclonal antibody

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The potential of DU-PAN II, monoclonal antibody (IgM), which was raised against
the human tumor cell line, was evaluated for radioimmunodetection of human pancreatic
tumors (PAN-5-JCK and EXP-58) grown in nude mice. 125I-labeled DU-PAN II was
accumulated into PAN-5-JCK producing DU-PAN II antigen with a tumor-to-blood ratio
of 2.72±3.00, but it did not localize in EXP-58 because of insufficient DU-PAN II. There
was no significant uptake of 125I-nonimmunized IgM in PAN-5-JCK. These facts indicated
the specific tumor uptake of DU-PAN II. Excellent images of the tumor PAN-5-JCK were
obtained 3 days after the injection of 125I-DU-PAN II. Gel chromatography was also in-
vestigated with respect to the plasma taken from mice injected with antibody, or incubated
with antibody in vitro. The results indicate that circulating antigen affected the tumor uptake
of DU-PAN II: The more the tumor grew, the higher the amount of antigen excreted into
the blood, leading to the degradation of DU-PAN II before it reached the tumor sites.
Consequently, the immunoscintigram of the small tumor was remarkably clear. The catab-
olism and the radiolysis of the labeled IgM injected are critical points in applying immuno-
scintigraphy.

Key words: Radioimmunodetection, Human pancreatic tumor, DU-PAN II

INTRODUCTION

DU-PAN II is the monoclonal antibody which was
developed and characterized by Metzgar as being
specific to a human pancreatic tumor cell line
(HPAF).† The isotype of DU-PAN II as determined
by immunodiffusion and immunofluorescence analy-
sis was IgM. It reacted with the adenocarcinoma
cells, and failed to react with the normal hepatocytes
or connective tissues. In 1984, pancreatic cancer-
associated antigen (DU-PAN II antigen) was
detected in serum and ascites of patients with adeno-
carcinoma. Subsequently, DU-PAN II antigen was
isolated and partially purified to prove that the
etiophe was expressed on a mucin-like molecule.2

The assay of DU-PAN II antigen has been devel-
oped, and some papers have evaluated the clinical
significance of the assay of serum DU-PAN II antigen
for the diagnosis of pancreatic cancer.4-6 Takami
reported that the sensitivity was 48.9% and that the
assay was efficient in monitoring tumor load and
response to therapy of pancreatic cancers, which are
hard to detect in the early stage.4

But when the murine immunoglobulins are used
in patients, the direct reactions of the administered
antibody with normal human cells, or the allergic
reactions caused by the antimouse immunoglobulins
are undesirable side effects. The development of
human monoclonal antibodies has, therefore, become
a subject of intense interest as the logical step in
clinical applications. The main immunoglobulin class
of the human antibody established so far is mostly
IgM.7 There is, however, little information on the
tumor localization potential of IgM antibodies,7-10
since the majority of antibodies so far evaluated for
their applications in in vivo diagnosis and therapy
have been mouse antibodies of IgG isotypes.

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These facts have led us to examine the potential of DU-PAN II for radioimmunodetection of human pancreatic tumors grown in nude mice. We recently reported that 125I-labeled monoclonal antibody (IgM), named NCC-ST-433, holds promise for the detection of gastric cancers because of its rapid clearance. In this paper we describe the efficiency of IgM in the radioimmunodetection of tumors.

MATERIALS AND METHODS

Monoclonal antibody, DU-PAN II and DU-PAN II antigen assay kit (DU-PAN II Enzyme immunoassay kit) were supplied by Kyowa Medex Co. Ltd. Non-immunized IgM (control IgM) was purchased from the Calbiochem Co., Ltd. Other reagents used throughout the experiments were of analytical grade.

The tumor xenograft model consisted of male mice which had been injected subcutaneously in the right flank with human pancreatic tumor tissues; PAN-5-JCK producing DU-PAN II or EXP-58 which did not react immunohistochemically with DU-PAN II. Both types of tumor tissues were kindly given to us by Dr. Ueyama of Tokai University. Mice developing tumors larger than 0.5 mm in diameter were used for the experiments.

