brief communication

Interrupting autocrine ligand-receptor binding: comparison between receptor blockers and ligand decoys

Kimberly E. Forsten and Douglas A. Lauffenburger
Department of Chemical Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

ABSTRACT Stimulation of cell behavioral functions by ligand/receptor binding can be accomplished in autocrine fashion, where cells secrete ligand capable of binding to receptors on their own surfaces. This proximal secretion of autocrine ligands near the surface receptors on the secreting cell suggests that control of these systems by inhibitors of receptor/ligand binding may be more difficult than for systems involving exogenous ligands. Hence, it is of interest to predict the conditions under which successful inhibition of cell receptor binding by the autocrine ligand can be expected.

Previous theoretical work using a compartmentalized model for autocrine cells has elucidated the conditions under which addition of solution decoys for the autocrine ligand can interrupt cell receptor/ligand binding via competitive binding of the secreted molecules (Forsten, K. E., and D. A. Lauffenburger. 1992. *Biophys. J.* 61:1–12.) We now apply a similar modeling approach to examine the addition of solution blockers targeted against the cell receptor. Comparison of the two alternative inhibition strategies reveals that a significantly lower concentration of receptor blockers, compared to ligand decoys, will obtain a high degree of inhibition. The more direct interruption scheme characteristic of the receptor blockers may make them a preferred strategy when feasible.

INTRODUCTION

Cell surface receptors bind corresponding ligand molecules from the surrounding medium to initiate a sequence of events resulting in a cell behavioral response such as proliferation, adhesion, or motility. Such receptor-mediated responses can be influenced by the source and transport properties of the ligand. Cells that synthesize and secrete ligand molecules, and then bind and respond to them, are termed autocrine cells (Sporn and Todaro, 1980). Acquisition of the ability to produce their own signals may be one means by which cells become transformed and lose normal regulatory control (Huang et al., 1984; Imanishi et al., 1989; Partridge et al., 1989; Yamada and Serrero, 1988).

Cell surface receptor/ligand complexes are typically the signal-generating species, though in some systems, intracellular complexes may generate the key signals from autocrine ligands (e.g., Williams, 1989). The proximal relationship between the secreted ligand and its target receptor on an autocrine cell makes signal inhibition a particularly difficult procedure. Interruption of the surface signaling compound may be aimed at either the cell receptor or the autocrine ligand. Both approaches have been attempted in vitro with some limited success (Sato et al., 1983; Cuttitta et al., 1985; Imanishi et al., 1989; Rodeck et al., 1990).

Previous theoretical work has addressed the issues of competing soluble receptors in a nonautocrine cell situation (Goldstein et al., 1989), inoculum cell density effects with autocrine cells (Lauffenburger and Cozens, 1989), and the role of autocrine cells in tumor growth (Michelson and Leith, 1991). Our interest has been to develop mathematical models which can be used to investigate inhibition of autocrine-ligand/cell-receptor complexes by including inhibitory molecules in the model framework.

An analysis of the effect of solution decoys for an autocrine ligand on cell surface receptor binding revealed that effective inhibition of autocrine ligand/receptor surface complexes may require surprisingly high concentrations of these decoys, with the required concentration dependent on ligand transport properties (Forsten and Lauffenburger, 1992). Using a similar modeling approach, we now present comparative results for the effect of receptor blockers on autocrine complex formation. We find that significantly lower concentrations of inhibitors can lead to inhibition of complex formation when attempted via direct receptor blocking rather than by trapping the ligand with solution decoys. This result is not necessarily intuitive, since transport limitations coupled with transient binding and depletion considerations could hinder blockers' effectiveness in competing with the secreted ligand for cell surface receptor binding.

