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Suppression of an Epidermal Growth Factor Receptor-hyperproducing Tumor by an Immunotoxin Conjugate of Gelonin and a Monoclonal Anti-Epidermal Growth Factor Receptor Antibody[1]

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ABSTRACT

An immunotoxin was made by conjugating a murine monoclonal antibody (B4G7) that recognizes the human epidermal growth factor (EGF) receptor with gelonin, a ribosome-inactivating protein. This B4G7-gelonin conjugate was shown to be specifically cytotoxic for EGF receptor-hyperproducing cells. The conjugate was tested in nude mice and shown to be capable of suppressing the growth of an EGF receptor-hyperproducing squamous carcinoma cell (A431) solid tumor. Nude mice bearing an A431 cell tumor that were given injections i.p. for 5 consecutive days with at least 10 μg of the conjugate showed significant suppression of tumor growth for about 7 days. On the other hand, an unconjugated mixture of B4G7 and gelonin showed no specific antitumor activity against the A431 cell tumor. The growth of an EGF receptor-deficient small cell lung cancer cell (H69) tumor was not suppressed by injection of the conjugate. No toxic effects were observed in histological examination of nontumorous tissues of mice treated with at least 250 μg of conjugate per mouse. These results suggest that the conjugate may be useful for targeting therapy to EGF receptor-hyperproducing squamous carcinoma.

INTRODUCTION

The human EGF[3] receptor, a M_r 170,000 transmembrane glycoprotein, was identified as the product of the protooncogene c-*erb*B (1-4).

The amplification and relative level of expression of the EGF receptor gene were determined in surgically resected fresh tumors of various origins by DNA hybridization and EGF binding studies. We found that the EGF receptor gene was amplified and overexpressed in squamous cell carcinomas of esophagus and lung (5, 6). Furthermore, the increase in expression level of the EGF receptor gene was suggested to be associated with the proliferation of these squamous cell carcinomas (7). Moreover, the patients with a high EGF receptor level and EGF receptor gene amplification died from recurrence of the cancer within several months after radical surgery (8).

The ideal features of a target for the targeting therapy include its absence in blood, high specificity to cancerous tissues, and presence in cell membranes. Since EGF receptors in squamous cell carcinomas fulfill these conditions, we have developed a new strategy using an immunotoxin prepared by conjugating gelonin with the monoclonal antibody B4G7 (9). B4G7 preferentially binds to human EGF receptors of the low-affinity class, which are found on many receptor-hyperproducing cancerous tissues (10). When B4G7 binds to an EGF receptor on the cell surface, a significant amount of antibody-receptor complex is internalized through the formation of an endocytic vesicle (11, 12). Gelonin, which is extracted from *Gelonium multiflorum*, is a 60S ribosome-inactivating glycoprotein with a molecular weight of 30,000 that inhibits protein synthesis (13-15). Gelonin has many advantages over the ricin, abrin, and diphtheria toxin A chains. It is stable to biochemical and physical treatment and is as potent as ricin A chain in a cell-free rabbit reticulocyte system (16). Gelonin conjugate is nontoxic up to 2 mg (total dose) per mouse (17).

We have previously examined the cytotoxic effect of the B4G7-gelonin conjugate in cell culture (9). The conjugate killed EGF receptor-hyperproducing squamous carcinoma cells (A431, NA, Ca9-22, TE5) and, to a lesser extent, human fibroblasts (HFO). It did not kill EGF receptor-deficient small cell lung cancer cells (H69) and mouse fibroblasts (Swiss/3T3). An unconjugated mixture of B4G7 and gelonin did not have a toxic effect. The number of EGF receptors on the cell surface was correlated with the cytotoxic effect at 10 nM conjugate. Nonspecific cell killing was not observed.

In this paper, we have investigated the antitumor effect of the B4G7-gelonin conjugate injected i.p. into nude mice bearing human squamous cell carcinoma, and we have predicted that a new immunotherapy targeted to cells overexpressing a specific oncogene will be useful.

