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Bismuth-212-labeled anti-Tac monoclonal antibody: α-Particle-emitting radionuclides as modalities for radioimmunotherapy

(interleukin 2 receptors/adult T-cell leukemia/diethylenetriaminepentaacetic acid chelation)

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ABSTRACT **Anti-Tac, a monoclonal antibody directed to the human interleukin 2 (IL-2) receptor, has been successfully conjugated to the α-particle-emitting radionuclide bismuth-212 by use of a bifunctional ligand, the isobutylcarboxycarbonic anhydride of diethylenetriaminepentaacetic acid. The physical properties of ^{212}Bi are appropriate for radioimmunotherapy in that it has a short half-life, deposits its high energy over a short distance, and can be obtained in large quantities from a radium generator. Antibody specific activities of 1–40 μCi/μg (1 Ci = 37 GBq) were achieved. Specificity of the ^{212}Bi-labeled anti-Tac was demonstrated for the IL-2 receptor-positive adult T-cell leukemia line HUT-102B2 by protein synthesis inhibition and clonogenic assays. Activity levels of 0.5 μCi or the equivalent of 12 rad/ml of α radiation targeted by anti-Tac eliminated >98% the proliferative capabilities of HUT-102B2 cells with more modest effects on IL-2 receptor-negative cell lines. Specific cytotoxicity was blocked by excess unlabeled anti-Tac but not by human IgG. In addition, an irrelevant control monoclonal antibody of the same isotype labeled with ^{212}Bi was unable to target α radiation to cell lines. Therefore, ^{212}Bi-labeled anti-Tac is a potentially effective and specific immunocytotoxic reagent for the elimination of IL-2 receptor-positive cells. These experiments thus provide the scientific basis for use of α-particle-emitting radionuclides in immunotherapy.**

Anti-Tac is a murine monoclonal antibody directed to the human interleukin 2 (IL-2) receptor (1). T cells express detectable IL-2 receptors 6–12 hr after activation with antigen or mitogen (2). While mitogen-activated T cells have been shown to express 30,000–60,000 receptor sites per cell, leukemic T-cell lines from patients with adult T-cell leukemia express 10 times more receptor sites per cell (3). Adult T-cell leukemia is an aggressive almost uniformly fatal disease in which leukemic T cells harbor the human type C retrovirus, human T-cell lymphotropic virus (HTLV-I) (4, 5). The rationale for using anti-Tac as a vehicle to deliver cytotoxic reagents to leukemic cells in these patients is based on the observation that these leukemic T cells, but not normal resting or precursor T cells, express IL-2 receptors. In addition, IL-2 receptors are not expressed by nonlymphoid tissues examined.

Anti-Tac alone is unable to inhibit the proliferation or protein synthesis of most leukemic T-cell lines (6). However, one of three patients with adult T-cell leukemia treated with anti-Tac showed two remission periods following two courses of therapy (7). A more effective treatment might be achieved by conjugating anti-Tac with toxins or radionuclides. Efforts to conjugate toxins to anti-Tac have demonstrated *in vitro* cytotoxic specificity. *Pseudomonas* exotoxin (8) and the A chain of the toxin ricin (6) have been conjugated to anti-Tac. Both reagents selectively inhibit protein synthesis in IL-2 receptor-positive cell lines but not in receptor-negative lines. The effectiveness of these toxins, however, relies on their ability to be internalized by the cell and released into the cytoplasm (9).

Anti-Tac bound to IL-2 receptors on leukemic T cells is both poorly and slowly internalized into endosomes (8). Furthermore, most of the toxin conjugate of anti-Tac does not pass easily from the endosome to the cytosol, as required for its action on elongation factor II. Thus, it was of value to develop an alternative cytotoxic reagent to conjugate to anti-Tac that would be effective while bound to the surface of the leukemic cells. Bismuth-212, an α-particle-emitting radionuclide, is well suited for this role. α-Particle-emitting radionuclides are very effective cyotoxic reagents in that they release their high energy (≈8 million eV; 1 eV = 1.602×10^{-19} J) over a very short distance (40–80 μm). This low penetrability necessitates the targeting by monoclonal antibody. The densely ionizing radiation requires only one or two particles to hit the nucleus of a cell for inactivation. ^{212}Bi is a decay daughter of thorium-228 with a 1-hr half-life, which makes it appropriate for rapid targeting to leukemic cells without prolonged radiation exposure to normal tissue.