MATHEMATICAL MODEL

A full description of our autocrine cell model was presented previously for the case in which ligand decoys have been added to the surrounding medium (Forsten and Lauffenburger, 1992). A schematic of the current receptor blocker model is shown in Fig. 1, with the corresponding differential equations, initial conditions, and an explanation of variables and parameters provided in the Appendix. In both models, diffusional transport and reversible binding between the added inhibitor and its target molecule are included as is autocrine ligand secretion and reversible binding of the ligand to the cell surface receptor. To explore a realistic example, parameter values for interleukin 2 and its high-affinity receptor are used, as previously (Forsten and Lauffenburger, 1992). Receptor blockers are assumed to have the same affinity

0006-3495/92/09/857/05 $2.00

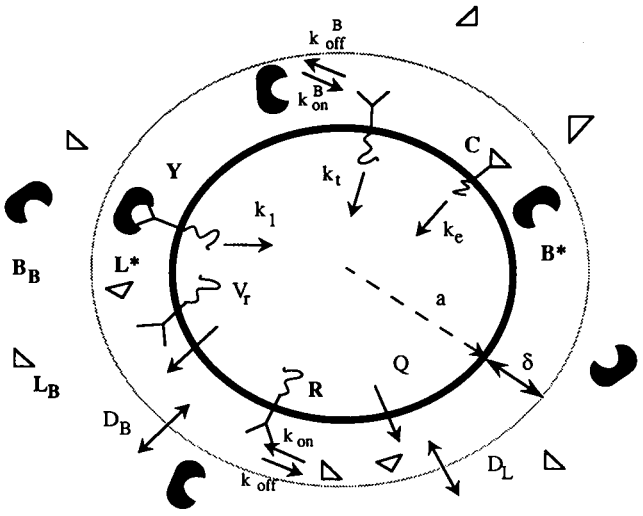

FIGURE 1 Diagram of model. Complete description of variables and parameters is included in the Appendix.

for the receptor as does the ligand. Values of the inhibitor's affinity for the receptor and its diffusion constant are varied to investigate property effects of the competitive inhibitors.

A transient solution of the system of coupled nonlinear equations was sought and obtained using LSODE, an implicit solver (Hindmarsh, 1980). Initial conditions used correspond to the initial moment of both secretion and the addition of receptor blockers. Hence, initially all surface receptors are unbound and unblocked (see Appendix). Inhibition plots found in Figs. 2 and 3 reflect the level of surface complexes found at the end of 30 h of both autocrine secretion and inhibitor exposure with the complex level being scaled by the number of surface receptors found in the absence of autocrine ligand secretion or receptor blocker exposure.

RESULTS AND DISCUSSION

Our foremost objective was to determine the concentration of receptor blockers needed to substantially prevent autocrine ligand-receptor binding, and to compare this result to that previously obtained for ligand decoys by Forsten and Lauffenburger (1992). As shown in Fig. 2 *A*, low concentrations of receptor blockers have essentially no effect upon the level of surface complexes after 30 h of both ligand secretion and blocker exposure. Once a threshold concentration of inhibitor molecules has been added, surface complexes are reduced with increasing concentration of inhibitor until near complete elimination of surface complexes is obtainable. The threshold concentration of blockers required to initiate inhibition is dependent on cell density. Essentially, at high cell densities in the absence of inhibitor, cell separation distances are sufficiently small that gradients driving the ligand flux away from the cell's immediate surrounding are reduced. Higher levels of ligand are available for binding and the level of surface complexes is thus, limited by receptor synthesis and endocytosis. At lower cell densities, the available ligand for binding is reduced such that it becomes a limiting factor for the level of surface complexes. Initiation of inhibition comes when receptor blockers can begin to compete with ligand for the available receptors. This is similar to the initial inhibition threshold response exhibited in the presence of ligand decoys (Fig. 2 *B*). The decoys begin to affect complex

