Effects of Temperature on Radiochemical Purity and Immunoreactivity of Radiolabeled Monoclonal Antibody 1H10

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Objective: This study was undertaken to investigate the effects of temperature on preserving the radiochemical purity and immunoreactivity of ^{125}I- and ^{131}I-labeled monoclonal antibody (MAb) 1H10—an antibody against human cervical carcinoma cell-surface antigen.

Methods: An antibody-irrelevant human melanoma cell line, H2269, served as the control group. Iodine-125 and ^{131}I radiolabeling of MAbs 1H10 and H2669 was performed by the chloramine-T method. All the prepared MAbs were divided into aliquots and stored at 4, −20, and −70°C for 2–14 d. The radiochemical purity and immunoreactivity of the labeled antibodies in set conditions were measured by thin-layer chromatography and a modified index, respectively, after a single freeze-and-thaw cycle.

Results: Reduced release of free radioiodide and better preservation of immunoreactivity were observed in the radiolabeled MAbs stored at −70°C than in those stored at −20°C or 4°C. The extent of free iodide dissociation and immunologic binding degradation of ^{125}I-labeled MAb 1H10 appeared milder than that of ^{131}I-labeled MAb under the same conditions. However, both ^{125}I- and ^{131}I-labeled MAb stored at −70°C or −20°C retained more than 90% radiochemical purity for at least 3 d.

Conclusion: Freezing provides an appropriate alternative for reducing radiolysis and preserving immunoreactivity of radioiodinated MAbs. MAb 1H10, labeled with either ^{125}I or ^{131}I and stored at temperatures of −20°C or below for 3 d after labeling, appeared stable in both radiolabeling and binding studies in vitro and was still acceptable for in vivo use.

Key Words: effects of temperature; immunoreactivity; monoclonal antibodies; radiochemical purity

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Monoclonal antibodies (MAbs), raised against specific tumor-associated antigens, have become valuable carriers to deliver agents such as radionuclides to tumor sites (*1*). The localization of radioactivity in tumor sites, either primary or metastatic, has provided a means for tumor diagnosis and perhaps treatment (*2*,*3*). While radiolabeled MoAbs are potential tools for tumor imaging and treatment, such as radioimmunodetection (RAID) and radioimmunotherapy (RAIT), several problems need to be resolved before they can become applicable on a large-scale clinical basis (*4*,*5*).

Iodine-125 is the most often used radionuclide to determine relative tissue distribution in animals, while ^{131}I is one of the basic isotopes for imaging and treating patients. Both are used frequently for radiolabeling of antibodies. Factors such as radiation from isotopes attached to antibodies and the interaction of chemicals used for iodination, or constituents of stock solutions of labeled antibodies, may deteriorate antibody molecular structure and result in dissociation of the isotope or a decrease in immunoreactivity of the radioiodinated antibodies (*6*). Unstable radiolabeling and poor binding studies in vitro usually exclude those antibodies from in vivo use (*7*). This is especially true when the time from radiolabeling to application is prolonged. Many techniques are available to stablize the labeling and immunoreactivity (*8–10*).

Despite recent advances in cancer management, cervical carcinoma is still a serious problem and is ranked first among malignancies affecting women in Taiwan (*11*). MAb 1H10, which recognizes 40% of human cervical carcinoma tissues, has been used for in vivo tumor localization (*12*). In this study, we investigated the efficacy of cryopreservation on stabilizing labeled MAb, by observing the changes in radiochemical purity and immunoreactivity of ^{125}I- or ^{131}I-labeled MAb 1H10 after periods of storage at different temperatures.

MATERIALS AND METHODS

Iodine-131 (40 mCi/mL) and ^{125}I (100 mCi/mL) were both purchased from a commercial source (Amersham Co., Buckinghamshire, UK). Human CaSki cell line was provided by RA Pattillo (Medical College of Wisconsin, Milwaukee, WI). MAb 1H10 was generated against CaSki human cervical carcinoma cell-surface antigen as previously described (*13*). The MAb 1H10 belongs to the immunoglobulin of the IgG_3 subclass. An

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IgG_3 antibody-irrelevant human melanoma cell line, H2669 (provided by I Hellstrom, University of Washington, Seattle, WA), served as the control. Both cell lines were maintained in RPMI-1640 medium supplemented with 5% heat-inactive fetal calf serum (FLOW Laboratories, Springfield, IL), with 100 U/mL penicillin and 100 µ/mL streptomycin. Iodine-131 and ^{125}I radiolabeling of MAbs was performed using the chloramine-T method (*14*), to a level of about 5 mCi/mg MAb for ^{131}I and 2 mCi/mg MAb for ^{125}I. The iodinated protein was separated from reactants using Sephadex G25 columns (Pharmacia, Piscataway, NJ) in phosphate-buffered saline (PBS) at a pH of 7.4, at room temperature (*15*). The preparations were each divided into 0.2-mL aliquots (adjusted to approximately 1 mCi/mL) and stored at −70, −20, and 4°C for various time periods. The labeled MAbs in aliquots were frozen and thawed for 1 cycle only.

