# Autocrine ligand binding to cell receptors

## Mathematical analysis of competition by solution "decoys"

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ABSTRACT Autocrine ligands have been demonstrated to regulate cell proliferation, cell adhesion, and cell migration in a number of different systems and are believed to be one of the underlying causes of malignant cell transformation. Binding of these ligands to their cellular receptors can be compromised by diffusive transport of ligand away from the secreting cell. Exogenous addition of antibodies or solution receptors capable of competing with cellular receptors for these autocrine ligands has been proposed as a means of inhibiting autocrine-stimulated cell behavioral responses. Such "decoys" complicate cellular binding by offering alternative binding targets, which may also be capable of aiding or abating transport of the ligand away from the cell surface.

We present a mathematical model incorporating autocrine ligand production and the presence of competing cellular and solution receptors. We elucidate effects of key system parameters including ligand diffusion rate, binding rate constants, cell density, and secretion rate on the ability of solution receptors to inhibit cellular receptor binding. Both plated and suspension cell systems are considered. An approximate analytical expression relating the key parameters to the critical concentration of solution "decoys" required for inhibition is derived and compared to the numerical calculations. We find that in order to achieve essentially complete inhibition of surface receptor binding, the concentration of decoys may need to be as much as four to eight orders of magnitude greater than the equilibrium disociation constant for ligand binding to surface receptors.

## INTRODUCTION

Binding of a polypeptide ligand to its corresponding cell membrane receptor can initiate a sequence of events resulting in a cellular response such as growth, adhesion, or migration. In vivo, regulatory ligands may originate from a distant organ, a neighboring tissue, or the actual target cell itself (Fig. 1). The term autocrine has been applied to ligands which cells produce capable of binding to their own receptors (Sporn and Todaro, 1980). Production of autocrine stimulatory factors may enable cells to escape exogenous controls and, hence, lead to apparently unregulated behavior such as that characteristic of tumor cells (Partridge et al., 1989; Smith et al., 1987; Yoshida et al., 1990).

One possible means of interrupting an autocrine-stimulated response is the exogenous addition of soluble receptors or antibodies targeted to the autocrine ligand. Acting as "decoys" for the secreted factors, these soluble receptors may inhibit ligand binding to cell surface receptors and interfere with the ligand-stimulated behavior. As an example, Cuttitta et al. (1985) investigated the inhibitory effect of antibodies against bombesinlike protein (BLP) on the growth of human small-cell lung cancer (SCLC) xenografts in vivo. SCLC cells produce and secrete BLP and, further, they express the appropriate cell surface receptor to BLP. Growth of SCLC tumors, as measured by tumor volume, was inhibited by anti-BLP antibodies while control antibodies had no effect. In another example, Imanishi et al. (1989) investigated the effect of antibodies to transforming growth factor-α (TGF-α) on the growth of human lung adenocarcinoma cells. Their in vitro study found that growth inhibition was dependent on antibody concentration and that it could be negated by addition of exogenous TGF-α.

Some debate has centered on whether the ligand-receptor interaction characterizing an autocrine response is an intracellular or cell surface encounter. Addition of exogenous antibodies or receptors has been proposed as a means of distinguishing between the two mechanisms because it should inhibit a cell surface receptor-ligand interaction but not an intracellular one (Heldin and Westermark, 1990; Huang et al., 1984; Lang et al., 1985; Yamada and Serrero, 1988).

A system in which autocrine ligand is secreted and then must find its appropriate surface receptor will be dependent not only upon the kinetics of the binding interaction but also on the extracellular transport properties of the autocrine factor. When examining inhibition due to the presence of competing soluble decoys, these transport considerations may become paramount. Mathematical modeling can provide a quantitative examination of these competing processes to aid interpretation of experimental observations and suggest further helpful experimental studies.

A previous mathematical model addressing autocrine systems investigated the effect of inoculum cell density


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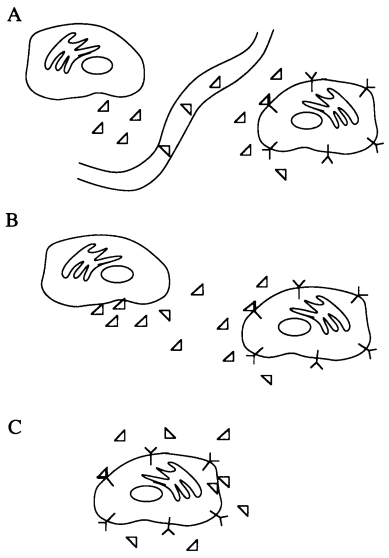


FIGURE 1 Schematic depiction of ligand secretion cell systems. (*A*) Endocrine system: ligand is secreted from the source cell or tissue and must than travel via the bloodstream and diffuse to the target cell. (*B*) Paracrine system: the secreting cell and the target cell are in close proximity. Ligand diffusion must occur over only a short distant. (*C*) Autocrine system: source and target cell are the same cell. Picture depicts extracellular ligand-receptor interactions although intracellular interactions have been proposed as a means of autocrine stimulation.

on anchorage-dependent cell growth with the key result being that the initial growth rate is not simply proportional to surface area coverage by the inoculum cells (Lauffenburger and Cozens, 1989). This result is consistent with experimental observations by Hu and Wang (1987) for cells cultured on microcarrier beads. It was further found that surface geometry could influence cell growth rate, with higher inoculum densities required for flat as opposed to spherical surfaces. Goldstein et al. (1989) considered the effect of competing soluble receptors on cell surface complex dissociation for nonautocrine cells. They showed experimentally that soluble receptors could interfere with diffusion-limited ligand rebinding indicating that ligand dissociation from membrane receptor complexes could be enhanced by the presence of these decoys. They also derived a theoretical expression for the soluble receptor concentration needed to significantly accelerate dissociation from the cell membrane.

In this paper, we focus on the effects of soluble decoys on cell receptor binding of autocrine ligand. Situations involving cells in suspension and cells on surfaces are each investigated. Illustrative computations are provided based on parameter values for the interleukin-2 (IL-2) system, a well-studied autocrine growth factor for T lymphocytes (Smith, 1990; Duprez et al., 1985). There is evidence that cell surface receptor-ligand complexes are the signal generating form of the receptor, at least for the epidermal growth factor (EGF) system (Wells et al., 1990), so that inhibition of this complex could lead to attenuation of the signal. Our analysis reveals that effective inhibition of surface complex formation can be attained by a sufficiently high concentration of soluble receptors but that ligand transport limitations play a key role in determining the required concentration.