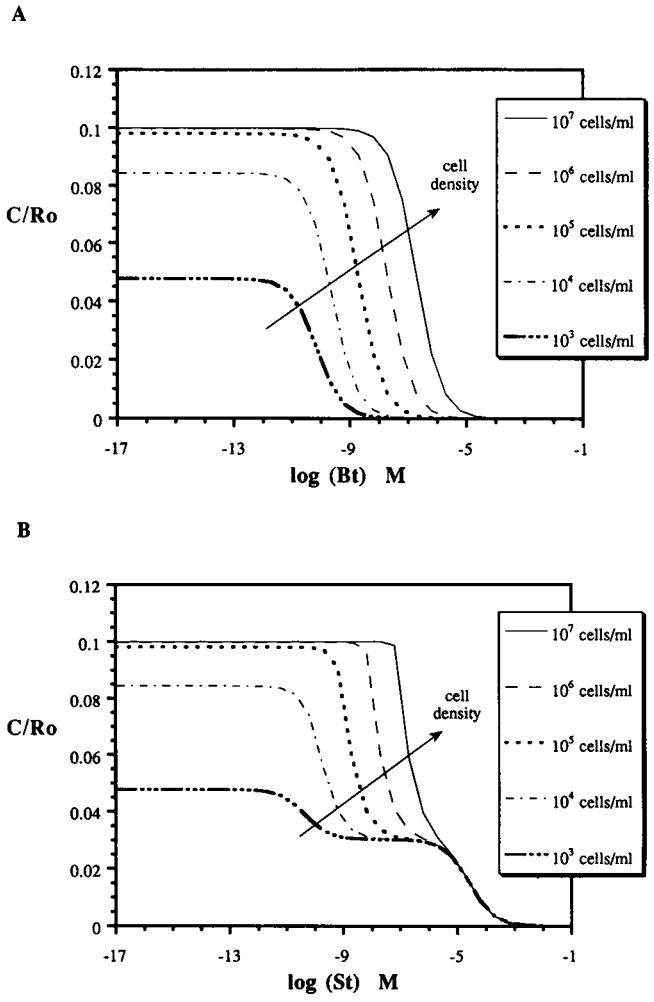

FIGURE 2 Effect of cell density. (*A*) Receptor blocker addition. Effect of increasing concentrations of receptor blockers on the level of surface complexes at various cell densities is shown. Surface ligand/receptor complexes, C, are scaled by R_0, the level of surface receptors found in the absence of ligand secretion. The total concentration of receptor blockers added to the cell environment, B_t, has been plotted logarithmically for visual ease. (*B*) Ligand decoy addition. The effect of increasing concentrations of ligand decoys on the level of scaled surface ligand/receptor complexes is shown as a function of cell density. Calculations for both inhibitor systems used the following interleukin-2 parameter values: $k_{on} = 3.1 \cdot 10^7\ M^{-1}\ s^{-1}$, $k_{off} = 2.3 \cdot 10^{-4}\ s^{-1}$, $k_{on}^B = k_{on}^S = k_{on}$, $k_{off}^B = k_{off}^s = k_{off}$, $R_0 = 2{,}000$ receptors/cell, $k_t = 0.0046\ min^{-1}$, $V_r = R_0 * k_t$, $k_e = 0.046\ min^{-1}$, $k_1 = 0.0046\ min^{-1}$, $D_L = 10^{-6}\ cm^2/s$, $D_B = 4.0 \cdot 10^{-7}\ cm^2/s$, $Q = 500$ molecules/min, $a = 5\ \mu m$, $\delta = 0.2\ \mu m$. For all subsequent figures, 10^5 cells/ml is the standard density used.

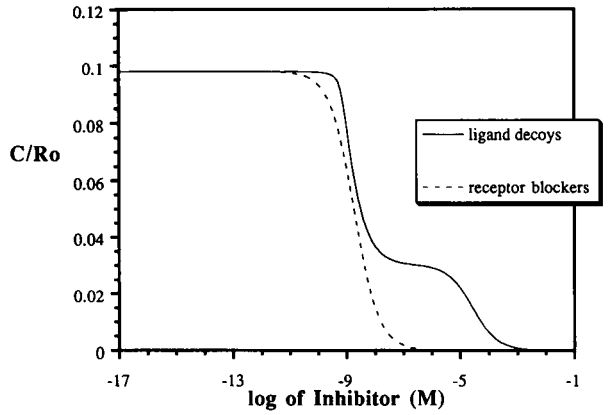

FIGURE 3 Direct comparison of inhibition schemes. Level of scaled surface ligand/receptor complexes as a function of inhibitor concentration for both receptor blockers and ligand decoys is shown for a cell density of 10^5 cells/ml. The concentration of receptor blockers required to initiation reduction in surface complex levels is about an order of magnitude less than that required by ligand decoys. To obtain over 99% inhibition of surface complexes, the required concentrations differ by nearly four orders of magnitude. Parameter values are listed with Fig. 2.

formation when their concentration is high enough to begin affecting the ligand concentration.