Assays for radiochemical purity of ^{125}I or ^{131}I iodinated MAb on Days 1, 2, 3, 5, and 8 after labeling were performed using instant thin-layer chromatography (ITLC; Gelman ITLC-SG, Ann Arbor, MI) using chloroform-methanol 0.25% KCl (30:15:4, v/v) as a developing agent (*16*). The percentage of the total activity remaining with the protein was determined by cutting the strip, followed by counting the radioactivity using a gamma counter (LKB 1272 Clinigamma gamma counter; Wallac, Turku, Finland).

Immunoreactivities for radiolabeled ^{131}I or ^{125}I antibody stored at −70, −20, and 4°C on Days 1, 3, 5, 8, and 14 after labeling were measured by in vitro binding assay. Briefly, labeled 1H10 MAb (about 10^6 cpm/50 µL) was incubated with 10^5 solid-phase glutaraldhyde-fixed CaSki or H2669 cells for 45 min at 37°C. After washing 3 times with PBS, the bound radioactivity was quantified using the gamma counter and expressed as CPM. The bound radioactivity in wells containing CaSki cells was considered as tumor binding (T) while that in wells containing H2669 cells was considered nonspecific binding (C). Radioactivity in a blank well was designated as background (B). The index of immunoreactivity was expressed as:

(CPM in T − CPM in BKG)/(CPM in NSB − CPM in BKG).

This equation was modified from a previous report (*17*), where T CPM = Tumor, B CPM = Background, and C CPM = Nonspecific binding.

RESULTS

For ^{125}I-labeled MAb 1H10, the radiolabeling yields at different temperatures showed the least free iodide release when stored at −70°C (Fig. 1). The bound radioiodine remained more than 90% from Day 1 to Day 8. The percentage, however, dropped slightly more during a comparable period in the −20°C group. A greater decrease in the percentage of bound radioiodide was found in samples stored at 4°C. A similar trend in the dissociation curves also was found in the ^{131}I-labeled MAb 1H10, but the percentages of decrease were amplified compared to the corresponding ^{125}I groups (78% versus 64%) (Figs. 1 and 2). Overall, it appears that, for both ^{125}I- and ^{131}I-labeled 1H10, storage at −20 or −70°C can preserve the radiochemical purity above 90% for 3 d.

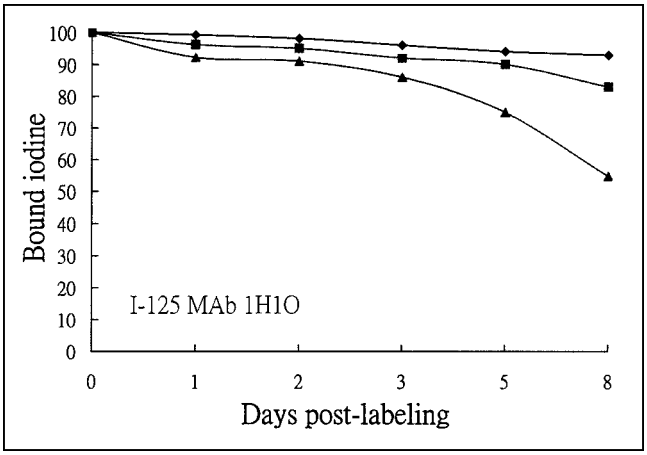

FIGURE 1. Radiochemical assay results. The percentage of bound ^{125}I, as measured by thin-layer chromatography, for ^{125}I-MAb 1H10 after storage at −70°C (◆), −20°C (■), or 4°C (▲) for various days (the x-axis) after radiolabeling. Each value plotted is the average of 3 measurements.

Both ^{125}I- and ^{131}I-labeled MAbs showed a progressive decrease of immunoreactivity after labeling. However, the changes of indices in various storage periods revealed that binding was more stable in the ^{125}I-labeled groups than those in the corresponding ^{131}I-labeled groups. Storage at −70°C or −20°C preserved more immunoreactivity than storage at 4°C in both ^{125}I- and ^{131}I-labeled groups. (Figs. 3 and 4).

