Localization of radiolabeled monoclonal antibodies in thyroid tumor xenografts

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Monoclonal antibodies to human thyroglobulin were produced using the hybridoma technique. Two monoclonal antibodies D51 and F91 were radiolabeled with $^{125}$I and used for radioimmunolocalization studies in an immunosuppressed animal model bearing xenografts of human thyroid tumor tissue. Biodistribution studies were carried out at various time intervals post-injection. Maximum tumor uptake was obtained at 72 hr after administration of the antibodies. The absolute tumor uptake (ATU) expressed as percentage of injected dose per gram of tissue (%ID/g) was 15.49 ± 2.47, 4.51 ± 0.69 and 2.50 ± 0.41 for D51, F91 and control Igs respectively. The tumor to blood ratios (T/B) obtained were 3.01 ± 0.43 for D51, 0.98±0.2 for F91 and 0.47 ± 0.12 for control Igs. ATU as well as T/B ratio obtained with D51 was significantly higher as compared to F91 and control Igs. The results indicated the potential application of radiolabeled monoclonal antibodies to human thyroglobulin for tumor targeting in patients of differentiated thyroid carcinoma, particularly those metastases which did not concentrate radioiodine.

Keywords: Monoclonal antibodies, Thyroglobulin, Thyroid tumor xenograft

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Conventional treatment of differentiated thyroid carcinoma (DTC) is thyroidectomy followed by ablation of the residual thyroid tissue by $^{131}$I. Follow-up of these patients involves imaging with diagnostic $^{131}$I scan to localize metastases and the measurement of serum thyroglobulin (Tg) after withdrawal of thyroid hormones. Further, patients with metastatic disease are treated by administration of therapeutic doses of $^{131}$I. Significant number of these patients become refractory to $^{131}$I therapy as the metastatic tissue fails to concentrate $^{131}$I. However, the cells continue to produce Tg which is an established tumor marker for DTC. Since most of the tumors concentrate $^{131}$I, this is the primary mode of treatment, but problem arises mainly when the metastases do not concentrate $^{131}$I. Treatment modalities are then limited to either external radiation or chemotherapy which do not seem to be very effective in controlling the disease.

Monoclonal antibodies (MAbs) produced by hybridoma technology are being labeled with various isotopes and used for radioimmunoscintigraphy (RIS) and radioimmunotherapy (RIT) in wide variety of human carcinomas. Use of radiolabeled MAbs to carcino-embryonic antigen in the management of medullary thyroid carcinoma has been reported. Few reports are available for targeting DTC with radiolabeled antibodies. A specific approach for the diagnosis and therapy of non-iodine concentrating metastases could be the use of radiolabeled MAbs against specific thyroid antigens for tumor targeting. The aim of this study was to evaluate the usefulness of MAbs to human Tg (H-Tg) produced in our laboratory for in vivo localization of thyroid tumor xenografts.

Materials and Methods

Production of monoclonal antibodies—Human Tg was purified from a single specimen of normal human thyroid by the method of Mouriz and Stanbury. Monoclonal antibodies to H-Tg were produced by hybridoma technique as described earlier. Briefly, Sp2/0 mouse myeloma cells were fused with spleen cells of Balb/c mice immunized with H-Tg. The clones obtained were screened for anti-H-Tg antibodies by an enzyme linked immunosorbant assay (ELISA). Two monoclonal antibodies D51 and F91 having high ELISA positivity were selected and single, stable clones were established by limiting dilution method and propagated in tissue culture flasks or grown as ascites in pristane primed mice. Immunoglobulins (Igs) from culture supernatants and ascitic fluid from mice were precipitated with 50% (NH$_4$)$_2$SO$_4$, pH 7.2 and dialyzed against 0.02M of phosphate buffered saline (PBS; pH 7.2). Affinity purification of MAbs was car-
ried out using MAPS II kits from Bio-Rad, USA as per the manufacturers protocol.