In this study, anti-Tac was conjugated with ^{212}Bi by use of a carboxycarbonic anhydride of diethylenetriaminepentaacetic acid. Selective cytotoxicity of an IL-2 receptor-positive T-cell line, but not receptor negative lines, was demonstrated by using protein synthesis inhibition and clonogenic assays. Specific cytotoxicity was inhibited by the addition of excess unlabeled anti-Tac. In contrast, a control monoclonal antibody of the same isotype labeled with ^{212}Bi was unable to specifically target α radiation to these cell lines.

MATERIALS AND METHODS

Cell Lines. The IL-2 receptor expressing HUT-102B2 and the receptor-negative lines MOLT-4, $1D_{11}$, and U-937 were used in these studies. HUT-102B2 is a cloned T-cell line derived from a lymph node of a patient with adult T-cell leukemia and harbors the type C retrovirus HTLV-I (10). These cells express $2–4 \times 10^5$ IL-2 receptors identified by the anti-Tac antibody per cell (3). MOLT-4 is an acute lymphocytic leukemia T-cell line. ID_{11} is an Epstein–Barr virus-transformed B-cell line established from a normal donor by transformation with the Epstein–Barr virus strain B95-8. U-937 is a monocyte-like line from a patient with diffuse histiocytic lymphoma. MOLT-4, ID_{11}, and U-937

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Abbreviations: IL-2, interleukin 2; DTPA, diethylenetriaminepentaacetic acid; ^{212}Bi–anti-Tac, ^{212}Bi-labeled DTPA–anti-Tac complex.

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were demonstrated to be IL-2 receptor negative by a ^{3}H-labeled anti-Tac binding assay. These data were also confirmed by indirect fluorescent flow cytometry.

Antibodies. The monoclonal antibody anti-Tac is a murine IgG_{2a} antibody that is directed to the human IL-2 receptor. Anti-Tac was purified either by protein-A Sepharose (11) or by DEAE-52 cellulose (Whatman) followed by sodium sulfate precipitation. RPC-5 is a control IgG_{2a}-purified monoclonal antibody obtained from Litton Bionetics. It does not bind specifically to T cells, B cells, or monocytes. Human IgG was purchased from Armour (Kankakee, IL).

^{212}Bi Labeling of the Anti-Tac Monoclonal Antibody. Labeling protocols are described in detail elsewhere (12). Briefly, a modification of the method of Krejcarek and Tucker (13) was used to link diethylenetriaminepentaacetic acid (DTPA) to anti-Tac, with ^{14}C-labeled DTPA used to quantify chelate/antibody ratio. DTPA (0.2 mM) was dissolved in 2 ml of H_2O by addition of triethylamine (1.38 mM) and lyophilized. The solid formed was taken up in 1 ml of acetonitrile at 4°C and treated with isobutylchloroformate (0.27 mM) for 30 min, centrifuged, and a 20-μl aliquot of isobutylcarboxycarbonic anhydride solution reacted with anti-Tac at 4°C for 1.5 hr (255 μl, 10.3 mg/ml in 50 mM $NaHCO_3$, pH 8.0). Sequential dialyses in metal-free buffer were used to purify protein. The first dialysis solution was 50 mM citrate, pH 5.5/0.15 M NaCl/0.1 mM ascorbic acid; the two subsequent dialyses were the same except the ascorbic acid was omitted; the final dialysis solution was 0.1 M phosphate-buffered saline (pH 7.4). About 2 ml of chelex ion exchange resin (Bio-Rad) was added to all citrate dialyses. Quantification of chelate-linked anti-Tac by scintillation counting and colorimetric assay (Bio-Rad) showed a protein concentration of 5.8 mg/ml with about three chelates per antibody molecule. For labeling with ^{212}Bi, DTPA–anti-Tac was dialyzed 2–4 hr into 20 mM 2-(*N*-morpholino)ethanesulfonic acid sodium salt (pH 6.0) (Calbiochem–Behring) just prior to use.