## Mathematical model

A homogeneous distribution of spherical suspension cells of radius $a$ form the basis for our mathematical model (Fig. 2). Ligand is secreted at a constant rate $Q$ into a secretion layer of thickness $\delta$ surrounding each cell. Cell receptors are synthesized at a rate $V_r$ and are homogeneously distributed over the cell surface. The receptor synthesis rate is considered to be constant and independent of ligand secretion, thereby setting $V_r$ equal to $R_o/k_t$, where $R_o$ is the total number of surface receptors found in the absence of ligand secretion and $k_t$ is the constitutive internalization rate for receptors. Receptor-ligand binding is reversible with on- and off-rates, $k_{on}$ and $k_{off}$. The binding of ligand to a cell receptor may promote a conformational change in the receptor which allows the complex to enter the cell via an induced pathway which is not utilized by free receptors. Consequently, internalization of bound and free

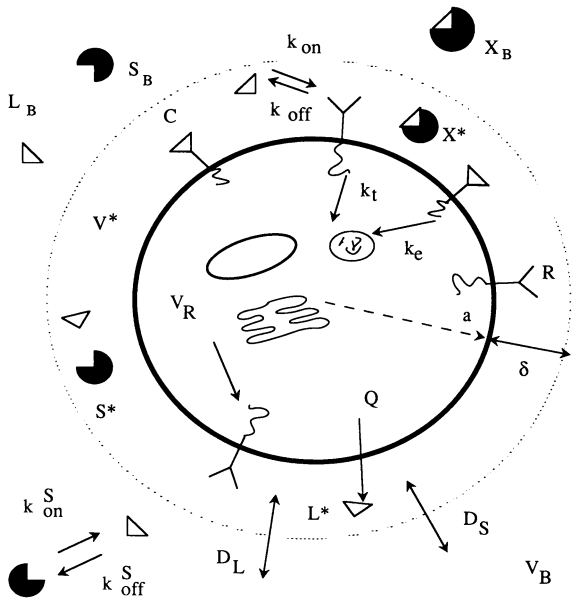


FIGURE 2 Schematic view of the suspension cell model including all variables and parameters.

receptors occur at rates $k_e$ and $k_t$, respectively, with the ligand-induced rate considered to be faster than the constitutive rate (Heldin and Westermark, 1990; Lund et al., 1990). Free ligand pinocytosis and receptor recycling are neglected here as second-order effects. Transient expressions for the change in bound and unbound surface receptors, $C$ and $R$ respectively, are:

$$\frac{dR}{dt} = -k_t R - k_{on} L^* R + k_{off} C + V_r, \quad (1)$$

$$\frac{dC}{dt} = -k_e C + k_{on} L^* R - k_{off} C. \quad (2)$$

Note that cellular receptor binding occurs only by ligand within the secretion layer at concentration $L^*$.

Transport of the ligand to and from the cell primarily occurs via diffusion. We model this transport as between adjacent discrete regions, or layers, in order to avoid cumbersome calculations inherent in a spatially-continuous model (cf. Lauffenburger and Cozens, 1989). With suspension cells, homogeneous bulk medium surrounds the secretion layer and it is through this bulk compartment that cell-to-cell communication via ligand transport must occur.

As stated above, the secretion layer surrounding the cell immediately receives all secreted ligand, and this ligand is assumed to distribute uniformly within the secretion layer volume, $V^*$. This ligand binds reversibly with both cellular and local soluble receptors, $R$ and $S^*$, and is transported by diffusion with diffusion coefficient, $D_L$. The soluble receptors' affinity for the ligand may differ from the cellular surface receptor's affinity and is characterized by the rate constants $k^s_{on}$ and $k^s_{off}$. Transport of ligand between the secretion layer and the surrounding media is characterized by the Smoluchowski diffusion-controlled rate constant to a sphere (Smoluchowski, 1917). The kinetic expression is as follows:

$$V^* \frac{dL^*}{dt} = Q - k_{on} L^* R + k_{off} C - V^* k^S_{on} L^* S^* + V^* k^S_{off} X^* + \Delta^* (L_B - L^*) \quad (3a)$$

$$\Delta^* = 4\pi D_L (a + \delta). \quad (3b)$$

In the bulk fluid region, reversible binding with the appropriate soluble receptors and diffusive transport of the ligand are included.

$$V_B \frac{dL_B}{dt} = -V_B k^S_{on} L_B S_B + V_B k^S_{off} X_B - \Delta^* (L_B - L^*). \quad (4)$$

Cell density is reflected in the volume of the bulk fluid layer, which is evaluated on a per-cell basis. Transport between neighboring bulk fluid regions is reciprocated.

Our main interest is inhibition of surface complex formation due to the presence of competing soluble decoys. Therefore, it is essential that the model incorporate kinetic expressions for diffusion and reversible binding of these receptors. Soluble receptors are present throughout the fluid phases surrounding the cell and each compartment, secretion layer and bulk medium, contains a well mixed homogeneous distribution of these receptors. Diffusion between the compartments occurs, with a diffusion coefficient $D_S$. Transport to the cell is again characterized by the Smoluchowski diffusion-controlled rate constant to a sphere. Kinetic expressions for the transient changes in the bound and unbound soluble receptors within the secretion layer, $X^*$ and $S^*$ respectively, are:

$$V^* \frac{dS^*}{dt} = -V^* k^S_{on} L^* S^* + V^* k^S_{off} X^* + \Delta^*_S (S_B - S^*) \quad (5a)$$

$$\Delta^*_S = 4\pi D_S (a + \delta) \quad (5b)$$

$$V^* \frac{dX^*}{dt} = V^* k^S_{on} L^* S^* - V^* k^S_{off} X^* + \Delta^*_S (X_B - X^*). \quad (6)$$

The kinetic expressions for the bulk medium solution receptors again include only the reversible binding and the diffusive flux between compartments and are given below for the unbound solution receptors:

$$V_B \frac{dS_B}{dt} = -V_B k^S_{on} L_B S_B + V_B k^S_{off} X_B - \Delta^*_S (S_B - S^*). \quad (7)$$

A mass balance is used in place of a kinetic expression for the bound soluble receptors in the bulk medium, $X_B$, with $St$ being the concentration of soluble receptors added to the medium.

$$X_B = \frac{St(V^* + V_B) - (S^* + X^*)V^* - S_B V_B}{V_B} \quad (8)$$

Computations were performed using parameter values for the IL2 system. The pertinent system values and appropriate references are listed below:

IL2:

| | | |
|---|---|---|
| $K_D$ | $0.7 \cdot 10^{-11}$ M | (Wang and Smith, 1987); |
| $k_{on}$ | $3.1 \cdot 10^7$ M$^{-1}$ $s^{-1}$ | (Wang and Smith, 1987); |
| $R_o$ | 2,000 receptors/cell | (Wang and Smith, 1987); |
| $k_t$ | $4.6 \cdot 10^{-3}$ min$^{-1}$ | (Smith and Cantrell, 1985); and |
| $k_e$ | $4.6 \cdot 10^{-2}$ min$^{-1}$ | (Smith, 1988). |

Our model segregates the diffusive transport of the ligand from the binding step. Dissociation rate constants in both systems were determined from the $K_D$ and $k_{on}$ values. The association and dissociation rate constant of the ligand for the soluble receptors, $k^S_{on}$ and $k^S_{off}$, are assumed to be equivalent to the ligand-surface receptor values. The diffusion coefficients for both the ligand and

the soluble receptor are estimated from their respective molecular weights using a semiempirical equation for large (molecular weight above 1,000) biological solutes in aqueous solutions as:

$D_L$ $10^{-6}$ cm$^2$/s (Geankopolis, 1983), and
$D_S$ $4.0 \cdot 10^{-7}$ cm$^2$/s (Geankopolis, 1983).

