Human IgG2 Constant Region Enhances in Vivo Stability of Anti-Tenascin Antibody 81C6 Compared with Its Murine Parent

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ABSTRACT

The in vivo properties of radiolabeled chimeric monoclonal antibodies (mAbs) with human IgG1 and IgG3 constant regions generally are similar to those of their corresponding murine construct. In contrast, we have observed that chimeric anti-tenascin mAb 81C6, which contains IgG2 constant regions, exhibits significantly higher localization in s.c. D-54 MG xenografts and prolonged retention in most normal tissues compared with its IgG2b murine parent. The purpose of the present study was to determine whether substitution of the murine IgG2b constant region domains in mAb 81C6 with those from human IgG2 enhanced the in vivo stability of the 81C6 mAb. Both mAbs were radiiodinated using Iodogen and administered to athymic mice bearing s.c. D-54 MG human glioma xenografts. The nature of the labeled species present in tumor and normal tissues over a 144-h period was investigated by trichloroacetic acid precipitation and SDS PAGE. In tumor and most normal tissues, a greater fraction of chimeric compared with murine 81C6 was present as intact IgG. For example, in tumor at 144 h, the fraction of radioactivity present as intact IgG was twice as high for chimeric compared with murine 81C6. A substantial fraction of murine but not chimeric 81C6 was present as a $\mu$, 70,000–90,000 molecule, which could represent the generation of Fab/Fc monomers through the reduction of the interchain disulfide bonds in the murine IgG2b molecule. These results suggest that the higher tumor and normal tissue levels of chimeric compared with murine 81C6 can be attributed in part to the enhanced in vivo stability of the IgG2 chimeric mAb. The chimeric construct also was demonstrated to be more stable than murine after incubation with cyst fluid obtained from glioma resection cavity patients. Chimeric mAbs containing human IgG2 constant region domains could be of particular value for certain radioimmunotherapeutic applications.

INTRODUCTION

The extracellular matrix antigen, tenascin, is an attractive target for radioimmunotherapy because it is overexpressed in several tumor types including glioma, melanomas, and breast carcinomas (1–3). We have been investigating the toxicity, pharmacokinetics, and potential efficacy of $^{131}$I-labeled 81C6, a mu (3) IgG2b anti-tenascin mAb, administered via non-i.v. routes for the treatment of patients with neoplastic meningitis, cystic glioma, and surgically created glioma resection cavities (4, 5). A recently completed Phase I dose escalation study of up to 120 mCi of $^{131}$I-labeled mu 81C6 administered into surgically created resection cavities has shown no significant toxicity at the maximally tolerated dose, and encouraging responses and increased survival have been obtained in patients with glioma and other brain tumors (6).

In anticipation of multidose treatment protocols, genomic cloning was used to generate a human/mouse ch construct consisting of the variable regions of mu 81C6 and the constant region domains of human IgG2 (7). Human IgG2 constant region domains were selected instead of the IgG1 and IgG3 constant regions used in most human/mouse ch mAbs to provide a construct with optimal properties for radioimmunotherapy. Human IgG2 has a low affinity for Fc receptors (8), a property that was desired to minimize radiation dose to normal tissues such as the liver, spleen, and bone marrow. As observed with ch mAbs constructed with other human subclass constant regions, the affinity and specificity of 81C6 IgG2 was the same as those of its mu parent (7).

In contrast to IgG1 and IgG3 ch mAbs (9–16), the in vivo behavior of ch 81C6 was considerably different from its mu counterpart in several important respects. Retention of activity in s.c. and intracranial D-54 MG human glioma xenografts for ch 81C6 (radioiodinated using Iodogen) was significantly higher than mu 81C6, and some normal organs had higher levels of ch 81C6 as well (7). Tumor localization indices for ch 81C6 compared favorably with those obtained using mu 81C6, indicating that its enhanced tumor uptake was specific (17). When mu and ch 81C6 both were labeled with SIB, a reagent shown previously to decrease deiodination and increase tumor uptake of mu 81C6 compared with Iodogen labeling (18), the ch mAb again exhibited higher retention in tumor and some normal tissues (17). Unexpectedly, paired-label experiments failed to demonstrate significantly enhanced tumor uptake for SIB labeling with ch 81C6.

