Self-Reactive Antibody Expression by Human Carcinoma Cells Engineered with Monoclonal Antibody Genes

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ABSTRACT

The purpose of this study was to determine if human colon cancer cells transduced with monoclonal antibody (MAb) genes become sensitive to immune destruction through coexpression of both the MAb and its reactive antigen. Murine retroviral expression vectors were constructed with the heavy or light chain genes of an anti-human colon carcinoma MAb, D612, that mediates antibody-dependent cell-mediated cytotoxicity (ADCC). Transduction of D612 MAb genes into the D612 antigen-positive (>95%) human colon carcinoma cell line, LS-174T, was carried out by sequential cocultivation with PA317 packaging cells producing infectious virions containing the light or heavy chain expression vectors. Six cultures survived drug selection, two of which were found to have elevated levels of both light and heavy immunoglobulin chain activity in their supernatants. IgG secretion levels (24 h) were 1-2 ng/1 x 10^6 cells. Low but definite antigen reactivity was also present in supernatants obtained from these LS-174T transductants. Immunocytochemical staining of transduced tumor cells revealed that >95% of the cells were positive for IgG expression. Thus, LS-174T transductants were capable of producing both the D612 MAb and D612-reactive antigen. Analysis of transductants by flow cytometry further revealed that >95% of the cells had murine immunoglobulin on their surfaces. ADCC mediated by human natural killer cells against nontransduced tumor cells was observed when the latter cells were cocultivated in the presence of transductants producing both D612 heavy and light chains but not in the presence of tumor cells transduced with light chain only. LS-174T cells transduced with both D612 heavy and light chains were more sensitive to cytotoxicity mediated by natural killer cells than were light chain only transductants. ADCC contributed to the greater sensitivity of the former transductants to cytotoxicity based on its inhibition by anti-FcγRIII antibody. Thus, these studies demonstrate that tumor cells transduced with genes encoding for MAb genes that can participate in ADCC reactions are able to sensitize nontransduced tumor cells to immune destruction as well as to direct killer cells against themselves. These studies may lead to a new immunotherapeutic approach for the treatment of cancer based on MAb gene therapy.

INTRODUCTION

Immunomodulation of cancer cells by the introduction of genes that promote immune responses has been used to augment antitumor actions. Genes encoding for lymphokines (1-3), cytokines (4-6), class I MHC antigens (7, 8), and foreign antigens (9) have been transferred into various cancer cells and have been shown to be effective in inducing protective immunity. As effector molecules, antibodies have certain unique attributes that may make them well suited for tumor cell immunomodulation through antibody gene transfer. MAbS can participate with both macrogaphes (10, 11) and natural killer lymphocytes (12, 13) in mediating cellular cytotoxicity against tumor cells, and it is believed that these killer cells have an important role in combination with MAbS in inhibiting the outgrowth of experimental tumors (14, 15). Although MAbS with the capacity to activate FcR+ killer cells have been used in clinical trials in which therapeutic responses were noted in some cases (16), antibody engineering efforts may lead to further improvements in clinical efficacy, especially where problems associated with immunogenicity can be mitigated.

We have described the production of a MAb, designated D612, that reacts with a membrane antigen expressed on the majority of human colon carcinomas (17, 18). The D612 MAb was shown to mediate ADCC with both human lymphocytes and macrophages, and stimulation of peripheral blood lymphocytes with IL-2 or IL-6 enhanced ADCC mediated by D612 (12, 19). In other studies (20), the D612 MAb inhibited the outgrowth of human colon carcinoma xenografts implanted in athymic mice. The present study was undertaken to determine if the D612 MAb would be expressed following D612 MAb gene transfer into human colon tumor cells that constitutively produce the D612-reactive antigen. We show that these cells are indeed able to secrete the D612 MAb and that the antibody produced by these cells elicits ADCC mediated by NK cells against themselves as well as against the unmodified parental cells. This work has been presented in preliminary form elsewhere (21).

