Inability of Anti-epidermal Growth Factor Receptor Monoclonal Antibody to Block "Autocrine" Growth Stimulation in Transforming Growth Factor-secreting Melanoma Cells*

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Mouse monoclonal antibodies to the human epidermal growth factor (EGF) receptor were raised by immunizing with plasma membrane vesicles prepared from A431 cells. This paper describes the characterization of one of the IgG anti-receptor monoclonal antibodies generated and its use to probe the role of transforming growth factor (TGF) in the autonomous growth of a melanoma cell line in culture. This antibody blocks: 1) the binding of EGF to the A431 EGF receptor; 2) the EGF stimulation of the EGF-dependent protein kinase in vitro; and 3) human fibroblast DNA synthesis and proliferation in culture. It can precipitate the EGF receptor from metabolically labeled A431 cells and human fibroblasts and these receptors have indistinguishable peptide maps. No EGF receptor could be detected by immunoprecipitation after fibroblasts were treated with EGF or conditioned medium from the melanoma cells which secrete EGF-like TGF (αTGF). The antibody itself did not downregulate the receptor but could block down-regulation caused by EGF and αTGF. Despite its ability to block EGF-stimulated growth and down-regulation in fibroblasts, the antibody was unable to block the growth and soft agar colony formation of αTGF-secreting melanoma cells, nor could the antibody detect EGF receptor in these cells under the conditions developed to prevent down-regulation and lysosomal degradation of the EGF receptor. These studies suggest that these melanoma cells do not have the intact EGF receptor and that the secretion of αTGF by these cells plays no role in their growth in culture. The absence of receptor cannot be explained by down-regulation by secreted αTGF.

A variety of transformed cell lines have been shown to secrete growth factors (1). A subset of these growth factors are capable of allowing nontransformed target cells to assume anchorage-independent growth. Such mitogens have been operationally defined as TGFs (for review, see Ref. 2). One class of TGFs are capable of competing with EGF for binding to the EGF receptor (3-8) and these have been termed αTGFs (2). Blocking of the EGF receptor by anti-EGF receptor antibodies can block the stimulation of anchorage-independent growth of target cells by exogenously added αTGF (9). Therefore, the EGF receptor plays an integral role in the mechanism of action of αTGF. The mechanism by which the EGF receptor contributes to the TGF-induced transformed phenotype is not known. However, the EGF receptor shares properties with the products of a variety of oncogenes in that it contains a domain with intrinsic protein kinase activity (10, 11) capable of phosphorylating its substrates at tyrosine residues (12). The activity of the kinase is stimulated similarly by EGF and αTGF (13, 14). The ability of this tyrosine kinase to phosphorylate antibodies to p60v c (15, 16) and sequence data revealing homology with the v-erb-B oncogene protein (17) imply a structural relationship between the gene for the EGF receptor and the src-related subset of oncogenes. Therefore, the hypothesis has arisen that a cell capable of synthesizing an αTGF and the EGF receptor would, by an "autocrine" route, stimulate the activity of the EGF-dependent protein kinase and initiate the cascade of events that would ultimately lead to autonomous growth independent of exogenous signals (2, 7).

To test whether we could intervene in this process of autostimulation, we made use of a monoclonal antibody we recently raised to the EGF receptor. In this paper, we show that this antibody precipitates the EGF receptor and behaves as a competitive inhibitor of EGF action. It competes with EGF for binding to the human EGF receptor, blocks EGF stimulation of the EGF-dependent protein kinase, blocks EGF stimulation of human fibroblast growth, and blocks EGF-stimulated down-regulation of its receptor. We determined the effect of this antibody on the growth of a human melanoma cell line that secretes αTGF (8, 18) and on the down-regulation of their EGF receptor. We show that the EGF receptor in these αTGF-secreting cells is not detectable by our antibody by biologic and biochemical criteria. The antibody cannot block the autocrine growth stimulation and the autocrine down-regulation of the receptor, nor can it detect the receptor in these cells under conditions where EGF-stimulated lysosomal degradation of the receptor is blocked. These findings are compatible with the interpretation that the growth of this melanoma cell line in culture does not depend on the interaction of secreted αTGF with the EGF receptor.

