Interaction of Viruses with Cell Surface Receptors

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I. Definition of Viral Receptor Sites

The definition of a receptor ultimately depends upon the structural and functional identification of a site that is specifically recognized by a ligand. The most rigorous characterizations of receptor–ligand interactions have been derived

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from binding studies of radiolabeled ligands in neuropharmacology and endocrinology. It has been more difficult to study the interactions of viral particles with the cell surface in this rigorous a fashion. Viral particles are several orders of magnitude larger than conventional ligands, making it impossible to perform many of the manipulations that are routinely done with hormones, for example. In addition, viruses contain repeating subunits and many copies of the viral attachment protein are present on the virion surface. Thus a single virion may interact with many receptor sites on the cell surface. Nonetheless, most of the principles of receptor–ligand interactions established for other ligands apply to viruses as well and this establishes a framework for the definition of viral receptors.

At the simplest level, a viral receptor is the structure on the membrane surface of a cell to which virus binds prior to entering the cell. Often the presence or absence of a viral receptor determines whether or not the cell can be infected by virus. It is also possible that there are structures on the cell surface to which a virus binds but which do not serve as conduits for viral entry into the cell. Viral binding to such sites could have other important biologic consequences such as affecting cell function by triggering surface structures which affect cellular metabolism or being improtant for the generation of an immune response against the virus. In the broadest sense, then, a viral receptor is a structure on the cell surface to which a virus binds, the binding of which is of biological importance and can be measured in a biologically relevant way.

The definition of viral recognition sites as "receptors" involves three major criteria which are derived from models of ligand–receptor interactions (Bennet, 1978). These include saturability, specificity, and competition.

Saturability. If virus interacts with the cell surface at discrete sites along the membrane, only a finite number of sites will be available for viral binding and high concentrations of virus should be able to fully occupy or "saturate" them. It should therefore be possible to increase the concentration of particles presented to the cell surface until no further binding results. This can be shown experimentally by determining viral binding as a function of increasing viral concentration. Saturation is demonstrated if a plot of the result is a hyperbolic curve (see Fig. 1A).

Specificity. Specificity is first demonstrated according to "biologic parameters." In other words, viral binding is observed only to cells that the virus infects or to cells where viral binding induces some other biologically measurable response. The second measure of specificity relates to the binding assay itself. Even when virus binds to a biologically relevant cell, a certain proportion of viral binding is nonspecific and unrelated to specific viral receptors. For example, electrostatic forces result in some of the nonspecific adherence of particles to the cell surface. Nonspecific binding is contained in any binding curve but the nonspecific component is usually not saturable and therefore it is a
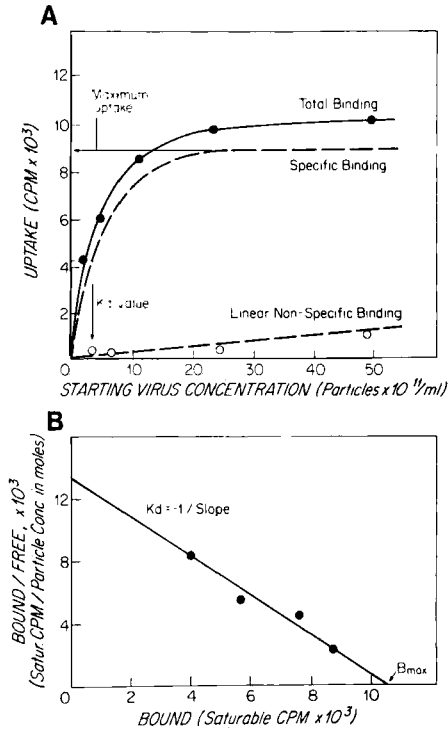
FIG. 1. (A) Binding of ^{125}I-labeled reovirus type 1 to L-cell fibroblasts. Each point represents the arithmetic mean of three separate determinations of uptake to 250,000 cells/sample equilibrated at 25°C for 60 minutes. The linear nonspecific binding was determined by mixing each concentration of labeled virus with an equal volume of high titer unlabeled virus containing 2.0×10^{13} particles/ml. The saturable binding curve results from subtraction of the linear component from the total binding curve. The maximum uptake is the uptake value at saturation, and K_d is the virus concentration at the half saturation point. (B) Scatchard plot of ^{125}I-labeled reovirus bound to L cells. After subtracting the linear component, bound/free was calculated by dividing the corrected cpm by the viral concentration expressed in particles divided by Avogadro's number (to result in a molar expression). Linear regression analysis of the data provides the slope to estimate K_d.

linear function which can be measured, and subsequently subtracted from total binding to reveal "specific" binding (Fig. 1A).

