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GRANT NUMBER DAMD17-96-1-6107

TITLE: Antibody-IL2 Fusion Protein Delivery by Gene Transfer

PRINCIPAL INVESTIGATOR: Charles M. Nicolet, Ph.D.

CONTRACTING ORGANIZATION: University of Wisconsin
Madison, WI 53706

REPORT DATE: October 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1997	Annual (1 Oct 96 - 30 Sep 97)

4. TITLE AND SUBTITLE
Antibody-IL2 Fusion Protein Delivery by Gene Transfer

5. FUNDING NUMBERS
DAMD17-96-1-6107

6. AUTHOR(S)
Charles M. Nicolet, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Wisconsin
Madison, WI 53706

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The purpose of the work described is to assess the feasibility of a gene therapy approach to deliver a specific antibody cytokine fusion protein called CC49-IL2 to a tumor expressing antigen reactive with the antibody. While gene therapy approaches to the delivery of immune stimulating compounds such as cytokines have been widely explored, less has been done investigating other molecules in these contexts. We further were interested in assessing a specifc type of gene therapy for this purpose called particle mediated gene transfer in which the DNA of interest is deposited directly into cells using gas propelled gold beads as a delivery vehicle. In our studies, we first showed that a commonly used retroviral derived vector was the most effective at driving expression of the fusion protein. We also attempted to establish an immunotherapy model in which antibody reactive tumors were engrafted into syngeneic immunocompetent murine hosts. Growth of these tumors was monitored following PMGT of the FP at the tumor site. Our initial attempts at exploiting this model were unable to document a statistically significant anti-tumor effect.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	17
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Charles Nicolet 12/16/97

PI - Signature Date

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INTRODUCTION

A promising area of breast cancer treatment involves the use of monoclonal antibodies directed against breast tumor associated antigens. Several classes of such antigens have been identified. These include the epidermal growth factor related HER-2/neu proto-oncogene and cell surface proteins in the mucin family. These proteins have been examined as targets for disease detection and immunotherapy. Numerous studies have demonstrated the value of antibodies against these antigens in prognostic and diagnostic applications, and have shown potential utility in animal, in vitro, and early clinical experiments. Using recombinant DNA technology, it has been possible to extend the utility of these molecules by altering their configuration to suit specific needs (1).

There has been extensive investigation in the use of antibodies to serve as delivery vehicles for particular cytolytic compounds or, alternatively, immune stimulating agents that can target tumor cells for destruction directly or by attracting and stimulating resident immune effector cells. We have been interested in examining the immunotherapeutic effectiveness of an anti-mucin directed antibody-cytokine fusion protein. The antibody portion recognizes the tumor associated glycoprotein antigen TAG-72. This antibody is covalently linked to IL-2, a potent immune cell stimulator (2). We are investigating the potential advantages of delivering this fusion protein by a novel gene therapy mechanism, using particle mediated gene transfer (PMGT) to transiently transfect murine epithelial cells at the site of delivery (3).

The TAG-72 antigen is expressed on many breast carcinomas. An anti-TAG 72 antibody in widespread use, and currently approved for imaging studies in certain types of cancer, was originally derived from mice immunized against a cell lysate from metastasized breast carcinoma tissue. This mAb, denoted B72.3, was initially shown to react with 46% of breast cancer biopsies analyzed. Subsequent studies have revealed TAG-72 expression to be more widespread on breast carcinoma (4). A different anti-TAG 72 mAb denoted CC49, having roughly eight-fold higher affinity for TAG-72 than the B72.3 antibody, showed strong reactivity with 6 of 6 breast cancer biopsies analyzed (5). The single chain human-mouse chimeric antibody-cytokine fusion protein we are using in the proposed studies is derived from the CC49 murine monoclonal. B72.3 reactivity is also positively correlated with the degree of malignancy, indicating the usefulness of the TAG-72 protein as a prognostic marker for high risk breast cancer. The goal of our approach is to provide effective concentrations of both tumor reactive chimeric antibody and IL-2 in the microenvironment of metastatic breast lesions.

