High-Specific-Activity $^{111}$In-labeled Anticarcinoembryonic Antigen Monoclonal Antibody: Improved Method for the Synthesis of Diethylenetriaminepentaacetic Acid Conjugates

Raymond J. Paxton, James G. Jakowitz, J. David Beatty, Barbara G. Beatty, William G. Vlahos, Lawrence E. Williams, Brian R. Clark, and John E. Shively

Division of Immunology, Beckman Research Institute of the City of Hope [R. J. P., J. E. S.], Department of General Oncologic Surgery [J. G. J., J. D. B., B. G. B., W. G. V.], Division of Radiological Sciences [L. E. W.], and Division of Pediatrics [B. R. C.], City of Hope National Medical Center, Duarte, California 91010

ABSTRACT

A new method has been developed for conjugating diethylenetriaminepentaacetic acid (DTPA) to proteins using the N-hydroxysuccinimide active ester of DTPA. The DTPA-active ester was prepared using disocpropylcarbodiimide in a simple single-step synthesis. DTPA-conjugated proteins were prepared by adding the DTPA-active ester reaction mixture to protein solutions (5 mg/ml) buffered at pH 7.0 and purified by Sephadex G-50 chromatography. A monoclonal antibody directed against carcinoembryonic antigen was reacted with four different amounts of the DTPA-active ester. Solid-phase enzyme immunoassay showed that the immunological activity of the antibody conjugate was not altered when the active ester:antibody molar ratio was 36:1 or 72:1; however, it decreased when the ratio was 180:1 or 360:1. The antibody heavy and light chains had slightly decreased electrophoretic mobilities when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a result consistent with the covalent attachment of DTPA to the protein. Sephadex G-200 chromatography showed that the native and conjugated antibodies were the same size. When the DTPA-conjugated antibody was incubated with 10, 50, and 100 µCi of $^{111}$In/µg of protein, specific activities of 9.8, 43.1, and 56.3 µCi/µg were obtained. Enzyme immunoassay and radiolmunoassay of the $^{111}$In-labeled antibody showed that it retained its full immunological activity. The high specific activity of the $^{111}$In-labeled antibody makes it suitable for imaging carcinoembryonic antigen-bearing tumors using low doses of antibody.

INTRODUCTION

In vivo detection of tumors can be accomplished with a variety of radiolabeled antibodies. Early studies utilized antibodies against CEA$^2$ radiolabeled with $^{131}$I (1, 2). Although antibodies can be labeled to high specific activities (>10 mCi/mg) by radioiodination with $^{131}$I, the long half-life (8 days), undesirable $\beta$-radiation, and loss of the label from the antibody by tissue dehalogenation are disadvantages of the method. In nude mouse xenograft studies using $^{131}$I-labeled monoclonal antibodies to CEA Haskell et al. (3) and Hedin et al. (4) demonstrated tumor localization with little liver and spleen uptake. The penetration of the antibody into tumors and therefore the tumor to blood ratios were improved by the use of F(ab) or F(ab')$_2$ antibody fragments (5, 6). The use of $^{131}$I-labeled anti-CEA polyclonal antibodies has required sophisticated background subtraction techniques in order to reliably detect tumors in humans (7, 8). In a human study by Mach et al. (9) the use of monoclonal antibodies (to another colorectal antigen) or their fragments gave equivalent results and also required background subtraction.

The use of $^{111}$In as a label has the advantages that it can be linked irreversibly to proteins via a bifunctional chelating agent such as derivatives of DTPA, has more favorable emission probability than $^{131}$I, does not emit $\beta$-radiation, and has a short half-life (67 h). Progress in the use of $^{111}$In-labeling has depended upon the development of appropriate bifunctional chelating agents. A commonly used reagent is the symmetrical bicyclic anhydride of DTPA prepared according to Hnatowich et al. (10-12) and Layne et al. (13). Anti-CEA labeled with $^{111}$In by this method gave substantial spleen and liver uptake in addition to the expected tumor uptake in the nude mouse system (10). A similar result was reported by Halpern et al. (14) utilizing a mixed anhydride reagent for conjugating DTPA to anti-CEA. The accumulation of the radiolabeled antibody in the liver and spleen has not been explained but it is potentially due to the modification of the antibody by the conjugating reagents. Paik et al. (15, 16) showed that both the symmetrical bicyclic anhydride of DTPA and the mixed anhydride of DTPA led to decreased antibody activity when large reagent to protein ratios were used. When increasing amounts of the symmetrical bicyclic anhydride were reacted with antibody, increasing amounts of cross-linked protein species were observed (15). At the suggested lower ratios of anhydride to protein the specific activities achieved with $^{111}$In-labeling were low (1-4 µCi/µg).