Competition. It should be possible to competitively inhibit specific binding using a second ligand which is known to bind to the same receptor. In neuropharmacology, for example, specific binding of acetylcholine to the muscarinic acetylcholine receptor is defined as that component of total binding which can be blocked by atropine. In viral systems, specific blockers of this type are usually unavailable and the only certain method for competition is to block the binding of

radiolabeled virus with unlabeled virus or, if possible, the viral attachment protein.

A variety of nomenclatures have been used to describe viral–receptor interactions, and depending on the context of the discussion several abbreviations will be used in this article. The protein by which the virus attaches to cells is the viral attachment protein (VAP). The "receptor" on the surface of the cell to which the virus binds is the cellular receptor site (CRS). Finally, it has been postulated that the CRS may be composed of multiple units and these units, which may bind a single VAP, are termed cellular receptor units (CRU) (Lonberg-Holm, 1981).

II. Biological Characteristics of Viral Attachment to Cells

A. TECHNIQUES USED TO STUDY VIRAL ATTACHMENT

A variety of approaches have been used to study the interaction of viral particles with cell surface receptors or reception sites. As described below, each approach has advantages and limitations. A rigorous study of viral–receptor interactions requires the use of more than one technique since different approaches provide complementary information about viral binding.

1. Infectivity Assays

These assays measure the number of infectious viral particles that either remain in the medium following viral binding to the cells or that have attached to cells. Attached virus may be detected by measuring infected cells or "infectious centers," or by dissociation of receptor bound infectious virus, providing that receptor-mediated or cell-mediated modification ("eclipse") of the virus particles can be prevented or accounted for. The advantage of infectivity assays is that binding of very small amounts of virus can be detected and viral replication, a biologic function, is being measured. Infectivity assays have the following limitations: (1) Only binding of *infectious* viral particles can be measured. Most viral preparations have particle to plaque-forming unit (PFU) ratios of 10 to 100 and it is likely that noninfectious virus also binds to cellular receptor sites. (2) The measurement of infectious particles by plaque assay is time consuming, cumbersome, and statistically error-prone, making the generation of quantitative data difficult. (3) When infectious centers are being measured, cells which bear membrane receptors but which are unable to support viral replication cannot be detected. Attachment to such cells must be detected by measurement of virus removal from the media. (4) Factors which modify penetration and replication can affect results, since the data reflect only the end product of infectivity.

In one series of experiments, investigators surmounted some of these limita-

tions. Infectivity assays were used to study receptor-determined host restriction for ecotropic and xenotropic murine leukemia viruses. In this approach, pseudotype virions were created which contained the envelope of one virus and the infectious genome of another. Pseudotype virions were generated by the mixed infection of VSV (for which the cells being studied were permissive) and various retroviruses (for which the cells were not permissive). Resultant virions that contained VSV envelopes were then inactivated by anti-VSV neutralizing antibodies. The remaining pseudotype virions (retrovirus envelope + VSV genome) bound to the cell surface receptor for the retrovirus being studied and resulted in VSV replication. Thus, VSV replication measured the presence of surface receptors for retroviruses on a variety of host cells, independent of their ability to replicate the virus (Besmer and Baltimore, 1977).

2. Hemagglutination

Hemagglutination assays depend on the ability of many viruses to agglutinate red blood cells. Although red cell receptors responsible for viral hemagglutination might not be the same as viral receptors on host cells, hemagglutination has provided important information about virus–receptor interactions. Of note is that hemagglutination depends upon lattice formation and is very dependent upon conditions (e.g., temperature). Thus, it does not measure "attachment" directly.

Studies using hemagglutination have detected differences in neuraminidase sensitivity of erythrocyte receptors for various enveloped viruses. For example, the erythrocyte receptor for parainfluenza virus type 3 appears to be neuraminidase resistant, while receptors for influenza virus serotypes A and B (Hirst, 1950) are inactivated by neuramininidase. Adenovirus type 9 does not bind to neuraminidase-treated erythrocytes while types 2 and 7 do (Wadell, 1969; Boulanger et al., 1972). For some nonenveloped viruses, hemagglutination is totally resistant to neuraminidase (e.g., reovirus, Gomatos and Tamm, 1962).