MATERIALS AND METHODS

MAbs and Cell Lines. The preparation and characterization of the D612 MAb (κ, IgG2a) are described elsewhere (17). The anti-FcγRIII MAb, 3G8, was obtained from Medarex, Inc. (West Lebanon, NH), while purified control murine myeloma protein, UC10 (IgG2a) was purchased from Organon Teknika (Durham, NC). The human colon carcinoma cell line, LS-174T, was obtained from American Type Culture Collection (Rockville, MD), and was maintained on Dulbecco's minimum essential medium (Irvine Scientific, Santa Ana, CA) supplemented with 2 mg/l-glutamine, 100 units/ml of penicillin, 100 µg/ml of streptomycin, and 10% heat-inactivated fetal bovine serum (Gemini Bioproducts, Calabasas, CA). The ecotropic retroviral packaging cell line 2φ and the amphotropic packaging cell line PA317 were obtained from Dr. Robert Bassin (National Cancer Institute, NIH, Bethesda, MD) and were maintained on the above medium.

Expression Vector Construction. The complementary DNA of the immunoglobulin genes of the D612 MAb was initially cloned at the EcoRI site of the phage vector, lambda ZapII (Stratagene, La Jolla, CA), and subsequently excised in the phagemid, pBlueScript (Stratagene, La Jolla, CA). The retroviral vector pLNCl2 (Tsang, 1993) was used to prepare the expression construct of the light chain gene. The retroviral vector pNO6 (22) was used to prepare the expression construct of the heavy chain gene.

Introduction of Expression Constructs into Helper Cells. Retroviral expression constructs were introduced into φ2 packaging cells by lipofectin following the manufacturer's recommendation (Bethesda Research Laboratories, Gaithersburg, MD). Briefly, 1 x 10^6 cells were seeded into 60-mm tissue culture dishes, and after incubation overnight, the culture medium was replaced with serum-free Optimem medium (GIBCO, Grand Island, NY). Lipofectin-recombinant expression constructs were introduced into the φ2 packaging cells by lipofectin treatment.

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2 To whom requests for reprints should be addressed, at the Division of Immunology, Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd., Duarte, CA 91010.
3 The abbreviations used are: MAb, monoclonal antibody; ADCC, antibody-dependent cell-mediated cytotoxicity; PBS, 0.01 M phosphate plus 0.15 M NaCl buffer, pH 7.2; CMF-PBS, Ca^2+ and Mg^2+-free PBS; EIA, enzyme immunoassay; FBS, fetal bovine serum; NC, natural killer; FITC, fluorescein isothiocyanate; CTL, cytotoxic T-lymphocytes; TNF-α, tumor necrosis factor α; IFN-γ, γ-interferon.
the medium was removed and 0.4 ml of conditioned medium obtained from productively transfected 62 cells and also containing 8 \( \mu \)g/ml polybrene was added to the cells. After a 2 h incubation at 37°C, complete medium was added to the cells, followed by selection medium 48 h post infection.

**Introduction of D612 Antibody Genes into LS-174T Cells.** D612 light and heavy chain genes were sequentially introduced into LS-174T cells. First, 4 \( \times 10^6 \) LS-174T cells were cocultivated with 0.5 \( \times 10^6 \) irradiated (4000 rads) PA317 packaging cells producing virions containing the D612 light chain expression vector. After 4 days, the cells were expanded and placed on selection medium containing 1000 \( \mu \)g/ml of G418. Cell cultures surviving selection were assayed for the presence of murine Ig light chain in the culture medium by EIA. Using an anti-\( \kappa \) antibody in EIA and intact D612 IgG as a standard, one line was found to produce 20 ng/ml of murine immunoglobulin and was used to introduce the D612 heavy chain gene. Transduction of the D612 heavy chain gene was carried in the same manner as described for the light chain gene except PA317 packaging cells producing virions containing the D612 heavy chain expression vector were used. Instead of G418, the selection medium contained 100 \( \mu \)g/ml of hygromycin.

**Enzyme Immunoassays.** Immunoglobulin and antibody activity in culture supernatants were determined in EIA. For immunoglobulin measurement, culture supernatants were added to the wells of 96-well EIA microtiter plates (CoStar, Cambridge, MA) previously coated with anti-mouse IgG F(ab')2 (Organon). After incubation and washing, the wells were exposed to alkaline phosphatase-conjugated anti-mouse \( \gamma \) or \( \kappa \) chain antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) followed by development with substrate solution consisting of 1 ml of \( p \)-nitrophenyl phosphate in 1.0 M diethanolamine, pH 9.8. Immunoglobulin levels in test samples were determined from standard curves generated with hybridoma-produced D612 IgG purified from ascites by protein A chromatography (12). Microtiter plates previously coated with extracts of cultured LS-174T cells (17) were used to measure antibody activity. Antibody binding was detected by indirect assay using alkaline phosphatase-labeled anti-mouse IgG antibody (Tago, Burlingame, CA).