The radionuclide generator used for ^{212}Bi production is a modification of one described elsewhere (14). The generator was eluted with 0.75 ml of 2 M HCl to produce ^{212}Pb–Bi, which was evaporated to dryness by vacuum centrifugation (Savant), redissolved in 250 μl of 0.1 M HCl, and ^{212}Pb was quantitatively absorbed on a 1 × 2.5 mm column of AG-50WX4 (200–400 mesh) H^+ form resin (Bio-Rad) and rinsed with 2 ml of deionized water. After 2 hr, ^{212}Bi was eluted with 0.15 ml of 0.1 M HI.

For protein incorporation, the ^{212}Bi/HI solution (≈3 mCi; 1 Ci = 37 GBq) was reduced to 50 μl and brought to pH 4–4.5 with 0.3 M phosphate/0.10 M acetate, pH 6.9, and immediately underwent reaction for 15 min with 30 μg of anti-Tac. The resulting ^{212}Bi-labeled anti-Tac was purified by high-performance liquid chromatography on a TSK-3000 column with 0.1 M phosphate-buffered saline eluant. Protein specific activity is, at maximum, 40 μCi/μg.

^{3}H-Labeled Anti-Tac Competitive Binding Assay. The monoclonal antibody anti-Tac was tested after each labeling for alterations in binding capacity to HUT-102B2, the IL-2 receptor-positive cell line. Binding integrity was evaluated by comparison of modified and untreated anti-Tac in their ability to compete with a nonsaturating (5.5 ng) amount of ^{3}H-labeled anti-Tac for binding to 10^6 HUT-102B2 cells. This competitive binding assay has been described (2).

Protein Synthesis Assayed by [^{3}H]Leucine Incorporation. Cytotoxicity of ^{212}Bi-conjugated anti-Tac was evaluated by measuring protein synthesis 48 hr after treatment of 2×10^5 cells per ml per well with various doses of radioactivity. The cells were in a total vol of 1 ml in 24-well plates (Costar, Cambridge, MA). The dose of ^{212}Bi added was calculated as the dose delivered per 1 ml of medium and is not a microdosimetric calculation. After treatment, 200-μl aliquots were set up in triplicate in 96-well round-bottom plates (Costar, Cambridge, MA). Cells were washed three times with 0.15 M phosphate-buffered saline by centrifugation and aspiration of supernatants. Cells were then resuspended in 100 μl of leucine-free RPMI 1640 medium (GIBCO) supplemented with 5% dialyzed fetal calf serum. Cells were pulsed for 4 hr with 0.5 μCi of [^{3}H]leucine per well (New England Nuclear), harvested on a Skatron (Sterling, VA) Mash unit, and counted in a liquid scintillation counter. Cycloheximide (50 μM) was used to establish background noninhibitable levels of [^{3}H]leucine incorporation, which was subtracted from the experimental values. Resulting values were then expressed as a percentage of the [^{3}H]leucine incorporated into the medium control cell lines that were not treated with ^{212}Bi conjugates.

Limiting Dilution Analysis. Cell survival after treatment with ^{212}Bi conjugated to anti-Tac was also evaluated by testing reproductive integrity by a clonogenic assay. Limiting dilution assay methods have been described in detail (15). Briefly, 18 hr after treatment with ^{212}Bi-labeled anti-Tac, cells (5×10^5 cells per ml) were diluted and plated in 96-well flat-bottom plates (Costar) at concentrations ranging from 10,000 to 0.6 cells per well. Twenty-four replicate wells were set up for each dilution. After 2 weeks, each well was scored positive or negative for growth based on microscopic examination of colonies of >20 cells. Based on the Poisson distribution, $P = e^{-fa}$, the aliquot of cells (a) that results in 37% negative cultures (P) is equal to the frequency (f) of responding units in that sample (15).

