Evaluation of a Remote Radioiodination System for Radioimmunotherapy

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This report describes a remote radioiodination system which is inexpensive, easy to assemble, disposable, and capable of radioiodinating curie levels of activity safely. In addition to the safety afforded by this system, an immobilized oxidant and anion exchange resin are used to generate electrophilic iodine and remove free iodine, respectively. Reducing agents are not used and, therefore, when radioiodinating F(ab')$_2$ fragments, degradation does not occur. In contrast, chloramine-T, sodium metabisulfite (CT/SMB) iodinations of F(ab')$_2$ fragments resulted in products with up to 40% Fab' fragments. Radiolabeling yields (65.8% ± 8.1%) and antibody immunoreactivity (68.8% ± 8.0%) were not statistically different ($p < 0.001$) from those obtained in remote CT/SMB iodinations. The system is currently being used to radioiodinate both IgG and F(ab')$_2$ monoclonal antibodies with up to 450 mCi ^{131}I for clinical radioimmunotherapy trials.

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The majority of radioimmunotherapeutic studies have employed iodine-131 (^{131}I) as the radioisotope coupled to monoclonal antibody. The amount of radioactivity associated with radiolabeling monoclonal antibodies with ^{131}I for radioimmunotherapeutic purposes may be several hundred millicuries (*1–4*). This amount of activity requires a degree of shielding and containment beyond that found in typical radiolabeling procedures. Radioisotope decay, associated energies, and volatility must be considered in the design of a safe, effective radiolabeling system.

Radioiodination systems previously described (*5–7*) for large amounts of activity are dependent on gel filtration columns to separate bound from free isotope and in-line peristaltic pumps to move reagents through reaction compartments. Such systems are inherently prone to leakage, difficult to shield, and require decontamination after use. A robotic system also has been reported (*8*), but is quite expensive. Weadock et al. (*9*) developed a remote labeling system which is inexpensive, disposable, easy to shield, and does not generate liquid waste. In this report, we describe a refined version of this method currently being used for radioimmunotherapy labels. In addition, we compare the quality of the products of this system to a remote procedure employing chloramine-T/sodium metabisulfite (CT/SMB) radioiodinations.

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MATERIALS AND METHODS

Antibody Purification

Intact IgG monoclonal antibodies NP-4, EPB-2, MA5, and their respective F(ab')$_2$ fragments were purified from mouse ascites as previously described (*10–12*). These antibodies are directed against carcinoembryonic antigen (CEA), a B-cell lymphoma antigen, and a milk-fat globule membrane antigen respectively. Antibodies were stored in PBS (40 m*M* sodium phosphate, pH 7.4) at concentrations of 10–20 mg/ml and radioiodinated at a specific activity of 60 μg antibody per millicurie Na^{131}I.

Chloramine-T Iodination

The chloramine-T method of radioiodination was based on the method described by McConahey et al. (*13*). Assembly of components used in this procedure was similar to that previously described by Ferens et al. for remote radioiodination of Fab' fragments (*5*). The isotope was obtained as a reductant-free solution of Na^{131}I in 0.1 *M* NaOH in a 3-ml vial containing a magnetic stir bar. The shielded vial was placed on a stir plate inside a ventilated glove box. Approximately 0.1 ml of a 0.5-*M* KPO_4 buffer, pH 6.0, was injected into the vial to neutralize the 0.1 *M* NaOH to a pH between 7.2 to 7.6. The antibody was then injected into the vial followed by 0.1 ml of a chloramine-T (5 μg/mCi ^{131}I) solution in 0.04 *M* $NaPO_4$ buffer, pH 7.4. After 2–4 min, 0.1 ml of a sodium metabisulfite (10 μg/mCi ^{131}I) solution in 0.04 *M* $NaPO_4$, pH 7.4, was added. Immediately after the injection of the sodium metabisulfite, a needle (20 g, 6.25 cm) was pushed to the bottom of the vessel so that air could be pumped into the vessel to force the reagent mixture to a shielded gel filtration column (PD-10, Pharmacia, Piscataway, NJ). It took ~ 3 min from the time of (SMB) addition (to stop the iodination reaction) to initiation of separation on the PD-10 column. The column was equilibrated previously with 0.04 *M* phosphate-buffered saline (PBS, 0.04 *M* phosphate, 0.15 *M* NaCl), pH 7.4, containing 1% human serum albumin (HSA). The first 5.8 ml

