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A Theoretical Model for Adhesion Between Cells Mediated by Multivalent Ligands

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ABSTRACT

A theoretical model is developed for cell-to-cell binding by bivalent ligands that can bind to mobile receptors on the cell surfaces. Monovalent inhibitors that can bind either to receptors or ligands are also included. For symmetrical ligands, that is, ligands in which both binding sites are the same, it is shown that crosslinking of receptors on each cell will interfere with intercellular bridge formation. At equilibrium, such interference is not drastic, but if the crosslinks can form before the cells are brought into contact, crosslinking may greatly impede the rate of intercellular binding. Comparison is made with experiments, and the importance of receptor mobility is discussed. It is noted that ligands can also bind a cell to itself or to a surface.

Key Words: Cell adhesion; cell agglutination; binding between cells; adhesion, cell-to-cell; ligands; lectins; agglutination, cell-to-cell.

INTRODUCTION

In many studies, cells are caused to stick to each other by multivalent ligands, such as lectins, antibodies, or antigen–antibody complexes. For example, the agglutination of cells by multivalent lectins that bind to specific cell-surface oligosaccharides has been studied by many workers (*1*), partly in an attempt to elucidate differences between transformed and normal cells. Agglutination of red cells by antibodies (*2*) is often used as a test for the presence of specific antibodies in serum, and antibodies or antigen–antibody

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complexes may be used to form rosettes (*3*), as in red cells surrounding a lymphocyte or macrophage. Moreover, there is evidence that multivalent ligands may mediate aggregation of cells in many natural systems, including the aggregation of cellular slime molds (*4*) and sponge cells (*5*), and in the development of embryonic tissues (*6*, *7*).

I have recently presented a theoretical framework (*8*) for treating adhesion between cells that have naturally complementary and mobile surface receptors such as antibody on one cell and antigen in the other. It was shown that a few bonds can establish effective binding between cells and that the bonds can form rapidly between adjacent cells provided that their receptors are sufficiently accessible, numerous, and mobile in the cell membrane.

The purpose of this paper is to extend the theory to treat the interactions between cells that are caused by soluble multivalent ligands. There are many kinds of possible ligands, and since the details of the analysis would depend on the number of binding sites per ligand molecule (and per receptor molecule) and whether the sites are all the same, it does not seem fruitful to try and construct a general model for interaction. Instead we shall restrict our attention to a simple but important special case that illustrates many general features. In particular let us consider agglutination of cells by a bivalent ligand in the presence of a monovalent inhibitor. For example, the ligand molecule could be a bivalent antibody and the inhibitor a monovalent antigen (hapten) or monovalent antibody (Fab fragment) or the ligand could be a lectin and the inhibitor a sugar or a monovalent lectin fragment. In either case, the ligands can both crosslink receptors on a single cell and form bridges between cells. For simplicity, the receptors will be assumed to be monovalent, i.e., to have but one site per receptor molecule for binding ligand or inhibitor.

It is useful to have in mind at the outset a molecular model of the cell membrane that is appropriate for eukaryotic cells. For this I adopt the fluid mosaic model (*9*, *10*) and take the membrane to be a phospholipid bilayer in which various glycoproteins are dispersed and retained by virtue of the favorable free energy of interaction of their hydrophobic portions with the lipid environment. Some of these glycoprotein molecules may serve as receptors for interaction with the ligands, although glycolipids or other cell surface molecules could serve as well. I will generally assume that the receptor molecules are more or less free to translate in the plane of the membrane and to rotate about an axis perpendicular to the membrane and that this motion is random, as in diffusion, rather than ordered (*11*). I also assume that receptor molecules move independently of one another except when linked by a ligand molecule.

It should be noted that this model neglects several effects that may be important when cells are exposed to multivalent ligands. In particular, receptors, especially multivalent receptors, that have been crosslinked on the cell surface by multivalent ligands may become non-uniformly distributed, both by diffusion and random lattice formation (patching) and also by gross

ordered motions coupled to the cell cytoskeleton (capping) (*12*). In addition, receptors that are crosslinked or bound to a surface (or another cell) may interact with the cytoskeleton so as to modulate the anchorage of other receptors and reduce their mobility (*13*). Our neglect of such effects is motivated by a desire to keep the model simple, but may be partially justified by noting that patching, capping, and anchorage modulation are consequences of crosslinking and bridge formation and hence may not be important in the early phases of these processes. Moreover, I will suggest experimental conditions in which these complicating effects can be minimized.