A modified version of hemagglutination is the rosetting technique. In this method, cells to be tested are added to aliquots of chronically infected cells. Cells which express viral receptors adhere to the surface of infected cells which express the the viral attachment protein (VAP). In order to be used, the rosetting technique requires that a sufficient number of VAPs be expressed on the surface of infected cells. Hemadsorption of erythrocytes to cultures of virus-infected cells is a variation of the rosetting technique. In this method rosettes are formed only if the erythroctyes have viral "receptors" on their surface.

The technique of viral-induced agglutination has also been used for cells other than erythrocytes, specifically, lymphocytes. Woodruff and Woodruff (1972, 1974) have used the agglutination of T lymphocytes by viruses to define receptors for myxo- and paramyxoviruses on these cells (discussed in Section III,D). These techniques also allow characterization of receptors by use of reagents

which block agglutination (e.g., enzymes or antibodies) (Bankhurst et al., 1979).

3. Use of Fluorochromes

Fluorescein- or rhodamine-labeled antiviral antibodies have been used to visualize the presence of virus bound to the surface of various cell types. For example, indirect immunofluorescence techniques were used to define B cells as the lymphocyte subset susceptible to EBV infection (Jondal and Klein, 1973). The major advantage of measuring virus binding by indirect immunofluorescence is that it affords a qualitative approach for the study of viral–receptor interactions. This is especially relevant for studying primary cell cultures which contain a mixture of cell types. For example, for the study of serotype-specific binding of reovirus to cells in the nervous system, we prepared cell suspensions from mouse brain which contained 75% ciliated ependymal cells; these cells are easily identified under phase microscopy by their cilia. Using indirect immunofluorescence, it was possible to demonstrate that reovirus type 1 (which has an *in vivo* affinity for ependymal cells) but not type 3 binds to these cells (see Fig. 2A and B, discussed in detail later). Similarly, indirect immunofluorescence was used to demonstrate that reovirus type 3 binds to a subset of murine and human lymphocytes (Fig. 2D) (Weiner et al., 1980a). "Co-capping" studies are an extension of the indirect immunofluorescence approach. These experiments take advantage of the property of physiologically active cells to modulate receptor sites bound by ligand to one pole of the cell (Fig. 2C). Using two different colored fluorochromes (one to mark bound virus and the other to label another cell surface structure) it is possible to determine if a cell surface component moves in association with the viral receptor when viral receptors are modulated to one pole of the cell. This approach demonstrated that EBV receptors on cultured cells were closely associated with complement receptors (Yefenof et al., 1976). Co-capping studies also demonstrated that reovirus receptors on murine lymphocytes are distinct from other surface antigens, such as C3 and Fc receptors (Epstein et al., 1981).

Viral particles themselves may be directly fluoresceinated or rhodaminated. McGrath and colleagues have used a direct fluorescence technique to study the binding of MuLV virions to thymic lymphoma cells (McGrath et al., 1978; McGrath and Weissman, 1979). These studies allowed a quantitative as well as a qualitative measurement of binding since the amount of fluorochrome per viral particle could be compared with the total fluorescence per cell, as measured with a fluorescence activated cell sorter (FACS).