RESULTS

Characterization of the Modified Anti-Tac Monoclonal Antibody. Chelate linkage and ^{212}Bi-labeling of the anti-Tac monoclonal antibody did not significantly compromise binding integrity to IL-2 receptor-positive cells. The monoclonal antibody anti-Tac was chelated with the bifunctional mixed anhydride of DTPA resulting in approximately three chelates bound per antibody, as determined by ^{14}C-labeled chelates in parallel experiments. Radiolabeling the DTPA–anti-Tac complex with 2–30 μCi of ^{212}Bi per μg did not significantly alter the binding capacity of anti-Tac monoclonal antibody to IL-2 receptor-positive cells as assessed in competitive binding assays with ^{3}H-labeled anti-Tac. A representative competition binding assay is shown in Fig. 1, in which increasing concentrations of unmodified anti-Tac, or anti-Tac labeled to high or low specific activity with ^{212}Bi, had equal potencies for inhibiting ^{3}H-labeled anti-Tac binding. The binding capacity of modified anti-Tac was tested after each new chelation procedure as well as 24 hr after the labeling of the chelated antibody with ^{212}Bi. Thus, after each modification the binding capability of anti-Tac remained relatively unaffected.

Cytotoxicity of ^{212}Bi-Labeled Anti-Tac. Cytotoxicity of the isotope–conjugate was measured by assaying cell viability, protein synthesis, and cell growth after treatment of cell lines with ^{212}Bi-labeled DTPA–anti-Tac. The ^{212}Bi-labeled DTPA–anti-Tac complex will be referred to hereafter as ^{212}Bi–anti-Tac.

The selectivity of the cytotoxicity achieved by using ^{212}Bi–anti-Tac conjugates was first examined by measuring protein synthesis in IL-2 receptor-positive and -negative cell lines after treatment with radiolabeled anti-Tac. The effect of ^{212}Bi–anti-Tac on [^{3}H]leucine incorporation in cells from three separate experiments, using HUT-102B2 as the IL-2 receptor-positive line with three IL-2 receptor-negative lines, is shown in Fig. 2. α radiation at >0.75–3 rad per well produced by 50 pM to 0.2 nM anti-Tac, from three separate ^{212}Bi antibody-labeling preparations, resulted in >50% inhibition of [^{3}H]leucine incorporation by HUT-102B2 cells. Twenty to 50 times more α radiation was required to inhibit protein

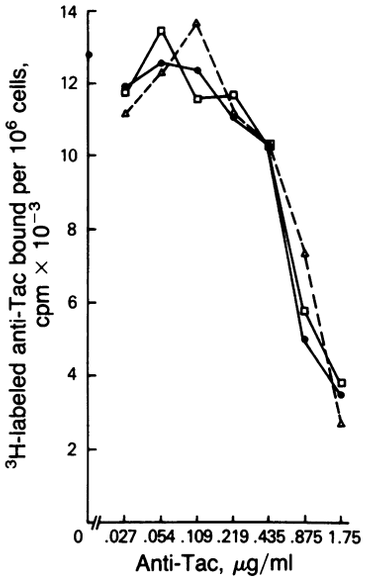

FIG. 1. Binding integrity of ^{212}Bi-conjugated anti-Tac assessed in a competitive binding assay was compared to unlabeled anti-Tac. HUT-102B2 cells (10^6) were incubated with 5.5 ng of ^{3}H-labeled anti-Tac alone or in the presence of increasing concentrations of unlabeled anti-Tac (△), ^{212}Bi-conjugated anti-Tac (2 μCi/μg) (□), or ^{212}Bi-conjugated anti-Tac (30 μCi/μg) (●). Cell-associated ^{3}H-labeled anti-Tac was measured by centrifugation through 1 M sucrose and the pellet was resuspended in 0.1 ml of 0.15 M phosphate-buffered saline and counted in a scintillation counter.

synthesis to the same half-maximum (ID_{50}) level in IL-2 receptor-negative T- and B-cell lines, while the monocyte line U-937 was resistant to nonspecific α cytotoxicity. Specific cytotoxicity of ^{212}Bi-anti-Tac on HUT-102B2 cells was blocked by the presence of excess anti-Tac. In contrast, unlabeled anti-Tac did not alter the nonspecific cytotoxicity observed on IL-2 receptor-negative lines (data not shown). Therefore, the level of nonspecific cytotoxicity does not appear to be due to Fc-mediated binding of ^{212}Bi-anti-Tac to these cell lines. Concentrations of ^{212}Bi-anti-Tac delivering 6–24 rad of activity per ml effectively reduced protein synthesis in HUT-102B2 cells >98%. Thus, ^{212}Bi-anti-Tac can selectively inhibit protein synthesis in IL-2 receptor-positive cells that bind this antibody isotope conjugate to the cell surface.