Our studies address the question of whether immunotherapeutic levels of an antibody-cytokine fusion protein can be achieved by gene therapy: delivering DNA encoding the fusion protein to tumor bearing animals. We utilized the transfection procedure known as particle mediated gene transfer, in which DNA-coated gold particles are accelerated by a compressed gas discharge into cells or organisms (6). Previously we had shown that substantial skin and detectable serum levels of the CC49-IL2 fusion protein were achieved following PMGT into murine epidermis (3). While expression levels were not as

high as those obtainable by parenteral administration, data from other studies has documented that in vivo delivery of an antibody-IL2 FP provides more efficient tumor destruction and more significant survival benefit in tumor-bearing mice than treatment with equivalent doses of IL-2 alone, mAb alone, or the combination of antibody and IL-2 given as separate molecules at equivalent doses to that provided by the FP (7). As little as one μg of FP injected intraperitoneally was sufficient to provide a significant therapeutic benefit. This corresponds to only about 1% of the maximal tolerated dose of IL-2 in mice, suggesting the highly bioactive nature of this type of fusion protein.

BODY

Task 1. Analysis of expression vector configurations and delivery regimens to provide augmented expression of CC49-IL2 Fusion Protein.

It was important to determine whether we could augment expression levels of the CC49-IL2 fusion protein (FP) beyond those seen with the current vector used, since expression is a critical parameter in any gene therapy model. Therefore we generated and analyzed a series of novel expression vectors.

The vector we have used for our initial studies on the CC49-IL2 fusion protein is derived from the commonly used pLNCX plasmid (8), and is denoted pLNC-CCIL. This vector is from a family of recombinant replication defective retroviruses, and utilizes the eukaryotic-selectable marker Neomycin. The gene of interest is cloned downstream of the CMV promoter, which is active in most mammalian cell types (9). As described in previous publications, we have used the pLNCX based expression system to generate information on in vitro and in vivo produced protein (3).

The expression vectors described below were created through standard recombinant DNA manipulations, including the use of PCR when required to clone particular regions. We pursued conventional cloning strategies and methodologies. Thus, we have not included here precise details of how each current recombinant construct was assembled. Constructs were analyzed by restriction digest and, when appropriate, sequenced, and purified.

The B16 murine melanoma cell line was used for the in vitro expression analyses, since these cells are effective recipients for particle delivery, and efficiently use the CMV promoter. Twenty four hours prior to particle delivery, 2 x 10^5 cells were plated in 35 mm tissue culture dishes. This results in plates that are approximately 75% confluent with cells at the time of PMGT. The medium was removed, the pulse gun barrel placed on the dish, and DNA coated particles accelerated by a pulse of helium (at 200 psi) into the cells on the dish. Medium was added, and the cells were incubated for 24 hours prior to assaying the medium for secreted FP expression. Four 35 mm dishes were assayed for each vector, as well as control dishes of cells receiving "naked" (non-DNA coated) gold particles.

To analyze expression in vivo, BALB/C mice were shaved on the belly, then the barrel of the gene gun placed to the exposed skin, and particles delivered at 350 psi. 24 hours following delivery, the animals were sacrificed. Skin biopsies of the recipient epidermal area were scissor-minced in PBS plus 0.5% Triton and aprotinin, then subjected to two freeze-thaw cycles. The remaining tissue debris was pelleted, and the supernatant withdrawn for analysis.

In both the in vivo and in vitro experiments, fusion protein levels were quantitated using an IL-2 ELISA, known to be specific for human IL-2. The assay is sensitve to approximately 3 pg/ml of IL-2, corresponding to a level of 15 pg/ml of the fusion protein.

The expression derived from pLNC-CCIL2 served as a baseline on which to compare the other vectors. Two additional promoters were tested: a bovine major histocompatibility class I gene promoter (active in murine cell lines [9]) and the promoter from the transcription factor IRF-1. Both promoters show high basal expression in numerous cell types and are further inducible by Type I and Type II interferons (the interferon inducibility was considered to be an advantage if we decided to explore a model using adjuvant therapy with these immune stimulating compounds). Two other separate vectors were created in which different sequence elements were cloned downstream of the CMV promoter but within the 5' untranslated region of the CC49-IL2 fusion protein gene. Both elements had been shown in other related expression systems to play a role in increasing transcript levels by post-transcriptional mechanisms. One of the elements tested was an intron from CMV ("Intron A"), and is widely used in expression constructs for its ability to increase expression (10). The other sequence element tested is a 119 bp region from the HSV thymidine kinase gene, shown by a colleague (J. Mertz) to have a similar effect on increasing steady state levels of mRNA (11). Finally, we tested expression of a Sindbis-virus derived self amplifying system studied by our colleagues in the Jon Wolff lab (12). Results from the in vitro (in B16 melanoma cells) and in vivo experiments are summarized in Figure 1.