passively collected from the column were passed through an in-line, 0.22 μ filter to a vial containing 200 ml of 2.5% HSA in 0.04 *M* PBS. We had empirically determined that storage of the final product at a concentration < 2 mCi/ml reduces the amount of product degradation (i.e., unbound radioiodine) when storage for 24 hr is required.

Iodogen Iodination

Iodogen iodinations were based on the method described by Fraker et al. (*14*), as modified by Haisma et al. (*15*). The system used is a modified version of the remote system described previously (*9*). Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenylglycouril, Pierce Chemical Company, Rockford, IL) was plated onto the inner surface of a 20-ml glass vial by evaporating 2 ml of chloroform containing 250 μg/ml iodogen. A magnetic stir bar (8 mm × 1.5 mm) was placed in the vial, the vial capped with a rubber stopper, and finally sealed with an aluminum closure. Anion exchange resin (AG1-X8) was obtained from Biorad Laboratories, Richmond, CA. This resin has a particle diameter of 149 μm and a molecular weight exclusion limit of 1,000.

The remote iodogen labeling system illustrated in Figure 1 was assembled in a ventilated glove box. With stopcock number 3 closed to the iodogen coated vial, antibody and 0.1 *M* borate buffer, pH 8.3 (volume of antibody : 0.1 *M* borate buffer mixture is 2.0 ml) is injected into the system through stopcock number 2, and the pump is turned on for 1 min. Stopcock number 1 is used as a safety valve so that injected reagents do not enter the pump line. Note that the spinal syringe in the iodogen vial is in the up (U) position, and stopcock number 3 is adjusted to allow flow to the isotope vial only. This manipulation forces the antibody through the vial containing the isotope and into the iodogen-coated vial. Typically, < 5% of the initial radioactivity remains in the isotope vial. After 10 min, stopcock number 3 is adjusted so that the isotope vial is by-passed and flow is directed to the iodogen-coated vial directly. Two milliliters of a 20% w/v dowex solution is then injected through stopcock number 2. After 3 min, the spinal syringe is pushed to the down (D) position. The pump is then turned on so that the entire reagent mixture can be sterile-filtered (Milli-Fil GS, IV GS0103F, Millipore, Eschborn, West Germany) into a vented final product vial containing 200 ml of 2.5% HSA in PBS. Finally, 2 ml of 0.1 *M* borate buffer, pH 8.3, are injected to rinse the iodogen vial of residual product. Note that the filter, which contains most of the radioactive waste in the form of ^{131}I-anion exchange resin, is housed in a lead syringe shield with a wall thickness of 1.25 cm. Housing the filter in this syringe shield enables easy disposal of high-level waste without exposure.

