Localization of the Monoclonal Antibody HMFG2 after Intravenous and Intraperitoneal Injection into Nude Mice Bearing Subcutaneous and Intraperitoneal Human Ovarian Cancer Xenografts

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ABSTRACT

Xenografts (s.c. and i.p.) of human ovarian cancer, shown to express the tumor associated antigen defined by the monoclonal antibody HMFG2, were used to investigate in vivo localization of the radiiodinated antibody after i.p. and i.v. injection. Following i.v. injection, maximum uptake (31.4 ± 3.5%/g s.c. tumor) was seen in s.c. tumors at 48 h after injection. Tumor:normal tissue ratios increased with time to 240 h. Uptake by ascites and i.p. tumors was less, with a maximum of 10.0 ± 8%/g ascites reached at 16 h and 11.4 ± 3.2%/g i.p. tumor at 96 h.

After i.p. injection uptake in s.c. xenografts was maximal (14.8 ± 2.0%/g) at 20 h. For ascites, the i.p. route of administration resulted in high uptake (27.7 ± 5.8%/g) early (2 h) with an ascites:normal tissue ratio of 69.3 and a specific antibody:nonspecific antibody ratio of 30.8. Except for data at one time point, uptake by i.p. solid tumor was similar to that seen for s.c. tumor.

These data strongly suggest that large concentration advantages can be achieved in ascites cells by regional i.p. injection, but that i.p. solid tumor may rely on i.v. delivery of antibody before uptake.

INTRODUCTION

Mouse monoclonal antibodies directed against tumor associated antigens have now established a role in the diagnosis and management of ovarian cancer. Thus, MoAbs1 have been shown to detect target cancer cells in vitro (1, 2) and in vivo (3, 4). This in vivo localization of labeled monoclonal antibodies has allowed the technique of immunoscintigraphy to be developed and ovarian cancers can be reliably imaged by this means (5, 6).

The success of immunoscintigraphy has led to the consideration of the use of MoAbs as carriers for toxic agents as therapy for this disease. Substances considered have been radionuclides (7), plant toxins (8), light unstable porphyrins (9), or conventional chemotherapeutic agents (10). Early studies of the use of 125I as the toxic agent have been reported (7, 11–13). To date, however, these studies have involved few patients and have been uncontrolled. Studies with patients are difficult to undertake as the numbers needed to investigate different parameters (such as antibodies, routes of administration, and disease patterns) are too great for most oncology centers. To overcome this difficulty, nude mouse xenograft models of human ovarian cancer were developed (14), whereby s.c. tumors, and i.p. solid tumors or ascites were available for these experiments.

MATERIALS AND METHODS

Nude Mouse Xenograft i.p. Models of Ovarian Cancer

Young (6–12 week old) female, random bred nu/nu nude mice from a colony of mixed genetic background (bred at the I.C.R.F. Animal Breeding Unit, Clare Hall, UK), were used to establish i.p. xenograft models of ovarian cancer as previously described (14). Briefly, fresh ovarian cancer tissue (ascites or minced solid tumor) was injected i.p. into recipient animals. Solid tumors i.p. and/or ascites developed in 4 weeks to 9 months and, to date, i.p. xenografts have been established from eight patients of which six were used in this study (Table 1). All studies were conducted at early passage numbers (<5), the technique for passaging of the i.p. tumors has been previously described (14). Tumors were used for experiments when palpable through the abdominal wall or visible on inspection (5–600 mg for solid tumor, <2 ml for ascites).

Xenografts s.c. in Nude Mice

Xenografts s.c. in identical mice were established by the injection into each flank of 0.3 ml of ascites cells from mice bearing the O.S. ascites i.p. xenograft. The resulting tumors were large enough for use in localization experiments (10–60 mg in weight) within 6 weeks.

Monoclonal Antibodies

HMFG2. The MoAb HMFG2 (15) defines a tumor associated antigen which is expressed in >90% ovarian carcinomas (16). This antigen is a high molecular weight (Mw, 80,000–200,000) glycoprotein (17) expressed mainly on the tumor cell luminal surface (16). It is not expressed by normal mouse tissues.

