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SOME MODELS FOR THE INTERACTION BETWEEN CELLS OF THE IMMUNE SYSTEM

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Introduction

It is by now abundantly clear that interactions between cells are of great importance in regulating immune responses. For example, it has been demonstrated by adoptive transfer experiments in vivo (Greaves, et. al.. 1974; Roit, 1974), that antibody production by B lymphocytes in response to an antigen, is generally regulated by T lymphocytes having specificity for the antigen. Similar regulation has been found in vitro (e.g. Feldmann, 1978) for all save a limited class of so-called thymus independent antigens. In addition, activation of any T-lymphocyte function in vitro seems to be regulated by and require the presence of macrophages or other adherent cells in the culture. (Immunological Reviews, 1978). Such examples could be enumerated indefinitely.

In formulating mathematical models of cell-cell interactions in the immune system, a key question is whether the interactions are entirely mediated by soluble factors which are secreted by one cell and are received by another cell at a distance, or whether they also involve cell to cell contact perhaps mediated by specific ligands such as antigen-antibody complexes. If the interaction is entirely by diffusable factors, then it is natural to consider models in which the dependent variables are the concentrations of cells in the various interacting populations and the concentrations of the diffusable factors. Some of the cells may secrete factors, others may bind the factors and have their own activity, both proliferation and secretion, modified by such binding. I believe it is fairly clear, in principle, how to formulate such models and examples will be presented at this conference (Bruni, et. al., 1979; Mohler and Hsu, 1979). In practice the models might be quite complicated, particularly if the number of interacting populations be large and/or the factors are multivalent and interact with each other and the immunizing antigen.

However, in this talk I wish to focus on interactions which require cell to cell contact and to describe some theoretical approaches that I have developed for treating contact interactions. It is clear that immunologists can devise experimental conditions in which contact interactions are obligatory as in haemagglutination (Solomon, et. al., 1965) or rosette assays (Mandache, et. al., 1978), and the study of these artificial systems may be useful in validating any theory for

First, it should be noted that cells of the immune system, especially the lymphocytes, are mobile, rather than fixed in tissue. Thus if two cell types of the immune system need to exchange signals, they do not have to rely on diffusable, hormone like molecules for relaying this information. For there is a possiblility that they may find each other by virtue of the relative cell to cell motion. I have discussed elsewhere (Bell, 1978a) the nature of the cell traffic during lymphocyte recirculation a process in which most lymphocytes engage. In this process, lymphocytes in the blood are continuously attaching to endothelial cells which line the venules in lymph nodes and then crawling between the endothelial cells to enter the lymph node where they mingle with other lymphocytes, macrophages, reticular and other cells for hours before leaving the node in the efferent flow of lymph. From experiments on sheep lymph nodes in vivo, about $3x10^7$ lymphocytes traverse an unstimulated one gram node per hour. Eventually, the lymph and lymphocytes will reenter the bloodstream to begin their recirculation anew. There are thus many opportunities for migrating lymphocytes to come into contact with other cells, including macrophages and lymphocytes. In particular, I have estimated that in a sheep node, a migrating lymphocyte will encounter ~200 other cells/hr and that in an unimmunized animal there may be ~400 encounters per hour between antigen specific T and B cells for each gram of lymphoid tissue. Although these numbers are rather uncertain they illustrate that most lymphocytes, which are to be found in lymphoid tissue, are more or less mobile and able to make contact with large numbers of diverse other cells.

Additional evidence for the importantce of cell-cell contact in regulating response has been obtained in vitro. Thus, Pierce and Benacerraf, 1969 and Mosier, 1969 reported that clusters of cells were required in order to activate B cells in the standard Mischell-Dutton assay. Moreover it is known that the activation of T cells requires the presence of macrophages or adherent cells and various investigators, e.g. (Nielsen, et. al., 1974) have observed, with electron microscopy, antigen-specific T lymphocytes tightly bound to macrophages.

Thus indirect arguments concerning the cell interactions in lymphoid tissue together with observations in vitro have indicated that cell-cell contacts are important in regulating immune responses. It is thus logical to try to understand the conditions under which contact between cells having receptors of complementary specificity, or cells of similar specificity in the presence of complementary ligand will lend to a binding between the cells or to an interaction which is likely to be important for the cells. The main body of this talk suggests some theoretical approaches to this understanding.