Labeling monoclonal antibody. To label monoclonal antibodies with 125I, the lactoperoxidase technique was employed using enzymeobeads (Bio-Rad). Briefly, 100 µl of 0.3 M phosphate buffer (pH 7.3) was added to the enzymeobeads in the reaction vial and incubated at 4°C for one hour at least. Monoclonal antibody (1 mg/ml), 10 µl of Na 125I, and 25 µl of 1% β-D-glucose solutions were added to the vial in this order. The vial was swirled gently and allowed to stand at room temperature for 30 min. To terminate the reaction, 10 µl of freshly prepared sodium-metabisulfite solution was added. The labeled molecules were separated from the unbound radioactivity by gel filtration chromatography (PD-10) using 0.9% NaCl plus 1% bovine serum albumin as eluant. 125I-labeled antibodies were more than 80% immuno-reactive as determined by the assay described in the “Quantification of serum- and tumor-derived DU-PAN II antigen,” where labeled antibody-coated plates were used instead of DU-PAN II-coated ones. Biodistribution and imaging studies. The biodistribution experiments were performed as described previously. Routinely, 10 µg per mouse of labeled antibody was injected intravenously. The mice were sacrificed 5 days after the administration of the antibody, and tissue samples were removed and weighed. The radioactivity was measured using a scintillation counter, and the percentage of injected dose per gram (%ID/g) and the tissue-to-blood ratios were determined.

For the imaging studies, a mouse was injected with 10 µg of the labeled DU-PAN II (ca, 70 µCi, 25.9 x 10^5 Bq). After 1, 3, and 5 days the mouse was anesthetized and scanned using a medium field-of-view camera fitted with a pinhole collimator. Quantification of serum- and tumor-derived DU-PAN II antigen. A mouse was anesthetized and the blood (generally over 1 ml) was collected and centrifuged at 3,000 rpm for 5 min. The serum was aspirated and the DU-PAN II antigen was assayed with the enzyme immunoassay kit: 100 µl of phosphate buffer and 20 µl of the test samples or control materials were added to the well of the DU-PAN II-coated plate, followed by incubation for 2 hr at 37°C. The plate was washed three times with 200 µl of saline. Then 100 µl of DU-PAN II-labeled HRP was added, and the mixture was incubated at 37°C for 2 hr. After washing the plate three times with 200 µl of saline, 100 µl of staining solution was added and incubated at 37°C for 30 min. Then 50 µl of the solution was added to stop the enzyme reaction, and the absorbance was measured at 660 nm.

The tumor was excised from the mouse, cleaned of all extraneous tissues and immediately frozen. At the time of assay, the tumor was thawed and the DU-PAN II antigen was extracted in the following manner: pieces of tumor of various sizes were sliced off with a scalpel and transferred to a grinding tube, together with saline. Following the grinding, the tube was centrifuged (10,000 x g) at 4°C for 15 min, and the supernatant was filtered through a 0.22 µm Millipore filter. This was assayed for DU-PAN II antigen in the same way as the sera.

Gel chromatography of labeled compounds, sera, tumor or liver homogenates. 125I-labeled monoclonal antibody before administration to the mouse, or that incubated with an equal volume of either DU-PAN II positive or negative serum at 37°C for 24 hr was applied to a Sepharose 6B column (1.5 x 75 cm, Pharmacia Fine Chemicals), and eluted with PBS. Radioactivity in a 2.5 ml fraction was counted by a gamma-counter. A plasma sample, or tumor or liver homogenates taken from the tumor-bearing mouse 1 day after injection was also passed through a Sepharose 6B gel and eluted in the same manner. The tumor or liver homogenate was prepared in the way described in the section on quantification.

RESULTS

Biodistribution of 125I-labeled DU-PAN II and non-immunized IgM. 125I-labeled DU-PAN II or non-immunized IgM was administered to PAN-5-JCK, EXP-58 or non-tumor-bearing nude mice, and 5 days later the tissue-to-blood ratios were compared (Fig. 1). There was a preferential localization of
Fig. 1 Biodistribution of $^{125}$I-DU-PAN II and non-immunized IgM in human pancreatic tumor, PAN-5-JCK or EXP-58, or in non-tumor-bearing mice 5 days after administration.

- PAN-5-JCK-bearing mice injected with $^{125}$I-DU-PAN II
- PAN-5-JCK-bearing mice injected with $^{125}$I-nonimmunized IgM
- EXP-58-bearing mice injected with $^{125}$I-DU-PAN II
- non-tumor-bearing mice injected with $^{125}$I-DU-PAN II

3–4 mice were used in each group.