U1J3A. The MoAb U1J3A (18) defines an antigen found exclusively on neuroectodermal tissue and is strongly expressed in certain central nervous system tissues. It is unreactive with normal mouse tissues.

H17E2. The MoAb H17E2 is directed against an epitope on the enzyme placental alkaline phosphatase (19). It is also unreactive with normal mouse tissues.

HMFG2, U1J3A, and H17E2 are all of the IgG1 immunoglobulin subclass.

Assessment of Antigen Expression

Solid Tumor. Expression of these antigens was assessed by the immunoperoxidase technique on both the xenografts and primary tumors from which they were derived, as previously described (16).

The HMFG2 antigen was present on tumor cells of all solid tumors used in this study, and HMFG2 was used in all experiments. Placental alkaline phosphatase was expressed by the D. O. xenograft but not by the others, U1J3A was used as negative control antibody for all solid tumor experiments.

Ascites Cells. Assessment of these antigens on washed ascites cells was by immunofluorescence. Cells were washed twice in PBS and incubated for 40 min in test antibody hybridoma supernatant (approximately 10 g antibody/ml). After further washing, cells were incubated for 1 h with a rabbit antimouse-fluorescein isothiocyanate conjugate (Cappell, USA) at 4°C, washed further, and fluorescence intensity assessed by fluorescence-activated cell sorting (Becton-Dickinson modified FACS I, interfaced with a Tektronics 4052 graphics computer). The O.S. ascites cells were shown to strongly express the HMFG2 antigen but not the U1J3A or H17E2 antigens.

Iodination of Antibodies

HMFG2 and U1J3A or H17E2 were conjugated to either 125I or 131I by the iodosogen method (20). Iodination efficiencies of 50–90% were achieved and iodination of these antibodies was shown by an enzyme-linked immunosorbent assay not to effect their specific binding, and
Table 1 Human ovarian cancer xenografts

<table>
<thead>
<tr>
<th>Designation</th>
<th>Original histology</th>
<th>Xenograft formed</th>
<th>Passage no. used</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. S.</td>
<td>Mod. diff. serous cystadenocarcinoma</td>
<td>Solid tumor s.c. i.p. ascites</td>
<td>1-5</td>
</tr>
<tr>
<td>H. U.</td>
<td>Mod. diff. mucinous cystadenocarcinoma</td>
<td>Solid tumor i.p.</td>
<td>1, 2</td>
</tr>
<tr>
<td>D. O.</td>
<td>Well diff. serous cystadenocarcinoma</td>
<td>Solid tumor i.p.</td>
<td>1-4</td>
</tr>
<tr>
<td>P. I.</td>
<td>Mod. diff. serous cystadenocarcinoma</td>
<td>Solid tumor i.p.</td>
<td>1</td>
</tr>
<tr>
<td>C. L.</td>
<td>Well diff. serous cystadenocarcinoma</td>
<td>Solid tumor i.p.</td>
<td>1</td>
</tr>
<tr>
<td>S. N.</td>
<td>Mod. diff. serous cystadenocarcinoma</td>
<td>Solid tumor i.p.</td>
<td>1, 2</td>
</tr>
</tbody>
</table>

* Laboratory identification code.
* Mod. diff., moderately differentiated; well diff., well differentiated.

Direct radiobinding assay demonstrated that over 80% of antibody was immunoreactive. Antibody-radioiodine conjugates were checked for immunoreactivity by these means after each iodination.

Double Label Counting

The technique of Pressman (21) was used to separately calculate the contribution of each isotope to the activity contained within a given sample.