Initial work in our laboratory confirmed the problems associated with the mixed anhydride and symmetrical bicyclic anhydride reagents. An alternate method of conjugating DTPA to proteins involves activation of only one carboxylic acid group per DTPA molecule using the $N$-hydroxysuccinimide active ester. While this study was in progress, a preliminary account of a similar method was described by Buckley and Searle (17). In this report, we present an improved protocol for conjugating DTPA to proteins. Antibody conjugates prepared using this method had higher specific activities when labeled with $^{111}$In than was previously reported and retained full immunological activity. The

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2 To whom requests for reprints should be addressed.

The abbreviations used are: CEA, carcinoembryonic antigen; MAB, monoclonal antibody; DTPA, diethylenetriaminepentaacetic acid; BSA, bovine serum albumin; DTPA-OSU, N-hydroxysuccinimide ester of diethylenetriaminepentaacetic acid; anti-CEA MAB-DTPA, DTPA-conjugated anti-CEA monoclonal antibody; BSA-DTPA, DTPA-conjugated BSA; ELISA, enzyme-linked immunosorbent assay; anti-CEA MAB-DTPA-$^{111}$In, DTPA-conjugated anti-CEA monoclonal antibody labeled with $^{111}$In; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Received 3/15/85; accepted 7/12/85.
**111In-Labeled Anti-CEA MAB Synthesis**

**Use of the 111In-labeled antibody in a nude mouse system is described in the companion paper (18).**

**Materials and Methods**

Reagents. The following reagents were used: DTPA, diisopropylcarbodiimide, and triethylamine (Aldrich Chemical Co., Milwaukee, WI); N-hydroxysuccinimide, BSA, Sephadex G-50 and G-200, and protein A-Sepharose CL-4B (Sigma Chemical Co., St. Louis, MO); sodium acetate (puratronic grade) and acetic acid (ultrapure) (Alfa Products, Danvers, MA). Acetonitrile (high performance liquid chromatography grade; J. T. Baker Co., Phillipsburg, NJ) was dried over molecular sieves and triethylamine was distilled from ninhydrin and calcium hydride before they were used.

The previously described anti-CEA monoclonal antibody (19, 20), clone TB4.66 A3.1 H11, was purified from mouse ascites fluid by protein A-Sepharose chromatography (21). Anti-cytochrome P-450 monoclonal antibody was obtained from Wayne Levin, Hoffmann-La Roche Inc., Nutley, NJ. 111InCl3 (research grade, 5 mcCi/0.1 ml in dilute HCl) was a gift from Mediphysics, Inc. (Emeryville, CA).

Synthesis of DTPA-N-Hydroxysuccinimide Ester. DTPA (12 mg, 0.0305 mmol) and triethylamine (0.017 ml, 0.122 mmol) were dissolved in 0.20 ml acetonitrile by stirring at 50°C for 1.5 h. After cooling the solution to 25°C, N-hydroxysuccinimide (1.76 mg, 0.0153 mmol) and diisopropylcarbodiimide (0.0024 ml, 0.0153 mmol) were added and the reaction was stirred for 1.5 h. The theoretical concentration of active ester is 0.06 M. The reaction mixture was used directly to prepare DTPA-conjugated proteins.

Synthesis of DTPA-conjugated Proteins. Lyophilized anti-CEA monoclonal antibody was dissolved in 0.10 M NaHCO3:0.20 M EDTA, pH 7.0 at a concentration of 5 mg/ml. DTPA-conjugated anti-CEA MAB was prepared by adding 1, 2, 5, or 10 µl of the DTPA-OSU reaction mixture to 50 µl of the protein solution and mixing every 20 min for 1 h. The anti-CEA MAB-DTPA conjugate was separated from free DTPA and other reaction by-products using either a 0.7-x 30-cm Sephadex G-50-fine column or a 0.7-x 50-cm Sephadex G-200 column equilibrated with 0.10 M sodium acetate, pH 5.6. BSA-DTPA and anti-cytochrome P-450 MAB-DTPA conjugates were prepared using the same methods.