Cell to Cell Binding

Let us consider binding of a lymphocyte to another cell which is mediated

Examples of interaction in vivo by complementary receptors may be the sticking of lymphocytes to endothelial cells in lymph nodes (Bell, 1978a, deSousa, 1976) or possibly to epithelial cells in the thymus as part of thymocyte education (Zinkernagle, et. al., 1978). In lymphoid tissue, it would appear that lymphocytes might bind to each other, either by antigen to which they both have receptors or by idiotype - anti-idiotype interactions. In addition, since several classes of antibodies are cytophilic for lymphocytes or macrophages, antigen-antibody complexes could cause the adherence of lymphocytes to each other or to macrophages.

Various questions may be raised concerning adhesion between cells mediated by interactions of specific molecules such as antigen and antibody. In this talk, I propose to address three of them. First, how many bonds are required in order to cause a firm adhesion between two cells? Second, how rapidly will the bonds form, once the cells are in contact? Third, how will adhesion between cells be expected to modify cell behavior?

The Strength of Specific Bonds

I have discussed elsewhere (Bell, 1978b) the force which is required in order to break a typical antigen-antibody bond. Of course such bonds break spontaneously. Each has a lifetime which is perhaps a second, give or take a couple of factors of ten. However if two cells are stuck together by many bonds, these bonds are most unlikely to all break at once and a bond that breaks may reform. Hence a force is required in order to separate the cells. I have found it useful to view the force as accelerating the rate constant for bond breaking. When this is done the following conclusions may be reached.

First, the force f_o, which is required in order to rapidly break any bond is of the order of the free energy change, E_o, in bond formation divided by the range, r_o, of the bond. With energy in kcal/mole and r_o in Å,

$$f_o = 7\times10^{-6} E_o / r_o \text{ dynes/bond.} \qquad (1)$$

For a typical antigen-antibody bond having an equilibrium constant $10^6 M^{-1}$, $E_o \simeq 8.5$. I have estimated that $r_o \simeq 5$ and thus $f_o = 1.2\times10^{-5}$ dynes/bond. Such a force will rapidly break the bond and I have estimated that a smaller critical force, f_c, around one-third this value per bond will suffice to separate cells. Hence a representative value for f_c is 4×10^{-6} dynes/bond.

This critical force has been compared (Bell, 1978b) with non-sepcific electrical forces between cells, which are estimated to be $\sim 10^{-5}$ dynes/μm^2, and thus unimportant compared to ~ 10 specific bonds per μm^2, with the force to extract a receptor molecule from a cell membrane, which is $\gtrsim f_c$, and with other forces in biology. In particular it was concluded that a lymphocyte could be held still in

cm/sec, could be achieved by about four of these typical bonds. Of course we don't know what the receptor molecules are in this case but the essential conclusion is that the adhesion could be mediated by relatively few bonds, say $\lesssim 10$.

In lymphoid tissue, relative cell velocities are small, $\sim 10^{-5}$ cm/sec, and their causes uncertain. However it would appear that once two cells had become stuck together by, say $\gtrsim 10$ bonds they would have difficulty separating again.

Rate of Bond Formation

Consider two cells which are in contact. I have considered elsewhere the rate of bond formation when the cells have complementary receptors (Bell, 1978b) or similar receptors for a soluble bivalent ligand such as an antigen (Bell, 1979a). In both cases, I assumed the receptors are free to translate in the plane of the cell membrane and to rotate about an axis perpendicular to the membrane, motions which are consistent with the fluid mosaic model of the membrane. Hence a receptor in the contact area can wander about on the membrane until it eventually finds a reactive partner on the opposite cell. For typical diffusion coefficients for integral membrane proteins ($D \sim 10^{-10}$ m^2/sec) and typical receptor numbers per cell ($\sim 10^4$-10^5), "eventually" is not all that long. A typical value is $\lesssim 1$ sec. An important reason for rapidity of such encounters is that the local concentration of receptors adjacent to a cell membrane, is likely to be very large. If, for example, the receptors are antibody molecules on a B cell, there are $\sim 10^5$ on a cell of radius $\sim 4\mu m$ and area $\sim 200\mu m^2$. Hence the number of receptors per unit area is $\sim 500/\mu m^2 = 5\times 10^{10}/cm^2$. If their binding sites are all confined to a 20 Å band adjacent to the membrane, the local concentration of molecules is $\sim 5\times 10^{10}/2\times 10^{-7} cm^{-3} = 2.5\times 10^{17} = 0.4\times 10^{-3} M$. This is a remarkably large concentration of antibody molecules.