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3 The abbreviations used are: mu, murine; mAb, monoclonal antibody; ch, chimeric; SIB, N-succinimidyl 3-iodobenzoate; TCA, trichloroacetic acid; % ID, percentage of injected dose.
**Table 1** Tissue distribution of radioiodine in athymic mice bearing D-54 tumor xenografts*  

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>mu 81C6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>4.00 (3.49-4.51)</td>
<td>2.84 (2.35-3.33)</td>
<td>1.42 (1.08-1.76)</td>
<td>0.76 (0.40-1.12)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.38 (0.33-0.43)</td>
<td>0.24 (0.24-0.24)</td>
<td>0.12 (0.08-0.16)</td>
<td>0.07 (0.05-0.09)</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.32 (1.25-1.39)</td>
<td>0.66 (0.49-0.84)</td>
<td>0.42 (0.37-0.47)</td>
<td>0.16 (0.10-0.23)</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.00 (0.93-1.07)</td>
<td>0.54 (0.46-0.62)</td>
<td>0.52 (0.40-0.63)</td>
<td>0.23 (0.11-0.35)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.88 (0.74-1.01)</td>
<td>2.02 (1.85-2.18)</td>
<td>1.52 (1.37-1.67)</td>
<td>1.95 (1.73-2.18)</td>
</tr>
<tr>
<td>Blood</td>
<td>13.7 (13.3-14.1)</td>
<td>7.22 (5.34-9.11)</td>
<td>1.94 (0.58-3.29)</td>
<td>1.88 (0.97-2.78)</td>
</tr>
<tr>
<td>Urine</td>
<td>0.52 (0.28-0.76)</td>
<td>0.35 (0.31-0.39)</td>
<td>1.35 (0.66-2.03)</td>
<td>0.04 (0.04-0.05)</td>
</tr>
<tr>
<td>Tumor*</td>
<td>26.5 (25.6-27.3)</td>
<td>31.3 (29.5-33.0)</td>
<td>26.1 (24.4-27.8)</td>
<td>21.0 (13.9-28.2)</td>
</tr>
<tr>
<td>ch 81C6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>4.70 (3.94-5.54)</td>
<td>4.41 (3.30-5.53)</td>
<td>2.20 (1.95-2.46)</td>
<td>0.69 (0.27-1.12)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.33 (0.33-0.35)</td>
<td>0.25 (0.20-0.29)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.05 (0.02-0.07)</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.80 (1.80-1.81)</td>
<td>1.18 (1.16-1.20)</td>
<td>0.71 (0.66-0.76)</td>
<td>0.19 (0.07-0.31)</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.81 (0.80-0.82)</td>
<td>0.48 (0.43-0.53)</td>
<td>0.42 (0.34-0.50)</td>
<td>0.15 (0.11-0.19)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.84 (0.73-0.95)</td>
<td>1.20 (1.16-1.23)</td>
<td>1.01 (0.98-1.03)</td>
<td>1.53 (0.92-2.14)</td>
</tr>
<tr>
<td>Blood</td>
<td>22.2 (21.3-23.2)</td>
<td>14.9 (14.1-15.7)</td>
<td>8.54 (7.67-9.41)</td>
<td>2.65 (0.81-4.49)</td>
</tr>
<tr>
<td>Urine</td>
<td>0.46 (0.28-0.65)</td>
<td>0.11 (0.07-0.14)</td>
<td>0.09 (0.06-0.13)</td>
<td>0.05 (0.03-0.06)</td>
</tr>
<tr>
<td>Tumor*</td>
<td>34.3 (31.1-37.4)</td>
<td>45.4 (39.5-51.3)</td>
<td>32.6 (26.4-38.7)</td>
<td>28.8 (19.6-38.0)</td>
</tr>
</tbody>
</table>

* Groups of two mice per time point were injected with 125I-labeled mu 81C6 or 131I-labeled ch 81C6.  
+ Average and range.  
% ID/g.