4. Enrichment for Receptor-Positive Cells

In an extension of the qualitative approach described above, it is possible to use the virus itself to select from a heterogeneous population those cells which
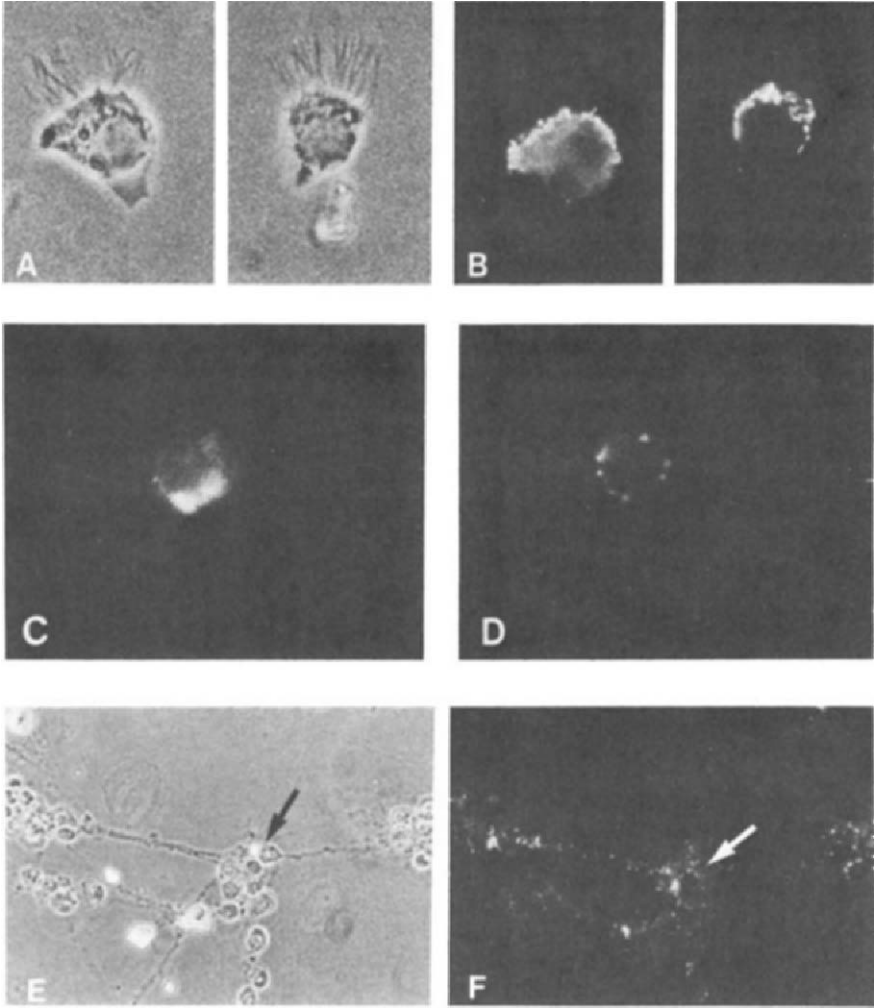
FIG. 2. Binding of reovirus to isolated ependymal cells, lymphocytes, and neurons demonstrated by fluorescence staining. (A and B) Unstained isolated ependymal cells examined by phase microscopy and the same fields seen by fluorescence microscopy showing bright labeling of the cells after incubation with reovirus type 1. Viral binding was demonstrated by indirect immunofluorescence with rabbit antibody to reovirus and with FITC-conjugated goat–anti-rabbit Ig. (C and D) Fluorescence microscopy showing labeling of lymphocytes with reovirus type 3 after capping of the receptors (C). (D) shows the appearance prior to capping. (E and F) Phase contrast photomicrograph of cultured neuronal cells (arrow) and of neuronal cells overlayed with reovirus type 3 and then labeled with FITC-labeled antireovirus antibody. Staining can be seen on the neuronal cell body surface and neuronal processes.

bind the virus. For example, cells that have bound virus and then have been labeled with fluoresceinated antiviral antibody can be sorted on the FACS. Another method uses plate-adherence. We have developed the plate-adherence technique for the study of reovirus–receptor interactions. In this technique, cells are first incubated with virus and then with a rabbit antiviral antibody. These cells are then plated on a Petri dish previously coated with a goat–anti-rabbit immunoglobulin and separated into adherent and nonadherent populations. Using the plate-adherence technique, the percentage of reovirus type 3-positive murine splenic T lymphocytes was enriched from 21% in the initial unseparated population to 88% in the plate-adherent population (Epstein *et al.*, 1982).

5. *Radiolabels*

Radiolabeled virions permit the most quantitative measurement of viral binding, either by measuring loss of radioactivity from media, or more usually, by measuring attachment of labeled virus to cells. Because of impurities in labeled preparations (i.e., labeled particles that do not bind), these two approaches are not identical (Richter, 1976), and the uptake of labeled virus to cells is preferred because it is the more direct approach. Furthermore, measurement of uptake is more accurate. For example, 2% uptake can be more accurately measured than 2% loss from the medium (100 to 98%).

For radiolabeled studies, many investigators have grown virus in the presence of ^{3}H- or ^{14}C-labeled amino acids (Lonberg-Holm, 1964; Lonberg-Holm and Whiteley, 1976; Fries and Helenius, 1979). These preparations had specific activities in the range of 10^{13} viral particles/μCi (Lonberg-Holm, 1981), and have been used to study the time course of viral–receptor interactions, and competition for receptors by different viruses. The physical conditions which affect viral binding, such as ionic strength, pH, temperature, and cell concentration have also been studied (Lonberg-Holm and Whiteley, 1976; Lonberg-Holm and Philipson, 1974, 1980).