MATERIALS AND METHODS

Mice. Six-wk-old female BALB/c mice and BALB/c (*nu/nu*) mice were obtained from Sankyo Laboservice Co. (Tokyo, Japan).

Tumor Cells. Squamous carcinoma A431 cells established from human squamous cell carcinoma of the vulva were obtained from Dr. S. Cohen. Human small cell lung cancer H69 cells were obtained from Dr. Y. Shimosato. A431 and H69 cells were grown as described previously (9).

Chemicals. *N*-Succinimidyl-3-(2-pyridyldithio)propionate was purchased from Pharmacia (Sweden). Gelonin was purchased from Pierce Chemical Co. (Rockford, IL). ^{125}I-EGF was prepared with Iodobeads (Pierce) as described previously (18).

Monoclonal Antibody (B4G7). The production and characterization of B4G7, a murine monoclonal IgG2a antibody, have been described (10). Ascitic fluid was produced by injection of pristine-primed BALB/c mice i.p. with 10^6 hybridoma cells. Total IgG was isolated from ascites by affinity chromatography on Protein A-Sepharose CL-4B (Pharmacia).

Preparation of B4G7-Gelonin Conjugate. Gelonin was conjugated to B4G7 by the procedure described previously (9, 16). Briefly, dithiopyridyl groups were incorporated into B4G7 by treatment with a 10-fold molar excess of *N*-succinimidyl-3-(2-pyridyldithio)propionate. Sulfhydryl groups were incorporated into gelonin by treatment with 2-iminothiolane. Modified B4G7 was mixed with an approximately equimolar amount of modified gelonin. The reaction was stopped by the addition of iodoacetamide and dialyzed against 5 mM sodium phosphate buffer, pH 6.5, containing 35 mM NaCl. The solution was applied to a column of CM-cellulose (CM-52; Whatman) equilibrated with the same buffer. The column was extensively washed with the solution used to equilibrate the column. Nonconjugated B4G7 was eluted from the

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[3] The abbreviations used are: EGF, epidermal growth factor; PBS, phosphate-buffered saline.

column. The conjugate was eluted with a linear concentration gradient of NaCl (from 35 to 300 mM) and was sterilized by filtration through a 0.22-μm membrane (Millex-GV; Millipore Co., Bedford, MA). The conjugate was stored at 4°C until use.

Transplantation of A431 and H69 Cells into Nude Mice. A431 or H69 cells were harvested, washed to remove fetal calf serum, and resuspended in Dulbecco's modified Eagle's medium at a cell concentration of 5×10^8 cells per ml. Cells (10^7/site) were injected into the subcutaneous tissues of nude mice. After injection, nude mice developed solid tumors in at least 5 days for A431 cells or 10 days for H69 cells.

Treatment of Tumor with Conjugate or Mixture. After the transplantation of A431 or H69 cells into the subcutaneous tissues of nude mice, the conjugate or an unconjugated mixture of B4G7 and gelonin in PBS was injected i.p. This treatment was repeated for 5 consecutive days.

Tumor Weight. During the time course of tumor growth, tumor weight was determined by measuring the major and the minor axes of the tumor which are perpendicular. Tumor weight was calculated by the formula W (mg) = $0.5 \times LS^2$, where L is the major axis (mm), and S is the minor axis (mm).

EGF Binding Assay. A431 cell tumors were removed from nude mice and frozen at −80°C until use. The EGF binding assay has been described previously (5). EGF binding was expressed as a percentage of ^{125}I-EGF bound on the tissues per mg of protein.

Protein Concentration. The determination of protein concentration was performed by the method of Lowry *et al.* (19) using bovine serum albumin as a standard.

RESULTS

***In Vitro* Effect of Conjugate.** The *in vitro* cytotoxicity of the B4G7-gelonin conjugate to the EGF receptor-hyperproducing A431 cells was tested by measuring cell survival as described (9). The toxic effect of the conjugate was shown to be dose dependent. The conjugate caused 50% cell death at 0.3 μg/ml and 90% cell death at 3 μg/ml. Gelonin alone at 50 μg/ml caused no cytotoxicity to A431 cells. These results (data not shown) were consistent with our previous observations (9).