The relative sensitivity of HUT-102B2 cells to focused α radiation as delivered by ^{212}Bi-conjugated anti-Tac is contrasted to cytotoxicity due to nonspecific α radiation and to γ radiation delivered by a cesium-137 irradiator (Fig. 3). The dose of ^{212}Bi-anti-Tac needed to reduce protein synthesis to 50% of control values was 2 rad, while 45 rad, or 20 times more activity, was needed to nonspecifically reduce protein synthesis to half-maximum when ^{212}Bi-anti-Tac was inhibited from binding to HUT-102B2 cells by the presence of excess unlabeled anti-Tac (200 μg/ml). Furthermore, 400 rad, or an additional 10-fold increase in dose was necessary for an equivalent effect by γ irradiation. These findings are consistent with the view that the relative biological effects of α radiation are 5 to 10 times more potent than γ radiation.

A final control used to demonstrate the specific targeting of ^{212}Bi-anti-Tac to IL-2 receptor-positive cells involved the use of a ^{212}Bi-conjugated, irrelevant isotype-identical (IgG_{2a}) monoclonal antibody called RPC-5 (Fig. 4). The ^{212}Bi-labeled RPC-5 had no selective biological effect on HUT-102B2 cells and was indistinguishable from ^{212}Bi-anti-Tac in its effect on the receptor-negative cell line MOLT-4. These findings provide evidence that the selective cytotoxicity of ^{212}Bi-anti-Tac for the IL-2 receptor-positive cell line is mediated by interaction with the IL-2 receptor rather than through Fc receptor binding. In accord with this conclusion, the addition of human IgG (1 mg/ml) did not inhibit the cytotoxicity of ^{212}Bi-anti-Tac for HUT-102B2 cells (data not shown).

We also examined the radiobiological effects of ^{212}Bi-anti-Tac by measuring the loss of viability and proliferative potential in cells after exposure to the α-particle-emitting anti-Tac conjugate by using a clonogenic assay, which is more sensitive than protein synthesis measurements for determining cell survival. HUT-102B2 and MOLT-4 cells (5 × 10^5 cells per ml) were treated for 18 hr at 37°C with various doses of ^{212}Bi-anti-Tac and then plated in a limiting-dilution assay ranging from 10,000 to 0.6 cells per well. Cell growth was determined after 2 weeks in culture and was evaluated as described in *Material and Methods*. The results are corrected for the cloning efficiency of the nontreated parental lines and are shown in Fig. 5. At a ^{212}Bi-anti-Tac dose of 6 rad, the receptor-negative line was unaffected, while the HUT-102B2 receptor-positive line was reduced by a factor of 50 in its ability to grow in culture. In contrast, MOLT-4 is 10 times more sensitive to γ radiation than is HUT-102B2 (data not shown); therefore, MOLT-4 is a cell line that is sensitive to nonspecific radiation damage. At a ^{212}Bi-anti-Tac dose of 24 rad, the survival fraction of the receptor-negative line was one-half that of untreated MOLT-4 cells, while the survival fraction of the receptor-positive HUT-102B2 line was reduced by a factor of 1000. Less than 1 in 10,000 HUT-102B2 cells survived at a dose of 48 rad. The clonogenic data thus corroborate the protein synthesis data in demonstrating the selectivity of the cytotoxicity of ^{212}Bi-anti-Tac-directed α radiation.