I have explained elsewhere (*14*) that when two cells establish an adhesive contact by bonding of complementary receptors, additional receptors, if still mobile, will tend to accumulate in the contact area, possibly forming a "contact cap." Such events may also follow ligand bridging between cells, but are not treated in this paper. They have also been discussed by Singer (*10*).

THE MODEL

Consider a solution that contains bivalent ligand molecules at concentration L, monovalent inhibitor molecules at concentration H, and cells. The cells are assumed to have mobile receptors, such as glycoprotein molecules in the cell membrane, which can bind to ligand or inhibitor molecules. Each receptor molecule can bind to one ligand molecule or to one inhibitor molecule.

Consider two cells which may be in contact and let N_i ($i = 1, 2$) be the number of receptors per unit area on cell i. For the present, assume that N_i is constant over the surface of cell i, although as shown elsewhere (*14*) receptors will tend to accumulate in an area of local cell contact. As shown in Fig. 1, receptors at position r, and time t on the surface of cell i, can exist in five different states of binding: (1) free receptors, unbound to any ligand, $N_{fi}(r, t)$ per unit area; (2) receptors bound to monovalent inhibitor, $N_{hi}(r, t)$ per unit area; (3) receptors bound to ligand molecules that are not attached to another receptor $N_{li}(r, t)$; (4) receptors that are crosslinked by ligand to nearby receptors on the same cell, $N_{ci}(r, t)$ pairs per unit area; and (5) receptors that are bridged by ligands to another cell, $N_b(r, t)$. Since each receptor must be in one of these five states,

$$N_i = N_{fi} + N_{hi} + N_{li} + 2N_{ci} + N_b \qquad (i = 1, 2) \qquad [1]$$

Kinetic equations can now be written for receptors in each of the states. In considering the reactions of free receptors with ligand or inhibitor molecules in solution, it is reasonable to use ordinary rate constants for molecules in solution, at least for ligands and inhibitors that are not large compared to the receptor molecules. Other reactions will occur between reactants bound in cell membranes, namely the crosslinking reaction between free and ligand-bound receptors on the same cell, and the bridging reaction

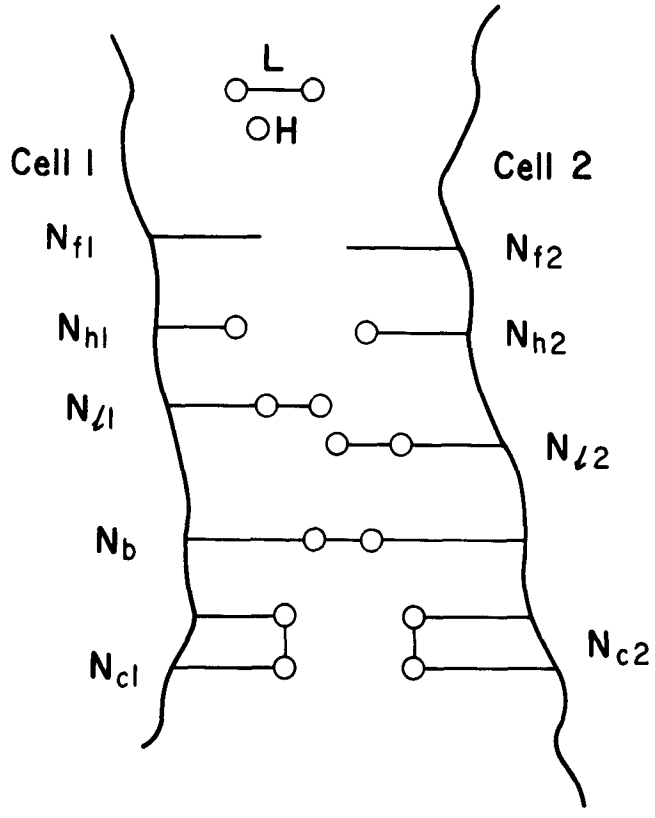

FIG. 1. Receptor states for cell to cell binding by bivalent ligands (L) in the presence of inhibitor (H). Receptors can be free (f), bound to ligand (l), bound to inhibitor (h), bridged to a receptor on the other cell (b), or crosslinked, (c).

between the same reactants on two different cells. For such reactions, we must use rate constants which are appropriate for membrane bound reactants (*8*, *15*). These rate constants differ from those for reactants in solution because the reactants that are tethered in membranes have reduced mobility and are confined to translational motion in two dimensions rather than three. We will distinguish the rate constants for solution and membrane bound reactants by superscripts s and m, respectively.