***In Vivo* Effect of Conjugate.** We examined suppression of tumor by i.p. injection of various concentrations of conjugate (Fig. 1). The logarithmic increase in tumor size began in PBS-treated control mice about 7 days after injection of A431 cells. However, this increase in tumor weight terminated on about Day 25. When nude mice were given injections for 5 consecutive days (Day 7 to Day 11) with 10 or 50 μg of conjugate per injection, tumor growth was significantly suppressed for about a week, followed by partial suppression. Injection with 1 μg of conjugate per injection caused only a slight tumor suppression. The lower limit to effectively suppress tumor growth was about 50 μg of accumulative dose per mouse. The unconjugated mixture of B4G7 (50 μg) and gelonin (50 μg) did not suppress tumor growth.

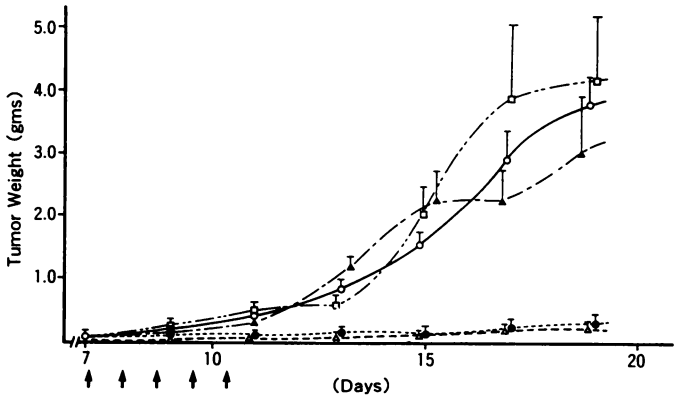

Fig. 1. Antitumor effect of the B4G7-gelonin conjugate or the nonconjugated mixture of B4G7 and gelonin. Nude mice were given injections of 10^7 A431 cells i.p. on Day 0 and on Days 7, 8, 9, 10, and 11 (indicated with *arrows*) with 200 μl of PBS alone (○), with 1 μg (▲), 10 μg (●), and 50 μg (△) of conjugate in 200 μl of PBS, or with a mixture of 50 μg of B4G7 and 50 μg of gelonin (□) in 200 μl of PBS. The *ordinate* represents the calculated tumor weight by W (mg) = $0.5 \times LS^2$. Values of tumor weight are means from PBS-treated mice (n = 5), 1-μg conjugate-treated mice (n = 4), 10-μg conjugate-treated mice (n = 8), 50-μg conjugate-treated mice (n = 3), and mixture-treated mice (n = 4). *Bars*, SE.

Tumors of A431 cells were removed from nude mice 8 days after the final i.p. injection of the conjugate or the unconjugated mixture of B4G7 and gelonin. The actual value of tumor weight was measured (Table 1). The conjugate at 1, 10, or 50 μg per injection suppressed tumor growth by 17, 87, or 88%, respectively. However, the mixture of 50 μg of B4G7 and 50 μg of gelonin did not suppress tumor growth. These results showed a good correlation with the calculated values obtained in Fig. 1.

The suppression of A431 cell tumor growth depends on not only the dose administered, but also the time of the initial injection of the conjugate. This time dependence was investigated by injecting 10 μg of conjugate i.p. for 5 consecutive days into nude mice with various tumor sizes (data not shown). When the initial injection was carried out in nude mice whose tumors weigh up to 100 mg, tumor growth was significantly suppressed. For tumors weighing from 100 to 300 mg, tumor growth was significantly suppressed for about a week and then partially suppressed. In the case of tumor weight exceeding 300 mg, the growth rate was suppressed but the tumor continued to grow. The antitumor activity decreased with increased tumor weight at the time of the initial injection. This may be due to reduced access of the conjugate through blood vessels to a large solid tumor mass.