A likely explanation for these observations, of importance for its potential utilization for radioimmunotherapy, is that the in vivo stability of ch 81C6 is higher than that of mu 81C6. To investigate this possibility, the present study was performed to compare directly the nature of the labeled catabolites generated after i.v. injection of radioiodinated ch and mu 81C6 mAbs in athymic mice bearing s.c. D-54 MG xenografts. Our results suggest that the higher retention of ch IgG2 81C6 in human glioma xenografts is due to its enhanced in vivo stability.

**MATERIALS AND METHODS**

**mAbs.** mu 81C6 is an IgG2b mAb specifically reactive with the extracellular matrix antigen tenasin present on a majority of glioma cell lines and xenografts but not in normal brain tissue (1). Genomic cloning was used to combine the mu 81C6 variable region genes with those for the human IgG2 constant regions, and the resultant ch mAb was characterized as described (7). Antibodies were grown in mouse ascites, purified initially using Protein A-Sepharose, and then as a final purification step, passed through an ion exchange chromatography column compatible with the isoelectric point of each mAb (17).

**Radioiodination.** Both mu and ch 81C6 were radiola-

**mitted.** Athymic mice bearing s.c. D-54 MG human glioma xenografts were used for these studies. Two mice per time point were injected via the tail vein with either 2.5 $\mu$Ci of 125I-labeled mu 81C6 or 2.5 $\mu$g of 131I-labeled ch 81C6. Prior to killing the mice by halothane overdose at 24, 48, 72, and 144 h after injection of the labeled mAbs, urine and blood were collected. Tissues of interest were harvested, washed, weighed, and counted in a LKB 1282 automated gamma counter. The % ID in the blood was calculated, assuming that the blood pool accounted for 6% of body weight. Tissue samples from two animals at each time point were pooled prior to being homogenized. Liver, spleen, kidney, and tumor tissue were homogenized using a hand-held glass tissue homogenizer in the presence of 1-2 ml of PBS containing the following protease inhibitors: pepstatin (70 $\mu$g/ml), phenylmethylsulfonyl fluoride (4 mM), leupeptin (50 $\mu$g/ml), antipain (20 $\mu$g/ml), and EDTA (5 mM). The homogenates were pelleted, and the tissue super-

**Table 2** TCA-soluble radioactive counts associated with tissue homogenates and the blood pool*  

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>mu 81C6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.1 ± 0.6</td>
<td>3.8 ± 2.3</td>
<td>4.1 ± 0.9</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.7 ± 0.4</td>
<td>2.1 ± 0.8</td>
<td>4.4 ± 0.5</td>
<td>6.9 ± 3.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.0 ± 0.6</td>
<td>5.9 ± 3.8</td>
<td>5.5 ± 1.1</td>
<td>5.7 ± 1.4</td>
</tr>
<tr>
<td>Blood</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.4</td>
<td>1.5 ± 0.5</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Tumor</td>
<td>9.3 ± 4.6</td>
<td>5.1 ± 0.7</td>
<td>5.0 ± 0.3</td>
<td>5.6 ± 1.0</td>
</tr>
<tr>
<td>ch 81C6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>2.8 ± 1.6</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.7 ± 0.6</td>
<td>0.8 ± 0.2</td>
<td>2.3 ± 1.1</td>
<td>8.6 ± 3.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.5</td>
<td>1.8 ± 0.5</td>
<td>5.8 ± 4.2</td>
</tr>
<tr>
<td>Blood</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Tumor</td>
<td>1.5 ± 0.5</td>
<td>4.1 ± 2.5</td>
<td>3.2 ± 0.5</td>
<td>2.5 ± 0.4</td>
</tr>
</tbody>
</table>

* The percentage of TCA-soluble radioactive counts is displayed. Mean ± SD of triplicate measurements.
natants were passed through a 0.45 μm filter (Millipore), pooled and saved for analysis.

**Analysis of Tissue Homogenates, Blood, and Urine.** The presence of low molecular weight catabolites in the tissue samples was determined by measuring the fraction of radioiodine activity that was precipitable in TCA. Aliquots of urine, blood, and tissue homogenate supernatants, as well as the radiiodinated mAb itself, were incubated in triplicate for 10 min at 4°C in 12.5% TCA. Precipitated counts were separated from soluble counts by centrifugation, and both fractions were counted for radioactivity. The results obtained with ch 81C6 and mu 81C6 were compared using an unpaired t test assuming equal variances.