The expression studies demonstrated that the pLNCX derived vector pLNC-CCIL generated the highest levels of CC49-IL2 fusion protein both in vitro and in vivo. Therefore, we decided to carry out further experiments using this vector.

Task 2. Analysis of the ability of CC49-IL2 Fusion Protein to localize specifically to TAG-72+ tumor, both at the site of gene delivery and at tumor sites distal from gene delivery.

Prior to the development of an in vivo immunotherapy model utilizing CC49-IL2 FP, we examined the ability of the protein to localize specifically to tumor sites that expressed the target antigen. This was done by two methods. The first involved the use of PMGT to deliver the gene encoding the CC49-IL2 FP into mouse epidermis overlying a TAG-72$^+$ tumor.

The TAG-72$^+$ tumor used in our studies was the human colon cancer cell line LS174T, since murine cells do not typically express CC49-reactive epitopes on normal or transformed tissue. 1-3 x 10^6 cells were engrafted intradermally into the belly of immunodeficient mice, typically nude mice (SCID mice were also used in some experiments, but as these strains spontaneously rejected the engrafted tumor over time, they were unsuitable for immunotherapy models and their use discontinued). Simultaneously at a site approximately 2 cm. away, 1-3 x 10^6 M21 cells were also engrafted intradermally. M21 is a human melanoma line that shows similar growth kinetics in nude mice as LS174T, but has no detectable expression of TAG-72. Once tumors were between 3 and 5 mm in diameter, they were blasted with beads containing the pLNC-CC49-IL2 expression vector DNA using the gene gun. A third site (denoted as "neutral") also served as a recipient for PMGT on the same animal. Tissue was then harvested from

sacrificed mice from areas encompassing the gene delivery site: over the LS174T tumor (TAG-72$^+$), over the M21 tumor (TAG-72$^-$), and over the neutral site (also negative for TAG-72 expression). Tissue samples were obtained at 24 and (from different animals) at 96 hours. The expectation was that at the 96 hour time point more of the FP would be retained in the antigen positive site (the LS174T tumor) than in the other 2 antigen negative sites. Table 1 shows the results from one experiment using 4 animals, 2 harvested at 24 hours and 2 at 96 hours. The data show that there is no evidence of fusion protein retention at the LS174T tumor compared to what is seen at the other 2 sites. Additional studies gave similar results (data not shown).

We also developed a model to assess the ability of the CC49-IL2 fusion protein to target tumor via the circulation. For these experiments purified protein was not simply injected into mice since tsuch a bolus administration was not representative of what would occur following PMGT. One potential advantage of PMGT, and other related gene therapy approaches, is that a constant level of protein is continually being released. To mimic this situation, the LS174T cell line was transfected with the expression vector, and stably transfected clones constitutively secreting the CC49-IL2 FP were isolated and characterized phenotypically. When these cells were engrafted into nude mice, they produced and released fusion protein into the circulation at a constant level, presumably similar to what would occur in skin cells following PMGT. One difference in this model is that higher serum levels of FP were achieved using the transfected cells. Concomitant with the engrafting of the FP expressing cells, other untransfected TAG-72$^+$ LS174T cells (i.e., cells not producing the CC49-IL2 FP) were engrafted at a distal site. Comparison of the amount of FP found in the untransfected tumor was compared to the amount found in the serum as well as in an adjacent "neutral" skin site, allowing an estimation of the ability of engrafted antigen positive cells to bind circulating CC49-IL2 FP. Fusion protein was quantitated in the serum and at the tissue sites by ELISA as described above. The results from 4 animals are shown in Table 2.