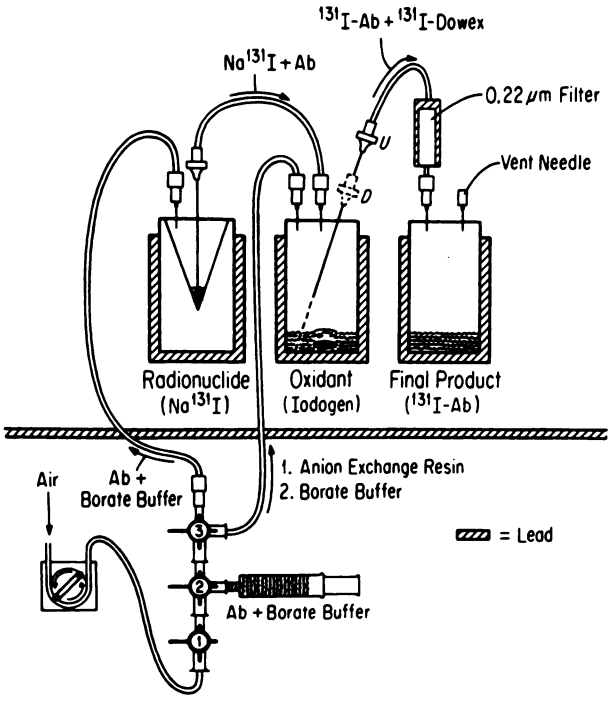

FIGURE 1
A schematic representation of the iodogen-based remote iodination system. The spinal syringe is initially in the up (U) position and the three-gang four-way stopcock set so that the antibody solution is pumped through the vial containing Na^{131}I. Following the injection of anion exchange resin, the syringe is pushed to the down (D) position and stopcock number 3 is set so that pressure is applied directly to the filtration process.

Radioimmunoconjugate Characterization

Radiolabeled antibodies were characterized by HPLC using a Zorbax GF-250 (9.4 × 250 mm, DuPont, Wilmington, DE) or TSK-250 (Biorad, Richmond, CA) gel filtration column. Radiolabeling yield was calculated by measuring radioactivity in the final product vial and the isotope vial prior to iodination. Immunoreactivity was determined by passing diluted samples over a CEA-Affi-gel immunoadsorbent. The immunoreactivity of ^{131}I-EPB-2 and ^{131}I-MA5 were not determined. For sterility assays, 50 μl of final product were incubated at 37°C in both trypticase soy broth and fluid thioglycolate medium for 24 hr. All products were sterile at the time of administration. In addition, all buffers tested negative for pyrogen as determined by the Limulus Amebocyte Lysate Assay (QCL-1000, Whittaker Bioproducts, Inc., Walkersville, MD).

RESULTS

Activated charcoal filters sampling the effluent from the glove box did not have any activity above background following either iodination procedure. A comparison of the iodogen versus CT/SMB remote radioiodinations of NP-4 F(ab')$_2$ is shown in Table 1. As determined by HPLC, the percent of product found as F(ab')$_2$, post F(ab')$_2$ (assumed to be Fab' based on elution time of Fab' standards), and free ^{131}I are included. The iodogen reaction resulted in a significantly higher (Students t-test, $p < 0.001$) fraction of product in the F(ab')$_2$ state. There was no statistical difference ($p < 0.5$) between the two methods with respect to yield. However, the CT/SMB radioiodination of F(ab')$_2$ fragments produced samples with 28.4% ± 8.9% degradation products in the post-F(ab')$_2$ fraction while the iodogen method produced virtually no activity in this fraction. No correlation between initial activity and immunoreactivity or yield was evident. Although both labeling methods yield products with <10% free ^{131}I,

TABLE 1
Comparison of the Two Different Methods of Iodinating NP-4 F(ab')$_2$

Initial activity (mCi)	Yield (%)	Immunoreactivity (%)	HPLC analysis (%) F(ab')$_2$	post F(ab')$_2$	^{131}I
Chloramine-T/Sodium Metabisulfite					
120	62.5	80.4	62.1	33.4	4.5
210	65.7	74.1	73.2	21.0	5.8
260	72.7	73.3	70.6	25.3	4.1
355	66.4	73.8	75.1	18.5	5.2
360	78.0	74.0	59.9	31.8	8.3
410	72.4	72.6	72.9	18.3	8.8
420	63.8	72.7	60.6	36.8	2.6
430	61.8	54.0	51.5	41.8	6.7
Iodogen					
140	77.1	76.8	97.4	0.0	2.6
175	69.7	67.4	90.8	0.0	6.4
180*	59.3	59.5	100.0	0.0	0.0
270*	53.9	81.4	96.9	1.9	1.2
300	72.0	70.0	97.3	0.0	1.4
340*	67.4	60.7	99.0	0.0	1.0
350	61.1	65.5	85.5	0.0	5.5

* Eight-minute anion exchange resin incubation.

samples containing more than 10 mCi of free ^{131}I are not used. When the incubation time for the anion exchange resin was increased from 4 to 8 min, the percentage of free ^{131}I decreased. The remote iodogen procedure also effectively radioiodinated intact IgG monoclonal antibodies used for radioimmunotherapy. The results are presented in Table 2.