Radiolabeled mu and ch 81C6 and tissue supernatants also were analyzed in triplicate by SDS-PAGE using 4–20% gradient gels (Bio-Rad) under nonreducing conditions. Images of the gels were obtained, and the radioactivity in the bands was quantified using a phosphorimage analysis system. Dried gels were exposed to a phosphor screen (Kodak) for 48 h. The screen was scanned using a Storm 860 Phosphorimager (Molecular Dynamics). The distribution of radioactivity among the different bands was analyzed using the ImageQuant analysis program developed by Molecular Dynamics.

**In Vitro Analysis of Tumor Cyst Fluid.** Tumor cyst fluid from seven glioma patients participating in the Phase I clinical trial of 131I-labeled 81C6 administered to surgically created resection cavities was obtained prior to therapeutic injection, sterile filtered, and stored at −170°C. Aliquots of radiiodinated mu and ch 81C6 were incubated in the presence of 100 μl of tumor cyst fluid for 4 h at 37°C. Samples were then assayed by TCA precipitability and SDS-PAGE, followed by autoradiography as above.

**RESULTS**

**Radiolabeled mAb Quality Control.** Size-exclusion HPLC profiles of 125I-labeled mu 81C6 and 131I-labeled ch 81C6 indicated that in both preparations, >95% of the radioactivity eluted with a retention time corresponding to monomeric IgG. The TCA precipitability of radiiodinated mu and ch 81C6 was >99%. Samples of both preparations were analyzed by SDS-PAGE, followed by autoradiography. Phosphorimage analysis of the resultant images indicated that >95% of the activity corresponded to a species with a molecular weight consistent with monomeric IgG (M, 150,000–160,000).

**Tissue Distribution of 131I-labeled mu 81C6 and 131I-labeled ch 81C6.** The in vivo distribution of radioiodine activity was measured in these mice to ensure that the differences in mu and ch 81C6 mAb biodistribution observed in previous studies also existed in the animals used for the catabolism measurements. Table 1 summarizes the average (range) % ID of radioiodine activity found in selected tissues at 24, 48, 72, and 144 h after injection. Consistent with our previous observations, levels of activity in tumor and normal tissues generally were higher for ch 81C6 compared with mu 81C6. The largest difference in tumor localization between the two mAbs occurred at 48 h after injection when a 45% enhancement in tumor uptake was observed for ch 81C6. Higher levels of the 131I-labeled ch 81C6 were observed at 48 and 72 h after injection for all of the normal tissues examined except the thyroid and stomach, tissues known to sequester free iodide. For example, at 48 h, thyroid retention was 2.02 (1.85–2.18) % ID for mu 81C6 compared with 1.20 (1.16–1.23) % ID for ch 81C6.

**Labeled Catabolite Analysis.** The TCA solubility of activity in the tissue homogenates, blood, and urine was measured to determine the presence of low molecular weight, non-protein-associated catabolites. The percentages of TCA-soluble counts measured in these tissues are summarized in Table 2. The percentage of TCA-soluble radioactivity for both mAbs was <10% in all tissues at all time points but generally was lower for ch 81C6. With both mAbs, the lowest percentages of TCA-soluble counts were observed in the blood pool. At 24 h, there was a significant enhancement (P < 0.05) in the percentage of TCA-soluble activity for mu compared with ch 81C6 in all
tissues, with differences of a factor of three or higher seen in liver, kidney, and tumor.

The nature of the higher molecular weight labeled catabolites present in D-54 MG human glioma xenograft, liver, spleen, kidney, and blood after i.v. injection of radioiodinated mu and ch 81C6 mAbs was investigated by SDS-PAGE, followed by autoradiography and phosphorimage analysis. The distribution of labeled species found in the 24-h tissue samples, along with molecular weight standards, is illustrated in Fig. 1. There was qualitative evidence of catabolism of both mu and ch 81C6 in the 24-h samples, and different patterns of labeled catabolites were found in the various tissues.