The results described above indicated that the conjugate can suppress the growth of A431 cell tumors. However, tumor suppression may have selected for a mutation in the tumor cells that decreased EGF binding. To test this possibility, the EGF binding capacity of tumor was measured on 19 days after injection of A431 cells. The EGF binding capacity of conjugate-treated tumors was not significantly different from that of PBS-treated tumors (33 and 36% of input/mg of protein, respectively). This result indicates that EGF receptor-hyperproducing cells maintain their high level of EGF binding despite suppression of tumor growth.

Specific Antitumor Effect of Conjugate. The cytotoxic effect of the conjugate was not observed in human EGF receptor-deficient H69 cells that grow as a suspension culture (9). We tested the antitumor effect of the conjugate in nude mice bearing a solid tumor of H69 cells. A431 cell tumors and H69 cell tumors were formed in subcutaneous tissues on the right flank and left flank of nude mice, respectively. A431 cells are more tumorigenic than H69 cells. Nude mice were injected i.p. for 5 consecutive days with 10 μg of conjugate (Fig. 2, *right*) or PBS

Table 1 *Antitumor effect of the B4G7-gelonin conjugate*

Treatment	Dose/injection[a]	Tumor wt (g)
PBS		2.61 ± 0.22[b] (1.00)[c]
Conjugate	1 μg	2.16 ± 0.62 (0.83)
Conjugate	10 μg	0.35 ± 0.23 (0.13)[d]
Conjugate	50 μg	0.32 ± 0.11 (0.12)[e]
Mixture	50 μg of B4G7 + 50 μg of gelonin	2.62 ± 0.73 (1.00)

[a] Injection was repeated for 5 consecutive days.
[b] Mean ± SE of tumor weight excised 19 days after transplant.
[c] Numbers in parentheses.
[d] PBS *versus* 10 μg of conjugate, $P < 0.01$.
[e] PBS *versus* 50 μg of conjugate, $P < 0.01$.

Fig. 2. Effect of the conjugate on A431 and H69 cell tumor growth. Mice were given s.c. injections of 10^7 H69 cells on the left flank on Day 0. After 5 days, mice were given s.c. injections of 10^7 A431 cells on the right flank. Mice were treated on Days 16, 17, 18, 19, and 20 i.p. with 10 μg of conjugate in 200 μl of PBS (*right*) or 200 μl of PBS alone (*left*). The photograph was taken on Day 29.

(Fig. 2, *left*). The photograph shows that tumor growth of A431 cells is significantly suppressed by conjugate, while H69 cell tumor growth does not respond.

In order to determine the antitumor activity of the conjugate on H69 cell tumors quantitatively, we examined the weight of the A431 cell and H69 cell tumors over time. In PBS-treated control mice (n = 3), A431 cell tumors grew rapidly, and H69 cell tumors grew at a nearly linear rate, starting at about 10 days after injection (Fig. 3). On the other hand, in 10-μg conjugate-treated mice (n = 3), A431 tumor growth was completely suppressed for several days (Fig. 3*A*), while H69 cell tumors grew at the same rate as in untreated control mice (Fig. 3*B*). H69 cell tumors weighed 0.74 ± 0.39 g (mean ± SE) in control mice and 0.66 ± 0.35 g (mean ± SE) in conjugate-treated mice 8 days after the final injection. Ten μg of conjugate significantly suppressed A431 cell tumor growth, as seen in Fig. 2. However, H69 cell tumors had the same growth rate and weight regardless of treatment. Thus, the conjugate was specifically toxic to tumors that overexpress the EGF receptor, but was nontoxic to tumors that are EGF receptor deficient. The possibility that cells may have been exchanged between A431 cell tumors and H69 cell tumors in the conjugate-treated or untreated mouse was ruled out by measuring their EGF binding capacity (data not shown).