Charging of DTPA-conjugated Proteins with 111In. An equal volume of 1.0 M sodium acetate, pH 6.0 was added to 111InCl3 (6 mcCi/0.1 ml). Aliquots of this mixture (10, 100, or 500 µCi) were incubated with 10 µg (0.2 mg/ml) of the anti-CEA MAB-DTPA for 2–16 h. EDTA (final concentration, 1 mw) was then added to complex any free 111In. Thirty min after adding EDTA, the efficiency of charging was examined by chromatography on a 0.7-x 30-cm Sephadex G-50-fine column equilibrated with 0.10 M sodium acetate, pH 5.0. BSA-DTPA and anti-cytochrome P-450 MAB-DTPA conjugates were charged with 111In using the same method.

Stability Testing. Anti-CEA MAB-DTPA (200 µg) was charged with 111In (10 µCi/µg of antibody) as described previously. EDTA was added to complex any free 111In; then the 111In-labeled antibody (100 µg) was added to 9.5 ml of fresh human plasma and incubated at 37°C. At 0.5, 5, 24, 48, and 72 h, 1-ml aliquots were removed and analyzed by protein A-Sepharose chromatography (21). The nonbound protein was washed from the column with 0.1 M sodium phosphate, pH 8, and IgG was eluted from the column with 0.1 M sodium citrate, pH 6 and 3.5. The column fractions from the different times were saved and counted after sufficient radioactive decay.

ELISA. Enzyme immunoassay microtiter plates (Costar, Cambridge, MA) were coated with CEA at 5 µg/ml, washed, and incubated with 1% BSA to block any remaining binding sites. The plates were incubated with serial dilutions of anti-CEA MAB, washed five times, incubated with goat anti-mouse IgG-alkaline phosphatase conjugate (Cappel Laboratories, Malvern, PA), washed five times, incubated with p-nitrophenylphosphate (Sigma), and the absorbance of each well was measured at 405 nm on a Dynatek MR 580 enzyme immunoassay reader linked to an Apple II Plus computer. Quantitation of anti-CEA activity was performed according to Beatty et al. (22). ELISA of anti-CEA MAB-DTPA and anti-CEA MAB-DTPA-111In were performed in the same fashion.

Electrophoresis. DTPA-conjugated proteins were analyzed by SDS-polyacrylamide gel electrophoresis, as described by Laemmli (23). Samples were boiled in a dissociation buffer (0.0625 M Tris-HCl, pH 7.0, 6% SDS, 20% glycerol, and 5% 2-mercaptoethanol) for 5 min and applied to 10% acrylamide slab gels. Proteins were visualized with Coomassie blue stain. DTPA-conjugated proteins labeled with 111In were analyzed using the same method except that 1 mw diethytherol replaced the 2-mercaptoethanol in the dissociation buffer and the samples were boiled for 3 min. 111In-labeled proteins were visualized by autoradiography with Kodak X-OMAT AR film (Eastman Kodak Co., Rochester, NY).

**Mass Spectrometry.** The mass spectra of the DTPA-OSU reaction mixture were obtained using a JEOL JMS-HX100 mass spectrometer equipped with a fast atom bombardment ion source. Samples were dissolved in glycerol and applied to a 2- x 5-mm stainless steel sample stage. Spectra were acquired on a JEOL DAS5000 data system.

**Results**

Preparation of BSA-DTPA and Anti-CEA MAB-DTPA Conjugates. The N-hydroxysuccinimide active ester of DTPA was prepared by a reaction commonly used in peptide synthesis. The DTPA-OSU active ester was not isolated; however, the reaction mixture was examined by mass spectrometry. Fast atom bombardment mass spectrometry (negative ion mode) of the reaction mixture showed the expected mass:charge ratio of 489.