The molecular weight distribution of tumor-associated radioactivity for both mAbs determined by quantitative image analysis is displayed in Fig. 2. For ch 81C6, a higher percentage of tumor-associated radioactivity was present as intact mAb at 24, 48, and 144 h compared with mu 81C6. At 24 h, 52% of the ch 81C6 counts were found in a band with a molecular weight corresponding to intact IgG compared with 38% for mu 81C6. Even more striking differences were observed at 144 h when the fraction of radioactivity present as intact IgG was more than twice as high for ch compared with mu 81C6. The primary labeled catabolites from ch 81C6 occurred in three molecular weight categories: $M_r$ 110,000–130,000, $M_r$ 40,000–50,000, and $M_r$ <30,000. These catabolites also were observed with mu 81C6; however, a substantial amount of mu 81C6-associated radioactivity was present in a band corresponding to a $M_r$ 70,000–90,000 molecule.

The molecular weight distribution of labeled species found in liver, spleen, kidney, and blood at 24 h is shown in Fig. 3. As seen in tumor, a higher percentage of ch compared with mu 81C6 associated activity was present as intact mAb in liver, spleen, and kidney. In blood, nearly 100% of the radioiodine activity for both mAbs was present as intact IgG. Again, the size distribution of the labeled catabolites differed for the two mAbs. The major protein fragments generated from mu 81C6 occurred in three categories: $M_r$ 70,000–90,000, $M_r$ 40,000–50,000, and $M_r$ <30,000. In contrast, processed ch 81C6 was present primarily as $M_r$ 110,000–130,000, $M_r$ 40,000–50,000, and $M_r$ <30,000 fragments, with very little of the $M_r$ 70,000–90,000 fragment evident. For example, $M_r$ 70,000–90,000 molecules were the major catabolite in the liver, kidney, and spleen for mu 81C6, whereas $M_r$ 70,000–90,000 fragments accounted for only a small fraction of ch 81C6 in the liver and were not detected in either the spleen or kidney. The percentage of radioactivity associated with intact mAb in liver, spleen, kidney, and blood decreased with time. Fig. 4 shows the distribution of radioactive catabolites at 144 h for ch and mu 81C6. With the exception of the blood, the majority of the activity was present as $M_r$ 40,000–50,000 or $M_r$ <30,000 catabolites. At 144 h, the $M_r$ 70,000–90,000 species only was seen in blood (mu, 20.6%; ch, 4.9%).

An in vitro analysis of the stability of radiolabeled mu and
**Fig. 3** Quantitative analysis of normal organ and blood-associated radioactivity in 24-h samples by SDS-PAGE, followed by autoradiography and phosphorimage analysis. ■, mu 81C6; □, ch 81C6.

ch 81C6 in the presence of tumor cyst fluid was performed. Samples of radiiodinated mu and ch 81C6 were incubated in the presence of the tumor cyst fluid for 4 h at 37°C. Catabolism of the mAb was examined by TCA precipitability and SDS-PAGE, followed by autoradiography. A majority of the activity remained protein associated in the presence of tumor cyst fluid for both mu 81C6 (97.5 ± 2.2%) and ch 81C6 (97.7 ± 1.3%). However, as shown in Fig. 5, the percentage of the ch 81C6 present as intact IgG, 94.7 ± 5.1%, was significantly higher (P < 0.001) than for mu 81C6 (79.2 ± 3.8%). In addition, a significantly larger fraction of mu 81C6 was present as Mr 70,000–90,000 (P < 0.001) fragments, and no Mr 40,000–50,000 fragments were observed for ch 81C6.

**DISCUSSION**

The in vitro binding characteristics of human/mouse ch mAbs generally are assumed to be identical to those of the mu parent; however, at least one ch IgG2 mAb has been reported to have decreased avidity (19). This characteristic was attributed to the relatively low flexibility of the hinge region of human IgG2. In contrast, avidity of ch 81C6 was virtually identical to that of mu 81C6 (7); therefore, it might be expected that the in vivo behavior of the two mAbs also would be the same. However, in both the present study and prior paired-label experiments, ch 81C6 exhibited higher tumor uptake and normal tissue levels compared with its mu parent (7, 17). Our expectation that the tissue distribution of mu and ch 81C6 would be similar also was based on numerous reports indicating nearly identical in vivo behavior for human/mouse ch mAbs and their mu counterparts (9–16). The fact that these ch mAbs contained IgG1 or IgG3 constant regions suggested to us that the unexpected in vivo behavior of ch 81C6 could be related to its IgG2 constant region domains.