DISCUSSION

The development of radionuclide-conjugated monoclonal antibodies as specific cytocidal reagents for radioim-

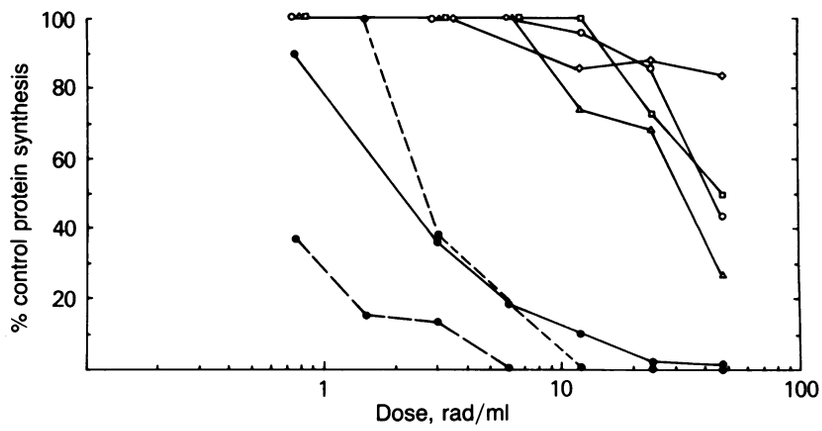

FIG. 2. Cytotoxicity of ^{212}Bi-anti-Tac on HUT-102B2 IL-2 receptor-positive cells compared to receptor-negative cell lines. Increasing concentrations of ^{212}Bi-anti-Tac were added to 2 × 10^5 HUT-102B2 cells (●), HUT-102B2 cells in the presence of excess anti-Tac (○), MOLT-4 (△), $1D_{11}$ Epstein–Barr virus-transformed B cells (□), and U-937 monocytes (◇) in 1 ml. Protein synthesis was measured by [^{3}H]leucine incorporation 48 hr later. The effect of three ^{212}Bi labelings are shown on HUT-102B2 cells and their matched control lines.

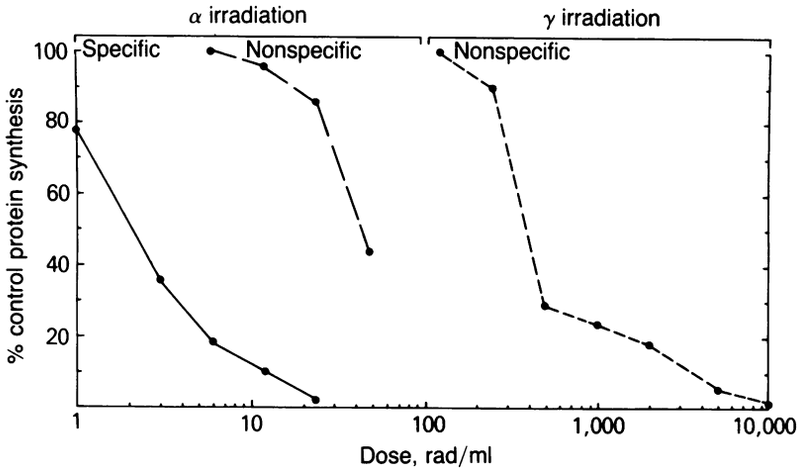

FIG. 3. Sensitivity of HUT-102B2 cells to α and γ radiation. Inhibition of protein synthesis of 10^5 HUT-102B2 cells 48 hr after exposure to increasing doses of ^{212}Bi-anti-Tac (●—●), ^{212}Bi-anti-Tac in the presence of excess anti-Tac (●---●), and γ radiation (●···●), produced by a ^{137}Cs irradiator.