The reaction rate for membrane bound reactants on two cells can be estimated (Bell, 1978b, 1979a) provided that certain assumptions are satisfied. First of all, the reactants must be accessible to each other, or else the reaction rate is clearly zero. Second, their motion in the membrane must be described by diffusion processes so that we can calculate the rate at which reactive partners encounter each other. Finally, the intrinsic reaction rates for reactants tethered in the membranes must be similar to those for reactants in solution, so that we may use experimental values for the latter. A method for making these estimates is described in Bell, 1978b.

There are some additional complications when the intercellular bonds are formed by soluble multivalent ligands such as antigens or antibodies. Such ligands can form not only intercellular bonds but they can also crosslink receptors on each cell, thereby tying up both receptors and ligand binding sites so that they

have concluded that at equilibrium, crosslinking and intercellular bond formation are equal competitors. However, insofar as crosslinks may begin to form before the cells come into contact with each other, crosslinking may greatly diminish the rate of intercellular bond formation. Even worse, crosslinking by multivalent ligands may lead to receptor redistribution in caps (Taylor, et. al., 1971) followed by loss of receptors from the cell surface.

For ligands having more than one kind of binding site for cellular receptors, the situation is somewhat more complex. Consider for example an antigen-antibody complex in which antigen epitopes can bind to one kind of cell and antibody Fc regions to the other. Although, at equilibrium these complexes could mediate a very tight binding between the cells, it is easy to imagine that at sufficiently high concentrations, the complexes might coat each kind of cell before they could come into contact. Moreover, since the complexes can bind multivalently to each kind of cell, it is likely that the complexes will bind to most of the cell receptors at concentrations which are far smaller than the reciprocals of the single site equilibrium constants (Bell, 1979a). Immunologists would say that this is because of the avidity of multivalent binding much exceeds the affinity of single site interaction. The theory of cell-cell binding by antigen-antibody complexes has not been worked out in any detail.

Effects of Binding on Cell Behavior

With the methods discussed in the preceding sections, we may calculate that when two cells come into contact and have mobile complementary receptors or mobile receptors for the same ligand, which is also present, then multiple bonds can rapidly form between the cells and cause a tight cell-cell adhesion. More details concerning the rates can be found in Bell, 1978b and 1979a. Major caveats are that excess ligand can greatly slow down the rate of bond formation and that multivalent ligands may modulate receptor expression prior to cell-cell contact.

These considerations would seem to suggest that various cells of the immune system would become more or less permanently stuck to each other, for example by antigen antibody complexes. However, while it does appear that antigen-binding lymphocytes are specifically retained in a lymph node for a couple of days following antigen presentation, they then emerge in efferent lymph in large numbers. It appears to me that this is because two cells which have become stuck together will find this contact an exciting event, which they will mobilize resources to exploit or to terminate. What is the evidence for this excitement and what theoretical appraoches can be taken to the problem?

As noted earlier, it is well known that many cells of the immune system find even the crosslinking of their own receptors by multivalent ligand to be exciting.

1978). When lymphocyte receptors are crosslinked by various ligands, a gross redistribution of the receptors into "caps" often ensues (Taylor, et. al., 1971). It seems that crosslinked receptors can couple to the cell's cytoskeleton to effect this redistribution (Bourguignon and Singer, 1977). Moreover it has been observed (Singer, 1979) that in regions of cell-cell contact there are accumulations of cytoskeletal elements. I believe that such rearrangements of the cytoskeleton indicate dramatic effects on cell behavior. This view is reinforced by, for example, the finding that sufficient binding of a lymphocyte to a surface (or another cell?) will immobilize receptors on the rest of the cell (Edelman, 1976) or by the observation of a macrophage eating a cap off a B lymphocyte (Griffin, et. al., 1976) an act which may terminate cell-cell contact.

Theoretical estimates can be made for one of the events following cell-cell binding, namely receptor redistribution. Suppose that two cells are stuck together over some surface area and that the receptors on the remainder of the cell remain mobile. Then receptors will tend to accumulate in the contact area because in this area they can encounter complementary or ligand bound receptors on the other cell and become stuck. We can estimate whether or not this redistribution is likely to be important. The situation is simplest when the cells have complementary receptors.