The reaction of DTPA-OSU with proteins is expected to produce an amide bond between the ε-amino group of lysine residues and the activated carboxyl group of DTPA. The modification of proteins with this large highly charged molecule might alter the electrophoretic mobility of the proteins in SDS-PAGE gels. To investigate this possibility, the test protein BSA was reacted with 1, 2, 5, and 10 µl of DTPA-OSU, giving reagent:protein molar ratios (theoretical) of 16, 32, 80, and 160, respectively, and the reaction mixtures were analyzed by SDS-PAGE. The electrophoretic mobility of BSA decreased as the amount of DTPA-OSU was increased (Fig. 1A). A minor protein band with an apparent molecular weight of 97,000 was detected when
BSA was reacted with 10 μl of DTPA-OSU. The nature of this band is not known; however, it possibly represents a uniquely modified form of BSA which migrates anomalously in SDS-PAGE gels (see "Discussion").

Anti-CEA MAB was also reacted with 1, 2, 5, and 10 μl of DTPA-OSU (theoretical reagent: protein molar ratios of 36, 72, 180, and 360, respectively), and the reaction mixtures were analyzed by SDS-PAGE. The electrophoretic mobility of both the antibody heavy (M, 50,000) and light (M, 30,000) chains decreased as the amount of DTPA-OSU was increased (Fig. 1B). The higher molecular weight forms of the light chain migrated as discrete species easily distinguishable from the native light chain. Minor protein bands with approximate molecular weights of 80,000, 120,000, and 200,000 were detected when the antibody was reacted with 10 μl DTPA-OSU and may represent cross-linked heavy and light chains (see "Discussion").

Purification and Characterization of Anti-CEA MAB-DTPA Conjugates. Anti-CEA MAB-DTPA conjugates were separated from free DTPA and other reaction by-products by Sephadex G-50 chromatography (Chart 1). Slow flow rates (<2 ml/h) improved resolution of the two peaks and prevented free DTPA from contaminating the antibody conjugates. The use of sodium acetate buffer allowed the anti-CEA conjugates to be charged with 111In immediately after chromatography (see below).

Anti-CEA conjugates were also purified by Sephadex G-200 chromatography. Chart 2 shows that the anti-CEA conjugate eluted from the column in exactly the same volume as did the unmodified anti-CEA MAB. This result suggested that the size of the native and conjugated antibodies were the same, a result consistent with the SDS-PAGE analysis.

Purified anti-CEA MAB-DTPA conjugates were tested for immunological activity using a solid-phase enzyme immunoassay. The ELISA results (Table 1) show that the anti-CEA conjugates prepared with 1 or 2 μl of DTPA-OSU retained 100% of their immunological activity, whereas the conjugates prepared with 5 and 10 μl of DTPA-OSU showed a decrease in immunological activity. All subsequent experiments were done with anti-CEA MAB-DTPA conjugates prepared with 2 μl of DTPA-OSU (theoretical reagent:protein molar ratio of 72:1).

Charging of BSA-DTPA and Anti-CEA MAB-DTPA Conjugates with 111In. BSA-DTPA conjugates (10 ng) were incubated with either 500 or 1000 μCi of 111In. EDTA was added to complex any free 111In and the efficiency of charging was tested by Sephadex G-50 chromatography. Table 2 shows that the specific activity of BSA increased as the amount of DTPA-OSU increased. Anti-CEA MAB-DTPA conjugates prepared with 2 μl of DTPA-OSU were incubated with 111In. EDTA was added to complex any free 111In and the efficiency of charging was tested by Sephadex G-50 chromatography. In the example shown in Chart 3, 89% of the 111In eluted with the protein peak. When unconjugated anti-CEA MAB was charged with 111In, 0% of the 111In eluted with the protein peak (data not shown). Table 3 contains
**Table 2**

Charging of BSA-DTPA conjugates with $^{111}$In

BSA-DTPA conjugates were prepared by adding the indicated amounts of DTPA-OSU (0.06 M) to a constant amount of BSA (50 µl, 5 mg/ml). After purification by gel chromatography, BSA-DTPA conjugates (10 µg) were incubated overnight with either 500 or 1000 µCi of $^{111}$In. EDTA was then added to complex free $^{111}$In and the $^{111}$In-labeled conjugates were purified by gel chromatography.