Experimental Design

Groups of normal mice or mice bearing s.c. or i.p. xenograft tumors were injected i.v. or i.p. with [125I]HMFG2 and [131I]UJ13A or H17E2 conjugates (10 μg antibody/10 μCi isotope). Alternatively, mice were injected simultaneously with [125I]HMFG2 i.p. and [131I]HMFG2 i.v.; for i.p. injection the antibody was diluted to a volume of 3 ml in PBS, and for i.v. injection the volume was 0.3 ml. At various time points to 240 h, mice were killed by cervical dislocation, dissected, and normal tissue (heart, lungs, liver, spleen, muscle, and skin), blood, and tumor removed. Samples were weighed and activity counted in a Beckman 4000 γ counter (Beckman UK). For ascites cells, the peritoneal cavity was lavaged with 20 ml PBS and the ascites cells separated by centrifugation (800 x g, 5 min). The ascites cell pellet was weighed and counted separately from the ascites fluid. Results were expressed as percentage injected activity per gram (milliliters) of tissue (blood).

Estimation of Free and Protein Bound Iodine in Biological Fluids

To determine that the data obtained from these studies referred to antibody-radioiodine conjugates and not to free iodine, the proportion of each was estimated by TCA precipitation. To a 1-ml sample of the fluid to be tested (e.g., serum, peritoneal fluid, and ascites) was added 1 ml of 10% TCA (Sigma; Poole, UK) in PBS. After standing for 15 min, centrifugation (8000 x g, 5 min) was performed and the activity in the supernatant and protein precipitate counted separately.

RESULTS

Antibody Localization in Normal Mice. In the absence of tumor, both HMFG2 and UJ13A were distributed and metabolized similarly in the nude mice after both i.v. and i.p. injection. After i.v. injection antibody concentration in blood fell to 27.4 ± 1.6% injected activity/ml by 20 h. Assuming a blood volume of 6.2 ± 0.5%/g at 20 h postinjection to 2.9 ± 0.3%/g at 240 h with a t½ value of 187 h. Thus, t½ in normal tissue closely reflected t½ in blood (Fig. 1A).

After i.p. injection in normal mice, antibody levels rose in serum to peak at 17.6 ± 1.1%/ml at 72 h and by 216 h had decreased to 4.6 ± 0.6%/ml with a clearance half-life of approximately 80 h. In the normal tissues, levels reflected serum levels: a peak of 4.6 ± 0.3%/g being reached at 72 h, this level decreasing to 1.0 ± 0.1%/g at 216 h. The tn value for removal of antibody from normal tissues was approximately 70 h. Thus, clearance from normal tissue reflected the clearance of antibody from the blood (Fig. 1B).

Antibody Localization in Mice Bearing O. S. s.c. Xenografts.

After i.v. injection, tumor levels of radioiodinated HMFG2 in the O. S. s.c. tumors reached a maximum of 31.4 ± 3.5%/g at 48 h. From 48 h tumor levels were consistently higher than blood levels. In contrast, levels of HMFG2 in normal tissue and levels of UJ13A in tumor tissue were invariably lower than the blood levels. The tumor:non-tumor ratio for HMFG2 rose from 5.3 at 20 h to 10.6 at 240 h. Complete data are presented in Table 2.

In contrast, when the i.p. route of administration was used, maximum tumor levels achieved were considerably lower (maximum 14.8 ± 2.0%/g at 20 h). This may be a reflection of the generally lower blood levels achieved (maximum 14.2%/g at 20 h). Tumor:non-tumor ratios were in the 3.2-4.1 range, however tumor:blood ratios were maintained about 1 for the course of the experiment. Specific:non-specific uptake ratios were also

![Fig. 1 A. serum and normal tissue levels of 125I-conjugated monoclonal antibody after i.v. injection in nontumor-bearing nude mice. Results, mean ± SD of four (serum) or 32 (normal tissue) samples from four mice at each point. B. serum and normal tissue levels of 131I-conjugated monoclonal antibody after i.p. injection in nontumor-bearing nude mice. Results, mean ± SD of four (serum) or 32 (normal tissue) samples from four mice at each point.](http://example.com/fig1.png)
lower than those achieved by the i.v. route. These data are presented in Table 3.