$^{125}$I-DU-PAN II in PAN-5-JCK xenografts, with a tumor-to-blood ratio of 2.72±3.00. This accumulation was clearly not due to an abnormal blood level in the tumor, since injected $^{125}$I-nonimmunized IgM did not appear significantly in tumor tissues (T/B = 0.75). Confirmation of the immunological nature of the uptake of DU-PAN II in PAN-5-JCK xenografts came from the results with EXP-58, which is lacking in DU-PAN II antigen. High tissue-to-blood ratios were found in some non-target organs, such as kidney, liver and spleen. High radioactivity observed in the kidneys of all groups examined indicated a dissociation of iodine from labeled antibody in vivo.

It should be noticed also that high tissue-to-blood ratios were found in the liver and spleen of the PAN-5-JCK-bearing mice injected with DU-PAN II. Immunoscintigraphy. $^{125}$I-DU-PAN II was administered intravenously to PAN-5-JCK-bearing nude mice. Figure 2 shows the immunoscintigrams 1, 3 and 5 days after injection. Strong activity was seen in the implanted tumors in the right flank at day 3. Excellent images of PAN-5-JCK were obtained when the tumor was small, about 150 mg in weight. High background activity was observed when the tumor was large. Specifically, in order to monitor the deiodination, we did not supress free iodine uptake of the thyroid in nude mice.

Effect of tumor size on the DU-PAN II antigen in serum and tumor, and on the uptake of $^{125}$I-DU-PAN II in tumor and liver. Figure 3 shows the correlation of the serum or tumor homogenate level of DU-PAN II antigen with the tumor burden of the mouse.

![Immunoscintigram of PAN-5-JCK-bearing mice injected with $^{125}$I-DU-PAN II. The tumor weights were 1,800 mg (2a) and 150 mg (2b).](image)

The serum DU-PAN II antigen rose continuously in 40 mg and 1,200 mg tumors, while the DU-PAN II antigen content per g of tumor was independent of the tumor size within the two ranges of 40–700 mg and 1,200–1,300 mg. When the tumor was 40 mg in weight, DU-PAN II antigen in the tumor was 8,200 U/g, although antigen was not detected in the serum. Necrosis was visible to the naked eye in
tumors more than 1,200 mg in weight. Studies were also performed to determine the effect of tumor size on the incorporation of labeled DU-PAN II into the tumors. The results of uptake of DU-PAN II in tumors and livers are included in Fig. 3. Increasing tumor weight tended to decrease tumor uptake, although few mice were studied. It is interesting that the per-gram uptake of labeled antibody, expressed by %ID/g, decreased as tumors increased in size, even though the antigen level in the tumors remained almost constant in the limited region described above. Liver-to-blood ratios were independent of tumor size.

**Gel chromatography.** Figure 4a shows the labeled compound profile in which only one peak appears corresponding to IgM. Incubation of equal volumes of DU-PAN II and serum from a tumor-bearing mouse, i.e., antigen-positive serum, and subsequent passage through a Sepharose 6B gel column shows two small peaks before and after the IgM peak, in addition to IgM and free 125I ion peaks (Fig. 4b). The radioactivity eluted in advance of the IgM peak suggests the presence of an immunocomplex formation, since this peak did not appear in the chromatogram of the mixture of the antigen-negative serum and labeled antibody. Another small peak behind the IgM peak, reflecting the label with a molecular weight form lower than radiolabeled IgM, was not definitely found in the profile of antigen-negative serum incubated with antibody in vitro. Figure 4c shows the elution of plasma samples taken from the tumor-bearing mice injected with DU-PAN II. The radioactivity corresponding to IgM was completely shifted to a lower molecular weight region located at the same level as the peak behind IgM shown in 4b. The elution of the plasma from a normal mouse injected with DU-PAN II, coincided with the IgM peak, indicating that the antibody remained as the polymer. Figure 4d shows the elution pattern of tumor or liver homogenates obtained.
DISCUSSION

Over the years, there has been much interest focused on the use of radiolabeled monoclonal antibodies for tumor imaging. Most of the work, however, has been done using the IgG subclass. In a few papers dealing with IgM, its use for immunoscintigraphy has been questioned because of the instability of labeled IgM in vivo. Labeling techniques also affected the stability of antibodies. In our experiments, DU-PAN II labeled with $^{125}$I by the chloramine T technique failed to accumulate in the tumor, although it retained at least 40% of its immunoreactivity (data not shown). However, as shown in Figs. 1 and 2, DU-PAN II labeled with $^{125}$I by the lactoperoxidase method became localized specifically in the pancreatic tumor xenograft, producing DU-PAN II antigen.