**Radiolabeling of MAb with**$^{125}$I**—** Affinity purified MAbs were labeled with $^{125}$I using iodogen method$^{24}$. A glass vial was coated with 200 μg of iodogen (Pierce chemicals, UK) dissolved in 200 μl of chloroform and air dried to form a thin film. Monoclonal antibody (10 μg) and 1 mCi of Na$^{125}$I (Amersham, UK) was added to the vial and the volume was made to 200 μl with 0.2M phosphate buffer (pH 7.2). The reaction was carried out for 20 min at room temperature with intermittent shaking. The $^{125}$I labeled MAb was separated from the free iodine by passing through a Sephadex G-75 column (Pharmacia, Uppsala, Sweden). The immunoreactivity of the $^{125}$I labeled antibodies was checked on H-Tg coated on solid phase. Normal mouse Igs purified and labeled using the same procedure as that used for MAb served as control Igs for biodistribution studies.

**Evaluation of immunoreactivity of $^{125}$I labeled antibodies**—Ninety-six wells polyvinyl plate was coated with 100 μl of H-Tg (10 μg/ml) by incubating overnight at 37°C. The plate was then washed with 0.02 M PBS 5 times, and uncoated sites were blocked with PBS containing 1% bovine serum albumin (BSA) for 1 hr at 37°C. After draining the BSA $^{125}$I labeled MAb was separated from H-Tg coated on solid phase. Normal mouse Igs purified and labeled using the same procedure as that used for MAbs served as control Igs for biodistribution studies.

**Establishing an experimental animal model**—Four to eight weeks old female Swiss-white mice were anesthetized and the thyroids was surgically removed. Two weeks after thymectomy, the mice were subjected to first dose of whole body irradiation of 2 Gy using a $^{60}$Co source. A total cumulative dose of 10 Gy comprising 5 doses of 2 Gy each were given at 2 weeks intervals$^{25}$. Total WBC as well as the lymphocyte counts were estimated, which were 60% lower than those of normal mouse. This confirmed the immuno-suppressed state of the mice. These mice were termed as T200×5R mice. Two weeks after the final dose the mice were used for xenografting.

Thyroid tumor tissue was collected from patients undergoing thyroidectomy for treatment of DTC. It was cleansed of the attached connective tissue, blood clots and then cut into small pieces of 100 mg each. The mice were anaesthetized and the tissue was grafted sub-cutaneously (sc) in the right flank of the mice. Two weeks after xenografting the mice were used for localization studies by administration of $^{125}$I labeled antibodies.

**Biodistribution studies using $^{125}$I labeled antibodies**—The T200×5R mice were given Lugol’s iodine (0.1% solution) in drinking water two days prior to the injection of $^{125}$I labeled antibodies and throughout the period of experiment to saturate the thyroid tissue and block the uptake of free $^{125}$I.

Radiolabeled monoclonal antibodies D5I and F91 specific for H-Tg were used for biodistribution studies. Normal mouse immunoglobulins were used as non-specific control antibody. Each of the above antibody preparation (0.1 ml) was injected (iv) using a 26 gauge needle in the tail vein of T200×5R mice bearing thyroid tumor xenograft (TTX). The amount of radioactivity injected per mouse was 10-20 μCi corresponding to 0.3-0.5 μg of each antibody. The biodistribution studies were performed at 1, 3, 5 and 7 days respectively after administration of the antibodies.

The animals were subjected to ether anesthesia and blood was collected by cardiac puncture, weighed and measured radioactivity content in a gamma counter (ECIL, Bangalore, India) calibrated for $^{125}$I energy against a reference sample for accurate measurement of the activity in each tissue. The mice were sacrificed, dissected open, the individual organs as well as the grafted tumor was removed, weighed and radioactivity in the organ/tumor was counted. The radioactive uptake in each organ was expressed as percentage of the injected dose per gram (% ID/g) of tissue. In case of the tumor graft it was termed as an absolute tumor uptake (ATU) and expressed as % ID/g of tumor tissue. The mean value and standard deviations were calculated for each group of mice.

**Statistical analysis**—ATU, the tumor to blood ratio (T/B) and the tumor to muscle ratio (T/M) of the three antibodies was compared using the Student’s $t$ test.