Histology. In order to examine the effect of the conjugate on the various tissues, hearts, kidneys, livers, lungs, and spleens

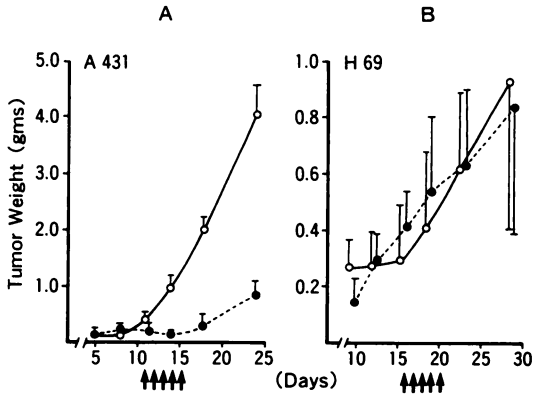

Fig. 3. Effect of the conjugate on A431 cell tumors or H69 cell tumors. In *A*, the weight of A431 cell tumors was examined in PBS-treated mice (n = 3) (○) and 10-μg conjugate-treated mice (n = 3) (●). In *B*, the weight of H69 cell tumors was examined in PBS-treated mice (n = 3) (○) and 10-μg conjugate-treated mice (n = 3) (●). *Bars*, SE.

Table 2 *Effect of the B4G7-gelonin conjugate on tissue weight*

Nude mice treated for 5 consecutive days with 10 μg of conjugate or PBS were sacrificed 19 days after injection with A431 cells. The weight of the tissues was determined.

Tissue	Wt (g)	
	Conjugate treatment (10 μg for 5 days)	PBS treatment
Heart	0.22 ± 0.02[a]	0.20 ± 0.02
Kidney	0.51 ± 0.04	0.49 ± 0.04
Liver	2.1 ± 0.2	2.0 ± 0.1
Lung	0.33 ± 0.03	0.30 ± 0.04
Both spleens	0.29 ± 0.08	0.25 ± 0.02
A431 cell tumor	0.35 ± 0.23	2.6 ± 0.73

[a] Mean ± SE from 4 mice.

were removed, weighed, and stained with hematoxylin-eosin. No difference in tissue weight between control mice and 50-μg conjugate-treated mice was observed (Table 2). Moreover, in a histological experiment, no toxic influence was found in any of the tissues from the 50-μg conjugate-treated mice (data not shown). These results indicated that the conjugate is specifically toxic to A431 cell tumors but not to normal tissue.

DISCUSSION

We have demonstrated that a conjugate formed by covalently linking gelonin to a monoclonal anti-EGF receptor antibody (B4G7) has a significant antitumor activity against EGF receptor-hyperproducing A431 cell tumors but no antitumor activity against EGF receptor-deficient H69 cell tumors in nude mice. An unconjugated mixture of B4G7 and gelonin had no effect on A431 cell tumor growth. This selectivity is due to the high affinity of B4G7 for the EGF receptor (10) and the lack of toxicity of gelonin to intact cells. The conjugate demonstrated a high antitumor activity and selectivity as predicted in the *in vitro* system (9).

A remarkable inhibitory activity against A431 cell solid tumors in nude mice was observed during the period of treatment with the conjugate via vessels. This fact is very important when considering a possible application to clinical cases. However, after cessation of treatment, the inhibitory effect became gradually reduced. This may reflect a reduction in concentration of the conjugate at A431 cell tumor sites. The B4G7 antibody accumulated at A431 cell tumor sites, but not in other tissues, such as liver, lung, and spleen as determined from localization of injected ^{125}I-B4G7 into a nude mouse.[4] Thus, the conjugate

[4] Nakajima *et al.*, unpublished observations.

probably also accumulates at A431 cell tumor sites. It has been reported that other antibody-gelonin conjugates accumulate in the liver and other tissues of the mouse through a nonspecific mechanism (20). The difference of tumor-specific accumulation must be dependent on the target. As EGF receptor localizes only in the cell plasma membrane but not in blood, the B4G7-gelonin conjugate would not make immune complex and hence would not accumulate in liver, spleen, and lung. Therefore, the reduction of antitumor action is most likely due to the consumption of conjugate that occurred by killing tumor cells. There is now a large body of data indicating that the disulfide bond in immunoconjugates is unstable *in vivo*. However, whatever the breakdown of the conjugate may have occurred is unimportant compared with the amount of intact conjugate localizing in the tumor.