Let k_{h+}^{s} and k_{h-}^{s} be the forward and reverse rate constants for binding of inhibitor to free receptors on either cell and let k_{l+}^{s} and k_{l-}^{s} be the corresponding rate constants for binding of ligand to free receptors. Moreover, let k_{+i}^{m} and k_{-i}^{m} be the rate constants for formation and breaking of crosslinks on cells ($i = 1, 2$) and let k_{+}^{m} and k_{-}^{m} be the corresponding rate constants for formation of bridges between the cells in contact. Finally let $\delta(r, t) = 1$ if the cells are in contact at (r, t), and hence potentially able to form bridges, and $\delta(r, t) = 0$ otherwise. Evidently if $\delta = 0$, $N_b = 0$.

With these definitions, the following model equations are assumed for ($i = 1, 2$ and $j = 2, 1$)

$$\frac{dN_{hi}}{dt} = k^s_{h+} N_{fi} H - k^s_{h-} N_{hi} \quad [2]$$

$$\frac{dN_{li}}{dt} = k^s_{l+} N_{fi} L - k^s_{l-} N_{li} + 2k^m_{-i} N_{ci} - k^m_{+i} N_{li} N_{fi}$$

$$+ \delta k^m_{-} N_b - \delta k^m_{+} N_{li} N_{fj} \quad [3]$$

$$\frac{dN_{ci}}{dt} = k^m_{+i} N_{li} N_{fi} - 2k^m_{-i} N_{ci} \quad [4]$$

$$\frac{dN_b}{dt} = \delta k^m_{+} [N_{li} N_{fj} + N_{fi} N_{lj}] - 2\delta k^m_{-} N_b \quad [5]$$

The interpretation of the equations is straightforward. For example, Eq. [3] gives the rate of formation of receptors bound to ligand as the sum of six terms: (1) binding of free receptor to ligand molecule; (2) loss of ligand molecule from receptor bound to ligand; (3) breakage of either receptor–ligand bond in a crosslinked pair; (4) formation of crosslinked pair; (5) breakage of bond between the cells, leaving ligand on the i^{th} cell; and (6) formation of bridge between the ligand-bound receptor on the i^{th} cell and free receptor on j^{th} cell.

In addition to these seven equations (Eqs. [2]–[4] for $i = 1, 2$ and Eq. [5]), we have Eq. [1] for $i = 1, 2$, and if the reactions deplete inhibitor and ligand molecules from the solution, two more kinetic equations for H and L. However, in this paper I shall assume that H and L are constants. Nevertheless, these seven nonlinear equations must in general be solved numerically.

EQUILIBRIUM CONSIDERATIONS

In some cases it will be a good approximation to assume that the reaction rates for reactants in solution are rapid compared to those for membrane bound reactants. It is then reasonable to assume that N_{hi} and N_{li} are in equilibrium, i.e., $dN_{hi}/dt = 0$ and $dN_{li}/dt = 0$. The first condition gives, from Eq. [2]:

$$N_{hi} = K^s_h N_{fi} H \quad [6]$$

where $K^s_h = k^s_{h+}/k^s_{h-}$. Similarly in Eq. [3], the first two terms will approximately balance each other, so that

$$N_{li} \simeq K^s_l N_{fi} L \quad [7]$$

where $K^s_l = k^s_{l+}/k^s_{l-}$.

Inserting Eqs. [6] and [7] into [1] and solving for N_{fi}, we find

$$N_{fi} = \frac{N_i - 2N_{ci} - N_b}{1 + K_h^s H + K_l^s L} \qquad [8]$$

Inserting Eqs. [8] and [7] into Eqs. [4] and [5], we obtain

$$\frac{dN_{ci}}{dt} = \frac{k_{+i}^m K_l^s L(N_i - 2N_{ci} - N_b)^2}{(1 + K_h^s H + K_l^s L)^2} - 2k_{-i}^m N_{ci} \qquad (i = 1, 2) \qquad [9]$$

and

$$\frac{dN_b}{dt} = \frac{\delta 2k_+^m K_l^s L(N_1 - 2N_{c1} - N_b)(N_2 - 2N_{c2} - N_b)}{(1 + K_h^s H + K_l^s L)^2} - 2\delta k_-^m N_b \qquad [10]$$

These equations give the rates of formation of crosslinked receptors on each cell and of intercellular bonds.