Immunoscintigrams of tumor-bearing mice injected with $^{125}$I-DU-PAN II reveal a strong accumulation of iodine in the thyroid at an early stage. The labeled antibody injected contained less than 2% iodine, suggesting that this deiodination occurred in vivo. The dissociation of iodine from labeled antibody was also implied by the fact that relatively high radioactivity was found in the kidneys of non-tumor-bearing mice as well as in tumor-bearing ones.

The stability of $^{125}$I-DU-PAN II in vivo can also be shown by gel chromatography of the plasma. In no plasma samples did a radioactivity peak appear behind the IgM peak in the absence of DU-PAN II antigen, suggesting that DU-PAN II antigen accelerated the catabolism of the labeled antibody. It was not clear, however, whether the degradation of IgM involved an immunocomplex formation or not. The protein derived from $^{125}$I-DU-PAN II was found in the tumor homogenates, although it was smaller in molecular weight than that in the plasma. The catabolism of IgM in the serum may be different from that in tumors. Antibody degraded in the serum may not be accumulated in tumors, meaning that antigen in the serum caused the decrease in tumor uptake of antibody.

The stability of $^{125}$I-DU-PAN II in the presence of antigen can be considered with regard to the effect of tumor size on the tumor uptake of antibody. As shown in Fig. 3, the smaller the PAN-5-JCK, the more it accumulated $^{125}$I-DU-PAN II. On the other hand, we reported previously that human gastric cancer H-111 growth was accompanied by a linear uptake of $^{125}$I-labeled monoclonal antibody, NCC-
ST-433, in a murine-human tumor system. It is possible that the difference between NCC-ST-433 and DU-PAN II was partly a matter of tumor necrosis. Less necrosis was observed in H-111 tumors, while PAN-5-JCK tended to become necrotic in large tumors. So there was no proportional correlation of tumor DU-PAN II antigen and tumor mass. This pattern is similar to colon cancer T-380 which produces CEA, as demonstrated by Martin. However, the irreversible correlation between tumor uptake of $^{131}$I-DU-PAN II and tumor size in the range between 40–700 mg cannot be explained by the tumor antigen-content, which remained relatively constant if expressed on a per-gram basis. Another reason, therefore, for the discrepancy between DU-PAN II and NCC-ST-433 lies in the fact that the DU-PAN II antigen is a circulating antigen. The antigen ST-433 in the model we used previously was not detected in the serum. The high circulating DU-PAN II antigen may result in a decrease in the absolute uptake of labeled antibody by the tumor, partly due to the degradation of antibody injected. It is not clear at present whether the degradation involved the nonspecific localization of radioactivity in the liver independent of the tumor size. Various factors may be involved in the high background radioactivity.

As displayed in the immunoscintigram, a small tumor, such as one 150 mg in weight, was clearly visible, probably because the circulating antigen was in a low enough concentration for the $^{131}$I-DU-PAN II to reach the tumor site before its degradation in the serum. It means that immunoscintigraphy is helpful in the diagnosis of small resectable tumors. In human studies it is also possible that smaller lesions may take up great amounts of radiolabeled antibody, making them more vulnerable to radioimmunotherapy. Our experimental results, however, suggested that immunoscintigraphy with labeled DU-PAN II may not work optimally when antigen circulates, especially if it does so in a high concentration. The rapid clearance of IgM is an advantage for the immunoscintigraphy as described by Mano or Scheinberg. The catabolism of the antibody, however, should not occur before it reaches the tumor sites. If the radiolabel is lost from the antibody it will not be detected, and if it is degraded in the serum it will not be fixed in the target-tissues. Finally, we need further studies to resolve the problems of $^{131}$I-DU-PAN II radioisotopes and its labeling techniques.

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