Only limited data are available concerning the in vivo properties of radiolabeled mAbs containing IgG2 constant regions because in most cases, ch mAbs were originally developed for complement-mediated and antigen-dependent cell-mediated toxicity, approaches that require IgG1 or IgG3 constant regions for optimal effectiveness. The results from the few studies evaluating the tissue distribution of radiolabeled IgG2 ch mAbs are consistent with a relatively high degree of in vivo stability of these constructs. For example, we have compared the tissue distribution of ch Me1-14, which reacts with the tumor-associated proteoglycan chondroitin sulfate, with its mu IgG2a counterpart (20). The ch mAb exhibited higher tumor retention, slower clearance from the blood pool, and lower thyroid uptake (an in vivo indicator of dehalogenation). Recently, we have constructed a ch mAb consisting of the human IgG2 constant domains and the variable region domains of the anti-epidermal growth factor receptor variant III mAb L8A4 (21). Normal tissue levels of ch L8A4 were significantly higher than mu at later time points. Tumor uptake of both mAbs was essentially
The goal of the present study was to determine whether the ch IgG2 81C6 construct was less susceptible to degradation than mu 81C6 and whether the spectrum of labeled catabolites generated in vivo from the two mAbs was different. Results from both the TCA and SDS-PAGE analyses indicate that ch 81C6 is more stable than mu 81C6. The fraction of TCA-soluble activity for the ch mAb was significantly lower in both tumor and normal tissues, suggesting that ch 81C6 (and/or its macromolecular catabolites) was less susceptible to dehalogenation than mu 81C6. Alternatively, the lower molecular weight catabolite could be moniodotyrosine created after mAb proteolysis. Reverse-phase HPLC analysis of the catabolites generated from Mel-14 F(ab'), labeled using the Iodogen method confirm that both deiodination and proteolytic degradation occur in vivo (22). It is difficult to discern the relative magnitude of the two processes because of the rapid deiodination of moniodotyrosine in the presence of tissue (23). In either case, TCA precipitability measurements indicated that >90% of the radioiodine activity from both mAbs was present in a protein-associated form throughout the 6-day experimental period. Thus, it appears that low molecular weight catabolites play only a minor role in determining normal tissue activity levels, which may in part reflect the rapid clearance of these low molecular weight species from tissue.

Because most of the radioactivity in tumor and normal tissue was protein associated, SDS-PAGE was performed to determine the molecular weight distribution of the TCA-insoluble species. With the exception of one time point, the catabolite profiles in tumor indicated that a higher percentage of the ch

Fig. 4 Quantitative analysis of normal organ and blood-associated radioactivity in 144-h samples by SDS-PAGE, followed by autoradiography and phosphorimage analysis. ■, mu 81C6; ■, ch 81C6.

Fig. 5 Quantitative analysis of radioiodinated mu and ch 81C6 in the presence of human glioma cyst fluid by SDS-PAGE, followed by autoradiography and phosphorimage analysis. Columns (■, mu 81C6; □, ch 81C6) are the mean of seven different cyst fluid samples; bars, SD.
compared with mu 81C6 remained as intact IgG. This was most apparent at 6 days when the percentage of radioactivity present as intact IgG was more than twice as high for ch 81C6. Except in blood, a higher fraction of ch 81C6 activity was present as intact IgG in normal tissues. An interesting finding was that a substantial fraction of radioactivity for mu but not ch 81C6 was present as a Mr, 70,000–90,000 catabolite in both tumor and normal tissues.

Consistent with the results reported herein are previous reports indicating that IgG2 is the human IgG subclass that is least sensitive to proteolysis by papain or pepsin (24). In contrast, F(ab')2 fragments cannot be generated from mu IgG2b with these enzymes because of the high sensitivity of its three interchain disulfide bonds to reduction, as evidenced by the production of Fab-Fc monomers (25). The molecular weight of the Fab-Fc monomer, Mr, ~80,000, is the same as the Mr, 70,000–90,000 species that we observed in the catabolism of mu 81C6 IgG2b but not ch 81C6. Relatively rapid degradation of mu IgG2b mAbs may account for the observation that their blood clearance is faster than seen with mu mAbs of other subtypes (26, 27).

