Analysis of gp74 Expression by Transformed Rat Fibroblasts from Experimental Pulmonary Metastases following Specific Ricin A-Chain Immunotoxin Therapy

Jack A. Roth, Sharon Thomsen, Kim D. Fry, Patrick Scannon, and Charlotte Chang

ABSTRACT

Ricin A chain immunotoxin (IT) 45-2D9-RTA mediates regression of spontaneous pulmonary metastases and lung colonies from K-ras transformed rat fibroblasts (TRF cells). However, residual metastases are frequently noted after IT therapy, and therefore, possible mechanisms mediating tumor cell escape were investigated. Individual lung colonies were dissected from lungs of BALB/c mice, and single-cell suspensions of fresh cells from short-term cultures (eight passages) were tested. Immunoperoxidase staining with 45-2D9 monoclonal antibody showed that stable loss of surface antigen by cells cultured from IT-treated mice did not occur after four injections of specific IT. Sensitivity to specific IT in vitro was equal for metastatic tumor cells from mice treated with either two or four doses of specific IT compared to cells from nonspecific IT-treated mice and to parental cells. Clones derived from metastases of IT-treated mice were not resistant to IT. Clones derived from metastases of specific IT-treated mice internalized bound antibody or IT at the same rate as untreated cells. Freshly disaggregated cells from specific IT-treated mice were as sensitive to specific IT as were cells from nonspecific IT-treated or untreated mice. Specific IT successfully mediated reduction of lung colonies derived from fresh suspensions of lung colony TRF cells from IT-treated mice. This reduction was equivalent to that seen for cells not previously exposed to specific IT. Immunoperoxidase stains of lung sections with 45-2D9 showed that colonies consisting entirely of unstained cells were present in both specific IT and phosphate buffered saline-treated mice. There was a trend toward a higher percentage of antigen-negative colonies in mice treated with IT, although 9 days following specific IT therapy, greater than 80% of lung colonies expressed gp74 antigen. When TRF cells were grown on agar plugs, which promoted three-dimensional growth, groups of cells showing absence of immunoperoxidase staining with antibody to gp74 were identified during 2 weeks of growth. Thus, stability of antigen-negative variants is favored by three-dimensional growth conditions and the selective pressure of IT administration. Our results also suggest that impaired trafficking of IT to antigen-positive cells may also contribute to escape from IT therapy.

INTRODUCTION

Potent cell toxins such as ricin may be selectively targeted to cancer cells by conjugation to monoclonal antibodies that recognize tumor-associated antigens. Several ITs have demonstrated selective toxicity for cancer cells in vitro (1-6). IT therapy of hematopoietic cancer and regional IT therapy have been successful in animal models (7-11). However, systemic administration of IT therapy for solid tumor metastases in human trials has not been as effective (12). Potential mechanisms for cancer cell escape from IT-mediated cytotoxicity include emergence of antigen-loss and toxin-resistant variants.

We previously described a ricin A chain IT, 45-2D9-RTA, that recognizes a 74,000 M<sub>r</sub> phosphorylated glycoprotein (13). The antigen recognized by the 45-2D9 murine monoclonal antibody is expressed by murine fibroblasts transformed by members of the ras oncogene family (14). Rat fibroblasts transformed with v-K-ras oncogene express high levels of gp74 antigen. They form spontaneous pulmonary metastases and lung colonies in irradiated BALB/c mice (15). However, most treated mice have residual metastases after single or multiple IT treatments. The purpose of this study was to investigate possible mechanisms for tumor cell escape from the toxic effects of IT.

MATERIALS AND METHODS

Purification of Monoclonal Antibody 45-2D9. The 45-2D9 antibody was purified from spent hybridoma supernatant as previously described (13).

Preparation of Immunotoxin. Ricin A chain and the 45-2D9-RTA IT were prepared by Xoma Corporation. A modification of the technique of Kernan et al. was used for purification of ricin A chain and synthesis of the antibody-toxin conjugate as previously described (15, 16).