Quantitative measurements of ligand secretion rates for autocrine cells are complicated by binding which occurs upon ligand release. Rosenthal et al. (1986) report a TGF-α secretion rate of ~600 molecules/cell-min for transfected rat fibroblasts while Smith et al. (1987) report a rate of ~0.3 molecules/cells-min for human pancreatic cancer cells. Leof et al. (1986), looking at mouse embryo-derived cells, report a secretion rate of 130 molecules/cells-min for platelet-derived growth factor (PDGF). These experimental estimates should be viewed as underestimates of secretion rates due to surface receptor binding. We chose to base our calculations on a secretion rate of 500 molecules/cell-min for IL2.

The appropriate volumes required are based on the cell size and density. Cells and the surrounding secretion layer are assumed to be spherical and uniformly distributed.

$a$ $5 \cdot 10^{-6}$ m
density $10^{-5}$ cells/ml.

Our model is compartment based with length scale δ characterizing the secretion layer. This parameter is an artifactual element built into the model and, thus, has no corresponding experimental measurements. Because each compartment is assumed to have no spatial gradients, the value chosen reflects the diffusional transport capability of the ligand. In accordance with Berg's assessment that flux lines to an absorbing disk on a sphere become radial at a distance away from the cell on the order of the distance between neighboring receptors (Berg, 1983), our secretion layer thickness reflects the distance between neighboring homogeneously distributed receptors. With a cell radius of 5 microns and a receptor density of 2,000 receptors per cell, an appropriate secretion layer thickness would then be:

$$\delta \quad 2 \cdot 10^{-7} \text{ m}.$$

Steady-state analysis of the model initially appeared desirable but a transient solution covering a time span of 30 h was instead chosen. Transient analysis revealed that multiple time scales governed the steady state solution for the multivariable system presented. Receptors and receptor complexes approach their steady-state values within hours while ligand concentration require weeks. Because cellular response has been linked with surface complex levels, we chose to look over a relatively shorter time period rather than when the entire system had reached steady state. Further, experiments designed to look at the effects of antibodies on cell growth in vitro are typically completed within 24 h (Huang et al., 1984; Johnsson et al., 1985; Yoshida et al., 1990). The computer subroutine LSODE, an implicit solver, was used to solve the system of nonlinear ordinary differential equations (Hindmarsh, 1980). Equations were scaled and the following initial conditions were used:

$R/R_o = 1.0$ (all receptors were initially unbound);
$C/R_o = 0.0$ (no complexes);
$L^*/K_D = 0.0$ (secretion has not begun yet);
$L_B/K_D = 0.0$ (secretion has not begun yet);
$S^*/St = 1.0$ (homogeneously distributed decoy concentration);
$X^*/St = 0.0$ (secretion has not begun yet);
$S_B/St = 1.0$ (homogeneously distributed decoy concentration);
$X_B/St = 0.0$ (secretion has not begun yet).

The boundary conditions were chosen to reflect the fact that secretion was initiated at time zero. Before secretion initiation, all receptors were unbound and the surrounding media was void of ligand. Soluble receptors had been added but were unbound, due to the lack of available ligand in the media.

## RESULTS

The primary thrust of our analysis is evaluation of the ability of solution binding molecules to inhibit cellular response using surface concentration of receptor complexes as our gauge. As shown in Fig. 3, for a given cell density, low concentrations of solution decoys have no effect upon the density of surface complexes present after 30 h of stimulation. A threshold concentration must be added to initiate inhibition and this threshold concentration of soluble receptors is cell-density dependent. A secondary plateau over which increasing concentrations of soluble receptors have no further inhibitory effect exists, in which the level of surface complexes present is independent of cell density. Total blockage of cellular complexes is only obtainable at very high concentrations of soluble receptors and, this too, is cell density independent. At a low cell density of $10^3$ cells/ml, the soluble decoy concentration required to initiate inhibition is about equivalent to the $K_D$ of the surface receptor-ligand interaction. Higher cell densities require larger concentrations of decoys to initiate inhibition, yet, to obtain essentially complete blockage of binding, given the parameter values for the IL2 system, would require a decoy concentration of about eight

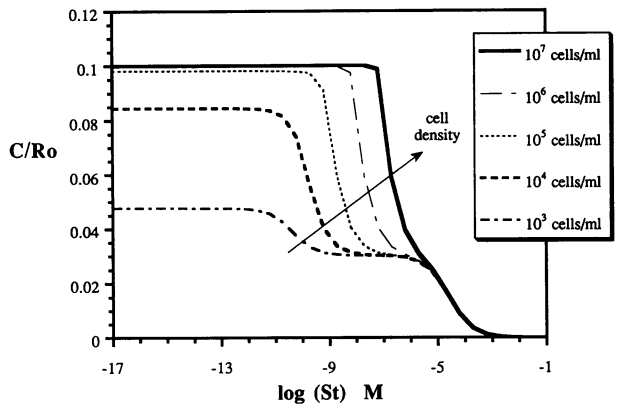


FIGURE 3 Effect of cell density, suspension cell model. Shown is the effect of increasing concentrations of soluble receptors on the level of surface complexes at various cell densities. Surface complexes are scaled by the level of surface receptors found in the absence of ligand stimulation. For visual ease, the concentration of soluble receptors added is plotted logarithmically. Parameter values for IL2 used for all calculations with the exception of Fig. A2: $k_{on} = 3.1 \cdot 10^7 M^{-1}s^{-1}$, $k_{off} = 2.3 \cdot 10^{-4}\ s^{-1}$, $k_{on}^S = 3.1 \cdot 10^7 M^{-1}\ s^1$, $k_{off}^S = 2.3 \cdot 10^{-4}\ s^{-1}$, $R_D$ = 2,000 receptors/cell, $k_t = 0.0046\ min^{-1}$, $k_e = 0.046\ min^{-1}$, $D_L = 10^{-6}\ cm^2/s$, $D_S = 4.0 \cdot 10^{-7}\ cm^2/s$, Q = 500 molecules/min, a = 5 μm, δ = 0.2 μm. For all subsequent figures, $10^5$ cells/ml is the standard density used.