Lactoperoxidase-catalyzed iodination labels viral surface components which contain tyrosine residues (Marchalonis *et al.*, 1971). Although it is more likely to result in a preparation containing inactivated virions (K. Lonberg-Holm and B. Korant, personal communication; Epstein *et al.*, unpublished data), iodination of virus yields labeled virus with higher specific activities. With reovirus, it is possible to obtain specific activities of approximatively 10^{12} particles/μCi. In general, the greater the specific activity of a ligand, the more accurate the quantitative measurements which can be made.

Radiolabeled purified viral attachment proteins (i.e., retrovirus spike glycoproteins or adenovirus fibers) have also been used to study binding to cellular receptors (DeLarco and Todaro, 1976; Choppin *et al.*, 1981).

Finally, ^{125}I-labeled protein A has been used for the indirect study of surface

interactions of mouse mammary tumor viruses with mouse and rat cells. Virus bound to the cell surface was detected using monoclonal–antiviral antibodies that then bound labeled protein A (Altrock *et al.*, 1981).

B. MATHEMATICAL ANALYSIS OF VIRAL BINDING

The interaction of viral particles with receptors is dependent upon a number of physical conditions, including receptor affinity and density, viral concentration, temperature, pH, and ionic strength of the bathing medium. It is useful to study binding interactions using standard values for these variables and then varying one parameter at a time. Mathematical models for receptor interactions have been derived from equations for enzyme kinetics which were developed by Michaelis and Menten (Lehninger, 1975). This approach assumes reversible bimolecular binding, as represented below by Eq. (1), a condition which is not necessarily true for viral binding to receptors. One approach which we have found useful for characterizing interactions of reovirus particles as ligands (L) with receptor sites (R) on cells has been to study binding under equilibrium conditions where a simple reversible bimolecular reaction holds:

$$R + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R \cdot L \tag{1}$$

where $R \cdot L$ represents viral particles bound to receptors (receptor–ligand complex), and k_1 and k_{-1} represent the forward or association and backward or dissociation *rate constants* for virus binding to receptors. The *equilibrium dissociation constant* for the reaction, K_d, describes the relative concentrations of these reagents at equilibrium, or the ratio of the *rate constants,* and is represented by the equation:

$$K_d = k_{-1}/k_1 = [R] [L]/[RL], \tag{2}$$

where the square brackets represent concentrations. For most binding interactions the concentration of $[R]$ is unknown, and the cell number rather than the number of sites can be manipulated in experiments. With cell number held constant in an experiment, and viral concentration varied, an uptake curve of virus binding to receptors may be obtained (see Fig. 1A). The saturable hyperbolic binding curve in Fig. 1A can be derived from Eq. (2) above, and from Eq. (3), indicating that the total number of receptors, R_{total}, is the sum of the free (R) plus bound (RL) receptors:

$$R_{total} = R + RL \tag{3}$$

Using Eq. (3) to eliminate $[R]$ in Eq. (2), one may express the number of bound virus receptors $[RL]$ as a function of viral concentration $[L]$ in terms of two

parameters, the K_d value and R_{total}, the total number of available binding sites in the preparation:

$$[RL] = [R_{total}] [L]/K_d + [L] \tag{4}$$

The concentration at which $[L] = K_d$ is a useful point on this curve, since it is the concentration of ligand at which 50% of the total receptor sites are occupied ($[RL] = \frac{1}{2} [R_{total}]$). Thus, K_d, the equilibrium dissociation constant, is a measure of the affinity of a receptor for its ligand, since a small K_d value indicates a tightly binding (high-affinity) ligand which saturates its receptor at a low ligand concentration (see Fig. 1A). Hence, K_d is a standard measure used to compare the binding of ligands to a variety of receptors. It is important to note, however, that these equations express K_d in terms of the concentration of free ligand when equilibrium has been reached. At this point, the value of L, the concentration of free ligand, must be very close to the value of L_{total}, since as for R, the value of $L_{total} = L + LR$. Therefore, binding studies are usually performed in a range where only small amounts of free ligand are removed from the solution, usually not more than 10–15%, so that L can be approximated to equal L_{total}. Otherwise the free ligand concentration must be measured directly at equilibrium. The dissociation and association rate constants for binding, k_{-1} and k_1, can also be estimated directly by measurement of cell-associated virus as a function of time. Rate equations for k_1 and k_{-1} in Eq. (1) can be derived from the same model of a bimolecular interaction, and can also provide estimates of K_d (see Bennett, 1978, for detail).