Lots routinely contained greater than 85% conjugates with a mean of 1.5 to 2 ricin A chains per antibody molecule. The absence of B chain was confirmed by identifying unchanged inhibitory activity of thymidine incorporation by TRF cells in the presence of 0.1 μM galactose. A control IT, IND-2-RTA, was prepared in a similar manner.

Inhibition of Thymidine Incorporation by 45-2D9-RTA Immunotoxin. Inhibition of thymidine incorporation was determined as described previously (13). Briefly, TRF or 196 melanoma cells were suspended in DMEM (Biofluids, Inc., Rockville, MD) and 2% dialyzed FCS. Various concentrations of antibody, immunotoxin, or ricin A chain were added. All cultures were maintained in quadruplicate for each test group. Control cultures consisted of medium alone added to cells or medium without cells. Plates were incubated at 37°C in a humidified 8% CO<sub>2</sub> and air atmosphere for 72 h. Twelve to 18 h before incubation was terminated, cultures were labeled with 0.5 μCi of tritiated thymidine. Five to 10% of cultures were counted in a liquid scintillation counter. The degree of inhibition of thymidine incorporation was expressed as follows: % control = cpm of cultures + experimental additive/cpm of control cultures.

Inhibition was invariably statistically significant (P < 0.05) by the two-sided Student's t test when the cpm for cultures containing IT was less than 70% of the cpm for control cultures.

Cell Lines and Culture Conditions. Rat fibroblasts transformed with the Kirsten sarcoma virus and expressing the K-ras oncogene (TRF cells) express high levels of the gp74 antigen recognized by 45-2D9. The TRF cell line is fully transformed by the following criteria: altered cellular morphology, focus formation in liquid culture, growth in soft agar, and progressive growth as a lethal tumor in nu/nu mice and irradiated (5 Gy) BALB/c mice. We selected this cell line for therapy because of its rapid growth rate, high level of antigen expression, and ability to form metastases from a small inoculum. DNA hybridization studies with the 0.6-kilobase SstI-EcoRI fragment of the H<sub>1</sub>H<sub>13</sub> v-K-ras oncogene demonstrated a single copy of the integrated v-K-ras oncogene.


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2 To whom requests for reprints should be addressed, at Department of Thoracic Surgery, P. O. Box 109, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030; and Xoma Corporation [P. S., C. C.], Berkeley, California 94710.

3 The abbreviations used are: IT, immunotoxin; PBS, phosphate-buffered saline; FCS, fetal calf serum; TRF cells, K-raJ transformed rat fibroblasts; DMEM, Dulbecco's modified Eagle's medium; ID<sub>50</sub>, 50% inhibitory dose; TCA, trichloroacetic acid.
ras plasmid demonstrated strong specific hybridization for DNA extracted from TRF cells (17, 18). Human melanoma cell line 196 expresses the p97 melanoma-associated antigen and grows as a lethal tumor in nu/nu mice. All cell lines were tested monthly for mycoplasma. Tests for murine viruses PVM, Poly, GDVII, Ectro, K, Reo3, Sendai, MHV, LCM, LDH, and MAD were negative (Animal Health Diagnostic Laboratory, Frederick, MD). Cell lines were grown in DMEM supplemented with 10% FCS, 2 mM L-glutamine (Biofluids), 2 mM sodium pyruvate (Biofluids), 100 µg/ml of penicillin, 100 µg/ml of streptomycin, and 0.25 µg/ml of (Fungizone, Biofluids). Cell lines were grown at 37°C in 8% CO₂ and air. TRF cells were grown on agar plugs as described by Hand and coworkers (19).