<table>
<thead>
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<th>DTPA-OSU (µl) used to prepare conjugate</th>
<th>Charging ratio (µCi/µg)</th>
<th>Specific activity (µCi/µg)</th>
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</thead>
<tbody>
<tr>
<td>1 (16)</td>
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</tr>
<tr>
<td>2 (32)</td>
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<td>85.1</td>
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<tr>
<td>10 (160)</td>
<td>100</td>
<td>92.7</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, reagent:protein molar ratios (theoretical).

the results obtained when anti-CEA MAB-DTPA was charged with increasing amounts of $^{111}$In. The efficiency of charging was 98 and 86% at 10 and 50 µCi/µg of protein, respectively. The charging efficiency at 100 µCi/µg of protein dropped to 56% implying that the anti-CEA conjugate was approaching saturation with $^{111}$In.

$^{111}$In-labeled anti-CEA MAB-DTPA conjugates were analyzed by solid-phase enzyme immunoassay and radioimmunoassay (Chart 4). When the results of the assay systems were compared at two levels of charging with $^{111}$In equivalent results were obtained. The curves were parallel when comparing unjugated versus DTPA-conjugated antibody in the EIA. This result suggested that the DTPA-conjugation had little effect on antibody activity.

Several attempts were made to analyze $^{111}$In-labeled anti-CEA conjugates by SDS-PAGE. If standard conditions were used, no radioactivity was associated with the antibody heavy and light chains. When 1 mM dithiothreitol was used in place of 5% (0.71 M) 2-mercaptoethanol in the sample dissociation buffer, both the heavy (M, 50,000) and light chains (M, 30,000) were radioactively labeled (Fig. 2).

Stability of $^{111}$In-labeled Anti-CEA MAB Conjugates. To measure the stability of the anti-CEA MAB-DTPA-$^{111}$In complex, the labeled antibody was incubated with fresh human plasma and analyzed by protein A-Sepharose chromatography. The amount of $^{111}$In-labeled antibody bound to the column decreased from 99% after 0.5 h of incubation to 75% after 72 h of incubation (Table 4). The majority of $^{111}$In-labeled antibody eluted at pH 6.
**In-Labeled Anti-CEA MAB Synthesis**

**Fig. 2. SDS-polyacrylamide gel electrophoresis of **\(^{111}\)In-labeled antibody. Anti-CEA MAB-DTPA (1 ng) was charged overnight with \(^{111}\)In (1 µCi) and analyzed by SDS-PAGE as described in "Materials and Methods." H, heavy chain; L, light chain.

(Fraction B), consistent with its being an IgG1. When the pH 3.5 eluent (Fraction C) was included in the calculations, the amount of labeled antibody retained on the column was only slightly higher. The experiment described by Table 4 was repeated, except that the free \(^{111}\)In (EDTA-complex) was first removed by gel filtration chromatography. The decrease in \(^{111}\)In-labeled antibody bound to the column was similar, from 93% at 0.5 h to 80% at 72 h.

**DISCUSSION**

Several methods are currently available for the preparation of DTPA-conjugated antibodies. The symmetrical bicyclic anhydride of DTPA is easy to use, but because it is a bifunctional reagent, the potential for antibody cross-linking is present (15). The DTPA-mixed anhydride reagent has been used successfully; however, several parameters must be carefully controlled to conjugate efficiently the antibody with DTPA while retaining antigen-binding activity (16).

Problems with both of these methods prompted us to develop a new method for conjugating DTPA to proteins. The stoichiometry of reagents used in preparing the DTPA-OSU active ester (DTPA:N-hydroxysuccinimide:diisopropylcarbodiimide, 2:1:1) was chosen so as to minimize the potential for producing a di-activated species of DTPA. The mass spectral results indicated that only a mono-activated species of DTPA was present in the reaction mixture. While this study was in progress, the synthesis of the same reagent was reported by other investigators using different reaction conditions (17).