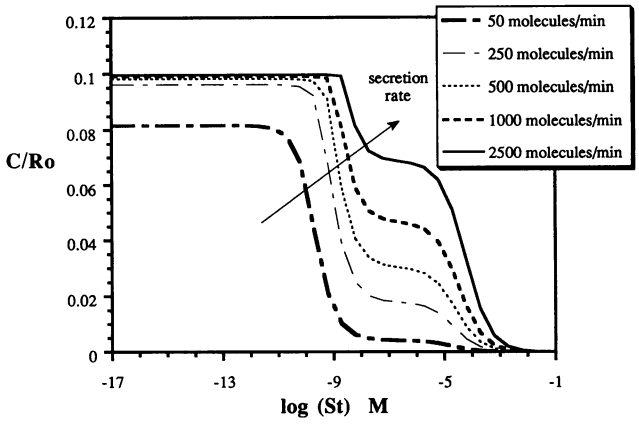


FIGURE 4 Effect of ligand secretion rate, suspension cell model. Level of scaled surface complexes is shown versus the concentration of soluble receptors added at various ligand secretion rates. Parameter values are listed in Fig. 3.

times the $K_D$ value. This concentration is independent of cell density, however, increased surface receptor-ligand affinities do increase the soluble receptor concentrations required for inhibition (Fig. 7).

The initial plateau of surface complex levels is due to kinetic limitations. A balance between degradation of receptors through endocytosis, diffusive transport of ligand away from the cell, and affinity of the receptor for the ligand places a natural upper boundary on the level of surface complexes. This level is dependent on the local concentration of ligand available to bind to the receptors. At low cell density, there is a greater concentration gradient driving secreted protein away from the binding surface so that maximum levels of complexes are not obtained. At high cell densities, smaller bulk phase volumes lead to decreased local concentration gradients but maximum complex levels are still limited by availability of unbound surface receptors. This point is emphasized by Fig. 4 in which surface complex levels increase with increasing ligand secretion rates but still are contained by the kinetic limit.

As depicted schematically in Fig. 5, soluble receptors begin to affect complex formation by binding ligand within the bulk phase thereby preventing diffusion of ligand between cells and, further, creating a gradient in the ligand concentration resulting in an increased driving force away from the cell. Cell receptor binding still occurs at this stage due to the influx of ligand from the secreting cell into the secretion layer. It is only when the number of solution decoys in the ligand secretion layer is high enough to directly compete with the surface receptors that complete inhibition is obtainable. Because the secretion layer volume is independent of cell density, the

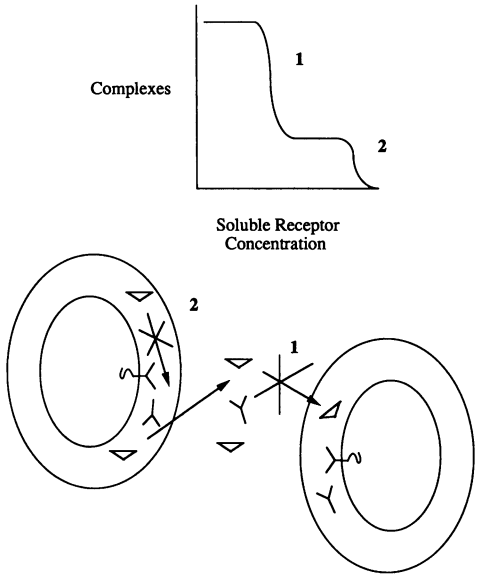


FIGURE 5 Schematic depicting inhibition process. Soluble receptors inhibit the level of surface complexes in two regimes. (1) Soluble receptors within the bulk compartment bind ligand which diffuses into this compartment thereby preventing further diffusion of this ligand back to the cell or to neighboring cells; further, this binding increases the driving force for ligand diffusion out of the secretion compartment. (2) Soluble receptors within the secretion compartment are now at a high enough concentration to compete directly with the surface receptors for the newly secreted ligand.

level of surface receptor complexes on the transport limited plateau and subsequent final inhibition requirement are cell density independent. Because complex formation is always dependent on the local secretion layer concentration of ligand, increased secretion rates lead to increased local concentrations, and subsequently, the level of inhibition obtained in the transport dependent plateau is decreased (Fig. 4).

Ligand binding can only occur when the ligand is in close proximity to the receptor. Hence, transport of the ligand is an important consideration. Transport occurs via diffusion only and, as shown in Fig. 6 *A*, increasing this transport rate greatly affects the transport plateau levels. For lower diffusivities, transport is retarded and the ligand remains in the vicinity of the cell longer. For higher diffusivities, the ligand quickly diffuses away from the cell and complex inhibition on the transport plateau level is greater. It is only within this transport plateau region in which ligand diffusion plays a role. To obtain complete inhibition, the number of solution receptors in the secretion layer must still be great enough to compete for the newly secreted ligand.

Because ligand transport played an important role in the inhibition levels obtainable in the transport plateau region, the effect of the transport properties of soluble receptors was considered. As shown in Fig. 6 *B*, the diffusive transport of the soluble receptors plays no role in the inhibition of the cellular complexes after 30 h of exposure. The analysis assumes that soluble receptors are uniformly distributed within the surrounding medium and, hence, gradients in their concentration may be minimized. The ligand is a much smaller molecule than the soluble receptor and its resulting quicker diffusion to the bulk medium may further minimize the effect of soluble receptor transport.