In the absence of intercellular bonds ($\delta = 0, N_b = 0$) Eq. [9] may be compared with the analysis of Dembo and Goldstein (*16*). These authors considered, however, bivalent receptors (IgE) and ligand (antigen) so that linear chains and rings of receptors and ligands could form on the cell surface. They were able to show that at equilibrium the number of ligands that are attached at both binding sites is a maximum when

$$L = \frac{1}{K_l^s}(1 + K_h^s H) \qquad [11]$$

This result may also be deduced from Eq. [9] by setting $dN_{ci}/dt = 0$, $N_b = 0$ and seeking that value of L that maximizes N_{ci}. Equation [9] then becomes

$$A_i(N_i - 2N_{ci})^2 = 2N_{ci} \qquad [12]$$

where

$$A_i = \frac{K_i^m K_l^s L}{(1 + K_h^s H + K_l^s L)^2} \qquad [13]$$

and $K_i^m = k_{+i}^m / k_{-i}^m$

Equation [12] may be solved for N_{ci} to obtain

$$N_{ci} = \frac{N_i}{2}\left[1 - \frac{1}{2A_i N_i}(\sqrt{1 + 4A_i N_i} - 1)\right] \qquad [14]$$

The value of L in Eq. [11] maximizes A_i and hence N_{ci}, according to Eq. [14] which has also been obtained by Perelson (*16a*).

The maximum possible density of crosslinked pairs is $N_{ci} = N_i/2$ and the ratio of N_{ci} to this limit is shown in Fig. 2 as a function of $A_i N_i$. In the

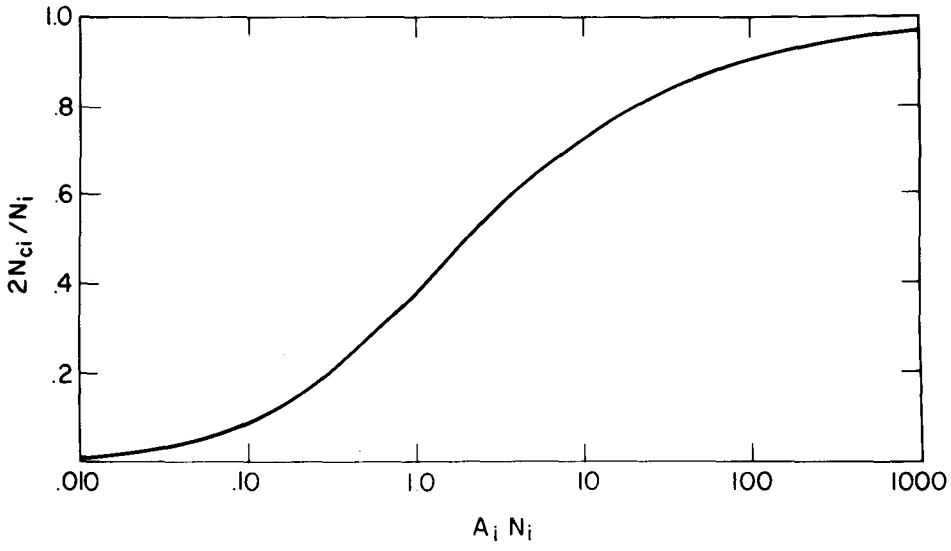

FIG. 2. Fraction of the receptors that at equilibrium are crosslinked is shown as a function of the parameter A_i [Eq. (13)] time N_i, the number of receptors per unit area.

absence of inhibitors ($H = 0$), the maximum value of A_i is $K_i^m/4$, so that Fig. 2 also shows the maximum density of crosslinks as a function of $K_i^m N_i$.

It is important to note that even when the concentration of ligand is low, so that $K_i^s L \ll 1$ and, according to Eq. [7], only a small fraction of the receptors are bound to single ligand molecules, nevertheless most of the receptors may be crosslinked. This will be the case if $A_i N_i \gg 1$ or, from Eq. [13] with $H = 0$, $K_i^m N_i \gg (K_i^s L)^{-1}$. As will be seen in the next section, $K_i^m N_i$ can be very large, so that for many systems, abundant crosslinks (and intercellular bridges) are to be expected for ligand concentrations that are small compared to their single site dissociation constants, $(K_i^s)^{-1}$. This effect is caused by the high *local* concentration of receptors on the cell surface (*14*).