Consider two cells which have N_1 and N_2 receptors per unit area and an equilibrium constant, K, for their binding to each other. If N_1 and N_2 are measured per unit area, e.g. μm^{-2}, then K must be measured in μm^2 as described in Bell, 1978b. Suppose that the cells are in contact over a local area and that free receptors can diffuse into or out of the contact area, as indicated in Fig. 1. If the diffusion

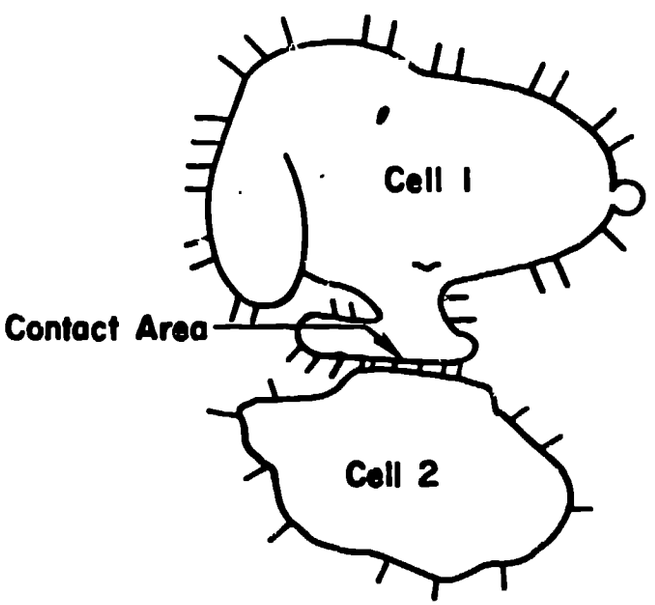

Fig. 1. Two cells in contact over an area are stuck together by intercellular bonds. Additional receptors may diffuse into the contact area and accumulate by binding complementary receptors on the other cell. This figure is meant to suggest

coefficients for the free receptors are independent of position on each cell, and in particular the same in contact and non-contact areas, that at equilibrium the number of free receptors will be the same in the contact area and outside it. If, in addition, the area of the contact area is realtively small so that only a small fraction of the receptors can accumulate therein, then the number of free receptors per unit area will be close to N_1 and N_2 respectively. It follows that under these conditions, the number of bound receptors, N_b will be

$$N_b = KN_1N_2 \qquad (2)$$

Thus substnatial receptor redistribution is to be expected on the first cell if $KN_2 >> 1$, for then $N_b >> N_1$, and on the second cell if $KN_1 >> 1$. Note that the redistribution need not be symmetrical, for if $KN_2 >> 1 >> KN_1$, receptors would accumulate in the contact area on the first cell but not on the second.

If we are considering antibody molecules on B cells as receptors on cell 1, having an affinity $\sim 10^6$-$10^7 M^{-1}$ for cell bound antigen, then $KN_1 \sim 10^3$-10^4 so that a gross redistribution of antigen on cell 2 and perhaps receptors on cell 1 is to be expected. In making this estimate for KN_2, we can use the local concentration of membrane receptors, estimated in the preceding section ($N_1 \sim 0.4 \times 10^{-3} M$) together with the solution equilibrium constants.

Other topics that can be addressed theoretically include the depletion of receptors from the remainder of the cell surface and the rate of receptor redistribution (Bell, 1979b). The former topic is readily treated by conserving receptors on each type of cell so that N_1 and N_2 in equation (2) are reduced to account for the bound receptors in the contact area. The rate of receptor redistribution will depend on the size of the contact area, diffusion coefficients for receptors, and reaction rate between receptors. For mobile immunoglobulin molecules as receptors and typical antigen-antibody reaction rates, redistribution times in the range 1 sec - 1 min have been estimated (Bell, 1979b).

From the foregoing theoretical discussion I conclude that receptor accumulations are to be expected in areas of cell-cell contact. Gross alterations in membrane properties in these "contact caps" may result. I suggest that receptor accumulation furnishes a natural link in the chain of events leading from cell-cell contact to cell excitation. Experimentally, it appears that subsequent steps are likely to involve the cell cytoskeleton and its coupling to the membrane (Bourguignon and Singer, 1977, Singer 1979).

Discussion

In this talk I have tried to indicate some of the theoretical approaches which can be taken to cell-cell interactions which are mediated by contact and the formation of specific intercellular bonds. The general conclusion is that cells

Moreover receptor accumulation on local contact areas is likely to grossly modify the cell membranes in such "contact caps" and thus to generate a variety of poorly understood effects on cell behavior.

The question remains whether such cell-cell contacts are important in the regulation of immune responses. At the very least, this possibility should be kept in mind while interpreting experiments both in vitro and in vivo. I believe that since such interactions are inevitable in lymphoid tissue, they will also be important.

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