The data indicate that high local levels and serum levels of the FP can be obtained in this model ("high" in a relative sense; the concentration of the FP in the serum ranges from about 3 to 50 ng/ml in these animal, but in animals receiving "conventional" PMGT serum levels rarely were greater than 1 ng/ml and usually substantially lower). However, there is no evidence of specific retention of FP in the untransfected antigen positive tumor--levels of FP were either at the threshold of detection, or essentially the same as a harvested neutral skin site of matched size.

During this period, we were also carrying out experiments probing the exact nature of the CC49-reactive TAG-72 antigen. We observed that Jurkat and several other hematopoietic cell lines showed reactivity with the original CC49 mAb. We determined that the CC49-reactive carbohydrate epitope was expressed on cell surface molecules besides the "classical" TAG-72 protein, which is most likely a high molecular weight epithelial mucin. This analysis led to a publication (13). We further determined that LS174T TAG-72$^+$ tumors grown in mice expressed readily detectable but not substantial amounts of

antigen, and even under the best in vitro conditions with strongly activated human immune effector cells and at a high concentration of antibody, were not efficiently killed by antibody dependent cellular cytotoxic mechanisms. The data we generated on CC49 cross-reactive antigens, coupled with our expression, retention, and targeting data following PMGT of the antibody cytokine protein, led us to re-evaluate our immunotherapy model as originally proposed.

Task 3: Evaluation of FP produced by gene delivery to exert anti-tumor effects in conjunction with adoptively transferred human effector cells.

As stated above, we re-evaluated the approach using adoptively transferred cells based on data generated in other experiments. We chose to attempt development of a syngeneic model in which TAG-72$^+$ murine tumors were grown in immunocompetent animals sharing the genetic background of the tumor. Two primary advantages could be exploited in this approach. The activity of the resident immune system in the immunocompetent tumor bearing animals would most likely show any subtle anti-tumor effects not observable in an adoptive transfer model. Furthermore, if anti-tumor effects were noted in the immunocompetent mice in conjunction with fusion protein gene delivery, a subsequent analysis of long-lasting tumor immunity could be carried out. In this way, the ability of antibody cytokine fusion proteins to produce a desired clinical response could be assayed in a gene therapy context.

The main difficulty with development of such a model is lack of a CC49-reactive murine tumor line. We attempted to generate a CC49-reactive line by transfecting murine tumor cells with a plasmid expressing a portion of the human MUC-1 gene. In human tumor cells (particularly in primary mammary tumor), the MUC-1 protein backbone can serve as a "scaffold" for addition of carbohydrate modifications strongly reactive with the CC49 antibody. The TSA tumor cell line was used as a recipient for transfection, since this cell line corresponds to a murine mammary adenocarcinoma line of a BALB/C genetic background. TSA cells were transfected with the expression plasmid, then several G418-resistant pools were generated and assayed by flow cytometry for reactivity with the CC49 antibody. A typical analysis is tabulated in Table 3. The data indicate that a substantial proportion of CC49 reactive cells are present in the transfected population only, some of which stain very brightly for antigen. However, the expression is polymorphic and shows a wide distribution of intensity. Efforts to sort a homogeneous brightly staining population were not effective, since the profile reverted to a broad based distribution following growth of the sorted pools. However, the polymorphic expression pattern observed is stable, since repeated passaging of the cells did not result in substantial alterations in the staining pattern.

The protocol for the immunotherapy experiment was as follows. 1-3 x 10^6 MUC-1 transfected (CC49 reactive) or untransfected (CC49 unreactive) TSA cells were injected intradermally into the shaved belly of 6-8 week old BALB/C females. When the tumors were approximately 3-4 mm across, day 4 following injection, PMGT was carried out at three sites surrounding the tumor and a fourth site right on

the tumor. A repeated series of gene deliveries were carried out 2 days later on day 6. PMGT was carried out either with the pLNCX vector alone, or pLNC-CCIL2, the expression plasmid driving production of the CC49-IL2 fusion protein (murine immune effector cells are able to recognize and use the human constant region encoded in this construct). Tumor measurements were carried out over the next several days to assess tumor growth. Each of the four groups, [transfected tumor blasted with vector alone, transfected tumor blasted with the CC49-IL2 expression vector, untransfected tumor blasted with vector alone, and untransfected tumor blasted with the CC49-IL2 expression vector], was comprised of 5 animals. Fig 2 shows the tumor growth curves of the mean tumor diameters in each of the 4 groups. The data indicate that there was not a significant difference in tumor growth kinetics among the four animals.