When intercellular bonds are considered they must compete with crosslinking on each cell. Some insight into this competition may be gained by considering the equilibrium situation for $\delta = 1$. There is further simplification if it is assumed that $k_{+i}^m = k_+^m$ and $k_{-i}^m = k_-^m$, which is reasonable if the receptors have comparable mobilities on the two cells. Then at equilibrium, Eq. [9] gives

$$A(N_i - 2N_{ci} - N_b)^2 = 2N_{ci} \qquad (i = 1, 2) \qquad [15]$$

while Eq. [10] gives

$$A(N_1 - 2N_{c1} - N_b)(N_2 - 2N_{c2} - N_b) = N_b \qquad [16]$$

where

$$A = \frac{K^m K_l^s L}{(1 + K_h^s H + K_l^s L)^2} \qquad [17]$$

Therefore, at equilibrium

$$N_b = 2(N_{c1} N_{c2})^{1/2} \qquad [18]$$

as can be seen by substituting the left sides of Eqs. [15] and [16] for $2N_{ci}$ and N_b in Eq. [18]. If $N_1 = N_2$, then $N_b = 2N_{c1} = 2N_{c2}$ so that *at equilibrium* a receptor is just as likely to be linked to a receptor on the other cell as to one on the same cell.

We thus see that, at equilibrium, crosslinks and intercellular bonds are quite competitive, assuming of course that the cells are in contact ($\delta = 1$). However, crosslinks will begin to form as soon as ligand binds to the receptors; intercellular bonds may or may not depending on experimental conditions and whether the cells are in contact. If crosslinks have an opportunity to form *before* the cells come into contact, then the rate of intercellular bond formation may be greatly reduced. Suppose, for example, that the crosslinks have reached equilibrium before the cells come into contact. Then when contact is made, N_{ci} will be given by Eq. [14]. If $A_t N_t \gg 1$, $N_{ci} \simeq N_t/2$ $(1 - (A_t N_t)^{-1/2})$ and most of the receptors will be crosslinked. Under these conditions, crosslinking will reduce the initial rate of intercellular bond formation by the factor $(A_t N_t)^{-1}$. This result follows from Eq. [10] by comparing the first term with and without initial crosslinks (and with $N_b = 0$). Finally, there is the problem noted earlier that crosslinks may induce receptor redistribution or anchorage modulation; either effect could inhibit or abolish intercellular bonding.

COMPARISON WITH EXPERIMENTS

Although countless experiments have involved the agglutination of cells or the sticking of cells to surfaces by multivalent ligands, few are suitable for theoretical interpretation. In order to apply the theory to a particular system, we should know: (a) the number of receptors per unit area on each cell and their mobilities, which are required in order to estimate reaction rates for membrane bound reactants (*8*, *15*); (b) forward and reverse rate constants for the ligand-receptor interaction, or at least the equilibrium constant; and (c) the number of binding sites per ligand and receptor molecule.

For most natural systems of aggregating cells, this information is lacking or qualitative at best. In many immunological systems the ligands (antibodies) are heterogeneous in their binding constants and even in the number of binding sites per molecule and the receptors (cell surface antigens) are

poorly characterized. However, it appears likely that some immunological systems should be appropriate for analysis. For example, a theoretical analysis has recently been made of the agglutination of red cells by antibodies (*17*), assuming, however that the red cell antigens are immobile. (We shall return to the question of receptor mobility in the concluding section.) Moreover it is now possible for immunologists to develop systems that are particularly suitable for comparison with theory. For example, various techniques can be used to obtain a population of cells that make homogeneous antibodies against definite antigens that can in turn be conjugated to cell surfaces. Binding constants and mobilities can also be measured. Other systems that may be appropriate at least for qualitative analysis involve cell agglutination by lectins. However, in both lectin and antibody induced agglutination one must worry about patching and capping of crosslinked receptors. Finally, in many experiments the conditions are unclear under which cells encounter each other; for example the frequency and duration of cell–cell collisions are really not known.