Antibody localization in mice bearing O. S. ascites xenografts. After i.v. injection to O. S. ascites bearing mice, levels of radioiodinated HMFG2 rose to a maximum of 10.0 ± 0.8%/g ascites cells at 16 h. Levels then decreased with time, such that by 48 h ascites cells at 16 h. Levels then decreased with time, such that by 48 h. Nonspecific antibody uptake by both tumor and normal tissue was low [tumor < 1.3%/g, normal tissue < 3.4%/g (Table 5)].

Antibody Localization in Mice Bearing i.p. Xenografts. After i.p. injection of radioiodinated HMFG2 antibody to mice bearing HMFG2 positive i.p. xenografts, circulating levels of antibody fell rapidly to approximately 14%/ml blood by 2 h. This level was then maintained up to 96 h after injection. Levels of antibody in normal tissue reflected this plateau concentration at approximately 3%/g tissue. Uptake into the i.p. tumors was rapid with 4.6% of activity localized/g tissue by 2 h. Maximum antibody uptake (11.4 ± 3.2%/g) was seen at 96 h after injection (Table 6).

After i.p. injection, blood levels rose to a maximum of ap-
proximately 16%/ml at 24 h and then declined with a half-life of 75 h. Tumor localization of HMFG2 conjugate was also maximal at this time and remained at a similar level for the course of the experiment.

At time points of 2, 24, 48, 96, and 144 h after i.p. injection, specific (HMFG2) antibody uptake was compared with nonspecific (UJ13A) antibody uptake. Specific:nonpecific ratios ranged from 1.0 to 1.8 in blood, 0.8 to 1.3 in normal tissue, and 1.1 to 2.7 in tumor (Table 7).

It can be seen from these data that no consistent advantage was gained for tumor localization when the antibody was given i.p. over i.v.

Levels of Free Iodine. Throughout these experiments, levels of free iodine in blood, ascites fluid, and residual i.p. fluid were <15% of total iodine, generally 5–10%.

DISCUSSION

The aims of these xenograft studies were to establish principles of antigen-antibody binding in vivo and to investigate the uptake of regionally injected antibody preparatory to uptake and therapy studies in patients. Iodine was used as the tracer isotope for two reasons. Firstly, 131I had been selected as the isotope for the final objective of these studies, i.e., antibody conjugated therapy, and therefore it was logical to perform the modeling experiments with this radioisotope.

Secondly, different isotopes of iodine were available, allowing for double label counting. For such experiments the same isotope for the final objective of these studies, i.e., antibody conjugated therapy, and therefore it was logical to perform the modeling experiments with this radioisotope.

In conjugated antibodies are localized differently from iodine conjugates. In normal mice, a basis for comparison with studies using s.c. xenografts. Using the i.v. route of administration, previous studies have reported maximal tumor levels of 5.4–40%/g tumor at times of 24–40 h (25–29). These results are in accordance with those reported in this study. Herlyn et al., using the i.p. route of injection with mice bearing s.c. colon cancer xenografts, reported maximal antibody uptake of 9%/g tumor at 48 h and blood levels of 10%, 6%, and 3%/ml at 48, 120, and 192 h, respectively. These results agree well with those reported here. When the O. S. ascites model was studied, the benefit of the regional i.p. route of administration of antibody conjugate was apparent. Specific:nonspecific antibody uptake ratios, tumor cell:normal tissue ratios, and the absolute antibody uptake were all maximal within the first 24 h, decreasing rapidly over 7 days. In contrast, these parameters rose more slowly when the antibody conjugate was given i.v., neither absolute levels nor specificity ratios achieving values approaching the i.p. route. Comparing the normal tissue levels of antibody conjugate uptake between both routes of administration in these and normal mice, levels were lowest in i.p. injected ascites-bearing mice. This suggested that entrapment of antibody within the peritoneal cavity had reduced the amount of antibody available to localize nonspecifically and is reinforced by the observation of greater levels of UJ13A (nonspecific) uptake by these tissues.