munotherapy results from the integration of three scientific disciplines: immunology, nuclear science, and inorganic chemistry. The field of immunology has provided the targeting vehicle for delivering the cytocidal reagents with the development of monoclonal antibodies (16) specific for unique cellular determinants. An understanding of the specificity, pharmacokinetics, and metabolism of these xenogenic and allogeneic proteins is important to assess potential toxicity and effectiveness of these reagents. The nuclear sciences provided radioisotopes available for linkage to monoclonal antibodies. There are numerous α-, β-, and γ-emitting radioisotopes that are suitable for different forms of therapy. Our selection of an α-particle-emitting radionuclide for immunotherapy was based on the radiobiological properties of α radiation. α-particles deposit high energy per unit path length and are thus referred to as high linear energy transfer particles. High linear energy transfer α-particles release very high energy (≈10 times greater than β or γ emitters) over a very short distance (40–80 μm), resulting in efficient killing of target cells and decreased penetrability to normal tissue (17). α radiation is also efficient at killing cells under hypoxic conditions with very little cellular repair possible. Suitable α-particle-emitting nuclides available for immunotherapy are astatine-211, lead-211, lead-212, and its daughter ^{212}Bi. Most other α-particle-emitting radionuclides have long half-lives or decay to longer-lived radionuclides, rendering them dangerous for use in patients. Astatine-211 has been used to label monoclonal antibodies. However, while cytotoxicity for target cells was demonstrated, free astatine was taken up by normal tissue, indicating that the conjugate was unstable (18). ^{212}Pb has a 36-min half-life limiting its application. ^{212}Pb as yet cannot be linked with a suitable chelate to protein. Thus, ^{212}Bi was used as our choice of an α-particle-emitting radionuclide. ^{212}Bi has a 1-hr half-life, can be obtained in large quantities from a radium-224 generator, and can form stable chelate complexes with antibodies (12). Finally, inorganic chemistry has provided the chelates and labeling methods for irreversible binding to the antibody while ensuring stability and antibody integrity. Chemically modified chelates must be able to bind the metal securely and must covalently link to the antibody. The modified metal chelate DTPA has been used to label polyclonal (19) and monoclonal antibodies (20) with indium-111, a γ emitter. In this study, ^{212}Bi was conjugated to anti-Tac by use of the isobutylcarboxylcarbonic anhydride of DTPA. Thus, with advances in these three areas of science, radionuclide-linked monoclonal antibodies can now be developed for use in radioimmunotherapy.

In the present study, ^{212}Bi-conjugated anti-Tac was shown to be an effective and specific cytocidal reagent for IL-2 receptor-positive cells in *in vitro* cultures. A dose equivalent of 6 rad/ml efficiently reduced protein synthesis of HUT-102B2 IL-2 receptor-positive cells by >80% without affecting IL-2 receptor-negative cells. Specific cytotoxicity was shown to be dependent on ^{212}Bi–anti-Tac binding to the cell surface IL-2 receptor, since cytotoxicity was inhibited upon addition of excess unlabeled anti-Tac but not by the addition of human IgG. In addition, a control murine isotype-identical monoclonal antibody linked with ^{212}Bi did not show any specific cytotoxicity. Fc receptor binding was therefore not responsible for the specific cytotoxicity. Furthermore, such binding did not mediate the nonspecific cytotoxicity effects of the conjugated antibodies in the Tac-negative lines studied, because the presence of unlabeled anti-Tac did not decrease the level of the toxicity observed. The nonspecific toxicity might be due to loss of label from the anti-Tac. Alterna-

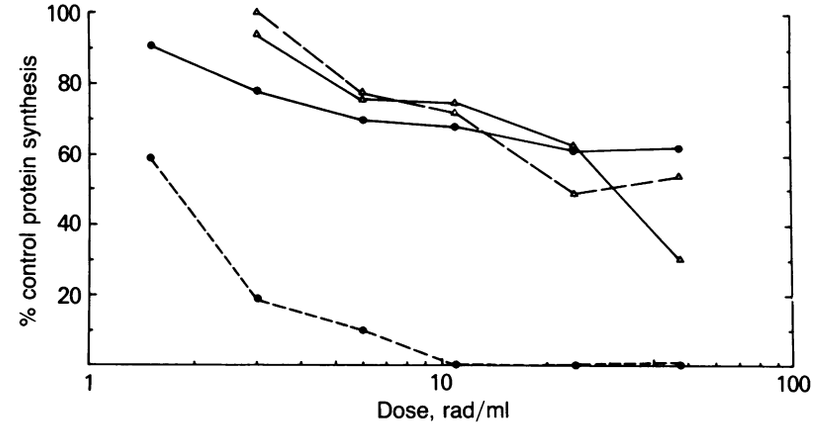

FIG. 4. Nonspecificity of cytotoxicity by ^{212}Bi-labeled RPC-5. Protein synthesis inhibition assessed 48 hr after adding increasing doses of ^{212}Bi-labeled RPC-5 to HUT-102B2 cells (●—●), or MOLT-4 cells (△—△), compared to inhibition by adding ^{212}Bi–anti-Tac to HUT-102B2 cells (●---●), or MOLT-4 cells (△---△).