What orders of magnitude are to be expected for the parameters in the model? These are probably best known in immunological systems in which the ligand is either antigen and the receptors are antibodies on, say, B lymphocytes, or the ligand is antibody, and the receptor is a cell surface antigen. Rate constants have been measured (*18*) for reactions between many different antibodies and antigens in solution. A typical value for an equilibrium constant is 10^7 L/mol, though variations by two orders of magnitude in either direction are not unusual. The estimation of rate constants for membrane bound reactants was described earlier (*8*) and it was seen that the equilibrium constant is the solution value, divided by a distance, R, within which the receptor binding site can be localized, relative to the membrane, i.e., $K^m \simeq K^s/R$. For example, if $R = 2nm$ and $K^s = 10^7$ L/mol, $K^m \simeq 8\ \mu m^2$/molecule. If the receptors are as abundant as immunoglobulin molecules on a B lymphocyte ($\sim 10^5$ molecules on a cell of radius $4\ \mu m$), $N_t \simeq 500/\mu m^2$ so that $K^m N_t = 4 \times 10^3$. As noted earlier, this large value means that we can find substantial fractions of the receptors crosslinked or bridged at relatively low ligand concentrations.

The forward rate constant for crosslinking or intercellular bonding can be written as (*14*):

$$K_+^m = 2\pi\epsilon(D_1^m + D_2^m) \qquad [19]$$

where D_1^m and D_2^m are diffusion constants for the two reactants in the membrane (of the same cell for crosslinking and of the two cells for bridging) and ϵ is a factor (< 1) by which k_+^m falls short of this simple diffusion limit. For various glycoproteins, $D \sim 10^{-10}$ cm^2/s (*19*) and if we take $\epsilon \sim 0.1$ (*8*), $k_+^m \simeq 10^{-10}$ cm^2/s $= 10^{-2}\ \mu m^2$/s.

From Eqs. [9] and [10] we see that an upper limit to the initial rate of crosslinking can be obtained by setting $K_l^s L = 1$, $H = 0$, $N_{cl} = 0$, $N_b = 0$.

Thus

$$\frac{dN_{ct}}{dt} \leq k_{+t}^{m} N_t^2/4 \qquad [20]$$

$$\frac{dN_b}{dt} \leq k_{+}^{m} N_1 N_2/2 \qquad [21]$$

Using the above values for k ($10^{-2}\,\mu m^2/s$), and $N_t = 500/\mu m^2$, we see that $dN_{ct}/dt \leq 600/\mu m^2$ s and $dN_b/dt \simeq 1200/\mu m^2$ s. If, however, crosslinks can form before bridging, then the rate of intercellular bond formation could be reduced by $A_t N_t = K_t^m N_t/4 \simeq 10^3$, to a value $\leqslant 1$ bond/μm^2-s. This could well be too small a value to suffice for establishing bonding on transient cell encounters.

The above numerical values are only a particular example. Variations in any of the model parameters by one or two orders of magnitude in either direction would not be unexpected for some other example.

In a series of experiments, Rutishauser and Sachs (*20, 21*), investigated cell-to-cell binding induced by various lectins, especially concanavalin A (Con A). In particular, they studed the lectin induced binding between a first population of cells that was stuck to a fiber and a second population of cells in solution. These studies showed the importance of receptor mobility in that binding could not be induced between gluteraldehyde fixed cells. Studies of cell agglutination in solution (*22, 23*) in which the temperature was reduced in order to reduce receptor mobility confirmed this result for Con A but not for two other lectins and another study (*24*), in which especially gentle conditions were used in a red cell agglutination assay, showed little effect of temperature. We shall return to this apparent discrepancy in the discussion.

Rutishauser and Sachs also showed that inhibition of crosslinking facilitated cell-to-cell binding, in agreement with the model. This conclusion was reached by comparing two experiments. In the first, the binding of untreated cells to lectin-coated, fixed cells was measured; in this case crosslinking was presumably minimal because the lectin was on the cell with immobile receptors. In the second experiment, binding between lectin coated cells and fixed cells was determined, conditions that permitted crosslinking of receptors by lectin. Binding was much better in the first case, even superior to that between two unfixed populations.

Lectin molecules bind to specific oligosaccharide molecules and lectin induced cell to cell binding is effectively inhibited by high concentrations [$\sim 0.01M$ (*21*)] of the appropriate free sugar molecules. Bound cells were dissociated by addition of free sugars (*20*) for two bivalent lectins but not for the tetravalent lectin, Con A. In neither case does the inhibitor interact with the cell-surface receptor; rather it binds to the ligand. However, it is shown in the Appendix that for bivalent ligands the effect of an inhibitor is

rather similar whether it acts on the receptor or the ligand. In particular, for large inhibitor concentrations the equilibrium number of intercellular bridges will be proportional to $(KS)^{-2}$ where S is the inhibitor concentration and K the inhibitor-ligand equilibrium constant. If for example $K = 10^6\ M^{-1}$ and $S = 10^{-2}\ M$ then $(KS)^{-2} = 10^{-8}$ and a large reduction in or elimination of intercellular bonding at equilibrium would be expected. The extent of this reduction depends on the ligand concentration and equilibrium constant.