**EXPERIMENTAL PROCEDURES**

_Materials_—Culture media and serum were from Gibco. Mouse EGF was purified from male Swiss Webster mice (Simonsen) submaxil-
of membrane protein in Freund's complete adjuvant was injected per ml insulin and 2 mM glutamine. Positively growing hybridoma clones were fused with the myeloma cell line Sp2/0 (kindly supplied by Dr. J. Falk, University of Toronto). The fused cells were seeded into five 96-well tissue culture plates containing an unimmunized spleen cell culture medium and screened for antibody capable of interfering with the EGF receptor.

Preparation of Monoclonal Antibodies to the EGF Receptor — The monoclonal antibody to the human EGF receptor on A431 plasma membrane vesicles was used as immunogen on BALB/c mice, and the immunization schedule was according to that reported for high frequency of antigen-specific hybridoma production (23). Briefly, 100 µg of purified membrane protein from Freund's incomplete adjuvant subcutaneously into the recipient mouse on day 0 as the primary dose, followed by 20 µg of membrane proteins from Freund's incomplete adjuvant injected subcutaneously and intramuscularly on days 14 and 28. From days 42-48, smaller amounts of membrane protein in PBS were injected intraperitoneally and intravenously by the schedule of Stihli et al. (22). On day 49, spleen cells from the in vivo immunized mice were fused with the myeloma cell line Sp2/0 (kindly supplied by Dr. J. Falk, University of Toronto). The fused cells were seeded into five 96-well tissue culture plates containing an unimmunized spleen cell feeder layer. The culture medium was further supplemented with 10% fetal bovine serum and 2% glutamine. Positive hybridoma clones were seen after 10 days in greater than 90% of the wells. The culture medium was screened for antibody capable of interfering with 125I-EGF binding to formaldehyde-fixed A431 cells as described below. Fifteen hybridoma clones were identified and these were cloned by limiting dilution at least twice prior to freezing. Three clones of these hybridomas were selected using the EGF radioreceptor assay as described above, then washed 3x with PBS containing 0.1% BSA. The labeled cells were then incubated with serum-free F12 medium previously conditioned by melanoma cells or with unconditioned F12 medium with or without B1D8 antibody.

Immunoprecipitation of EGF Receptor — After labeling the cells with [35S]methionine using the protocols described above, the monoclonal antibody and EGF were removed by trypsinization of the monolayer and trypsin was added to each well and incubation continued for 30 min. The labeled proteins on a 7.5% SDS-polyacrylamide gel were then visualized by fluorography. Autoradiographic exposure was carried out at -75°C.

Two-dimensional Tryptic Peptide Analysis — Tryptic peptide mapping of [35S]methionine-labeled EGF receptor from A431 cells and human foreskin fibroblasts was performed on receptor protein eluted from heat- and vacuum-dried unstained 7.5% polyacrylamide gels. The eluted protein was concentrated and digested with trypsin as described by Beemon and Hunter (27) and 15,000 cpm of 3H-EGF was added to each well. The labeled cells were harvested and used for immunoprecipitation. The unbound counts were then washed 3x with PBS containing 0.1% BSA. The labeled cells were then incubated with serum-free F12 medium previously conditioned by melanoma cells or with unconditioned F12 medium with or without B1D8 antibody.

Cell Growth Assays — Human foreskin fibroblasts from confluent tissue culture plates were distributed into 96-well plates (Costar) at a density of 5000 cells/well in F12 with 10% fetal bovine serum. After 18 h, the medium was replaced with F12 containing various concentrations of EGF and monoclonal antibody and EGF. NO serum was used. The cells were then incubated for 5 days, and the number of viable cells was determined using a Coulter Counter (Hialeah, FL), model ZB.
final concentrations of 0, 1, 10, 100, or 200 μg/ml in F12 culture medium containing 10% fetal bovine serum, 0.3% Agar Noble, and 2 × 10⁶ A2058 cells and added to 35-mm culture plates on top of a 1-ml base layer of 0.5% agar in F12 containing 10% fetal bovine serum. The plates were incubated at 37 °C in a humidified atmosphere containing 95% air and 5% CO₂. The plates were refed after 7 and 14 days with 1 ml of 0.3% agar in the same medium. After 21 days, the colonies were counted or photographed unfixed and unstained at low-power magnification. Colonies greater than 20 cells were counted as positive.

RESULTS

Three hybridomas producing monoclonal antibody raised against A431 plasma membrane vesicles and selected for their ability to interfere with ¹²⁵I-EGF binding to EGF receptors were stabilized and characterized. One of the hybridomas produced IgG₁ and two IgG₂a. In this communication, we describe in some detail the characterization of an anti-EGF receptor IgG₂a secreted by hybridoma B1D8.