Preparation of Single-Cell Suspensions of Fresh Tumor Cells. Tissue culture cells were injected s.c. into 6-8-week-old female NIH nu/nu mice. Tumors grew to 1-2 cm in diameter within 2-3 weeks and were excised under sterile conditions. Lung colonies were visible 10 days to 2 weeks following i.v. injection of TRF cells and were dissected free of normal lung. The tumors were finely minced and suspended in a solution of 0.1% collagenase (Type IV, Sigma), 0.01% hyaluronidase (Type V, Sigma), and 0.002% DNase (Type I, Sigma) with continuous stirring overnight at room temperature. Tumor cells were then washed twice with medium, and the debris and nonviable cells were separated with density gradient centrifugation using an LSM gradient (Liton Bioincetics, Kensington, MD). Viability was determined by trypan blue exclusion. The cells were cryopreserved and stored in a liquid nitrogen freezer.

Iodination of 45-2D9 and 45-2D9-RTA. 45-2D9 antibody and 45-2D9-RTA were labeled by the 1,3,4,6-tetrachloro-3-a,6-a-diphenylglycouril (lodogen) technique (20).

Animal Model and Evaluation of Results. The TRF cells grow as s.c. tumors and lung colonies in both nu/nu and irradiated (500 cGy) 6-8-week-old female BALB/c mice. The injected tumor cells were derived from a single-cell suspension of s.c. growing tumors cryopreserved in liquid nitrogen as described above. Mice injected i.v. via the tail vein with 5 x 10⁶ to 2 x 10⁷ TRF cells develop 50 to >250 lung colonies, which can be easily counted 10 days after injection. Metastases appear as discrete white nodules on the blackened surface of the lungs (insufflated with a 15% solution of India ink) when “bleached” with Fekete’s solution (21). Therapy was begun 2 days after tumor cell injection so that only established tumors were treated. Monensin administered i.p. in doses of 0.33-3.3 µg/mouse potentiated the IT therapeutic effect in a previous study and was therefore administered in this study at a dose of 3.3 µg to obtain maximum effect (22). After the mice were killed, the lungs were assigned codes and randomly distributed. The metastases were then counted “blindly” by a second observer. Groups were compared using the Wilcoxon rank-sum test, with P < 0.05 considered statistically significant.

Immunohistochemistry. Lungs from animal groups selected for immunohistochemical studies were fixed by infusion of the bronchial tree with 10% phosphate buffered formalin. Representative pulmonary lobes were embedded in paraffin and 4-µm sections prepared. After the removal of the paraffin and hydration, the sections were treated with methanolic hydrogen peroxide to block the endogenous peroxidase activity. Slides were transferred to PBS and were incubated for 15 min with 10% normal horse serum to block nonspecific antibody binding. The avidin-biotin complex technique was used for immunostaining (Vector Laboratories, Burlingame, CA). MOPC 21 IgG1, mouse monoclonal antibody was used as an isotype-identical control at the same concentrations as 45-2D9. The sections were exposed to biotinylated horse anti-mouse immunoglobulin, washed for 10 min with PBS, and then exposed to avidin-biotin-complex horseradish peroxidase H, followed by a 15-min PBS wash. A 5-min incubation with 3,3-diaminobenzidine tetrahydrochloride was performed, followed by a 10-min PBS wash. The slides were counterstained with Harris hematoxylin, dehydrated, and mounted with glass coverslips.

Radiolabeled Antibody and Conjugate Internalization. Internalization of antibody and conjugates was determined by a modification of the technique described by Sivam and coworkers (23). Antibody or conjugates were radiolabeled with ¹²⁵I, a quantity giving 3 x 10⁶ cpm was added to 5 x 10⁸ cells in centrifuge tubes, and cells were incubated at 4°C for 30 min. The supernatant was harvested, and cells were washed gently with PBS and incubated in DMEM with 10% FCS at 37°C for periods ranging from 3 to 20 h. At various times after centrifugation, the medium was removed and the pellets were treated with 200 µl of 0.1% trypsin. This trypsin concentration was shown to remove >99% of specifically cell surface bound 45-2D9 antibody (data not shown). Cell suspensions were centrifuged, and the disintegration from the trichloroacetic acid-precipitated cell pellet, the supernatant from the trypsinized cells, and the spent culture medium of triplicate samples were counted. The presence of cpm in the trypsin supernatant was considered evidence that antibody or conjugate was bound to the cell surface, whereas cpm recorded in the trypsin-insensitive TCA-precipitated cell pellet were considered evidence of internalized antibody or conjugate.