Given the parameters of our example system (interleukin-2 and its high-affinity receptor), the initial threshold concentrations required by the two inhibition schemes can differ by about an order of magnitude (Fig. 3). At a cell density of 10^5 cells/ml, a concentration of $4*10^{-10}$ M ligand decoys would be required to initiate surface ligand/receptor complex inhibition, whereas a concentration of only $1*10^{-11}$ M receptor blockers could achieve the same initial inhibition level. Depletion of decoys by ligand binding in the bulk medium, coupled with diffusion limitations for decoy transport into the secretion layer, causes an increase in the total decoy concentration which must be added to the system to obtain inhibition by decreasing the level of available decoys within the secretion layer. Receptor blockers within the secretion layer directly interrupt ligand-receptor complex formation via receptor blocking, with bulk phase blockers merely acting as a reservoir; a similar diffusion limitation for movement into the secretion layer still exists for the blockers but the overall effectiveness is not compromised by events far from the cell surface.

More importantly, the inhibitor concentration required to achieve almost complete inhibition (e.g., <1% of maximum possible surface complexes) can be dramatically lower for receptor blockers than for ligand decoys (Fig. 3). At a cell density of 10^5 cells/ml, our model calculations indicate a concentration requirement of $3*10^{-7}$ M for the receptor blockers as opposed to a concentration requirement of $1*10^{-3}$ M for the soluble decoys. However, with receptor blockers, the concentration required is cell density dependent which is not the case for the ligand decoys (see Fig. 2, *A* and *B*). Bulk medium influences are primarily responsible for these differences. Ligand decoys trap secreted ligand within the bulk medium, thereby increasing the gradient driving the ligand from the cell surface and, further, preventing the bulk ligand from diffusing to neighboring cells. Once the concentration of added decoys is sufficiently great to deplete the bulk ligand, a transport-limited regime in which further addition of decoys has essentially no effect on surface complex levels is exhibited (see Fig. 2 *B*). Newly-secreted ligand is still capable of binding to the surface receptors, so it is only when the concentration of ligand decoys close to the cell surface is high enough to directly compete with the surface receptors that further inhibition of surface complexes can be obtained. With receptor blockers as the inhibiting molecules, the transport-limited plateau and secondary threshold concentration characteristic of the ligand decoy inhibition profile are eliminated. Here, bulk medium interactions have no role in the process. When the concentration of receptor blockers within the secretion layer is high enough to bind available cell receptors, surface complexes are essentially eliminated. The required concentration is dependent on the ligand concentration and, hence, is cell density dependent.

It is the transport-limited regime aspect of inhibition which truly distinguishes between the inhibitory schemes. While transport properties of the ligand, as characterized by its diffusion coefficient, have a strong effect upon the level of inhibition obtainable with the ligand decoys, no effect upon the receptor blockers inhibition profile is exhibited (not shown). The affinity of the ligand for the cellular receptor has essentially the same effect for both inhibitory schemes—the association rate constant has a much more pronounced effect than the dissociation rate constant (not shown). However, the level of inhibition attainable within the transport-limited regime is significantly influenced by the receptor-ligand affinity when ligand decoys are added, but, again, this characteristic region is not evident with the receptor blockers (Forsten and Lauffenburger, 1992).