Initially BSA was used to test the effectiveness of the reagent. The change in mobility of BSA on SDS-PAGE gels clearly showed that increasing amounts of DTPA-OSU caused an increased modification of the protein (Fig. 1A). The decreased electrophoretic mobility of the modified protein was in agreement with other studies (24) which showed that treatment of proteins with succinic and maleic anhydrides caused a similar decrease in electrophoretic mobility. At the highest reagent:protein ratio tested, a trace amount of a new protein species (M, ~ 97,000) was observed. This molecular weight is clearly less than that expected for a BSA dimer (M, ~ 136,000) and most probably represents a specific modification of BSA distinct from the majority of the protein. One possibility is that an intramolecular cross-link has occurred due to the formation of a di-activated species of DTPA-OSU. We have reduced this probability by utilizing an N-hydroxysuccinimide:DTPA ratio of 0.5:1.0. After being purified by Sephadex G-50 chromatography, the BSA-DTPA conjugates were charged with \(^{111}\)In. The results of Table 2 show that the increase in specific activity of the \(^{111}\)In-labeled BSA-DTPA conjugates paralleled the increase in the amount of DTPA-OSU used to prepare the conjugates. Therefore it can be concluded that the reagent does produce a covalent attachment of DTPA to the protein. The increase in specific activity also paralleled the decrease in electrophoretic mobility of the BSA-DTPA conjugates (Fig. 1A). This further suggests that the decreased electrophoretic mobility was due to the covalent attachment of DTPA to the protein.

Anti-CEA MAB-DTPA conjugates were prepared using the same method. The SDS-PAGE results for the antibody conju-
gates (Fig. 1B) were similar to those for the BSA conjugate (Fig. 1A). The antibody heavy (M, 50,000) and light (M, 30,000) chains showed a decrease in electrophoretic mobility as the amount of DTPA-OSU increased. Trace amounts of higher molecular weight species were present at the highest reagent:protein molar ratio. Species consistent with the observed molecular weights are HL, H2, and H2L2 complexes (H, heavy chain; L, light chain). Since the two heavy and two light chains are in close contact in the IgG molecule, cross-linking between heavy and light chains could have occurred if a di-activated species of DTPA-OSU were present.

After purification, the antibody conjugates were tested for immunological (antigen-binding) activity. Table 1 shows that 2 μl of DTPA-OSU could be used without decreasing the immunological activity of the antibody. Theoretically this amount gives a DTPA-OSU:antibody molar ratio of 72:1. The actual ratio may be less than this due to incomplete formation and/or hydrolysis of the reagent. In comparing the results of Fig. 1B with Table 1, it can be seen that the loss of immunological activity with 5 and 10 μl of DTPA-OSU was accompanied by an increase in the amount of protein modification. With 2 μl of DTPA-OSU, there was only a slight amount of protein modification and the higher molecular weight species were not observed.

Anti-CEA MAB-DTPA conjugates were charged with 111In and analyzed by gel filtration chromatography. A specific activity of 43.1 μCi/μg was obtained at a charging ratio of 50 μCi/μg. This specific activity was substantially higher than those reported previously (10, 11, 14, 17). We have also tested the immunological activity of 111In-labeled antibody conjugates. Chart 4 shows that 111In-labeled conjugates had antigen-binding properties similar to either conjugated or unconjugated antibody. Therefore the presence of 111In had no measurable effect on the antibody activity. The stability of the anti-CEA MAB-DTPA-111In complex was tested by incubating it with fresh human plasma. The amount of labeled antibody bound to protein A-Sepharose decreased from 95 to 75% after a 72-h incubation. Using a DTPA-conjugated antibody prepared with the mixed anhydride of DTPA, Halpern et al. (14) reported an 89 to 73% decrease in the immunoreactivity of the antibody after incubating in human serum for 72 h. Although the methods used to test the stability of the antibody-DTPA-111In complex were different, the results of the two experiments were similar. These results provide further evidence that the DTPA-OSU active ester produces a stable covalent attachment of DTPA of proteins.

In conclusion, we have developed and systematically tested a new method for synthesis of DTPA-conjugated proteins. The method is both effective and reproducible in producing high specific activity antibodies that retain 100% of their immunological activity. The higher specific activities obtainable with this method should be clinically useful. By using a smaller dose of antibody, the potential for an immunogenic response is lessened.

REFERENCES

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