**Flow Cytometry.** Tumor cell monolayers were washed with CMF-PBS and then exposed to cold 0.02% EDTA. All staining procedures were carried out at 4°C. Released cells were centrifuged, resuspended in CMF-PBS containing 1% FBS, and distributed at 106 cells/tube. The cells were pelleted and then resuspended in unlabeled antibody (indirect method) or in FITC-labeled anti-mouse IgG antibody (Jackson Immunoresearch, Newark, DE) for the direct method. Following incubation at 4°C for 30 min, the cells were washed and either analyzed directly using a FACScan (Becton Dickinson, San Jose, CA) or were stained with cold 0.02% EDTA. All staining procedures were carried out at

**Preparation of Human Effector Cells.** Mononuclear cells were separated from leukapheresed blood obtained from healthy human donors by density gradient centrifugation on lymphocyte separation medium (Organon). Mononuclear cells collected from the gradient interface were washed repeatedly with CMF-PBS containing 2% FBS to remove platelets. Mononuclear cells were either used fresh or stored by freezing in a 10%-90% dimethyl sulfoxide:FBS mixture. Fresh or quickly thawed frozen lymphocytes were cultured for 18-24 h at 5 \( \times 10^6 \) cells/ml prior to use.

**Cytotoxicity Assays.** Cytotoxicity assays using target cells labeled with \( ^{31} \)Cr or \( ^{111} \)In were carried out as described previously (12, 20). The ability of transduced LS-174T cells to sensitize nontransduced LS-174T cells to ADCC was analyzed by first plating 2.5 \( \times 10^5 \) unlabeled, transduced LS-174T cells into 96-well flat bottomed tissue culture microtiter plates (CoStar). After 24 h, 1 \( \times 10^4 \) \( ^{111} \)In-labeled nontransduced LS-174T cells were added along with effector cells at different effector:target cell ratios. The plates were then incubated for 24 h and 48 h prior to harvesting.

**Immunocytocchemistry.** Tissue-cultured transduced LS-174T cells were released from flasks by brief trypsinization. The cells were washed in complete medium followed by CMF-PBS, and then the cell pellet was snap-frozen at -70°C. The cell pellet was embedded in OCT compound (Miles, Elkhart, IN) and 5-\( \mu \)m sections were cut, fixed in cold acetone (4°C), and stained with peroxidase-labeled anti-mouse IgG (Jackson Immunoresearch) or peroxidase-labeled D612 according to procedures described elsewhere (18, 23). Background nonspecific staining was determined by adding a 50-fold excess of mouse IgG or D612 to the enzyme-labeled anti-mouse IgG or D612, respectively.

**Isolation of IgG from Transduced LS-174T Cells.** Cultured supernatants were diluted one-half in 0.01 M phosphate buffer, pH 8.0, and then passed through a 1-mg MASS protein A filter (Nygene, New York, NY). After the filter was washed, bound IgG was eluted with 0.1 M citrate buffer, pH 3.5, and immediately neutralized with 1.0 M Tris, pH 8.0. The IgG was dialyzed against PBS prior to assay.

**RESULTS**

**D612 MAB Production by Transduced LS-174T Cells.** Following cocultivation of immunoglobulin light chain secreting LS-174T transductants with PA317 cells producing the heavy chain retroviral vector, 6 separate cultures survived selection with hygromycin. Supernatants from these cultures were tested in EIA for the presence of murine \( \gamma \) and \( \kappa \) chain. Two of the cultures, T1S2 A and T1S2 B, showed elevated levels of both light and heavy chain activities while in the remaining 4 cultures, both of these activities were absent or very low (Fig. 1A). In this initial assay, the EIA values obtained from 0.1 ml of culture supernatant from the T1S2 lines was similar to that produced by 1.25 ng of hybridoma-derived purified D612 MAB. Unconditioned culture medium (shown) or supernatants from nontransduced LS-174T cells tested negative for both light and heavy chain activity.
The previous experiments were carried out on spent supernatants obtained from cultures that differed in confluency. The production of murine immunoglobulin heavy and light chain by the T1S2 B line was assessed in 24-h supernatants obtained from confluent cultures. The amount of cell-associated heavy and light chain was also measured by assay of cell extracts. Table 1 shows that the production of immunoglobulin by the LS-174T heavy and light chain transductant remained relatively stable over 27 passages encompassing approximately 7 months in culture. The amount of heavy chain in supernatant medium varied from 0.39 to 2.78 ng/1 x 10⁶ cells while the amount of light chain activity was 12 to 38 times higher (Table 1). Similar levels of cell-associated heavy and light chain activity were also detected. As expected, the ratio of heavy to light chain activity in hybridoma-derived purified D612 MAb approximated 1.0 since it was used as a standard in both the light and heavy chain assays. The T1S2 B cell line was then cloned by limiting cell dilution. Four clones was further studied for IgG production and were found to produce levels of light and heavy chain activity similar to those of the uncloned parent (data not shown). Consequently, all the remaining studies were carried out with the T1S2 B line.