Despite the fact that no significant differences were noted in this first experiment, the kinetics of the response indicated a subtle slowdown in tumor growth in the experimental group of interest, namely the transfected tumor exposed to the CC49-IL2 expression plasmid (MUC-pLNCCIL). However, this decreased growth rate seen at days 6 to 10 was not maintained over the course of the experiment. A repeat of this experiment would involve additional blasting at later time points in the experiment, e.g., on day 8 as well as on day 4 and 6. Such additional gene deliveries have been shown to be necessary to observe anti-tumor effects in related PMGT experimental models (14).

CONCLUSIONS

PMGT has been shown to be an effective method of delivering genes that can recruit host immune effector cells to tumor sites to exert anti-tumor effects. Cytokine genes have primarily been used to engender such responses (15). We attempted to extend these observations using a gene encoding an antibody-cytokine fusion protein. Though we were able to generate readily detectable expression of the antibody cytokine fusion protein at the delivery site and in the serum, we were unable to show specific targeting or retention of the fusion protein in antigen positive tumor. Furthermore, despite our ability to create CC49 reactive murine cells, we could not show a statistically significant anti-tumor effect in syngeneic tumor engrafted animals following PMGT. Nevertheless, a variety of PMGT delivery regimens can be easily assayed and it is probable with additional experimentation effective alternative strategies can be developed. Such investigation appears warranted given the strong anti-tumor effects noted following parenteral administration of related antibody-cytokine fusion proteins.

REFERENCES

1. DeVita, VT, Hellman, S, and Rosenberg, SA. Biologic Therapy of Cancer. Philadelphia: J.B. Lippincott Co., 1995.

2. Shu, L, Chen-F. Qi, Schlom, J, and Kashmiri, SVS. Secretion of a single-gene-encoded immunoglobulin from myeloma cells. Proc. Natl. Acad. Sci. (USA) 90: 7995-7999, 1993.

3. Nicolet, CM, Burkholder, JK, Gan, J, Culp, J, Kashmiri, SVS, Schlom, J, Yang, N-S, and Sondel, PM. Expression of a tumor-reactive antibody-IL2 fusion protein following in vivo particle mediated gene delivery. Cancer Gene Therapy 2: 161-170.

4. Thor, A, Ohuchi, N, Szpak, CA, Johnston, WW, and Schlom, J. The distribution of oncofetal antigen TAG-72 defined by monoclonal antibody B72.3. Cancer Res. 46: 3118-3124, 1986.

5 Muraro, R, Kuroki, M, Wunderlich, D, Pool, DJ, Colcher, D, Thor, A, Greiner, JW, Simpson, JF, Molinolo, A, Noguchi, P, and Schlom, J. Generation and characterization of B72.3 second generation monoclonal antibodies reactive with the tumor-associated glycoprotein 72 antigen. Cancer Res. 48: 4588-4596, 1988.

6. Yang, N-S, De Luna, C, and Cheng, L. Gene transfer via particle bombardment: applications of the Accell gene gun. In: Gene Therapeutics: Methods and Applications of Direct Gene Transfer. J. Wolff, ed., Boston: Birkhauser, 1994, pg. 193-209.

7. Sabzevari, H, Gillies, SD, Mueller, BM, Pancook, JD, and Reisfeld, RA. A recombinant antibody-interleukin 2 fusion protein suppresses growth of hepatic human neuroblastoma metastases in SCID mice. Proc. Natl. Acad. Sci. 91: 9626-9630, 1994.

8. Miller, AD, and Rosman, GJ. Improved retroviral vectors for gene transfer and expression. BioTechniques 7: 980-989, 1989.

9. Boshart, M, Weber, F, Jahn, G, Dorsch-Hasler, K, Fleckenstein, B, and Schaffner, W. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 41: 521-530, 1985.

10. Buchman, AR, and Berg, P. Comparison of intron-dependent and intron-independent gene expression. Mol. Cell. Biol. 8: 4395-4405, 1988.