The ability of the B1D8 antibody to compete with ¹²⁵I-EGF for binding to formaldehyde-fixed A431 cells is shown in Fig. 1. Half-maximal inhibition of binding occurred at an antibody concentration of 2 × 10⁻⁸ M (3 μg/ml). A molar concentration of 30-fold more antibody than cold EGF was required for equivalent displacement of ¹²⁵I-EGF.

The antibody was capable of precipitating the M₁ = 170,000 receptor from A431 cells labeled with [³⁵S]methionine and from normal human foreskin fibroblasts (Fig. 2). However, no EGF receptor could be precipitated from human melanoma cells even when conditions were arranged to obtain 300-fold more label in protein than was used for A431 cells and 50-fold more than for the fibroblasts. To determine whether the fibroblast and A431 EGF receptors are structurally similar, peptide maps were made of the M₁ = 170,000 proteins isolated by immunoprecipitation and SDS-polyacrylamide gel electrophoresis (Fig. 3). The two-dimensional peptide maps of the A431 and fibroblast receptors were virtually indistinguishable. The antibody could precipitate a nonglycosylated M₁ = 130,000 protein from A431 cells labeled in the presence of tunicamycin indicating that the antibody recognizes the protein backbone of the receptor (data not shown).

Because B1D8 was capable of competing with EGF for binding to the A431 EGF receptor, we determined the effect of the antibody on the EGF-dependent protein kinase (Fig. 4). A431 plasma membrane vesicles were incubated with various doses of antibody with or without EGF and the ability of

FIG. 1. The effects of antibody B1D8 and EGF on the binding of ¹²⁵I-EGF to formaldehyde-fixed A431 cells. Various concentrations of EGF (○) or monoclonal antibody B1D8 (×) were incubated with ¹²⁵I-EGF on formaldehyde-fixed A431 cells for 2 h at 22 °C in triplicate wells. After washing the cells to remove unbound counts, the counts bound were determined. Each point is the mean of triplicate determinations. The standard error of the mean was <6% of the mean at each point.

FIG. 2. Immunoprecipitation of EGF receptors from A431 cells, fibroblasts, and A2058 melanoma cells. Immunoprecipitation of EGF receptor with B1D8 antibody (lanes 1, 3, and 5) and purified normal mouse IgG (lanes 2, 4, and 6) were performed on detergent extracts of 1.5 × 10⁶ A431 cells (lanes 1 and 2), 5 × 10⁶ foreskin fibroblasts (lanes 3 and 4), and 5 × 10⁶ melanoma cells (lanes 5 and 6) that had been labeled with [³⁵S]methionine as described under “Experimental Procedures.” The precipitated proteins were separated by SDS-polyacrylamide electrophoresis (7.5% gel). Shown is the autoradiogram resulting from exposure of the fixed, stained, and dried gel. On the left of the figure are given the positions of the molecular weight standards with M₁ × 10⁻³.

FIG. 3. Two-dimensional peptide maps of the A431 and human fibroblast EGF receptor. A431 cells and human fibroblast were metabolically labeled with [³⁵S]methionine. The immunoprecipitated receptors were further purified by SDS-polyacrylamide electrophoresis as shown in Fig. 2. The receptor protein, eluted from the gel, was digested with trypsin and the resultant peptides were separated, first by high voltage electrophoresis (horizontal axis, +electrode at the left) and subsequently by thin-layer chromatography (vertical axis). Shown are the autoradiographs of the separated ³⁵S-receptor peptides from A431 cells (A) and human fibroblasts (B).

the EGF-dependent protein kinase to phosphorylate the EGF receptor in vitro was assessed. At a concentration of 100 μg/ml (0.7 μM), the antibody could completely block the stimulation of the kinase activity by EGF.

EGF has been shown to cause the down-regulation of its own receptor (29). After binding of EGF to the receptor, the EGF-receptor complex is internalized with ultimate degradation of the ligand receptor in lysosomes (30–33). To determine the effect of the antibody on this process, normal human fibroblasts were incubated with or without antibody and EGF
in the presence of [35S]methionine. After incubation with 10^{-9} M EGF overnight, no receptor could be immunoprecipitated. Overnight incubation with antibody at 100 μg/ml had no effect on the receptor. However, when cells were incubated with EGF and antibody, receptor down-regulation was completely blocked (Fig. 5).