Expression of Murine IgG by LS-174T D612 MAb Transductants Determined by Flow Cytometry and Immunocytochemistry. EIA assays of culture supernatants and cell extracts obtained from LS-174T cells transduced with D612 heavy and light chain genes demonstrated that MAb was produced by at least some of the drug-resistant cells. The latter assays also showed that antigen-reactive antibody was present in the culture medium. Additional analyses by flow cytometry were carried out to determine whether immunoglobulin could be detected on the surface of the transduced LS-174T cells. LS-174T cells were gently released from flasks with EDTA and then

Table 1 Production of IgG by LS-174T colon carcinoma cells transduced with D612 monoclonal antibody genes

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Passage no.</th>
<th>IgG content (ng/1 x 10⁶ cells ± SE)</th>
<th>Cell Extract</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>κ chain</td>
<td>γ chain</td>
<td>κ chain</td>
</tr>
<tr>
<td>T1S1 B Heavy + Light chain</td>
<td>6</td>
<td>14.8 ± 0.56</td>
<td>0.39 ± 0.04</td>
<td>15.3</td>
</tr>
<tr>
<td>20</td>
<td>14.5 ± 0.64</td>
<td>0.67 ± 0.05</td>
<td>7.4</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td>34.1 ± 2</td>
<td>2.78 ± 0.13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Light chain only</td>
<td>30</td>
<td>20.5 ± 0.94</td>
<td>Neg</td>
<td>4.65</td>
</tr>
<tr>
<td>Nontransduced</td>
<td>152</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

*Mean ± SE of quadruplicate wells for immunoglobulin content of culture supernatants. Cell extracts obtained from pool of cells from wells used for determination of supernatant activity.

**ND, not determined; Neg, <0.1 ng/1 x 10⁶ cells for both κ and γ chain assays.
stained directly with fluoresceinated anti-mouse IgG. As shown in Fig. 3, over 95% of the cells were positive when stained with the latter reagent. This was in comparison to cells that were not stained, stained with fluoresceinated anti-human IgG, or stained with fluoresceinated anti-mouse IgG in the presence of excess mouse IgG, all of which showed a similar level of background staining. The percentage of transduced cells positive for surface IgG was comparable to the number of nontransduced LS-174T cells positive for the D612 antigen (Fig. 4B). Likewise, when exogenous D612 MAb was used to stain the transduced LS-174T cells, the fluorescence intensity pattern was very similar to that of the nontransduced cells (Fig. 4A; mean channel fluorescent intensity, 500 versus 645 for transduced and nontransduced, respectively), suggesting that most of the transduced cells were also D612 antigen-positive. The higher fluorescence intensity obtained when the UPC-10 negative control antibody was reacted with the transduced cells as compared to that of the nontransduced cells was due to the presence of endogenous mouse IgG on the surface of the former cells as shown in Fig. 3. Furthermore, the staining intensity of the transduced cells increased in the presence of added D612 (mean channel fluorescent intensity, 95 versus 500 in the absence or presence of added D612, respectively; compare Figs. 3 and 4A), suggesting that not all of the antigen sites on the transduced cells were occupied by constitutively produced D612 or that some of the latter was removed during sample processing.