DISCUSSION

Our data further demonstrate the usefulness of freezing in preventing autoradiolysis and in preserving the immunoreactivity of radioiodinated MAb. The results are consistent with observations reported by Wahl et al. (*18*). MAb 1H10 labeled with ^{125}I had better radiochemical purity and immunoreactivity than with ^{131}I-labeled MAb 1H10 at various temperatures and storage periods. Hinkle et al. suggested that both radioiodinated

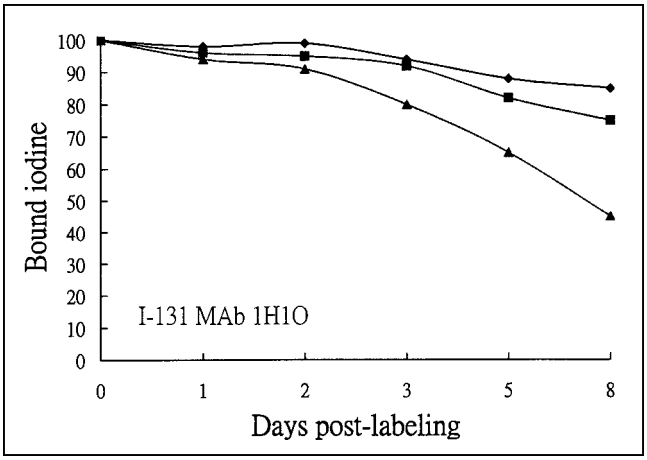

FIGURE 2. Radiochemical assay results for ^{131}I-labeled MAb 1H10 after storage at −70°C (◆), −20°C (■), or 4°C (▲) for various days (the x-axis) after radiolabeling. Each value plotted is the average of 3 measurements.

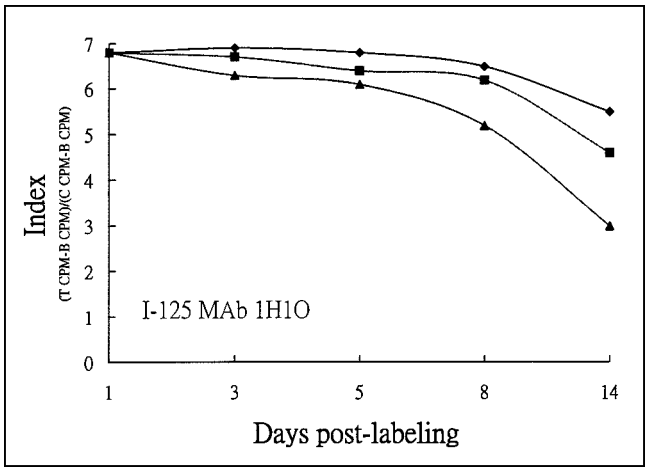

FIGURE 3. Immunoreactivity assay results for ^{125}I-labeled MAb 1H10 after storage at −70°C (◆), −20°C (■), or 4°C (▲) for various days (the x-axis) after radiolabeling. Each value plotted is the average of 3 measurements. The index was calculated as: T CPM − B CPM/C CPM − B CPM, where T CPM = tumor counts per minute, B CPM = background counts per minute, and C CPM = control counts per minute.

antibodies can be used for biologic studies only when the radiochemical purity is more than 90% (*19*). Storage of the labeled MAb 1H10, either ^{125}I or ^{131}I, at a temperature of −20°C or below can fulfill this requirement for at least 3 d.

Radioiodine is attached covalently to thyrosine residues of the antibody molecule. Although antibodies can be labeled easily with iodine isotopes by the chloramine-T procedure (*14*), problems remain for optimal recognition of antigens by the iodinated antibodies (*1*,*20*). Alteration of the biological activity is one of the major problems.

Changes in biological activity can be caused by either direct contact of the protein with iodine (*1*) or by exposure to oxidizing and reducing agents during iodination, both of which can damage the other functional groups of the protein, such as tryptophan, sulfhydryl, and hydroxy groups (*1*,*8*). Moreover,

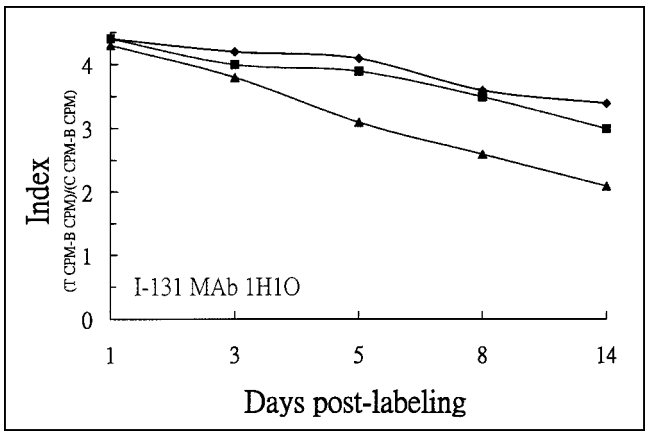

FIGURE 4. Immunoreactivity assayresults for ^{131}I-labeled MAb 1H10 after storage at −70°C (◆), −20°C (■), or 4°C (▲) for various days (the x-axis) after radiolabeling. Each value plotted is the average of 3 measurements. The index was calculated as: T CPM − B CPM/C CPM − B CPM, where T CPM = tumor counts per minute, B CPM = background counts per minute, and C CPM = control counts per minute.