RESULTS

A single 100-µg i.v. injection of 45-2D9-RTA IT reduced lung colonies by 54% compared with the control IT (IND-2-RTA), which recognizes an antigen not expressed by TRF cells, and by 62% compared with PBS (Fig. 1). However, as typically seen with this experiment, all mice in the 45-2D9-RTA group had residual pulmonary metastases. Increasing the single dose to 200 µg or giving up to four alternate-day 100-µg injections did not reduce the number of lung colonies further (data not shown). Unconjugated 45-2D9 antibody has no effect on lung colony formation (15). Therefore, a model system was devised to study potential mechanisms of tumor cell escape from specific IT toxicity.

A single-cell suspension of 10⁵ cryopreserved TRF cells derived from a fresh s.c. tumor was injected i.v. in 10 BALB/c mice. Mice were treated i.v. with 100 µg of 45-2D9-RTA 2 days and 10 days after tumor cell injection. Another group was treated with control IT IND-2-RTA at the same dose and schedule. Mice were killed 14 days after i.v. injection, and the individual residual lung colonies were dissected separately and dissociated into single-cell suspensions. Cells were cryopreserved and cultured for analysis of antigen expression and sensitivity to IT. The freshly prepared single-cell suspension of cells from lung colonies was then injected i.v. in another group of 10 mice who were treated with an additional two doses of IT as described above. After 14 days, the mice were killed and residual lung colonies were dissociated into single-cell suspensions, cryopreserved, cultured, or cloned at limiting dilution following short-term culture (≤ 8 passages).

gp74 Antigen Expression by TRF Cells from Lung Colonies Escaping 45-2D9-RTA Therapy. Immunoperoxidase stains of lung colony cells cultured for eight passages (8 weeks) from

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*J. A. Roth, unpublished observations.*
mice treated with two or four IT injections demonstrated expression of gp74 on more than 95% of cells (data not shown). This pattern of expression was not different for either cells from mice receiving the same number of injections of IND-2-RTA or for un.injected TRF cells. The degree of antigen expression was assessed by antibody titration from 0.2 to 200 μg/ml. No quantitative differences between 45-2D9-RTA- and IND-2-RTA-treated mice were observed. Thus, repeated doses of specific IT did not induce an increase in stable antigen-loss variants that were detectable by cell-culture techniques.

Susceptibility of Cells from Lung Colonies Escaping Therapy to 45-2D9-RTA in Vitro. TRF cells from lung colonies of IT-treated mice were placed in culture and, after eight passages, were tested for sensitivity to 45-2D9-RTA. After mice injected with TRF cells received two IT treatments in vivo, the titration curves for 45-2D9-RTA were identical for cells from 45-2D9-RTA-treated mice (ID₅₀ = 0.6 nM) and from mice receiving control IT IND-2-RTA (ID₅₀ = 0.6 nM). Single-cell suspensions of TRF cells from residual pulmonary metastases from 45-2D9-RTA-treated and IND-2-RTA-treated mice were reinjected i.v. into mice who subsequently received two additional injections of IT. The cells from residual lung colonies were cultured for eight passages, and then titration curves with 45-2D9-RTA were determined. TRF cells from 45-2D9-RTA-treated mice were equivalent to TRF cells from IND-2-RTA-treated mice and to cultured cells not exposed to IT in their susceptibility to specific IT (data not shown).

Cells from lung colonies of mice in the second group (exposed to four treatments of IT) were immediately cloned by limiting dilution after death to determine if resistant clonal cell populations could be identified. Individual clones were tested for sensitivity to 45-2D9-RTA and compared with a clone of TRF cells not previously exposed to IT (45-342-4). All of the 22 clones tested from IT-treated mice were as sensitive to the specific IT as the untreated TRF clone (Fig. 2). Thus, no IT-resistant clones were detected.