The observations that the catabolic rate and size distribution of labeled catabolites for ch and mu 81C6 are different suggest that the two mAbs may differ with regard to the nature of their proteolytic degradation sites. These could arise from differences between the two IgG molecules with regard to C4,2 and C4,3 domains as well as the hinge region. Several groups have shown that IgG C4,2 and C4,3 domains play a role in determining their in vivo stability (27–29). There has also been evidence that IgG metabolism is influenced by the presence of protective receptors (29–31) as well as by glycosylation (32).

Perhaps the most important structural feature of the IgG molecule that should be considered in interpreting the results of the present study is the nature of the mAb hinge region. The possible influence of the hinge region on in vivo stability can be inferred from a comparison of the tissue distribution of ch and mu F(ab')2 fragments (33), molecules that include the hinge region but not the C4,2 and C4,3 domains. The F(ab')2 derived from a human/mu IgG2 ch mAb exhibited the highest tumor uptake and most prolonged normal tissue clearance half-lives compared with F(ab')2 generated from other subclass ch mAbs or the mu parent.

A recent review has related hinge region flexibility to the ability of the four human IgG subclasses to bind antigen and then activate complement (34). The flexibility of the hinge region also is relevant to mAb catabolism because it can influence the solvent accessibility of various regions of the molecule to proteases (32). It is interesting to note that the mean rotational correlation time (high values indicate more rigid molecules) for IgG2b, 55 nanoseconds, is the lowest of the mu isoforms, while the value for human IgG2, 120 nanoseconds, is the highest for human IgG (35). Therefore, one would expect that the proteolytic breakdown of mu IgG2b would be relatively fast and ch IgG2 would be relatively slow, predictions that are consistent with the in vivo fate of mu and ch 81C6.

The results of this study demonstrate that the nature of the IgG molecule is a factor that should be considered in the design of reagents for use in radioimmunotherapy. Substitution of mu IgG2b constant region domains with those from the human IgG2 molecule not only decreased proteolytic degradation of the mAb but also decreased lower molecular weight catabolites. Labeling ch 81C6 using SIB, a reagent that minimizes recognition by endogenous deiodinases, decreased thyroid uptake by a factor of 5–17 compared with mAb labeled using iodogen (17); in contrast, SIB labeling decreased thyroid uptake of the mu mAb by two orders of magnitude (18). These observations could reflect decreased access of deiodinases to labeled tyrosine residues because of the rigidity of the IgG2 hinge region. Alternatively, proteolysis of the IgG molecule first may be required to generate a labeled mAb fragment of a size that is susceptible to attack by deiodinases.

In attempting to exploit the increased stability of human IgG2 in clinical radioimmunotherapy, it is important to select applications in which enhanced tumor doses might be obtained without concomitant gains in normal tissue levels. Settings that might be ideal are those in which the labeled mAb is administered non-i.v., such as our ongoing clinical protocols in patients with neoplastic meningitis, cystic gliomas, and surgically created glioma resection cavities (4–6). The results of the present study demonstrate a significantly lower rate of degradation in vitro for radioiodinated ch compared with mu 81C6 in the presence of fluid obtained from glioma resection cavities. One can only speculate that the enhanced stability of ch 81C6 would lead to a prolonged residence time of radioactivity in the cavity because of a higher fraction of intact, immunoreactive mAb. On this basis, we have decided to use the ch construct in our recently initiated clinical protocol evaluating 211At-labeled 81C6 administered directly into patients with glioma resection cavities.

In summary, the increased tumor and normal tissue levels of ch 81C6 IgG2 compared with its IgG2b mu parent are due to an enhanced stability of the ch mAb in vivo. We speculate that this behavior may reflect the relatively high rigidity of the hinge region of the human IgG2 constant region. Because of their enhanced stability and low Fc receptor binding, ch mAbs containing the IgG2 constant region domains could be valuable reagents for use in radioimmunotherapy.

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