Using 125I-EGF binding as a means of detecting EGF receptors, it has been observed that cells that secrete αTGFs do not bind EGF (34). It has been suggested that the inability to observe receptors in αTGF-secreting cells results from “autocrine” down-regulation of the receptors (25). Since B1D8 antibody is capable of blocking EGF-stimulated down-regulation in fibroblasts, we determined whether we could block the down-regulation of the fibroblast receptor by melanoma-conditioned medium. The serum-free conditioned medium of the melanoma cells collected after 4 days of incubation of confluent cells contains EGF-displacing activity, as determined in a radioreceptor assay, equivalent to 10^{-10} M EGF (data not shown). This melanoma-conditioned medium causes only partial down-regulation of fibroblast EGF receptors (Fig. 5). When the melanoma-conditioned medium was concentrated 10-fold, down-regulation of fibroblast EGF-receptor was nearly complete and B1D8 could block this process (Fig. 5). While the antibody could block down-regulation of fibroblast receptors in the presence of 10^{-9} M EGF and in the presence of αTGF (concentrated melanoma cell-conditioned medium), incubation of antibody with melanoma cells did not result in the appearance of immunoprecipitable EGF receptor (Fig. 5).

The mechanism by which EGF causes the disappearance of EGF receptor involves lysosomal degradation of the internalized protein. Methylamine has been shown to completely block this proteolysis (33). We therefore attempted to observe melanoma EGF receptor in cells treated with methylamine. No M_r = 170,000 protein could be specifically precipitated by B1D8 under these conditions (Fig. 5). The inability to detect receptor in melanoma cells with B1D8 antibody raised the possibility that the melanoma receptor is immunologically distinct from the A431 and fibroblast receptors. We used our two other monoclonal antibodies in a similar attempt to detect EGF receptor in these cells without success (data not shown).

The effect of B1D8 on EGF-stimulated DNA synthesis in human fibroblasts was assessed. Fibroblast DNA synthesis was stimulated in a dose-dependent manner by EGF (Fig. 6). Co-incubation of these cells with varying doses of antibody and EGF resulted in a shift in the dose-response curves such that higher doses of EGF were required in the presence of antibody to give DNA synthesis comparable to cells without antibody.

The antibody could also block EGF-stimulated fibroblast proliferation. Fibroblasts in 2% fetal bovine serum stimulated with 10^{-10} M EGF achieved a higher cell density than cells not exposed to EGF. Antibody blocked this EGF-stimulated proliferation in a dose-dependent manner with 200 μg/ml (1.3 μM) blocking the EGF effect by greater than 70% (Fig. 7). In contrast, the same doses of antibody were unable to block the 10-fold increment in melanoma cell density achieved after 10 days of incubation in serum-free medium (Fig. 7).