Another approach to analyze the ligand–receptor interaction shown in Fig. 1A is to transform the data into a form which can be represented as a linear equation. One such form, the Scatchard plot shown in Fig. 1B, plots [bound/free ligand] against [bound ligand]. The purpose of this analysis is to allow the binding parameters to be directly estimated from the linear plot. Rearranging Eq. (4) to this form one obtains the expression:

$$[RL]/[L] = - [RL]/K_d + [R_{total}] \tag{5}$$

If the resulting plot is a straight line, the x intercept is the maximum binding of the ligand at saturation $[R_{total}]$, and the slope is the negative reciprocal of the K_d. Of concern, however, is that the manipulation of data for Scatchard analysis propagates errors in uptake measurements to both the x and y axis and changes the relative weighting of various regions of the binding curve. Since measurement of viral binding already has significant uncertainty, this magnification of errors further reduces the accuracy of binding data. Although it is used extensively to analyze ligand interactions in neuropharmacology and endocrinology, the Scatchard plot has not been used in the viral receptor field (see Incardona,

1981). Nonetheless, quantitative viral binding data can be expressed using Scatchard analysis (see Fig. 1B).

III. Membrane Components Which Interact with Viruses

A. DENSITY AND AFFINITY OF VIRAL RECEPTORS

Estimates of the density of cell membrane receptor sites for certain viruses have used either growth assays or radiolabeled binding techniques. Despite the variety of different cell types and viruses used, these estimates have been remarkably consistent, with values in the range of 10^4 to 10^5 sites per cell. For instance, Lonberg-Holm and Philipson (1974) estimated 1×10^4 receptor sites for poliovirus on HeLa cells, and found a similar value for adenovirus virions and other enteroviruses on several different permissive cell lines. Similarly, Birdwell and Strauss (1974) arrived at an estimate of 10^5 sites per cell for binding of Sindbis virus. Recent studies from our laboratory using ^{125}I-labeled reovirions have provided estimates in this range for reovirus type 1 and 3 receptor sites on L-cell fibroblasts, and for reovirus type 3 receptors on murine lymphocytes (Epstein et al., unpublished). McClintock et al. (1980) arrived at a slightly higher estimate ($1–5 \times 10^5$ sites/cell) for EMC virus binding to HeLa cells.

Studies using purified subviral binding components, e.g., the fiber protein of adenovirus, have usually led to higher estimates of receptor density than studies using whole virions. For example, Lonberg-Holm and Philipson (1974) demonstrated a 1 log increase in receptor site density when purified adenovirus fibers were used in place of virions (from 10^4 to 10^5 sites per cell). Similarly, 5×10^5 sites per cell were estimated when DeLarco and Todaro (1976) studied binding of the gp71 binding glycoprotein from Rauscher murine leukemia virus to NIH/3T3 cells. Such studies have supported the concept that viral receptor sites consist of multiple receptor "units" which can each bind an individual viral attachment protein, and that viruses can bind to the cell surface in a multivalent fashion.

Few studies have quantitatively measured the affinity of viral binding to receptors in terms of the K_d of the equilibrium binding reaction (see Section II,B). However, it has long been assumed that the virus–cell interaction is of very high affinity, both because of the rapid time course of viral binding and the difficulty in disrupting bound virus by physical means (Lonberg-Holm and Philipson, 1976). Although it has been suggested that in certain cases viral binding is virtually irreversible, some dissociation of bound virus probably occurs for most interactions (Lonberg-Holm, 1981). Rapid penetration after binding is one reason that dissociation has been difficult to measure. Initial studies from our laboratory have estimated very low K_d values for reovirus binding to L cells and

lymphocytes, demonstrating extremely high-affinity binding sites for reovirus (Epstein *et al.*, unpublished data).

B. RELATIONSHIP OF VIRAL RECEPTORS TO OTHER SURFACE COMPONENTS

It is unlikely that the structures on the cell surface which serve as viral receptors evolved merely for the purpose of virus binding. "Viral receptors," most probably, serve other biological functions for the cell. It is known, for example, that bacteriophage receptors are components of transport systems for low-molecular-weight sugars (Hazelbauer, 1975).