As stated earlier, ligand-receptor affinity is a key factor in determining the kinetic-limited binding level. As shown in Fig. 7, *A* and *B*, the intrinsic on- and off-binding rate constants of the receptor also play a role in determining the extent of inhibition obtainable in the transport-dependent regime. Varying either the cellular receptor's association and dissociation rate constants revealed that increasing affinity decreased the inhibition levels obtainable within that regime. However, the effect of the association rate constant is much more pronounced than that of the dissociation rate constant. The autocrine cell model includes a constant secretion rate which allows for newly synthesized ligand to be constantly available for receptor binding. An increased association rate constant increases the rate of binding, thus decreasing the available ligand for transport away from the cell. Although a decreased dissociation rate constant similarly affects the equilibrium affinity of the complex, it does so not by increasing the levels of initial

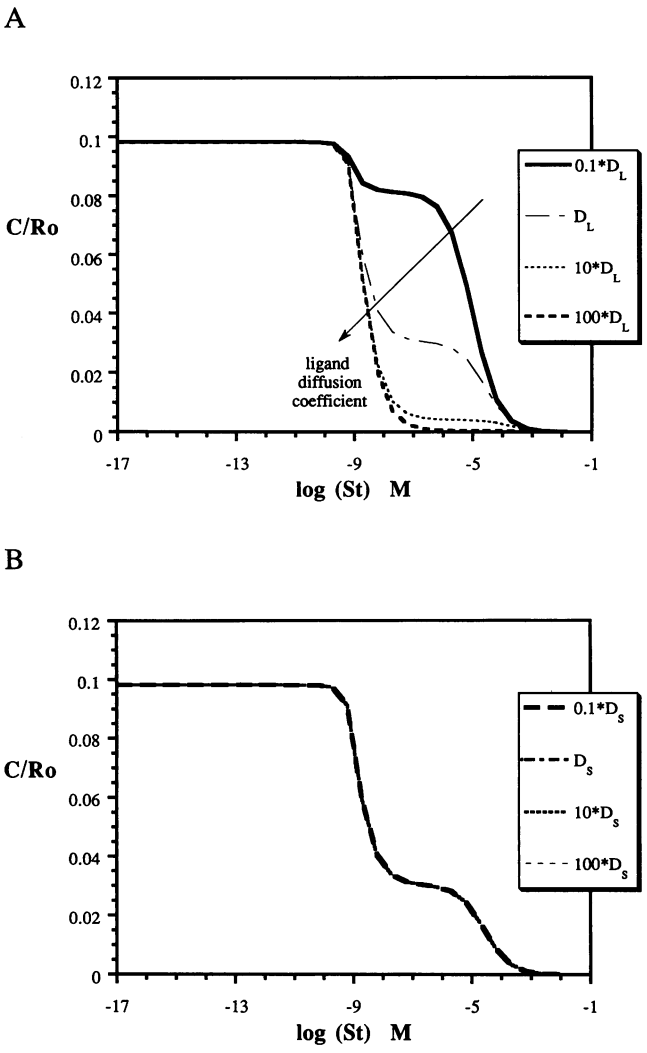


FIGURE 6 Effect of transport on inhibition levels, suspension cell model. (*A*) Scaled surface complexes are plotted against the concentration of soluble receptors at various ligand transport rates. Increasing values of the ligand diffusion ceofficient correspond to increasing transport rates. (*B*) Scaled surface complexes versus concentration of solution receptors at increasing solution receptor transport rates. Parameter values used for calculations are shown in Fig. 3.

binding but rather by increasing stability of formed complexes. Apparently, it is the direct kinetic competition between binding and diffusion which is of paramount importance.

In contrast to the solution receptor's transport characteristics, the affinity of the solution ligand-receptor complex does have an effect on the inhibition levels obtainable (Fig. 8, *A* and *B*). Increasing the decoy's affinity, by varying either the association or dissociation rates, has no effect on the kinetic limited binding level but does influence inhibition in the transport limited

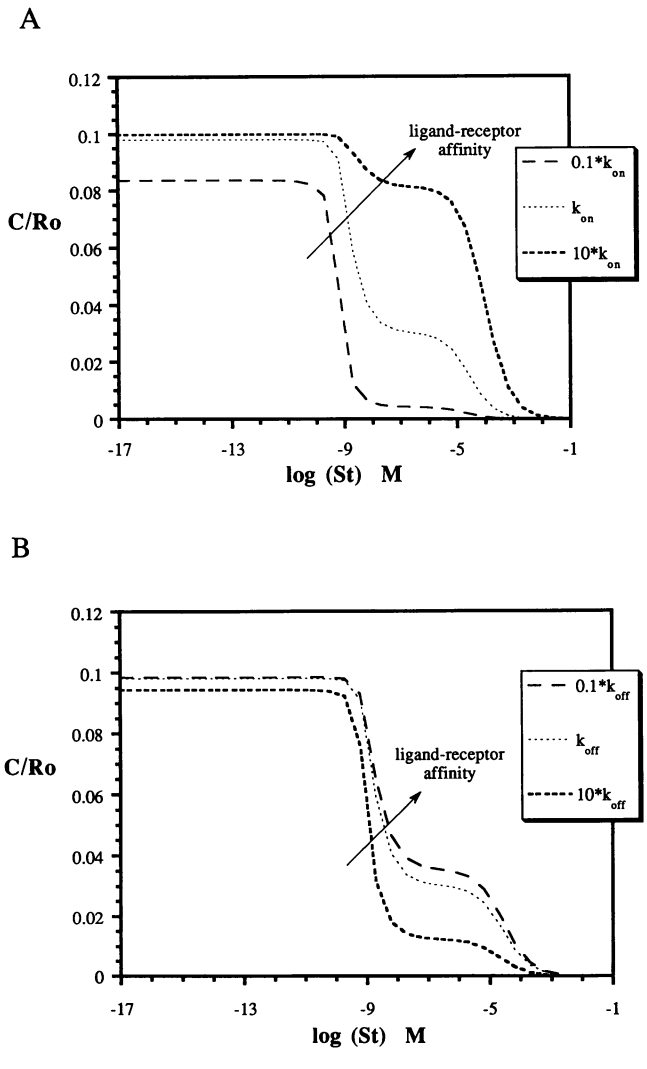


FIGURE 7 Effect of binding rate constants on inhibitory effect of solution receptors, suspension model. (*A*) Scaled surface complexes versus the concentration of solution receptors is shown at increasing values of ligand-receptor association rate constant. Dissociation rate constant of the surface complex was held constant. (*B*) Scaled surface complexes versus solution receptor concentration at increasing ligand-receptor dissociation rate constants. Association rate constant was held constant. Parameter values are listed in legend for Fig. 3.