**Results**

All the three antibody preparations could be successfully labeled with $^{125}$I using the iodogen method. The labeling efficiency of N-Igs, MAb D5I and MAb
F9I was 73.81 ± 8.92, 66.38 ± 19.87 and 62.60 ± 19.62% respectively. The specific activity expressed as mCi/mg was 10.54 ± 2.18, 34.94 ± 12.70 and 27.66 ± 13.86 for N-Igs, MAb D5I and MAb F9I respectively. Both the monoclonal antibodies retained their immunoreactivity to H-Tg, post-labeling. The per cent binding was 33.24 ± 5.14 for MAb D5I and 21.45 ± 4.31 for MAb F9I respectively.

The blood clearance of the three radiolabeled antibody preparations has been shown in Fig. 1. N-Igs remained in circulation for a longer time as compared to MAb D5I and MAb F9I. At 7 days post-injection the blood pool activity of N-Igs was 3.78 ± 0.18 % ID/g, which was higher than MAb D5I (2.82 ± 1.48 % ID/g) and MAb F9I (0.49 ± 0.18 % ID/g). Amongst the two MAbs used, MAb F9I showed a faster clearance as compared to MAb D5I. T1/2 values for N-Igs was 8 days, for MAb D5I was 4.8 days and that for MAb F9I was 1.3 days. The rate of clearance of the three 125I labeled antibody preparations was in the following order MAb F9I > MAb D5I > N-Igs.

Biodistribution of N-Igs in the animal model has been depicted in Table 1. The uptake in the tumor xenografts was 2.50 ± 0.41 % ID/g and 1.19 ± 0.12 % ID/g at 3 and 7 days post-injection. The tumor to blood ratios were < 0.5 at all the time intervals studied.

Biodistribution of MAb D5I has been shown in Table 2. Maximum ATU of MAb D5I in TIX (15.49 ± 2.47 % ID/g) was observed 3 days post-injection.
On the 7th day the ATU was 2.82 ± 1.19 % ID/g, whereas the uptake in other organs had reduced to < 2 %ID/g and the circulating activity in the blood was similar (2.82 ± 1.68 %ID/g) to that of the xenograft. The thyroid uptake was 4.12 ± 0.14 %ID/g at 3 days post-injection which reduced as a function of time. Maximum tumor to blood ratio (T/B) 3.01 ± 0.43 was obtained on 3rd day after injection of MAb DS1. As the muscle uptake of MAb DS1 was less (1.11 ± 0.42 %ID/g), the tumor to muscle ratio (T/M) was 13.95 ± 2.13 on 3rd day post-administration of MAB DS1.

Biodistribution of MAb F91 has been summarized in Table 3. Maximum uptake in the tumor was obtained on 3rd day which reduced significantly on 7th day. ATU was 4.19 ± 0.54 %ID/g on 1st day and remained nearly same 4.51 ± 0.69 %ID/g on 3rd day after administration of MAb F91. The thyroid gland of the mice also showed high uptake 6.0 ± 1.48 %ID/g at 3 and 7 days post-injection, the uptake was 3.5 ± 0.95 %ID/g. This was higher as compared to the other organs. The circulating activity in the blood was 8.36 ± 1.13 %ID/g at 24 hr, which reduced to 4.60 ± 0.23 %ID/g on 3rd day and further reduced to 0.49 ± 0.18 %ID/g on 7th day after administration of MAb F91.

T/B ratio was highest at day 3 post-injection (0.98 ± 0.20) but was much less than that observed with MAb DS1 (3.01 ± 0.43). The T/M ratio obtained was 6.94 ± 1.81 at day 3, indicating that there was no significant uptake in the muscles.

Comparison of various biodistribution parameters of three 125I labeled antibodies has been shown in Fig.2a, b. Maximum ATU obtained was at day 3 after administration of MAb F91 as well as for MAb DS1 as compared to N-Igs. ATU was maximum with MAb DS1 as compared to MAb F91 (p< 0.001) and N-Igs (p< 0.001). Both the MAb i.e. MAb DS1 and MAb F91 showed a significantly high uptake in the tumor xenograft as compared to N-Igs (p< 0.001 for both the MAb).

T/B and T/M ratios obtained with MAb DS1 and MAb F91 were significantly higher (p< 0.001 for MAb DS1 as well as for MAb F91) as compared to N-Igs. T/B ratio of MAb DS1 at 3 days post-injection was greater than that obtained with MAb F91 and was highly significant (p< 0.001).