Cells from freshly excised metastases were prepared as single-cell suspensions, cultured overnight, and titrated with 45-2D9-RTA. The freshly prepared cells from both 45-2D9-RTA- and IND-2-RTA-treated mice were somewhat more sensitive to 45-2D9-RTA than were cultured cells not previously exposed to IT (data not shown).

Susceptibility of Lung Colony Cells from IT-treated Mouse to a Second IT Treatment in Vivo. A single-cell suspension of freshly disaggregated TRF lung colony cells from mice that had received two injections of 45-2D9-RTA was injected i.v. into mice that were subsequently treated with a single 100-μg i.v. injection of 45-2D9-RTA or IND-2-RTA. The number of lung colonies present after 10 days was compared with that in mice injected with cultured TRF cells not previously exposed to IT (Fig. 3). Although fewer lung colonies were formed by the fresh cells, the percentage of reduction in lung colonies mediated by the specific IT was similar for the fresh cells pretreated with IT (78% reduction) and the nonpretreated cultured cells (53% reduction). Residual metastases were noted in both groups.

Effect of IT Pretreatment on Immunoconjugate Internalization. The internalization of the 45-2D9 antibody and the 45-2D9-RTA immunoconjugate was studied to determine if cells derived from metastases that escaped IT therapy were altered in their ability to internalize antibody or conjugate. Unconjugated 45-2D9 antibody was internalized slowly with approximately 20% of the total bound ¹²⁵I cpm detected in the trypsin-insensitive fraction at 20 h (Fig. 4). A higher percentage of conjugated 45-2D9-RTA was internalized both initially and following the 20-h incubation period (Fig. 5). The degree of internalization and rate of internalization were similar for cells from clonal populations derived from a single cell suspension of IT-treated metastases and for the untreated clone of TRF cells.

Immunohistochemical Analysis of gp74 Expression following 45-2D9-RTA Treatment. Experiments were performed to investigate alterations in antigen expression in vivo that might not be detected with cloning studies in vitro. Immunoperoxidase staining of lung paraffin sections with 45-2D9 antibody was performed for mice treated with a single 100 μg i.v. dose of 45-2D9-RTA or PBS and sacrificed at varying times following injection of the cells. The immunoperoxidase reaction product was determined. TRF cells from 45-2D9-RTA treated mice (ID₅₀ = 0.6 nM) and from mice receiving control IT IND-2-RTA were internalized both initially and following the 20-h incubation period (Fig. 5). The degree of internalization and rate of internalization were similar for cells from clonal populations derived from a single cell suspension of IT-treated metastases and for the untreated clone of TRF cells.
in positive cells was concentrated at the plasma membrane as dense, thick linear deposits. Necrotic cellular debris showed nonspecific reaction with the immunoperoxidase reagents but no other tissue or cellular constituents of the lung parenchyma reacted with the 45-2D9 antibody reflecting a high specificity of the antibody for the transformed cells. The lung colonies in the 45-2D9-RTA and PBS groups were variably composed of predominantly immunoperoxidase positive cells, mixed positive and negative cells, or totally negative cells.

Mice injected with 10⁵ TRF cells i.v. were treated 48 h later with 100 µg of 45-2D9-RTA or PBS in equal volume i.v. Groups of three to five mice were killed at the indicated number of days after cell injection. Paraffin sections of the lungs (4 µm) were stained with either 45-2D9 or MOPC21 control antibody (200 µg/ml) using the immunoperoxidase technique described in "Materials and Methods." Each set of lungs was divided into lobes and a section of each lobe was made so that the distribution of colonies throughout the lung could be determined. Colonies of TRF cells were scored as ≥50% of cells stained 1+ or greater, <50% of cells stained 1+ or greater, or all cells in the colony unstained. All slides were scored without knowledge of the experimental group. Significant differences in the distribution of stained colonies between treatment groups was determined by the Wilcoxon rank sum test comparing the percentage of colonies in the ≥50% staining group for individual mice.