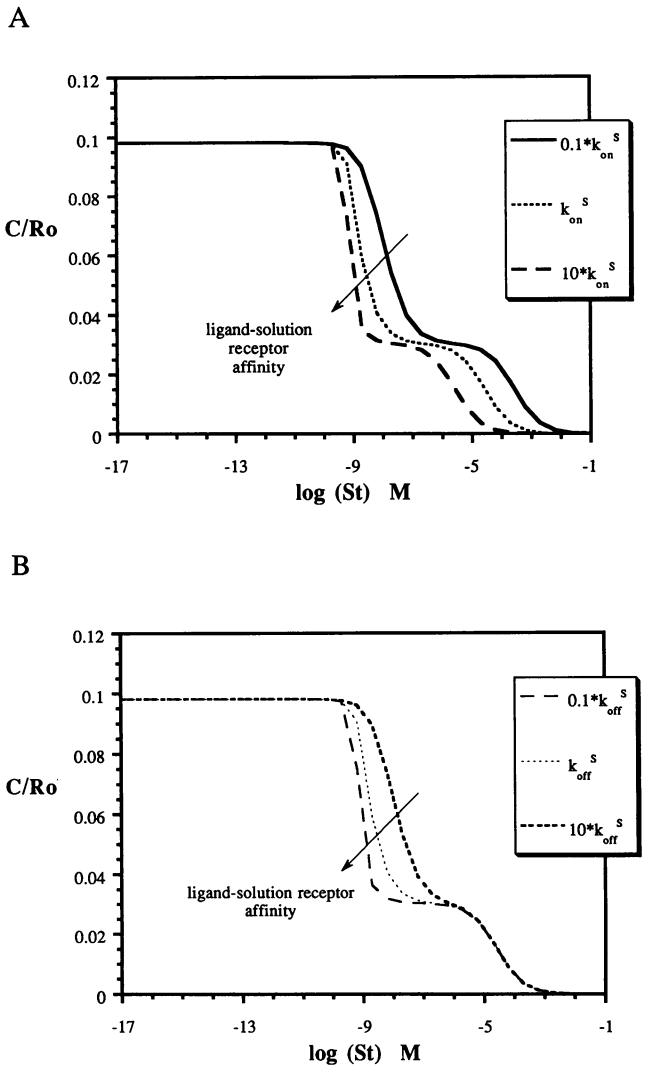


FIGURE 8 Effect of binding rate constants on inhibitory effect of solution receptors, suspension model. (*A*) Ligand-surface receptor $K_D$ was held constant while the solution receptor association rate constant was varied. The dissociation rate constant for the ligand-solution receptor complex was held constant. (*B*) The dissociation rate constant for the ligand-solution receptor complex was varied while holding constant the association rate constant. Scaled surface complexes versus the concentration of solution decoys is shown. Parameter values are listed in legend for Fig. 3.

regime. Within this regime, increased affinity does not affect the transport limited plateau level only the threshold concentration required to reach this plateau. Increased affinity for the ligand enables the soluble receptors to compete with the cellular receptors more effectively. Variations in the association rate constant, therefore, lead to changes in the soluble receptor concentration required to directly compete for the newly secreted ligand and, hence, obtain complete complex inhibition. While still affecting the threshold concentration required to initiate inhibition, decreases in the dissociation rate constant do not affect the levels of complex inhibition outside the transport regime. The effect is a secondary one, not clearly connected to the direct inhibition of newly synthesized material.

A length scale appropriate for the secretion compartment, δ, was selected based on the separation distance between neighboring receptors, assuming a uniform homogeneous arrangement. As stated earlier, this parameter is artificial and, as such, its choice is somewhat

arbitrary. We varied the parameter over a reasonable range to investigate its effect on the models' results. As shown in Fig. 9, the secretion layer thickness, δ, does influence the level of inhibition obtainable in the transport-influenced regime. With increased δ values both the concentration of soluble receptors needed to obtain complete inhibition and the range of the intermediate plateau is reduced. The increased volume of the secretion compartment at large δ values leads to an increased number of soluble receptors capable of directly competing with the surface receptors for the newly secreted ligand, hence reducing the surface receptor's binding advantage due to autocrine secretion. Until the solution receptor concentration is high enough to bind the majority of the bulk ligand, the secretion layer length scale plays no role. However, because complete inhibition is desirable, a closer look at the diffusion pathway within the region close to the autocrine cell itself will be focused on in future work. It should be noted that even when δ is sufficiently large that the intermediate, transport-limited plateau disappears, the concentration of decoys needed to achieve essentially complete inhibition may be four to six orders of magnitude greater than $K_D$.

## DISCUSSION

Autocrine ligand stimulation of cell receptors is thought to be one means of unmitigated response characteristic of malignant cells (Sporn and Todaro, 1980). Inhibition of complex formation on the cell surface via solution decoys, whether soluble receptors or antibodies, is one potential means of interrupting the cell signal of these

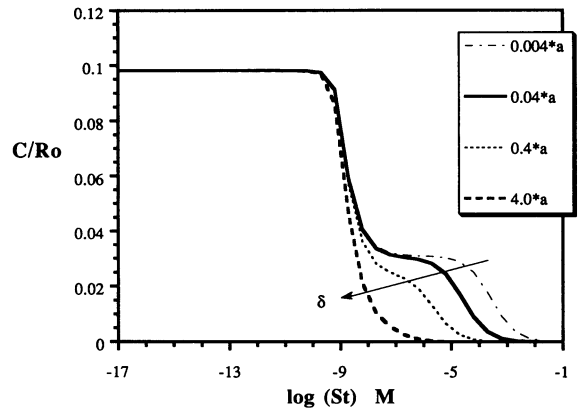


FIGURE 9 Examination of the sensitivity of the model to δ. Suspension cell model using standard IL2 parameter values (see Fig. 3) but varying δ over four orders of magnitude. Values of δ are shown as a function of $a$, the cell radius. 0.04* $a$ corresponds to the standard value for δ used in previous calculations.

ligand secreting cells. Ligand-receptor binding is dependent not only on the intrinsic rate constants of the complex but also on the local concentrations of the primary factors. It is in these local concentrations where synthesis and diffusion play key roles. This is especially true for autocrine cells whose response may be amplified by the proximal location of ligand and receptor synthesis.

We present a mathematical model which investigates the effect of a number of parameters on the level of complex inhibition obtainable via the addition of solution decoys on anchorage-dependent and suspension cells (see Appendix for anchorage-dependent cell model). Our models demonstrate that inhibition is obtainable but the critical concentration of soluble receptors required is dependent on the binding affinities of the competing receptors. Ligand transport plays a key role in the process and places a limitation on the inhibition at lower soluble receptor concentrations.

Investigators looking at the effect of antibody addition on response of autocrine cells have primarily assayed for response changes from control cells (Betsholtz et al., 1984; Huang et al., 1984; Imanishi et al., 1989). Reduction in response may be considered evidence that the presence of the antibody is affecting the cell. However, to effectively interrupt the autocrine cycle, complete inhibition of response may be necessary. Huang et al. (1984) examined the effect of several concentrations of antiserum on the inhibition of growth of Simian Sarcoma Virus (SSV)-transformed cells. They found that 50% inhibition was obtainable at low concentrations of antiserum and that a four-fold increase in concentration had no increased effect. As shown by our results, the concentration of competing antibody may not have been high enough to overcome transport limitations. A higher concentration of antiserum may be able to completely eliminate the response. It would be helpful to predict what concentration will be necessary, and a simple expression relating inhibition to antibody concentration would be desirable.