11. Liu Xuedong, and Mertz, J. hnRNP L binds a cis-acting RNA sequence element that enables intron-independent gene expression. Genes and Development 9: 1766-1780, 1995.

12. Herweijer, H, Latendresse, JS, Williams, P, Danko, I, Schlesinger, S, and Wolff, JA. High level gene expression in vitro and in vivo using a plasmid based self-amplifying Sindbis virus vector. Human Gene Therapy 6: 1161-1167, 1995.

13. Nicolet, CM, Siegel, DH, Surfus, JE, and Sondel, PM. TAG-72 reactive antibody CC49 recognizes molecules expressed by hematopoietic cell lines. Tumor BIology 18: 356-366, 1997.

14. Rakhmilevich, AL, Turner, J., Ford, MJ., McCabe, D, Sun ,WH, Sondel, PM, Grota K, and Yang NS. Gene gun-mediated skin transfection with interleukin 12 gene results in regression of established primary and metastatic murine tumors. Proc. Natl. Acad. Sci. (USA) 93:6291-6, 1996.

15 Sun, WH, Burkholder, JK, Sun, J, Culp, J, Turner, J, Lu, XG, Pugh, TD, Ershler, WB, and Yang, N-S. In vivo cytokine gene transfer by gene gun reduces tumor growth in mice. Proc. Natl. Acad. Sci. (USA) 92: 2889-2893, 1995.

Table I Retention of CC49-IL2 FP by Different Tissue Sites in Nude Mice

	Neutral (TAG 72-)	M21 (TAG 72-)	LS174T (TAG 72+)
24 hr.,A	113.90	48.10	24.00
24 hr.,B	96.50	79.00	34.00
96 hr.,A	0.10	0.07	<0.05
96 hr.,B	0.11	0.28	0.12

Data from each animal,sacrificed at different time points following PMGT with the pLNC-CC49 IL2 expression vector, are presented in ng/ml of FP. TAG-72 antigen status was defined by reactivity with the CC49 antibody. Approximately 100 mg of tissue are disrupted per 0.5 ml lysis buffer.

Table II. Targeting of Systemic CC49-IL2 FP to Antigen Positive Sites

Site:	Animal 1	Animal 2	Animal 3	Animal 4
Transfected (FP secreting) Tumor	321.0	1120.0	1093.0	249.0
Serum	4.7	6.7	49.8	2.8
Untransfected Tumor	0.1	0.3	0.6	<0.05
Neutral	0.6	<0.05	0.3	<0.05

Data are presented in ng/ml of FP. Nude mice were engrafted with antigen positive LS174T cells transfected or not with an expression vector producing the CC49-IL2 FP. Tumors were harvested at a diameter of 3-5 mm, and processed in 0.5 ml of lysis buffer.

Table III. CC49-Reactive Antigen Expression in Transfected TSA Murine Mammary Adenocarcinoma Cells

	Primary Antibody	Gated Population	% Gated Cells	Mean Fluorescence Intensity
Untransfected TSA	MOPC 21	All	100	4.4
		M1	0.4	28.4
	CC49	All	100	6.4
		M1	2.8	45.3
Transfected TSA	MOPC 21	All	100	7.9
		M1	4.6	39.5
	CC49	All	100	42.62
		M1	20.5	176.4

TSA Cells transfected or not with a MUC-1 expression plasmid were analyzed for reactivity with the CC49 antibody by flow cytometry. MOPC-21 is used as a non-reactive isotype control. "M1" corresponds to a marker defining a region of more brightly staining cells, and is established at the same coordinates in all experimental samples.

Figure Legends.

Figure 1. Relative expression levels of various plasmid constructs, all driving production of the CC49-IL2 FP, are shown, with the expression of the pLNCX parental vector set as 1.0. The salient features of the different vectors are described in the text. Whether the experiments were done in vitro, assaying production in B16 melanoma cells, or in vivo, assaying production in mouse skin, is indicated.