After tumor-bearing nude mice were treated 5 times with the conjugate (50 μg) during 5 days, we examined the weight and the histology of various tissues including heart, kidney, liver, lung, and spleen by staining with hematoxylin-eosin. No differences were observed between conjugate-treated mice and PBS-treated control mice. Thus, the dose administered had no undesirable effects on mice. However, the tolerance dose has not yet been determined.

There are only a few reports about the antitumor effect on solid tumors of immunotoxins containing toxins such as gelonin (21) and *Pseudomonas* exotoxin (20) in animal models. Monoclonal antibodies conjugated to toxins were prepared by using human cancer cells as target antigens. In some of these reports, immunotoxins showed unsatisfactory antitumor activity. The poor potency is probably due to the low efficiency of targeting cancer tissues in an animal. An immunotoxin made from a monoclonal antibody to the transferrin receptor on a human ovarian cancer cell surface was accumulated in not only cancer tissues, but a number of normal tissues (20).

In this study, we used an antibody against overexpressed oncogene products to target the toxin to cancer cells. The carcinogenesis of squamous cells in esophagus or lung was correlated with the amplification and overexpression of the c-*erb*B protooncogene (5). This finding indicated that the c-*erb*B protein, the EGF receptor, could serve as a target antigen to detect cancer cells for immunotherapy. We investigated this possibility in nude mice bearing A431 cell tumors which overexpress c-*erb*B protooncogene or H69 cell tumors which do not express c-*erb*B by using a conjugate composed of an anti-EGF receptor antibody (B4G7) and gelonin. The B4G7 antibody binds only to the human EGF receptor and not to the mouse receptor, but EGF receptors widely distribute in normal human tissues. Thus, the therapeutic usefulness of this reagent in humans might be limited. However, B4G7 antibody preferentially binds to the EGF receptor of low-affinity type (10), which is abundant in the EGF receptor-hyperproducing cancer tissues. The receptor of high-affinity type was found mainly on normal tissues. This fact suggests that the accumulation of the conjugate in normal human tissues may be insignificant. We are now in the process of making temperature-sensitive liposomes which contain the conjugate. This liposomal conjugate coupled with hyperthermia will facilitate the accumulation of the conjugates in tumors and aid in reducing side effects. Taking these into consideration, the conjugate can be expected to be therapeutically useful.