Influenza virus receptors on erythrocytes have been extensively characterized (Kathan *et al.*, 1961). These are sialoglycoproteins (called glycophorins) which have multiple functions, including M or N blood group activity (Springer *et al.*, 1966) and lectin binding activity (Jackson *et al.*, 1973). Extensive biochemical characterization and purification of these receptors (see Burness, 1981, for review) and analogous studies on other types of cells have confirmed that sialic acid-containing glycoproteins are important structural features of myxo- and paramyxovirus reception sites.

Receptors for the gp71 proteins of Rausher MuLV (previously described as the VAP) appear to be lipoproteins, since binding activity to fibroblast membranes is destroyed by protein-denaturing agents or treatments with chymotrypsin or phospholipase (Kalyanaraman *et al.*, 1978). McGrath *et al.* (1978) have suggested that T-lymphoma cell surface receptors for MuLV might be identical to the T cell antigen-binding receptor. However, a recently isolated 190,000 dalton dimeric protein from thymocytes which retains Maloney MuLV binding activity differs in size from previously reported values for T-cell idiotype receptors (Binz and Wigzell, 1976; Schaffar-Deshayes *et al.*, 1981). It also differs from immunoglobulin and Fc receptors which have also been suggested as candidates for the MuLV receptor.

In other studies, co-capping experiments have shown a relationship (though not identity) between EBV receptors and receptors for complement components C3b and C3d (Yefenof *et al.*, 1976). Helenius *et al.* (1978) reported that solubilized receptors for Semliki Forest virus (SFV) were enriched in HLA antigens but subsequent studies by Oldstone *et al.* (1980) showed binding and growth of SFV in cells lacking HLA antigens, indicating that HLA antigens were not biological receptors for SFV binding.

More recently, acetylcholine receptors (AChR) have been proposed to function as rabies virus receptors on mouse muscle cells. This conclusion was based on the similar anatomic distributions of bound virus and acetylcholine antagonists as observed by fluorescence microscopy. In addition, blocking studies showed that pretreatment of cells with AChR blockers (α-bungarotoxin or *d*-tubocurarin) decreased viral replication in susceptible cells (Lentz *et al.*, 1982).

It is possible that more than one surface structure might function as a viral receptor under special different circumstances. Daughaday *et al.* (1981) studied dengue virus binding to human macrophages and found that, although viral receptors were destroyed by trypsin, addition of specific nonneutralizing antibodies allowed penetration and replication of virus in the cell. They postulated that Fc receptors served as "nonneutralized viral receptors" in this instance by allowing binding of nonneutralized viral–antiviral antibody complexes to the cell via the Fc portion of the immunoglobulin molecule that was attached to the virus.

C. Definition of Cellular Receptor Units by Monoclonal Antibodies

Monoclonal antibodies directed against viral receptors represent a new approach for the characterization of viral cellular receptor sites. The fine specificity of monoclonal antibodies should permit elucidation of the precise cell surface antigenic domains involved in viral binding. Although minimal data using this approach are currently available, it is timely to review some of the technical options and difficulties associated with this approach.

One avenue is to screen a panel of monoclonal antibodies raised against the entire cell membrane, in hopes of isolating one which is specific for the viral receptor. These antibodies could be screened for based upon their ability to inhibit viral binding, viral growth, or hemagglutination. We have attempted this approach with reovirus. In order to find an antibody specific for the reovirus type 3 receptor on lymphocytes, a large panel of monoclonal antibodies against murine lymphocytes produced by Springer and colleagues (Springer, 1980) were tested for their ability to bind to lymphocytes which had been enriched for those bearing reovirus type 3 receptors (see Section II,A). Monoclonal antibodies which showed significantly more binding to viral receptor-positive lymphocytes were then tested for their ability to inhibit the binding of ^{125}I-labeled reovirus type 3 to lymphocytes. Only one antibody was found which had some effect: it minimally reduced viral binding. In an analogous approach, Campell and Cords (1982) generated monoclonal antibodies against HeLa cells and have identified monoclonal antibodies which block binding of coxsackievirus but not poliovirus to HeLa cells.