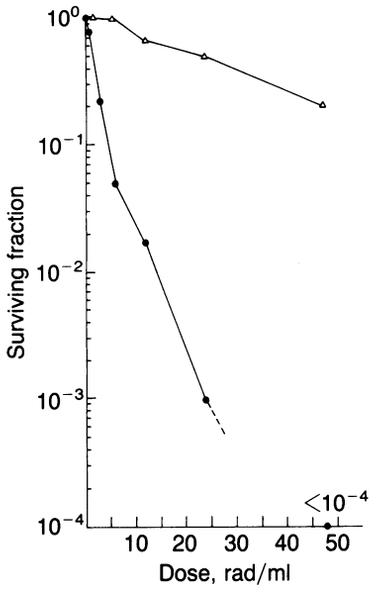

FIG. 5. Surviving fraction of HUT-102B2 (●) and MOLT-4 cells (△). Cells (5×10^5 cells per ml) were incubated for 18 hr with various concentrations of ^{212}Bi–anti-Tac and with 0.6–10,000 cells per well plated in 24 replicate cultures for each dilution. After 2 weeks, the wells were examined microscopically for growth and, based on the Poisson distribution, were evaluated for frequency of viable proliferating cells for each dilution. The values are corrected for the plating efficiency of each cell line. One in 3.5 HUT-102B2 cells and 1 in 2 MOLT-4 cells grew into colonies from untreated control cultures.

tively, it might be caused by the production of free radicals generated by β emissions into the medium. Ten percent of radiation dose from ^{212}Bi is by β rather than α radiation.

Specificity of ^{212}Bi-conjugated anti-Tac was also demonstrated by selective inhibition of reproductive integrity of IL-2 receptor-positive cells compared to receptor-negative cells, as determined by limiting-dilution analysis. The cell survival curve for HUT-102B2 demonstrated a linear curve for low doses of α radiation resulting in virtually no shoulder with an extrapolation number of 1. Clonogenic analysis corroborated the protein synthesis data with α radiation at 6 rad/ml reducing the survival fraction of the HUT-102B2 receptor-positive cell by a factor of 50 without affecting the receptor-negative line. At a ^{212}Bi-conjugated anti-Tac dose of 24 rad/ml, the survival fraction of receptor-negative line was reduced to one-half of the controls in growing potential, while only 1 in 1000 plated receptor-positive cells survived.

The advantages of radionuclide-conjugated antibodies is that α-particle emitting radionuclides have only to be focused to the surface of a cell in order to kill the target. Toxins, however, must be internalized and released from endosome vesicles into the cytoplasm in order to kill the cells. Furthermore, in light of a physical decay half-life of 1 hr, by 6 hr >98% of the ^{212}Bi has decayed to microquantities of inert lead (1 pg of lead per mCi of ^{212}Bi) with no residual radiation to normal tissues, while toxins can be concentrated within tissues and have prolonged effects. Thus, ^{212}Bi is particularly well-suited for the treatment of circulating leukemic cells where ^{212}Bi can be targeted quickly and effectively kill cells binding isotope-conjugated antibody. ^{212}Bi-conjugated anti-Tac is therefore of potential value as a cytocidal reagent for radioimmunotherapy of patients with adult T-cell leukemia, since their leukemic cells express large numbers of IL-2 receptors, whereas normal resting cells are receptor negative. Furthermore, tissue-destructive T cells become activated and express the IL-2 receptor (Tac antigen) in certain autoimmune diseases and during allogeneic organ graft rejection. ^{212}Bi–anti-Tac may therefore be effective in eliminating these unwanted Tac-expressing T cells and thus may be of value in the treatment of such autoimmune diseases and in organ transplantation protocols. Thus, α-particle-emitting radionuclides coupled to specific monoclonal antibodies such as anti-Tac represent a modality of potential use in immunotherapy.

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