TGFs can cause appropriate target cells to grow in an anchorage-independent manner. The melanoma cells, like many tumor cell lines, are also capable of colony growth in soft agar. Because these cells secrete αTGF, it has been assumed that αTGF imparts this ability to these cells. Since anti-EGF receptor antibodies have been shown to block αTGF-stimulated anchorage independence when TGF is added exogenously to the cultures (9), we tested whether our anti-receptor antibody could block the autocrine effect of αTGF. Various doses of antibody were added to the melanoma cells suspended in soft agar and the effect on colony number and size was noted. Up to a dose of 200 μg/ml (1.3 μM)
In this communication we present evidence that the monoclonal antibody B1D8 is directed at a determinant in or near the binding site of EGF on the human EGF receptor. Thus, this antibody can interfere with the binding of EGF to the receptor, preventing a variety of effects of EGF on its target cells. In vitro, the antibody can block the activation of EGF-dependent tyrosine-specific protein kinase by EGF and in normal human fibroblasts can block EGF-stimulated DNA synthesis, proliferation, and EGF receptor down-regulation. This antibody differs from those described by Schreiber et al. (35) which mimic EGF action. The antibody can also be used to precipitate the EGF receptor from A431 human epidermoid carcinoma cells and normal human fibroblasts. Peptide maps of the receptor from the cancer cells and normal fibroblasts are indistinguishable. Others have shown that the EGF receptors from A431 cells and human placenta have similar peptide maps (17). Thus, the human EGF receptor appears to be similar in normal and cancer cells and does not appear to vary with the embryologic origin of the tissue. Despite this apparent conservation of the receptor, no receptor could be immunoprecipitated from human melanoma cells. It had been hypothesized that these melanoma cells lack $^{125}$I-EGF cell surface binding sites because the receptors were occupied by $\alpha$TGF which resulted in autocrine growth stimulation and autocrine down-regulation of the receptor (34). However, conditioned medium from these melanoma cells, which contains $\alpha$TGF, could cause only a barely detectable loss of EGF receptors in normal fibroblasts. When the $\alpha$TGF was concentrated, it could cause complete down-regulation of the fibroblast receptor. Thus, the concentrations of EGF-displacing activity in the unconcentrated conditioned medium of the melanoma cells, although at a level adequate to give near-maximal fibroblast growth, was insufficient to cause disappearance of fibroblast receptors. Furthermore, B1D8 antibody could block down-regulation of fibroblast receptor by $10^{-8}$ M EGF and the $\alpha$TGF secreted by the melanoma cells. If the absence of EGF receptor in melanoma cells was a result of interaction of secreted $\alpha$TGF with cell surface EGF receptor, then it could be predicted that antibody could block this down-regulation. Nevertheless, incubation of melanoma cells with the antibody did not result in the emergence of the EGF receptor. Since newly synthesized receptor and $\alpha$TGF are processed as secretory proteins, it is possible that the receptor and $\alpha$TGF interact within the secretosomes, initiating the process of down-regulation and mitogenic stimulation prior to emergence on the cell surface. However, the subsequent degradation of the receptor would be expected to proceed as if the growth factor-receptor interaction had occurred on the cell surface, that is via the lysosomes. Methylamine, through its action on lysosomes, has been shown to prevent the disappearance of immunoprecipitable EGF receptor in fibroblasts incubated with EGF (33). However, incubation of melanoma cells with methylamine also failed to allow the detection of the receptor, indicating that these cells do not express sufficient levels of receptor to be detected by immunoprecipitation. Recently, a wide variety of human tumor cells probed for the expression of several oncogenes was found not to
express the erb-B gene (36). Since this gene codes for a portion of the EGF receptor (17), it can be concluded that these cancer cells were not expressing the erb-B domain of the EGF receptor even though their parental cells probably did. It would be important to confirm, using hybridization techniques, whether this line of melanoma cells has also discontinued expressing the gene for the erb-B and EGF-binding domains of the EGF receptor.

Our inability to detect EGF receptor synthesis in these melanoma cells by immunoprecipitation may have resulted from a level of receptor below our detection limits (about 500 receptors per cell). However, if these cells were synthesizing cell surface receptor which was necessary for their autonomous growth, then blocking of the EGF-binding site of the receptor, regardless of number, with antibody should have impaired the growth of these cells in serum-free medium or as soft agar colonies. The failure of the antibody to block the growth of these αTGF-secreting cells suggests that there were no EGF receptors on these cells accessible to the monoclonal antibody or that, if present, blocking of EGF receptors by antibody had no effect on growth. The possibility that αTGF acts through a mechanism distinct from the EGF receptor has been suggested. Affinity labeling of αTGF membrane binding proteins labeled both the EGF receptor and a M, = 60,000 membrane protein, the latter not compatible with EGF (37). This finding suggested the existence of an αTGF receptor distinct from the EGF receptor. However, subsequent experiments using more highly purified αTGF have failed to reveal the M, = 60,000 protein (38) and antibodies to the EGF receptor are able to block colony formation by target cells stimulated by the addition of purified αTGF to the medium. The data now suggest that the effects of αTGF are mediated entirely through the same receptor as for EGF.

We interpret the failure to detect EGF receptor in αTGF-secreting melanoma cells by immunoprecipitation or biologic methods to imply that these cells do not synthesize the receptor or at least the domain of the receptor that binds EGF. Alternatively, the receptor synthesized in these cells is immunologically distinct from the receptor in A431 cells and fibroblasts. However, the similarity of receptor from different human sources and the failure of three distinct monoclonal antibodies to detect the receptor makes this possibility somewhat less likely. If indeed this cell line does not synthesize EGF receptor, the autonomous production of the EGF-like growth factor may not be necessary for the autonomous growth of these cells in culture. However, these cells probably produce other growth factors which do not act through the EGF receptor (βTGF) and these may play an autocrine role in the autonomous growth. In the host, however, the production of αTGF may play an important paracrine role for the tumor by recruiting the normal surrounding cells to provide support for tumor cell growth such as the induction of angiogenesis.