In many experiments on lectin induced cell-to-cell binding, equilibrium conditions are probably not achieved. For one thing the forward rate constants for ligand interaction with receptors or inhibitors are fairly slow, $k_{l+}^{s} \sim 10^4 - 10^5 M^{-1}\text{-s}^{-1}$ (*24, 25*). Moreover, in the experiments of Rutishauer and Sachs, once lectin had been bound to one of the two cell populations free lectins were removed from the medium. Thereafter lectin molecules must have dissociated from the cell surface, but they probably never reached equilibrium with the resulting low solution concentrations.

DISCUSSION

In this paper a model has been developed for cell-to-cell binding mediated by multivalent ligands that can bind to mobile receptors on the cells. It has been emphasized that crosslinking of receptors on the same cell by these multivalent ligands is a reaction that competes with intercellular bridge formation, and that this competition may be severe both because it may have a chance to occur prior to intercellular contact and also because it may lead to altered receptor distributions on the cells. In addition, multivalent ligands must be able to mediate the sticking of one part of a cell to another portion of the same cell, for example, the tip of a microvillus to the cell body. Experimental evidence of such cell-to-self sticking might be sought in electromicrographs, but may be difficult to disentangle from artifacts of the micrograph preparation.

Certain experiments on the degranulation of mast cells by multivalent ligands (*26*) can be interpreted as evidence for cell-to-self sticking. It is believed that degranulation is caused by a clustering of IgE receptors on the cell surface (*16*) that is normally induced by multivalent antigen to which the IgE molecules are reactive. This multivalent antigen crosslinks the IgE and receptors causing an influx of calcium ions and degranulation. It is found however, that when the cell is exposed to IgE directed against some of the mast cells own surface molecules, namely its H-2 antigens, degranulation also results. This could be caused by IgE molecules which are bound to receptors on one portion of the cell surface, contacting and sticking to the H-2 molecules on another portion. This explanation requires, of course, further experimental testing.

We have already noted the paucity of experiments which are appropriate

for testing the model, partly because of the complications of receptor crosslinking. Conditions which minimize crosslinking may ease the interpretation of experiments. One possibility, noted earlier is to immobilize the receptors on one of the cells, expose it to ligand and study the binding of such liganded cells to untreated cells. Essentially one thereby studies the binding of ligand bearing particles to cells.

Another possiblity is to use unsymmetrical ligand molecules or complexes, one portion of which binds to one kind of cell and another portion to a second cell. Hybrid antibody molecules are one possiblity and cytophylic antibodies are another. The latter are molecules that bind non-specifically, via their *Fc* portions, to certain cells (e.g., macrophages or lymphocytes) and specifically, via *Fab* portions, to antigens that may be on cell surfaces. However, unless special precautions were taken, since normal antibody molecules have two or more antigen binding sites per molecule, such ligands could crosslink receptors on the antigen bearing cell.

In the present model it has been implied that cell surface receptors must be mobile in order for cell-to-cell binding to be established. However if the ligand and/or receptor molecules are fairly flexible this is not a necessary condition. For consider two cells in contact. A flexibile ligand molecule bound to the first cell can explore a small area on the second cell and by chance locate and bind to a free receptor on the second cell. The probability of multiple bond formation would then depend on the number of receptors per unit area on each cell. A theory of red cell agglutination has been worked out on this basis by Chak and Hart (*17*) and shown some agreement with experiment.

Suppose that each ligand molecule that is bound to the first cell can explore an area δA on the second. If N_2 is the mean number of receptors per unit area on the second cell, the probability of here being at least one receptor (whether bound or free) within δA is $1 - e^{-N_2 \delta A}$. The number of bridges, N_b, per unit area of intercellular contact cannot exceed N_1 times this probability

$$N_b < N_1(1 - e^{-N_2 \delta A}) < N_1 N_2 \delta A \qquad [22]$$

Thus a significant fraction of the receptors (on cell 1) can be bridged if $N_2\, \delta A \gtrsim 1$ and the ligand concentrations and equilibrium constants for receptor ligand binding are sufficiently large. For example, if the ligand is a flexible chain that can span $R = 10$ nm and $\delta A = \pi R^2 = 300\ \text{nm}^2$, $N_2\, \delta A = 1$ when $N_2 = 3.10^3\ \mu\text{m}^{-2}$.