radiation can cause the release of free radicals and disturb the molecular integrity of antibodies (*18*) due to the abundance of water in the radioiodinated MAb solution. Irradiation by ionizing radiation leads to radiolysis of water and results in the formation of free radicals such as $H^{\bullet}$, $HO^{\bullet}$, and highly active ionic and molecular species such as H_2O^+, H_2O^-, and H_2O_2 are produced (*6*). These species can cause conformational changes in proteins and produce specific protein fragments or oxidative products (damaged products (*21*,*22*)).

Applying this to radiolabeled MAbs, it is assumed that these biological consequences may result in a decrease of the immunoreactivity of the MAbs. Larson et al. (*23*) reported that the higher the specific activity achieved in iodination, the greater the loss of immunoreactivity.

Freezing has been used widely to preserve biological samples and may keep labeled MAbs from further dissociation and degradation. Wahl et al. (*18*), using high-performance liquid chromatography, demonstrated that the products of autoradiolysis also were decreased by freezing. This suggests that cryopreservation of radiolabeled MAb may be due to decreases in the notorious effects of peroxide substances on antibodies by immobilization of radioisotope decay-induced free radicals in the ice crystal lattice (*18*). While freezing antibodies is thought to diminish their immunoreactivity, this did not appear prominent in this study. Our data indicated that a single freeze-and-thaw cycle had little adverse effect on the immunoreactivity of the frozen MAbs (*18*).

The specific activities of ^{125}I- or ^{131}I-labeled antibody are moderate (2–6 mCi/mg). The radioactivity in the final stock solution was only about 1 mCi/mL. It is not known to what extent such radioactivity levels induce radiolysis. Obviously, the effect of this radiation level on the immunoreactivity is far lower than the 10 mCi/mg reported by Wahl et al. (*18*). More quantitative measurements of radiation effects on radiolabeled MAbs are needed. Our data indicated no apparent changes in either radiochemical purity or immunoreactivity of either ^{125}I- or ^{131}I-labeled MAb 1H10 when stored at −20°C or −70°C for 3 d. The results imply that the labeling efficiency and biologic activity of radioiodinated MAb could be sufficiently preserved at temperatures of −20°C or below, allowing biologic studies during this period. The reason labeling with ^{125}I seemed to preserve more bound radioiodine and binding immunity than that of ^{131}I is not fully known. Iodine-125 may exert a lower radiation effect on the antibody molecule than ^{131}I, and this may be responsible in part for the difference.

Using our indices to express immunoreactivity of both ^{125}I- and ^{131}I-labeled antibodies was an attempt to avoid the error of traditional enzyme-linked immunoadsorbant assay (ELISA) (*4*). Although ELISAs are effective, they fail to differentiate the immunoreactivity between labeled and unlabeled antibodies. The indices we used represent only the binding changes of the radiolabeled antibody, and may thus reflect more about the actual immunoreactivity of the labeled antibody. Since immunoreactivity of radioiodinated antibody is expressed as an arbitrary unit, the indices shown in this study do not necessarily represent the authentic immunologic response in vivo.

MAb 1H10 has been characterized initially (*13*). While its

relatively high K_a value (4×10^8/M) makes it a potential carrier for radioimmunodetection or perhaps radioimmunotherapy (*23*), many hinderances to clinical application still need to be resolved. Freezing appears to be a rational alternative to the preservation of radiochemical purity and immunoreactivity, however. Due to the immunologic heterogeneity of different subclasses of MAbs, the effect of cryopreservation presented may not be true for the other subclasses of radiolabeled antibodies. Available data indicate that the addition of human albumin at a concentration of 2%–5% weight/volume or the addition of a free radical scavenger also may slow radiolysis of radioantibody (*18,21*).

CONCLUSION

Our initial results show that freezing at −70°C results in a reduction of radiolysis and the preservation of more immunoreactivity of radioiodinated MAb 1H10 than freezing at 4°C or −20°C. Iodine-125-labeled MAb had more stable immunoreactivity and less free iodide dissociation than ^{131}I-labeled MAb at the same conditions. Freezing may provide an alternative for stablizing the radiochemical purity and immunoreactivity of radioiodinated MAbs.

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