To obtain such an expression relating the level of complex inhibition to the required concentration of soluble competing receptors which must be added, several simplifying assumptions are made. Within the transport region, essentially all the bulk phase ligand becomes bound to the competing soluble receptors and the free ligand concentration within this regime becomes negligible. Further, to obtain complete inhibition, the free soluble receptors within the secretion boundary layer must be able to directly compete with the surface receptors. Therefore, it will be assumed that the concentration of bound soluble receptors within the secretion

layer is negligible and the unbound concentration is equivalent to a constant.

Scaling Eqs. 1, 2, and 3 *a*, and assuming a quasi-steady state, the level of unbound surface receptor is:

$$u = \frac{\theta + v}{\theta + y^*} \tag{9}$$

where

$$\begin{aligned} u &= R/R_o \\ v &= C/R_o \\ y^* &= L^*/K_D \\ \theta &= k_t/k_{off}. \end{aligned} \tag{10}$$

The quasi-steady state level of surface complexes is:

$$v = \frac{uy^*}{\theta\eta + 1}, \tag{11}$$

where:

$$\eta = k_e/k_t.$$

Recalling that all ligand concentrations except for the secretion boundary layer are considered negligible, the ligand concentration within this layer is:

$$y^* = \frac{\sigma + \rho v}{\rho u + \alpha + \beta\omega_t}, \tag{12}$$

where:

$$\begin{aligned} \sigma &= Q/(V^* k_{off} K_D) \\ \rho &= R_o/(V^* K_D) \\ \beta &= k^s_{on}/k_{on} \\ \omega_t &= S^*/K_D \\ \alpha &= \Delta^*/(k_{off} K_D). \end{aligned}$$

Assuming that the transport flux of ligand away from the cell and the secretion of ligand from the cell itself into the secretion boundary layer are the dominate terms in the expression for $y^*$, an expression relating the scaled concentration of soluble receptors, $\omega_t$, with the surface complex level, $v$, is:

$$\omega_t = \frac{\sigma - v\alpha(1 + \eta\theta) - v\eta\sigma}{v\beta(1 + \eta\theta)} \tag{13}$$

Given the previous results and discussion, it is not surprising that the approximate relationship derived above should be a function of these particular parameters given the regime for which the equation is appropriate.

Knauer et al. (1984) showed that a linear relationship exists between the steady-state level of receptor occupancy and the mitogenic response for the EGF system. With human fibroblasts, 10% of the maximum cell response can occur with only ~5% of the initial receptors occupied. For complete inhibition of a cellular response, an extremely low occupancy level would be desired. A $v_{critical}$ of 0.1% may, therefore, be quite appropriate. Given the parameter values for IL2 and selecting a $v_{critical}$ value of .001, the concentration of soluble receptors which must be added is quite similar to the concentration necessary using the full system of differential equations.

$$\begin{aligned} &S_t = 8.5 \times 10^{-4}\ \text{M} \\ &(\log S_t = -3.1) \end{aligned} \quad \text{approximation,}$$

$$\begin{aligned} &S_t = 4.5 \times 10^{-3}\ \text{M} \\ &(\log S_t = -2.4) \end{aligned} \quad \text{full system of ODEs.}$$

The approximate solution differs by less than an order of magnitude from the full system of equations result. Eq. 12 may offer, therefore, a quick estimate of the level of inhibiting receptors required. It is interesting to note that the concentration required to obtain this level of inhibition is about eight orders of magnitude higher than the IL2 $K_D$ value ($0.7 \times 10^{-11}$ M). However, both the approximate expression and the full system of equations include an autocrine secretion rate, whose value is crucial to the level of inhibition obtainable. Because this quantity is currently very difficult to measure experimentally increased attention to its experimental determination is in order.

A key feature of our model is its emphasis on spatial heterogeneity for autocrine ligand access to cell surface receptor binding. In systems where ligand is added exogenously to the bulk medium, binding to surface receptors has been treated successfully as a boundary condition for a ligand diffusion equation (e.g., De Lisi and Wiegel, 1981; Shoup and Szabo, 1982). For ligand introduced directly at the cell surface, as in the autocrine case, the length scales of the binding and secretion sites are more difficult to smear into an average boundary condition within a continuously distributed model. We have therefore chosen to recognize and account for spatial distribution of ligand by a simple compartmentalized model, in which ligand close to the cell surface (the secretion layer) and far from the surface (the bulk medium) do indeed possess different kinetic possibilities for receptor binding. The length scale of binding site distribution is reflected in the value chosen for the secretion layer thickness, $\delta$, as suggested by Berg (1983). We believe that this compartmentalized model generates predictions allowing increased intuitive understanding, as well as improved quantitative guidelines, for binding inhibition by solution decoys. Although the sharpness of the transport-limited inhibition plateau is

overestimated by the discrete compartmentalization approach, we suggest that there ought indeed to be often found such an intermediate level of inhibition. The experimental results of Huang et al. (1984) may indeed be evidence of this. Ultimately, however, the most rigorous analysis of autocrine ligand binding to cell surface receptors will probably require a noncontinuum modeling approach, such as application of Brownian dynamics simulation techniques for the region close to the cell surface.

## SUMMARY

Many cellular responses governed through surface receptor-ligand binding might be inhibited through the addition of decoy receptors or antiligand particles aimed at disrupting this initial binding interaction. This interruption may be especially crucial in controlling autocrine cells because production of receptors, secretion of the ligand, and binding between the two molecules occur within the same cell's sphere of influence and other means of signal interruption may be more difficult.

A kinetic model was presented incorporating the reversible binding of the soluble receptors with the autocrine factor into its framework. Suspension and anchorage-dependent (see Appendix for comparison between the two model cell types) cells were treated separately, but the primary results were independent of the geometric configuration of the system. At low concentrations of soluble receptors there was essentially no inhibition of surface complexes. Once a threshold concentration had been added, partial inhibition could be obtained with the level of inhibition being dependent on such factors as receptor affinity, transport rate of the ligand, and secretion rate. The cellular density and affinity of the soluble receptor played roles only in the threshold concentration required but not in the inhibition level obtainable. However, this initial inhibition level is transport limited. Complete inhibition is not obtainable until all bulk ligand has been bound and the concentration of soluble receptors within the secretion layer is high enough to directly compete with the surface receptors for the secreted ligand. This second threshold is dependent on the association constants of the competing receptors. An important observation obtained through the models is the independence of inhibition on the transport of the competing receptors. Primarily, inhibition is dependent on cellular-derived parameters. Modified soluble receptors prove beneficial in as far as the ratio of their association constant with that of the cellular receptor's is high.