Figure 2. Growth kinetics of tumors following PMGT delivery of the pLNC-CCIL expression vector or the parental vector control, pLNCX. Tumors were either antigen negative (i.e., CC49 unreactive) in the case of the VEC-transfected TSA cell line, or antigen positive (CC49 reactive) in the MUC-transfected TSA cell line (see Table III). The arrows indicate the time points at which PMGT was carried out. The growth curves represent the mean of tumor sizes in 5 different animals for each of the 4 groups, with the standard error indicated.

Fig. 1. Activity of Expression Constructs

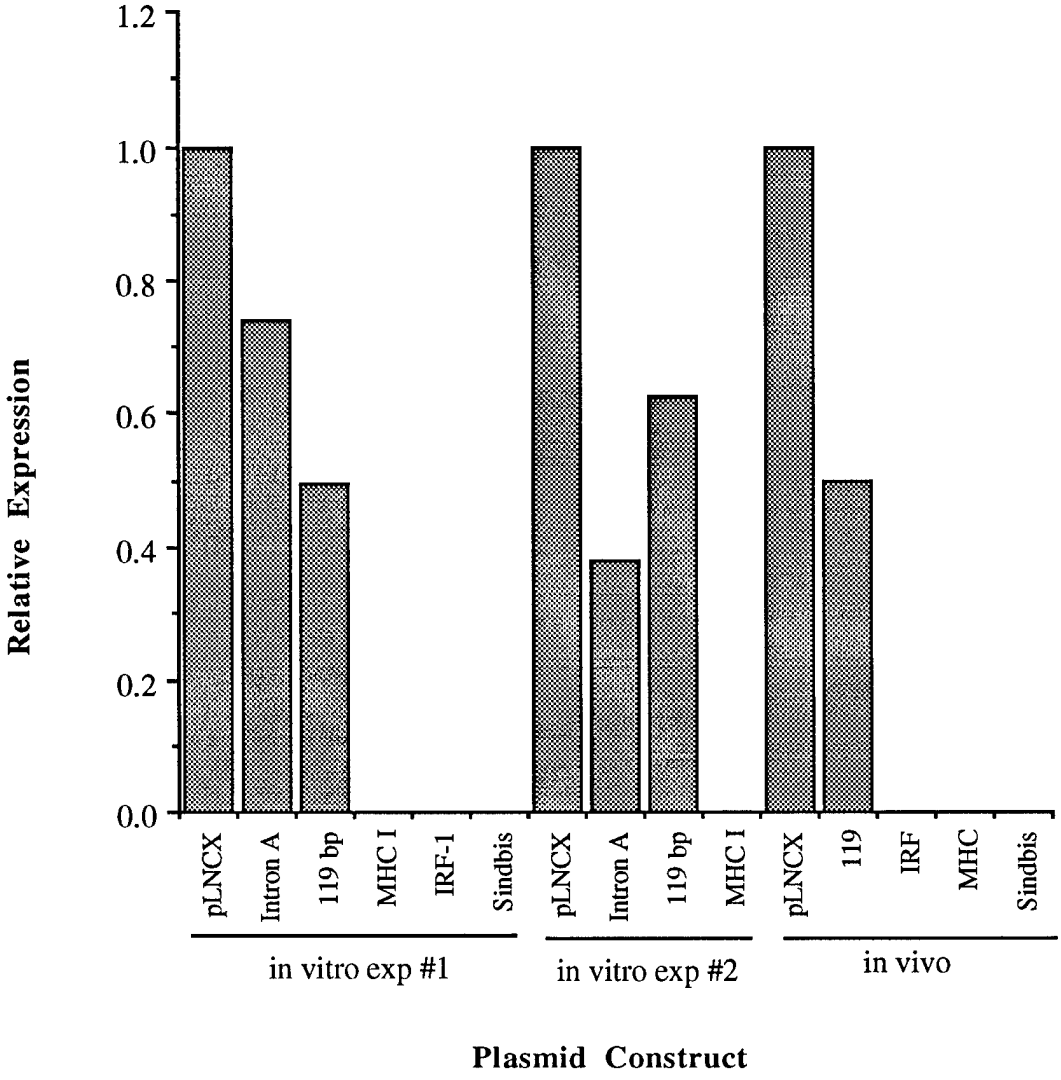

Fig. 2. Tumor Growth Kinetics Following FP Delivery by PMGT