The characteristics of the inhibitory molecule itself have roughly the same effect on the inhibition profiles in each scheme. The transport of the inhibitor, as characterized by its diffusion coefficient, has no effect on the inhibition profile in either case (not shown). The inhibitor's affinity for either the receptor or the ligand, depending on the scheme examined, has a small effect on both the concentration required to obtain initial inhibition and the subsequent concentration required to eliminate surface complexes. With the ligand decoys, the association rate constant has a much more dramatic effect on the inhibition profile than the dissociation rate constant, while it is the overall affinity change, not the individual rates, that influences the receptor blocker profile (not shown). For the decoys, complete inhibition occurs when the secretion layer molecules are able to compete

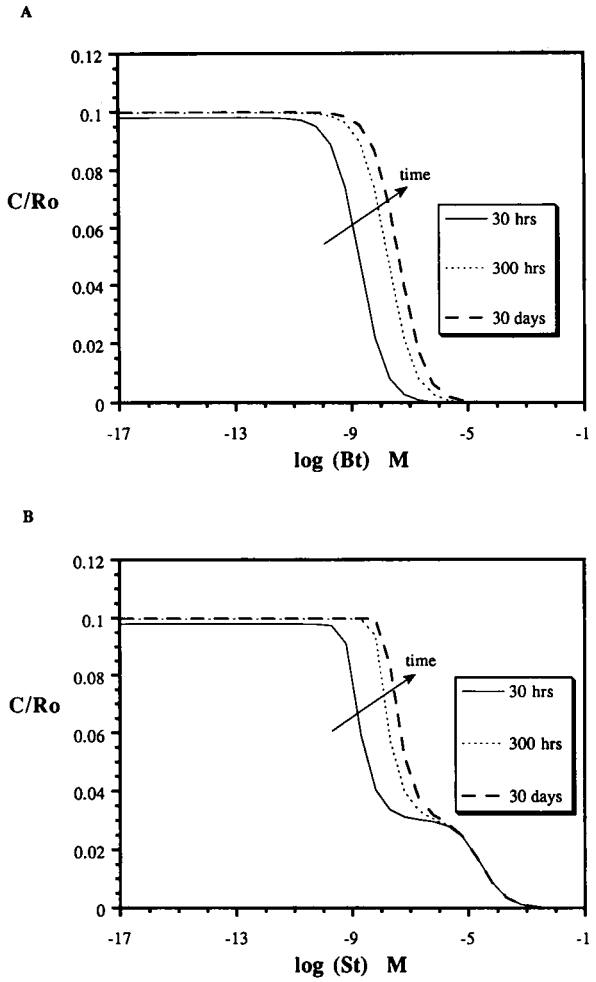

FIGURE 4 Inhibition of surface complexes as a function of time (A/B) (receptor blockers/ligand decoys). Scaled level of surface ligand/receptor complexes present as a function of both the total concentration of inhibitor added and the time following initiation of secretion and inhibitor addition. Parameter values are listed with Fig. 2.

and absorb all available ligand, even those newly secreted. It is the initial binding which is paramount. With the receptor blockers, ligand is readily available for binding and it is the hold of the receptor blocker on the receptor which is critical; the dissociation and association rates are both important.

In accord with our earlier work (Forsten and Lauffenburger, 1992), transient solutions rather than steady-state solutions were sought with time zero representing the simultaneous initiation of secretion and inhibitor addition. Receptor blockers can be removed from the cell environment via endocytosis whereas no degradation of either inhibitor while in solution is included. Hence, depletion of receptor blockers but not ligand decoys can potentially occur. However, given the parameter choices of our system, significantly lower concentrations of receptor blockers are still required to obtain near complete surface complex inhibition even after 30 d of exposure (Fig. 4, A and B). Increasing the time span does result in higher concentrations requirements to initiate inhibition for both schemes, but the concentration required to obtain near complete inhibition is only increased for the receptor blockers. The concentration of ligand decoys required to directly compete with the surface receptors is so high that any time-dependent binding depletion is insignificant. It is important to note that the assumption of no degradation of either ligand or inhibitor in solution may be inappropriate for long time spans, but the key result that receptor blockers offer a better means of complex inhibition should still hold.