Although flow cytometry showed that most transduced LS-174T cells expressed surface mouse IgG, it was possible that this was derived from a lower percentage of cells secreting D612 MAb into the culture medium that became secondarily bound to antigen-positive...
carried out to determine if nontransduced LS-174T cells would be

Since the D612 MAb mediates ADCC, additional experiments were

obtained with the D612 MAb that disappeared when an excess of

D612-reactive antigen (Fig. 5C). A uniform cytoplasmic staining was

gate showed that the majority of LS-174T cells were positive for the

(Fig. 55). As expected, direct staining with the D612 enzyme conju

reaction mixture blocked coloration due to the peroxidase enzyme

addition, prominent membrane staining was visualized around or be

their cytoplasm when stained with anti-mouse IgG alone (Fig. 5A ). In

constitutively expressing the D612 MAb. Frozen sections were cut

from cell pellets and stained directly with peroxidase-conjugated anti-

was used to determine whether the majority of LS-174T cells were

effector cells for 24 h prior to measuring lysis. Bars. SE.

Cultures were incubated with normal human peripheral blood lymphocytes as a source of

transductant cells (2.5 X 10^4) (O), (ß) Radiolabeled unmodified LS-174T cells (1 X 10^4)

was over twice that observed in control cultures. For comparison, Fig.

6B depicts the lysis occurring in cultures containing only labeled

nontransduced targets and effector cells, but to which exogenous

D612 MAb was also added. Nonspecific killing as measured in cultures

to which the control myeloma IgG2a protein, UPC-10, was

added was higher than the nonspecific killing observed during cocul-
tivation with light chain gene only transductants (Fig. 6A). This dif-

ference in nonspecific cytolyis was most likely due to the added total

number of potential cold and labeled targets in cocultivation assays.

This also explains the higher apparent lysis that appeared following

addition of exogenous D612 MAb as compared to that achieved by

cocultivation with transductants producing the D612 MAb.

Since transduced LS-174T cells were capable of sensitizing non-

transduced cells to ADCC, it seemed likely that the transduced cells

themselves would be coated with D612 MAb and elicit ADCC. To test

this possibility, assays were carried out that compared the sensitivity of heavy and light chain gene transduced cells to cell-mediated cy-
tolysis to that of light chain gene only transductants. In addition, the

cytolysis of heavy and light chain transduced cells in the absence or

presence of anti-FcRγIII receptor antibody was compared in order to
differentiate ADCC from antibody-independent cell mediated lysis of these transduced cells. An anti-FcRγIII receptor antibody was used

since our previous studies had shown that natural killer cells were the

major cell type responsible for mediating ADCC in the effector cell

population (12). Fig. 7 shows that the T1S2 B cells were more sen-
sitive to cell-mediated lysis than the light chain gene only transduc-

tants. At a 50:1 effector:target cell ratio, over 40% lysis was observed

against the T1S2 B cells; while at the same ratio, 18% lysis of the light

chain gene only transductants was observed. The difference in lysis

between the two cell types increased to 57% at the 100:1 ratio. When the

anti-Fc receptor antibody was added to cultures containing labeled

T1S2 B targets, cell-mediated lysis decreased to the level observed in
cultures containing the light chain gene only transductants. As an

additional control, ADCC against LS-174T cells in the presence of exogenously added D612 MAb was also blocked by the anti-FcRγIII

receptor antibody (data not shown).

DISCUSSION

The present studies have shown that it is possible to elicit, through

transfer of MAb genes, the production of MAb from colon carcinoma

cells coincidentally expressing the MAb-reactive antigen. Concordant

expression of both the D612-reactive antigen and D612 MAb was

demonstrated in more than 90% of the transduced cells. Moreover, the

antibody secreted by these cells retained its ability to bind antigen as

well as to mediate cellular cytotoxicity. It was found that the trans-
duced cells had the capacity to sensitize antigen-positive nontrans-
duced cells to ADCC during cocultivation with LS-174T tumor

cells transduced with D612 MAb genes. For these experiments, 2.5 X

10^4 T1S2 B cells were incubated overnight with 1 X 10^4 nontrans-
duced LS-174T cells that were labeled with ^111In. Human peripheral

blood lymphocytes were added as a source of killer cells. It was found

that the lysis of nontransduced cells cocultivated with tumor cells

transduced with both D612 heavy and light chain genes was aug-

mented as compared to labeled target cells incubated with LS-174T

cells transduced with the light chain gene only (Fig. 6A). In cultures

containing a 100:1 effector:labeled target cell ratio, approximately

25% cell lysis was observed. Cell lysis in the presence of antibody-

producing transductants increased to 40% at the 200:1 ratio, which

was over twice that observed in control cultures. For comparison,

transduced LS-174T cells can secrete active D612 MAb following

retroviral mediated transfer of D612 light and heavy chain genes.