**Discussion**

Purpose of this study was to assess the potential use of anti-H-Tg monoclonal antibodies for in vivo localization of thyroid tumor tissue in an animal model. Therefore, an animal model of human thyroid carcinoma was established in our laboratory to carry out immunolocalization studies with 125I labeled anti-H-Tg MAbs. Animal models bearing transplants of differentiated thyroid carcinoma as well as anaplastic thyroid carcinoma have been used for targeting with radiolabeled antibodies.

Earlier studies have used either polyclonal or monoclonal antibodies directed against H-Tg membrane antigen to target thyroid tumor tissue in vivo. We have used MAbs against H-Tg as this is a value of 0.001.

### Table 3 — Biodistribution of 125I labeled MAb F91 in T200 × 5R mice bearing thyroid tumor xenografts

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Days post-injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Blood</td>
<td>8.36 ± 1.13</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.92 ± 0.60</td>
</tr>
<tr>
<td>Thyroid</td>
<td>13.5 ± 2.19</td>
</tr>
<tr>
<td>Liver</td>
<td>3.57 ± 0.35</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.16 ± 0.41</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.63 ± 0.46</td>
</tr>
<tr>
<td>Large intestine</td>
<td>1.88 ± 0.05</td>
</tr>
<tr>
<td>Kidneys</td>
<td>5.00 ± 1.08</td>
</tr>
<tr>
<td>Tumor (ATU)</td>
<td>4.19 ± 0.54</td>
</tr>
<tr>
<td>Tumor/Blood ratio</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>Tumor/Muscle ratio</td>
<td>2.18 ± 0.92</td>
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</tbody>
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Values are expressed as % of the injected dose taken up per g of the tissues.
proven tumor marker for DTC patients. Preservation of immunoreactivity after labeling is also a key to the successful use of radiolabeled antibodies for tumor localization. Since MAbs D51 and F9I used in this study retained their immunoreactivity to H-Tg as proved by binding to H-Tg on solid phase, it was possible to use them for in vivo localization in tumor xenografts. Loss of immunoreactivity post-labeling has been reported even at low concentrations of the isotope used if the modified labeled residues comprise the part of the antibody combining site.

MAbs used in this study belonged to IgG1 subclass of immunoglobulins. Due to their ideal size and suitable kinetics in vivo, most of the radioimmunolocalization (RIL) studies are carried out with MAbs belonging to the IgG class, either whole IgG molecules or their fragments.

The two important parameters for deciding the final suitability of MAb for RIL are the rate of clearance of the antibody from circulation as well as the specific uptake of the antibody in tumor tissue. For any antibody, a high ATU with minimum blood pool activity is best suited for RIL. Monoclonal antibody D51 cleared slowly from the blood as compared to MAb F9I. N-Igs used as controls remained in circulation for longer time as compared to MAbs D51 and F9I. In case of MAb F9I, though the circulating radioactivity was less, ATU was not very high as compared to MAb D51 and the tumor to blood (T/B) ratio was also low, indicating that it might not be an ideal antibody for tumor localization. MAb F9I showed localization in the thyroid of the animal model used, since it also showed positivity on immunohistochemistry to murine Tg. This uptake could be due to cross-reactivity between human and murine Tg.

Monoclonal antibody D51 showed maximum T/B and T/M ratios as compared to MAb F9I and N-Igs. Though the rate of clearance of MAb D51 was slow as compared to MAb F9I, the T/B and T/M ratios were higher because of the high ATU of the antibody.

ATU of any antibody depends on various parameters like antibody affinity, antigen availability, and tumor antigen density. Tumor vascularity, blood flow and vascular permeability are other factors that affect the localization and distribution of radiolabeled antibodies in tumors. While the high ATU obtained with MAb D51 could be due to any of these factors, the higher affinity $2.4 \times 10^9 M^{-1}$ of MAb D51 as compared to MAb F9I $4.3 \times 10^8 M^{-1}$ may have resulted in high ATU in the tumor tissue.

In conclusion, the studies indicated high tumor to non-tumor ratio and a significant ATU of MAb D51 in the thyroid tumor tissue xenografted in T200 × 5R mice indicating its future use for targeting tumors in patients of DTC especially those who produce H-Tg but do not concentrate radioiodine.

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References