We thus see that *if* the ligand is sufficiently long and flexible when bound to a receptor, it is possible to obtain intercellular bonds even between fixed receptors. From the experimental results mentioned earlier it seems that in some instances receptor mobility is important, while in others it is not. Theory could be used to clarify the conditions under which receptor mobility is critical, but this will not be attempted in the present paper.

It should be noted that effective cell-to-cell binding for cells with mobile receptors does not require a large number of receptors per unit area; a

reduced receptor density can be compensated by an increased ligand–receptor binding constant. Moreover mobile receptors can come from all over the cell surface to accumulate in a contact area (*14*).

In this paper, we have considered bivalent ligands interacting with monovalent receptors. Generalization to multivalent receptors and ligands could be made using techniques developed in this paper and by other authors (*15*). If the receptor–ligand complexes can be treated as linear chains of molecules, the considerations are relatively straightforward. If cyclic complexes can also form, they may be important, but the theory is more difficult because molecular structure and steric effects are involved. Nevertheless, experimental and theoretical studies should be able to clarify these more complicated cases.

APPENDIX

Inhibition by Soluble Inhibitors that Bind to the Ligand is, at Equilibrium, Equivalent to Inhibition by Binding to the Receptor

Consider a bivalent ligand at concentration L which can bind to a soluble inhibitor at concentration S. A ligand site that is bound to S cannot interact with a receptor on a cell surface. For example, L might be a lectin and S a complementary sugar or oligosaccharide, or L might be an antibody and S a complementary soluble antigen. Let k_+ and k_- be the forward and reverse rate constants for binding of S to L. Then, in solution, a ligand molecule can exist in three possible states, free, bound to one or two molecules of S. Let the corresponding concentrations be L_0, L_1, and L_2. The kinetic equations for reactions between ligand and inhibitor molecules are

$$\frac{dL_0}{dt} = -2k_+L_0S + k_-L_1 \qquad [A.1]$$

$$\frac{dL_1}{dt} = 2k_+L_0S - k_-L_1 + 2k_-L_2 - k_+L_1S \qquad [A.2]$$

$$\frac{dL_2}{dt} = k_+L_1S - 2k_-L_2 \qquad [A.3]$$

At equilibrium it is easy to see that

$$L_0 = \frac{L}{(1 + KS)^2} \qquad [A.4]$$

where $K = k_+/k_-$.

It is now straightforward to write kinetic equations for receptor ligand complexes. The states are as before shown in Fig. 1, except that ligand-bound receptors can either be unbound to inhibitor (N_{l0}) or bound to an inhibitor molecule (N_{l1}). Evidently only the former can react with free receptors to form crosslinks or bridges to another cell. By considering the reaction

$$N_{l0} + S \rightleftharpoons N_{l1}$$

it is seen that at equilibrium

$$N_{l1} = KSN_{l0} \qquad [A.5]$$

In addition, as before (see Eq. [7])

$$N_{l0} = K_l^s N_f L_0 \qquad [A.6]$$

where we have suppressed the subscript i for simplicity of notation. Since the total number of receptors, N, per unit area is

$$N = N_f + N_{l0} + N_{l1} + 2N_c + N_b \qquad [A.7]$$

we have from equations [A.4]–[A.7]

$$N_f = \frac{N - 2N_c - N_b}{1 + K_l^s L/(1 + KS)} \qquad [A.8]$$

instead of Eq. [8].

Since the formation of crosslinks and bridges involves reactions between N_f and N_{l0} on one or different cells, we will obtain the same kinetic and equilibrium conditions as before except that quantities such as A in Eq. [17] will be given by

$$A = \frac{K^m K_l^s L_0}{(1 + K_l^s L/(1 + KS)^2} = \frac{K^m K_l^s L}{(1 + KS + K_l^s L)^2} \qquad [A.9]$$

Thus KS has simply replaced $K_h^s H$. We thus conclude that when this replacement is made, the inhibition by inhibitor binding to receptor becomes equivalent to inhibitor binding to ligand.

In this analysis, we have assumed that the ligand and inhibitor come to equilibrium in binding to each other and the receptor. If the time scales for ligand and inhibitor binding are comparable to those for crosslinking and bridging, then an analysis of the receptor kinetics would be more complicated.

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