Since the D612 MAb mediates ADCC, additional experiments were

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well as to mediate cellular cytotoxicity. It was found that the trans-
duced cells had the capacity to sensitize antigen-positive nontrans-
duced cells to ADCC during cocultivation of the two cell types. In

addition, lysis by natural killer cells of the transduced cells themselves

was augmented as expected by the observation that over 90% of the

cells in the transduced population expressed surface IgG.
Gene transfer of cytokine genes or genes encoding for foreign antigens can abolish tumorigenicity as well as elicit T-cell-dependent protection against the parental tumor cells (24). Although unproven as yet, vaccination with gene-modified tumor cells may engender a more effective therapeutic response than other antigen-specific vaccines or by systemic or even local cytokine administration (25, 26). We have shown in the present study that MAb gene-transduced colon carcinoma cells are indeed sensitive to ADCC mediated by NK cells and moreover that they were able to confer this sensitivity upon cotransfected nontransduced cells. It is likely that the ADCC activity of the MAb produced by gene-modified cells could be enhanced even further through the use of mouse/human IgG1 chimeric constructs (27, 28). However, it will be of interest to determine what effects cytokotoxic MAb gene modification of tumor cells has on the growth of these cells in vivo as well as on nontransduced tumor cells growing locally or at a distant site. In studies to be reported elsewhere, we have shown that transduced LS-174T carcinoma cells have reduced tumorigenicity in athymic mice, suggesting that the ADCC effects reported here may be functionally relevant in activating similar anti-tumor responses in vivo.

The multiple effector activities of MAbs that are locally produced by gene-modified tumor cells could promote the generation and proliferation of antitumor CTLs that recognize antigens native to the tumor cell (29). There are at least two pathways by which ADCC could augment CTL activity: (a) MAb enhancement of tumor cell lysis and/or phagocytosis (30–33) by macrophages could result in improved antigen presentation. It is well established that macrophages have a central role in the generation of CTL which can occur in the absence of T-helper cells (34, 35); (b) activation of NK cells through their Fc receptors results in the liberation of TNF-α, IFN-γ, and macrophage colony-stimulating factor (36–38). TNF-α and IFN-γ both have been shown to have an effect on the proliferation and generation of CTL (39–42). In addition, IFN-γ, TNF-α, and macrophage colony-stimulating factor can activate granulocyte- macrophage-mediated ADCC (43–45) and phagocytosis (46, 47) as well as chemotaxis (48) while enhancement of antigen expression and adhesion molecules by IFN-γ and/or TNF-α can promote cell-mediated killing of target cells (48–50). It should be mentioned that effective tumor cell vaccines have been produced with TNF-α or IFN-γ genes while locally activated NK cells have a major role in the rejection of interleukin 2 transfected tumors (4). Finally, a role for complement activation by MAb cannot be excluded and could be very important in initiating inflammatory reactions whereby macrophages and other effector cells are targeted to the virus site.

Although the MAb produced by the transduced LS-174T cells was able to elicit ADCC, the amount of antibody secreted was low. Transfectomas using myeloma recipients typically produce 5–100 μg/ml of MAb (51, 52). One possible reason for the lower production of MAb by the LS-174T cells is the coincident synthesis of the D612-reactive IgG1 MAb (51, 52). One possible reason for the lower production of MAb by the transduced LS-174T cells was in the study secreted IgG that had a light-heavy chain ratio 12 to 38 times higher than that associated with hybridoma-produced MAb. This suggests that improved production of intact MAb may be attained by using different expression vectors such as the one used for the light and heavy chain genes in the same expression vector or are combined as single chain Fv (53, 54).

These studies have shown that tumor cells transduced with genes encoding for cytokotoxic MAb produce self-reactive MAb that can participate in ADCC reactions. In addition to the antitumor effects associated with ADCC, class I MHC-restricted antitumor immune responses may be precipitated or enhanced through the latter activity. Thus, MAb gene transfer into autologous tumor cells appears to offer new and alternative applications in the use of MAbs for the immunotherapy of cancer.

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REFERENCES


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