Table 1 Expression of gp74 by TRF lung colony cells in vivo following IT treatment

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The effect of three-dimensional growth on the expression of the gp74 antigen was studied by propagating TRF cells on agar plugs. TRF cells formed spherical growths on the plugs. Immunoperoxidase staining of paraffin-embedded sections of the agar plugs demonstrated colonies of TRF cells that did not stain even with a high concentration (200 µg/ml) of the 45-2D9 monoclonal antibody when stained 1 or 2 weeks after seeding (Fig. 6). In contrast, TRF cells grown on microscope slides demonstrated homogenous staining of >95% of TRF cells with 45-2D9, with the appearance of only an occasional isolated unstained cell during the same time period (data not shown).

**DISCUSSION**

A previous study demonstrated that a single dose of 45-2D9-RTA could mediate a reduction in both spontaneous and experimental pulmonary metastases in mice (22). However, in most cases, residual metastases were present after therapy. The purpose of this study was to define mechanisms that mediate escape of pulmonary metastases from IT therapy. Experiments were designed to examine alternations in target cells after administration of IT in vivo. Factors related to IT chemistry and interaction with host tissues have previously been shown to influence IT therapy. IT efficacy may be influenced by the IT preparation. Also, contamination with free antibody or instability of the ricin A chain-antibody linkage may reduce efficacy (24, 25). Recognition of mannose and fucose receptors by Kupffer cells and sinusoidal endothelial cells results in rapid clearance of ricin A chain IT from serum (25). In this study, we focused on possible alterations in the target cell that would either facilitate escape from IT binding or render the cell resistant to IT toxicity.

A model system was established to generate stable variants that were resistant to IT toxicity. However, cells from mice receiving up to four cycles of IT continued to express the gp74 antigen in culture and to be sensitive to the toxic action of ricin A chain. The major mechanism of ricin resistance is thought to be related to loss of receptor or altered processing rather than to generation of internal resistance (26, 27). Although such resistant variants have been induced in vitro, we were unable to identify stable variants in culture.

Internalization of IT also does not appear to be altered after
IT therapy in vivo. All isolated clones processed both unconjugated antibody and IT equally. IT was more readily internalized than free antibody, confirming previous observations by Sivam and coworkers (22).

These studies suggest that the generation of tumor cells that do not express the gp74 antigen recognized by IT may be a mechanism for escape from IT toxicity. It is interesting that this was not apparent from the experiments performed in vitro. Antigen-negative cells were only detected following staining of metastases in vivo. Stable antigen-loss variants could not be generated in monolayer culture. However, they could be detected when cells were grown in a three-dimensional configuration. This suggests that the alteration in antigen expression is phenotypically derived. Previous studies showed that expression of gp74 was cell-cycle dependent (23). It is possible that the tumor cell’s environment, growth pattern, or both may influence antigen expression. Hand and coworkers described increased tumor antigen expression under conditions promoting three-dimensional growth (19). Raz and Ben-Ze’ev described increased metastatic potential with reversible reduction in accessibility of cell surface proteins to external iodination and decreased vimentin synthesis for B16 cells grown as spheroids as compared with flat configurations (28). Ames and coworkers described an inverse correlation between expression of gp74 and metastatic potential in a rat mammary carcinoma system. Thus, loss of gp74 may favor formation of lung colonies. It appears that both IT treatment and the spatial configuration of TRF cells in vivo contribute to the development of stable antigen-loss variants. Colonies consisting exclusively of cells not expressing gp74 were observed in both IT-treated and PBS-treated mice. However, IT treatment resulted in increasing numbers of antigen-negative colonies at longer postinjection times. Further studies to clarify factors that regulate antigen expression and the development of IT “cocktails” that recognize multiple antigen specificities may improve IT efficacy. Most of the lung colonies remaining after IT treatment showed high levels of gp74 expression. Cells cloned from these colonies continued to be susceptible to IT-mediated toxicity. This suggests that enhancement of trafficking of IT to tumor cells and penetration into solid tumor nodules may also increase their therapeutic efficiency.

REFERENCES

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