A simplified expression was derived relating the concentration of inhibitory receptors to surface complex levels. Good agreement was obtained between the full computer model results and the approximation solution, Eq. 12, indicating that the simple expression may allow quick estimates to be made before experimentation. Calculations indicate that soluble receptor concentrations as high as between four and eight orders of magnitude greater than the receptor-ligand $K_D$ value may be necessary to completely interrupt the autocrine secretion signal.

## APPENDIX

Many autocrine cell systems involve cell types which are anchorage-dependent and will not grow in suspension (Imanishi et al., 1989; Van de Vijver et al., 1991). Cellular arrangement might, therefore, play a pivotal role in response inhibition. We modified our suspension cell model to examine the response of these types of anchorage-dependent systems.

Primarily, the two models differ in their compartmentalization. The suspension cell model contains a secretion layer and a bulk fluid phase and it is only through this outer compartment that cell to cell communication can occur. In the plated cell model, fluid mixing properties close to the solid surface will be influenced by this physical boundary leading to a mass transfer layer. Hence, this model contains a boundary layer connecting the secretion layers of each cell and separating these secretion layers from the bulk fluid phase. Ligand diffusive transport through the mass transfer boundary layer is characterized by a transfer coefficient which incorporates appropriate length scales $\delta_i$, the height of the layer, and $\psi$, one half the distance between the centers of neighboring plated cells.

A direct comparison between the inhibition effect of solution receptors on surface complex levels in both the anchorage-dependent and -independent models is shown in Fig. A1. In both models, parameter values were based on the IL system and qualitatively, both show the same pattern of inhibition. At low cell densities, neither configuration allows for maximal levels of surface complexes indicating, perhaps, a suboptimal response. However, plated cells do show a

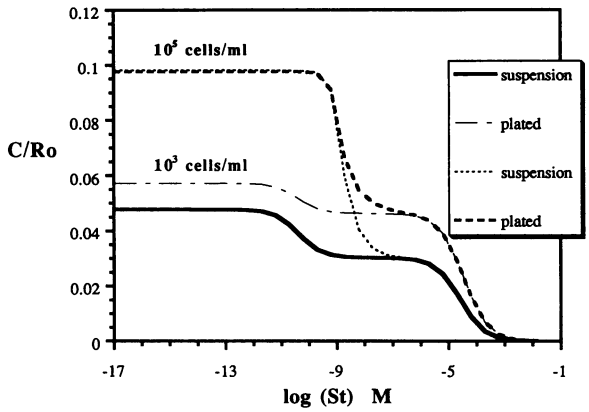


FIGURE A1 Comparison of geometrical effects. At both $10^3$ and $10^5$ cells/ml, inhibition of surface complexes due to the presence of competing solution receptors is shown for both the suspension and plated cell model. IL-2 parameter values were used in both models to allow direct comparison of purely geometric effects.

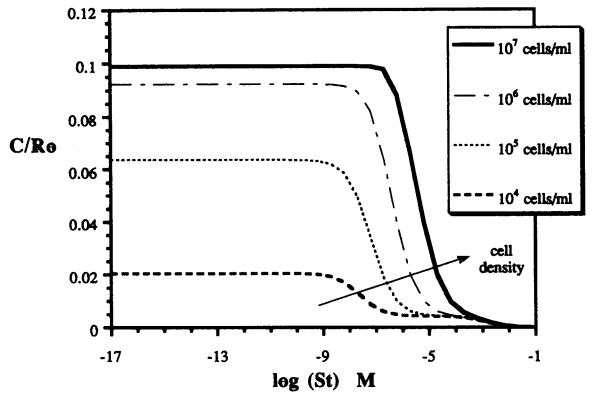


FIGURE A2 Effect of solution receptor addition on autocrine plated cells, EGF parameter values. Scaled surface complexes versus the concentration of inhibitory solution receptors added at various cell densities. EGF parameter values used in calculations: $k_{on} = 3 \cdot 10^6\ M^{-1}\ s^{-1}$, $k_{off} = 1.4 \cdot 10^{-2}\ s^{-1}$, $k^S_{on} = 3 \cdot 10^6\ M^{-1}\ s^{-1}$, $k^S_{off} = 1.4 \cdot 10^{-2}\ s^{-1}$, $R_o = 10^5$ receptors/cell, $k_t = 0.03\ min^{-1}$, $k_e = 0.3\ min^{-1}$, $D_L = 10^{-6}\ cm^2/s$, $D_S = 4 \cdot 10^{-7}\ cm^2/s$, Q = 5,000 molecules/min, a = 5 μm, δ = 0.3 μm, $\delta_i$ = 25 μm, total plate volume = 10 ml.

higher level of surface complexes than do the suspension cells. The close proximity of neighboring cells offers a further resistance to diffusion into the bulk fluid and an attraction towards the cell. This same geometrical binding advantage conveyed on plated cells is evident in the presence of soluble receptors throughout the transport dependent region although both models do exhibit complete complex inhibition when exposed to sufficiently high soluble receptor concentrations.

High density cultures are situated close enough to each other that the binding advantage of plated cells over suspension cells is not evident. However, with the addition of increasing concentrations of competing receptors, plated cells show a higher resistance to inhibition than do suspension cells. This is especially evident in the transport dependent regime. Previous mathematical analysis comparing autocrine cell growth on spherical and flat surfaces demonstrated that geometrical differences can affect growth behavior primarily through ligand transport differences (Lauffenburger and Cozens, 1989). As shown in Fig. 5, a transport dependent plateau level of surface complexes is obtained when a sufficiently high enough concentration has been added to bind bulk phase ligand and prevent diffusional communication or back diffusion to the cell itself. However, plated cells still have a growth advantage due to their reduced rate of transfer to the bulk phase.

To facilitate comparison of geometrical effects between the two mathematical models, IL2 parameter values were incorporated into both. However, IL2 is an autocrine factor for lymphocytes, an anchorage-independent cell type, so parameter values for EGF, a growth factor of fibroblasts, would yield more physiologically-meaningful results. Using parameter values for the EGF system, comparable calculations to those shown in Fig. 3 for the suspension cell model were evaluated. Comparison between Figs. A1 and A2 reveal that the EGF system yields qualitatively the same pattern of inhibition seen in the IL2 system. However, the transport dependent regime in the EGF system shows strikingly reduced levels of surface complexes when compared with the IL2 system. This is primarily due to the higher association rate constant of IL2 for the IL2 receptor as opposed to the EGF/EGF receptor complex. As depicted in Fig. 7*A*, the association rate constant greatly influences the level of surface complexes found within the transport dependent regime. Parameter values used for the EGF/EGFR system are shown with Fig. A2.

The length scale for the mass transfer boundary layer, $\delta_i$, is an artificial parameter which cannot be experimentally determined. However, varying this parameter over five orders of magnitude did not affect either the surface complex inhibition levels or the soluble receptor concentration profile (results not shown).

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