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REFERENCES

1. Cohen, S., Ushiro, H., Stoscheck, C., and Chinkers, M. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. J. Biol. Chem., *257:* 1523–1531, 1982.
2. Ushiro, H., and Cohen, S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A431 cell membranes. J. Biol. Chem., *255:* 8363–8365, 1980.
3. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D., and Seeburg, P. H. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature (Lond.), *309:* 418–425, 1984.
4. Shimizu, N., Hunts, J., Merlino, G., Wang-Peng, J., Xu, Y. H., Yamamoto, T., Toyoshima, K., and Pastan, I. Amplification and rearrangement of EGF receptor gene in translocation chromosome 7 (M4) of human epidermoid carcinoma A431 cells. Cytogenct. Cell Genet., *40:* 743–744, 1985.
5. Hunts, J., Ueda, M., Ozawa, S., Abe, O., Pastan, I., and Shimizu, N. Hyperproduction and gene amplification of the epidermal growth factor receptor in squamous cell carcinomas. Jpn. J. Cancer Res. (Gann), *76:* 663–666, 1985.
6. Kaseda, S., Ueda, M., Ozawa, S., Ishihara, T., Abe, O., and Shimizu, N. Expression of epidermal growth factor receptors in four histologic cell types of lung cancer. J. Surg. Oncol., *42:* 16–20, 1989.
7. Ozawa, S., Ueda, M., Ando, N., Abe, O., Hirai, M., and Shimizu, N. High incidence of EGF receptor–hyperproduction in esophageal squamous cell carcinomas. Int. J. Cancer, *39:* 333–337, 1987.
8. Ozawa, S., Ueda, M., Ando, N., Shimizu, N., and Abe, O. Prognostic significance of epidermal growth factor receptor in esophageal squamous cell carcinomas. Cancer (Phila.), *63:* 2169–2173, 1989.
9. Ozawa, S., Ueda, M., Ando, N., Abe, O., Minoshima, S., and Shimizu, N. Selective killing of squamous carcinoma cells by an immunotoxin that recognizes the EGF receptor. Int. J. Cancer, *43:* 152–157, 1989.
10. Behzadian, M. A., and Shimizu, N. Monoclonal antibody that immunoreacts with a subclass of human receptors for epidermal growth factor. Cell Struct. Funct., *10:* 219–232, 1985.
11. Das, M., and Fox, F. Molecular mechanism of mitogen action: processing of receptor induced by epidermal growth factor. Proc. Natl. Acad. Sci. USA, *75:* 2644–2648, 1978.
12. Das, M., Miyakawa, T., Fox, C. F., Pruss, R. M., Aharonov, A., and Merschman, H. R. Specific radiolabeling of a cell surface receptor for epidermal growth factor. Proc. Natl. Acad. Sci. USA, *74:* 2790–2794, 1977.
13. Stirpe, F., Olsnes, S., and Pihl, A. Gelonin, a new inhibitor of protein synthesis, non-toxic to intact cells. Isolation, characterization, and preparation of cytotoxic complexes with concanavalin A. J. Biol. Chem., *255:* 6947–6953, 1980.
14. Barbieri, L., and Stirpe, F. Ribosome-inactivating proteins from plants: properties and possible uses. Cancer Surv., *1:* 489–520, 1982.
15. Falasca, A., Casperi-Campani, A., Abbondanza, A., Barbieri, L., and Stirpe, F. Properties of the ribosome-inactivating proteins gelonin, *Momordica charantica* inhibitor, and dianthins. Biochem. J., *207:* 505–509, 1982.
16. Lambert, J. M., Senter, P. D., Yau-Young, A., Blattler, W. A., and Goldmacher, V. S. Purified immunotoxins that are reactive with human lymphoid cells. J. Biol. Chem., *260:* 12035–12041, 1985.
17. Sivam, G., Pearson, J. W., Bohn, W., Oldham, R. K., Sadoff, J. C., and Morgan, A. C., Jr. Immunotoxins to a human melanoma-associated antigen: comparison of gelonin with ricin and other A chain conjugates. Cancer Res., *47:* 3169–3173, 1987.
18. Miskimins, W. K., and Shimizu, N. Uptake of epidermal growth factor into a lysosomal enzyme-deficient organelle: correlation with cell's mitogenic response and evidence for ubiquitous existence in fibroblasts. J. Cell. Physiol., *118:* 305–316, 1984.
19. Lowry, O. H., Rosebrough, N., Farr, A. L., and Randall, R. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265–275, 1951.
20. Fitzgerald, D. J., Willingham, M. C., and Pastan, I. Antitumor effects of an immunotoxin made with *Pseudomonas* exotoxin in a nude mouse model of ovarian cancer. Proc. Natl. Acad. Sci. USA, *83:* 6627–6630, 1986.
21. Scott, C. F., Jr., Goldmacher, V. S., Lambert, J. M., Jackson, J. V., and McIntyre, G. D. An immunotoxin composed of a monoclonal antitransferrin receptor antibody linked by a disulfide bond to the ribosome-inactivating protein gelonin: potent *in vitro* and *in vivo* effects against human tumors. J. Natl. Cancer Inst., *79:* 1163–1172, 1987.