SUMMARY

An autocrine cell model has been developed which incorporates the addition of molecules aimed at interrupting a cell surface ligand-receptor interaction via blockage of the receptor binding site. To facilitate calculations, a simple compartmentalized model was developed in which all spatial variations were limited to two length scales: a short range cellular binding region and a bulk fluid phase. A similar approach was used previously to develop an autocrine cell model which incorporated decoy molecules capable of interrupting the surface complex by drawing secreted ligand away from the cell surface (Forsten and Lauffenburger, 1992).

Analysis reveals that receptor blockers may be more efficient, by one to a few orders of magnitude in concentration, in eliminating autocrine ligand/cell receptor complexes than are ligand decoys. Further, the inhibition profile for the blockers is quite distinct from the complex inhibition profile for the decoys. Decoys exhibit a transport-limited regime which is not evident for receptor blockers. Consequently, the diffusional transport of the autocrine ligand has essentially no effect on the blocker's inhibition profile while it does have a significant influence on that of the decoys. Variations in other system parameters, including characteristics of the inhibitor molecule itself, have similar affects in both systems.

The important findings from these studies are predictions for the concentrations of inhibitory molecules needed for prevention of cell surface binding by secreted ligands, and an understanding of how key system parameters influence the required levels. It has been shown that both receptor blockers and ligand decoys can be successfully used for this purpose, but that receptor blockers may require significantly lower concentrations to obtain an equivalent degree of surface complex inhibition. By interacting directly with the cell receptors, blockers bypass bulk medium interactions which serve to diminish the effectiveness of ligand decoys. This advantage predominates over the diffusion limitations that retard the transport of both blockers and decoys into the secretion layer, leading to the prediction that receptor blockers should be preferred to ligand decoys for efficiency in surface receptor/ligand complex inhibition.

APPENDIX

The autocrine cell model basis is a suspension cell of radius a which is secreting ligand at a constant rate Q. Receptors are synthesized at a rate V_r and homogeneously distributed over the surface. In the absence of ligand secretion, R_0 receptors are found on the cell surface. Newly secreted ligand is released into a secretion layer surrounding the cell of volume V^* and thickness δ. Ligand within this layer, L^*, is capable of binding reversibly with free cell receptors, R, to form receptor-ligand complexes, C. This binding is characterized by rate constants k_{on} and k_{off}. Internalization of both bound and unbound receptors can occur and is characterized by rate constants k_e and k_i, respectively. Unbound receptor blockers are assumed to be initially uniformly distributed throughout the extracellular fluid phase. Blockers within the secretion layer, B^*, are capable of reversibly binding to unbound cell receptors with this binding being characterized by rate constants k^b_{on} and k^b_{off}. Receptor-receptor blocker complexes, Y, are internalized with rate constant k_i which may differ from both the constitutive and endocytic rate constants k_i and k_e. Diffusion of both ligand and receptor blockers between the secretion layer and the bulk fluid phase is characterized by the Smoluchowski diffusion-controlled rate constant to a sphere (Smoluchowski, 1917). The model is written on a per cell basis with all cells assumed to be uniformly distributed and all transport of bulk fluid phase ligand and receptor blockers, L_B and B_B, respectively, between neighboring cell regions being reciprocated.

The seven ordinary differential equations describing the model are listed below. The initial conditions used to solve the system of equations follow. Parameter values choices are listed with Fig. 2 with selection references listed elsewhere (Forsten and Lauffenburger, 1992).

$$\frac{dR}{dt} = k_i R + V_r - k_{on} L^* R + k_{off} C - k^B_{on} B^* R + k^B_{off} Y$$

$$\frac{dC}{dt} = k_{on} L^* R - k_{off} C - k_e C$$

$$V^* \frac{dB^*}{dt} = -k^B_{on} B^* R - k^B_{off} Y + \Delta_B (B_B - B^*)$$

$$\frac{dY}{dt} = k^B_{on} B^* R - k^B_{off} Y - k_i Y$$

$$V^* \frac{dL^*}{dt} = -k_{on} L^* R + k^B_{off} C + \Delta_L (L_B - L^*) + Q$$

$$V_B \frac{dL_B}{dt} = -\Delta_L (L_B - L^*)$$

$$V_B \frac{dB_B}{dt} = -\Delta_B (B_B - B^*),$$

where:

$$\Delta_L = 4\pi D_L (a + \delta) \quad \Delta_B = 4\pi D_B (a + \delta)$$

$$\frac{R}{R_0} = 1 \quad \frac{C}{R_0} = 0 \quad \frac{Y}{R_0} = 0$$

$$\frac{B^*}{B_t} = 1 \quad \frac{B_B}{B_t} = 1$$

$$\frac{L^*}{K_D} = 0 \quad \frac{L_B}{K_D} = 0,$$

where B_t is the concentration of receptor blockers added and K_D is the equilibrium dissociation constant, k_{off}/k_{on}, for the ligand-cell receptor complexes.

This work has been partially supported by a grant from the National Science Foundation Biotechnology Program. The authors would like to express appreciation for computational work done by Chris Kocher.

Received for publication 3 February 1992 and in final form 14 April 1992.

REFERENCES

Cuttitta, F., D. N. Carney, J. Mulshine, T. W. Moody, J. Fedorko, A. Fischler, and J. D. Minna. 1985. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. *Nature (Lond.)*. 316:823–826.

Forsten, K. E., and D. A. Lauffenburger. 1992. Autocrine ligand binding to cell receptors: mathematical analysis of competition by solution decoys. *Biophys. J.* 61:1–12.

Goldstein, B., R. G. Posner, D. C. Torney, J. Erickson, D. Holowka, and B. Baird. 1989. Competition between solution and cell surface receptors for ligand. *Biophys. J.* 56:955–966.

Hindmarsh, A. C. 1980. LSODE and LSODI, two new initial value ordinary differential equation solvers. ACM-Signum Newsletter. 15:10–11.

Huang, J. S., S. S. Huang, and T. F. Deuel. 1984. Transforming protein of simian sarcoma virus stimulates autocrine growth of SSV-transformed cells through PDGF cell-surface receptors. *Cell.* 39:79–87.

Imanishi, K., K. Yamaguchi, M. Kuranami, E. Kyo, T. Hozumi, and K. Abe. 1989. Inhibition of growth of human lung adenocarcinoma cell lines by anti-transforming growth factor-α monoclonal antibody. *J. Natl. Cancer Inst.* 81:220–223.

Lauffenburger, D. A., and C. Cozens. 1989. Regulation of mammalian cell growth by autocrine growth factors: analysis of consequences for inoculum cell density effects. *Biotechnol. Bioeng.* 33:1365–1378.

Michelson, S., and J. Leith. 1991. Autocrine and paracrine growth factors in tumor growth: a mathematical model. *Bull. Math. Biol.* 53:639–656.

Partridge, M., M. R. Green, J. D. Langdon, and M. Feldmann. 1989. Production of TGF-α and TGF-β by cultured keratinocytes, skin and oral squamous cell carcinomas: potential autocrine regulation of normal and malignant epithelial cell proliferation. *Br. J. Cancer.* 60:542–548.

Rodeck, U., N. Williams, U. Murthy, and M. Herlyn. 1990. Monoclonal antibody 425 inhibits growth stimulation of carcinoma cells by exogenous EGF and tumor-derived EGF/TGF-α. *J. Cell. Biochem.* 44:69–79.

Sato, J. D., T. Kawamoto, A. D. Lee, J. Mendelsohn, J. Polikoff, and G. H. Sato. 1983. Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. *Mol. Biol. M.* 1:511–529.

Sporn, M. B., and G. J. Todaro. 1980. Autocrine secretion and malignant transformation of cells. *N. Engl. J. Med.* 303:878–880.

Williams, L. T. 1989. Signal transduction by the platelet-derived growth factor. *Science (Wash. DC)*. 243:1564–1570.

Yamada, Y., and G. Serrero. 1988. Autocrine growth induced by the insulin-related factor in the insulin-independent teratoma cell line 1246-3A. *Proc. Natl. Acad. Sci. USA.* 85:5936–5940.