Examination of serum radioactivity of i.v. injected antibody, showed it to be considerably shorter than in nontumor-bearing mice. This would suggest that an active movement of antibody from the vascular compartment to the i.p. cavity was occurring. On the other hand, after i.p. injection, antibody, localized to the tumor cells at 2 h, became dissociated from these cells over a short period (t1/2, approximately 33 h). With the absorption of antibody from the i.p. into the vascular compartment, the favorable i.p.:i.v. concentration kinetics achieved with the i.p. injection would decrease. The i.p. concentration of unbound antibody conjugate would decline, promoting dissociation of bound antibody conjugate from its antigen.

For the study of antibody localization in i.p. tumors, xeno-

| Table 6 HMFG2 localization in i.p. tumor-bearing mice after i.v. injection | All points are mean ± SD of samples from three to six mice. |
|---|---|---|
| Time (h) | Normal tissue | Blood | Tumor tissue |
| 2 | 3.5 ± 0.8 | 13.9 ± 2.7 | 4.6 ± 2.2 |
| 24 | 3.5 ± 0.7 | 13.0 ± 4.6 | 5.7 ± 1.0 |
| 48 | 3.5 ± 0.5 | 11.4 ± 1.3 | 7.0 ± 1.2 |
| 96 | 3.0 ± 0.4 | 13.5 ± 3.6 | 11.4 ± 3.2 |
| 144 | 1.4 ± 0.4 | 3.9 ± 0.9 | 7.1 ± 2.6 |

| Table 7 Specific (HMFG2) and nonspecific (UJ13A) localization in i.p. tumor-bearing nude mice after i.p. injection | Points are mean ± SD of samples from three to six mice. |
|---|---|---|---|---|
| Time (h) | HMFG2 | UJ13A | HMFG2 | UJ13A | HMFG2 | UJ13A | HMFG2 | UJ13A |
| 2 | 1.1 ± 0.3 | 1.3 ± 0.2 | 7.1 ± 2.4 | 6.0 | 2.6 ± 0.4 | 2.4 ± 0.3 | 1.1 | 1.6 |
| 24 | 3.8 ± 0.8 | 3.5 ± 0.7 | 15.7 ± 2.5 | 13.0 ± 4.6 | 8.1 ± 2.7 | 3.0 ± 0.3 | 2.7 | 2.1 |
| 48 | 3.4 ± 0.8 | 3.7 | 10.8 ± 2.4 | 8.6 | 5.9 ± 0.8 | 3.5 | 1.7 | 1.7 |
| 96 | 2.2 ± 0.6 | 1.8 | 5.2 ± 1.1 | 4.6 | 5.4 ± 2.0 | 4.2 | 1.3 | 2.5 |
| 144 | 2.1 ± 0.7 | 1.7 | 5.9 ± 1.9 | 4.6 | 7.8 ± 5.4 | 4.4 | 1.8 | 3.7 |

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clinical studies. When i.p. injected, radiolabeled HMFG2 was basically an enclosed cell suspension, was reflected in rapid, ical studies are underway, the mouse models have allowed measured in normal tissue, tumors, and ascites removed at after i.v. or i.p. injection. With the exception of localization in the ascites cells, i.p. injected antibody appeared to act after uptake by i.p. tumors was similar to that seen by s.c. tumors. This suggested that the i.p. instilled antibody was being absorbed into the venous system and acting primarily as circulat This suggested that the i.p. instilled antibody was being ab sorbed into the venous system and acting primarily as circulat ingtion. In summary, specific localization of HMFG2 antibody was demonstrated in s.c. tumor, i.p., and ascites xenograft models after i.v. or i.p. injection. With the exception of localization in the ascites cells, i.p. injected antibody appeared to act after absorption into the vascular compartment. In the case of O. S. ascites, the free availability of HMFG2 antigen in what is basically an enclosed cell suspension, was reflected in rapid, specific and avid binding to the HMFG2 antibody. These data have been reflected in the results of early, limited clinical studies. When i.p. injected, radiolabeled HMFG2 was measured in normal tissue, tumors, and ascites removed at laparotomy from patients with ovarian cancer, localization sufficient for safe antibody guided isotope radiotherapy was demonstrated only in the ascites cells (31). While further clinical studies are underway, the mouse models have allowed investigations which may save lengthy clinical trials.

REFERENCES


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