REFERENCES
1. Kaplan, P. L., Anderson, M., and Ozanne, B. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 485-489
2. Roberts, A. B., Frolik, C. A., Anziano, M. A., and Sporn, M. B. (1983) Fed. Proc. 42, 2621-2626
3. DeLarco, J. E., and Todaro, G. J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4001-4005
4. DeLarco, J. E., and Todaro, G. J. (1980) J. Cell. Physiol. 102, 267-277
5. Todaro, G. J., Fryling, C., and DeLarco, J. E. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5258-5262
6. Ozanne, B., Fulton, R. J., and Kaplan, P. L. (1980) J. Cell. Physiol. 105, 163-180
7. Sporn, M. B., and Todaro, G. J. (1980) N. Engl. J. Med. 303, 878-880
8. Marquardt, H., and Todaro, G. J. (1982) J. Biol. Chem. 257, 5229-5225
9. Carpenter, G., Stoscheck, C. M., Preston, Y. A., and DeLarco, J. E. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 5627-5630
10. Buhrow, S. A., Cohen, S., and Staros, J. V. (1982) J. Biol. Chem. 257, 4019-4023
11. Kudlow, J. E., and Leung, Y. (1984) Biochem. J. 220, 677-683
12. Ushiro, H., and Cohen, S. (1980) J. Biol. Chem. 255, 8363-8365
13. Reynolds, F. H., Todaro, G. J., Fryling, C., and Stephenson, J. R. (1981) Nature (Lond.) 292, 259-262
14. Pike, L. J., Marquardt, H., Todaro, G. J., Ballis, B., Casnelli, J. E., Bornstein, P., and Krebs, E. G. (1982) J. Biol. Chem. 257, 14659-14661
15. Chinkers, M., and Cohen, S. (1981) Nature (Lond.) 290, 516-519
16. Kudlow, J. E., Buss, J. E., and Gill, G. N. (1981) Nature (Lond.) 290, 519-521
17. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ulrich, A., Schlessinger, J., and Waterfield, M. D. (1984) Nature (Lond.) 307, 521-527
18. Marquardt, H., Hunkapiller, M. W., Hood, L. E., Twardzik, D. R., DeLarco, J. E., Stephenson, J. R., and Todaro, G. J. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 4684-4688
19. Savage, C. R., Jr., and Cohen, S. (1972) J. Biol. Chem. 247, 7609-7611
20. Cohen, S., Ushiro, H., Stoscheck, C., and Chinkers, M. (1982) J. Biol. Chem. 257, 1523-1531
21. Galfre, G., and Milstein, C. (1981) Methods Enzymol. 73B, 3-46
22. Stahl, C., Staehelin, T., Migliano, V., Schmidt, J., and Haring, P. (1980) J. Immunol. Methods 32, 297-304
23. Goding, J. W. (1978) J. Immunol. Methods 20, 241-253
24. Bruck, C., Portetelle, D., Glineur, C., and Bollen, A. (1982) J. Immunol. Methods 53, 313-319
25. Kudlow, J. E., and Kobrin, M. S. (1984) Enocrinology, in press
26. Lasmulli, U. K. (1970) Nature (Lond.) 227, 880-885
27. Beemon, K., and Hunter, T. (1978) J. Virol. 28, 551-566
28. Hunter, T., and Sefton, B. M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1311-1315
29. Carpenter, G., and Cohen, S. (1976) J. Cell Biol. 77, 159-171
30. Haigler, H., Ash, J. F., Singer, S. J., and Cohen, S. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 3317-3321
31. Schlessinger, J., Schechter, Y., Cuartocasas, P., Willingham, M. C., and Pastan, I. H. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2659-2663
32. Pastan, I. H., and Willingham, M. C. (1981) Science (Wash. D.C.) 214, 504-509
33. Stoscheck, C. M., and Carpenter, G. (1984) J. Cell Biol. 96, 1048-1053
34. Todaro, G. J., DeLarco, J. E., and Fryling, C. M. (1982) Fed. Proc. 41, 2996-3003
35. Schreiber, A. B., Lax, I., Yarden, Y., Eshhar, Z., and Schlessinger, J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7535-7539
36. Slamon, D. J., DeKernion, J. B., Verma, I. M., and Cline, M. J. (1984) Science (Wash. D.C.) 224, 256-262
37. Massague, J., Czech, M. P., Iwata, K., DeLarco, J. E., and Todaro, G. J. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 6822-6826
38. Massague, J. (1983) J. Biol. Chem. 258, 13614-13620