DISCUSSION

Radioiodinated monoclonal antibodies to tumor antigens are currently being evaluated worldwide for diagnostic and therapeutic efficacy (*1–4*). In these studies, the quantity of radioactivity coupled to antibody may be several hundred millicuries. Typical radiolabeling procedures must, therefore, be modified to account for such levels of activity. Exposure to personnel must be minimized while a dose of sufficient yield and immunoreactivity is produced. This report describes a remote labeling system which enables personnel to perform safe, reproducible, and efficient radioiodinations of monoclonal antibodies. In addition, the labeling reaction inherent to this system does not result in F(ab')$_2$ fragment degradation to Fab'. Intact IgG were also labeled with excellent results.

TABLE 2
Iodinations of Intact IgG Monoclonal Antibodies Using the Remote Iodogen Method

Antibody	Initial activity (mCi)	Yield (%)	HPLC analysis (%) F(ab')$_2$	post F(ab')$_2$	^{131}I
MA5	16	70.3	100.0	0.0	0.0
EPB-2	40	80.1	96.1	0.0	0.6
EPB-2	63	72.7	99.3	0.7	0.0
EPB-2	69	69.6	99.0	1.0	0.0
EPB-2	88	72.2	94.8	4.1	1.1
EPB-2	150	85.5	100.0	0.0	0.0
NP-4	300	73.7	97.3	0.0	0.4

Radiolabeling yield with the iodogen labeling system was similar to that obtained in the CT/SMB procedure. The remote system reported by Ferens et al. (*5*) was designed for radioiodinating Fab' molecules. When radioiodinating F(ab')$_2$ fragments by the CT/SMB method, up to 40% of the product may be Fab' molecules. Radiolabels of < 10 mCi are performed "manually" and the length of time that antibody is exposed to SMB is ~ 30 sec. In these preparations, Fab' molecules were not present. However, radiolabels involving 50 mCi or more are performed in the remote mode. As a result, the amount of Fab' molecules in the final preparation may be significant since the antibody is exposed to SMB for at least 3 min. Therefore, degradation is a function of the length of time the antibody is exposed to the reducing agent, SMB, since the concentration of SMB is approximately the same for all iodinations (data not shown). Since a reducing agent is not used in the iodogen reaction, generation of Fab' fragments is virtually absent (Table 1). Consequently, the percentage of radiolabeled antibody in the Fab' state is significantly less ($p < 0.001$) than that of the iodogen radioiodinated product.

Unlike gel filtration columns, ion exchange resins provide a quick and efficient means of removing free ^{131}I. In these studies, increasing the amount of time that the reaction mixture is exposed to anion exchange resin from 4 to 8 min resulted in a decrease in free ^{131}I. We have also found that the ion exchange resin can effectively remove free iodine from products stored for 24–48 hr containing an excess of free ^{131}I. However, immunoreactivity and sample sterility should also be analyzed to insure that the sample is still within quality assurance guidelines prior to its use.

An interesting observation from these trials is that the immunoreactivity of samples with up to 20%–30% NP-4 Fab' was not significantly less than products with little or no Fab'. Thus, if a quality assurance assay is designed to characterize only the immunoreactivity and amount of unbound radionuclide, products may be prepared with varying amounts of F(ab')$_2$.

Human serum albumin should not be used as a rinsing reagent in the iodogen procedure, since electrophilic iodine (I^+) will react with it to form a significant fraction of radiolabeled HSA aggregate. This finding was not reported by Weadock et al. (*9*) for a similar, less refined system. Our current system has several important modifications; HSA is not used as a rinse, the filter is housed in a lead syringe shield, the reaction

vial contains a stir rod and is placed on a stir plate, and the anion exchange resin is injected into the reaction vial (instead of a separate vial for the anion exchange reaction). Shielding is increased in this version and exposure is reduced even further. Several trials with dyed water should be performed before working with radioactive reagents. The system is currently being used in clinical radioimmunotherapy studies at our institution.

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