A second approach we have used takes advantage of the natural regulation of the immune response through the idiotype–antiidiotype network. To explain how this network might function during the normal immune response to a virus, assume that the virus has one antigenic determinant. That determinant will bind to B lymphocytes with an appropriate receptor or "idiotype" on the cell surface. The antiviral antibodies produced by the B lymphocytes bear idiotypic determinants that can bind the virus. These idiotypic determinants are themselves immunogenic and serve as antigens, so that antiidiotypic antibodies are made. These

antiidiotypic antibodies do not bind virus, but may bind to the cell surface structure that originally bound the virus. Thus, in theory, they could be used as specific antireceptor antibodies. This approach was first used in endocrinology. Antiidiotypic antibodies were raised against antibodies to retinol binding protein and to insulin. These antiidiotypic antibodies were shown to recognize the cell surface receptor for either retinol binding protein or insulin. Furthermore, it was found that antiidiotypic antibodies made against insulin antibodies mimicked the biological effects of insulin on adipocytes (Sege and Peterson, 1978).

We have attempted a similar approach to obtain an antibody against the reovirus type 3 receptor. In these experiments a polyclonal xenogeneic antiidiotype antiserum was made by injecting rabbits with hemagglutinin-specific mouse antireovirus type 3 antibodies (the viral hemagglutinin is the reovirus VAP) (Nepom et al., 1982). The antiidiotype antiserum was then absorbed with normal mouse immunoglobulins, and purified by using a monoclonal antibody to the neutralization site on the hemagglutinin of reovirus type 3. These purified antiidiotypic antibodies mimicked the virus in their binding patterns to various cell lines. Moreover, they bound to primary cultures of murine neuronal cells (which bind reovirus type 3) but did not bind to freshly prepared ependymal cells (which bind reovirus type 1 but not type 3). In addition, they appear to mimic the virus in its interaction with murine T lymphocytes (see Section V,A) (Nepom et al., in preparation; Tardieu et al., 1982). Thus, it appears that these antiidiotypic antibodies might recognize the CRS for reovirus type 3 on neurons and lymphocytes. Work is in progress to further characterize the properties of these antiidiotypic antibodies in terms of blocking viral growth and determining structures on the cell surface which they recognize.

Using a similar approach, McGrath and Weissman, studying a spontaneous murine B cell lymphoma (BCL1) recently produced a monoclonal antiidiotypic antibody against the BCL1–IgM (which binds BCL1-associated retrovirus) and demonstrated that the monoclonal antiidiotypic antibody blocks the binding of the retrovirus to BCL1 cells (M. S. McGrath and I. L. Weissman, personal communication).

D. SPECIFICITY OF CELL RECEPTOR SITES FOR VIRUSES

In some instances, structures on the plasma membrane which serve as viral receptors have specificity for a single virus, and in other instances, different viruses may share the same receptor. The concept of "viral receptor families" was introduced by Lonberg-Holm et al. (1976). In their experiments, they were able to block the binding of one virus to the cell surface by preincubating the cells with an unrelated virus (see Boulanger and Philipson, 1981, for review). Binding was measured either by infectivity (using UV-inactivated virus for blocking), by radiolabeled virus (using unlabeled virus for blocking), or by

immunofluorescence (using specific antiviral antibody which recognized the blocking virus). These studies established that HeLa cells have receptors for a variety of picornaviruses, and that some viruses share common receptors. Thus, the three poliovirus serotypes compete for a common receptor, which is distinct from the receptors that bind the six groups of Coxsackie B viruses (Crowell, 1966, 1976). Coxsackie viruses A13, A15, and A18 have a distinct receptor from the other two groups and Echoviruses and human rhinoviruses have separate receptors from the other picornaviruses (Crowell and Siak, 1978). Moreover, receptors were shown to be shared between viruses from different groups. Thus, the binding of adenovirus type 2 to HeLa cells was blocked by Coxsackie B3, and binding of human rhinovirus type 14 was blocked by Coxsackie virus A21 (Lonberg-Holm *et al.*, 1976). Confirmation of "receptor families" as defined in these experiments will ultimately depend upon biochemical characterization of the viral receptor sites.

The serotype specificity of some viruses is also associated with serotypic differences in their receptors. For example, two serotypes of reovirus, types 1 and 3, differ in their ability to bind to primary cell cultures. Reovirus type 3 binds to neurons and lymphocytes whereas reovirus type 1 binds to ependymal cells (see below). In addition, binding experiments using ^{125}I-labeled virions suggest different binding patterns of the two reovirus serotypes to L cells even though both serotypes do bind to and grow in this continuous cell line. (Epstein *et al.*, unpublished). Specific receptors for the two serotypes of Herpes simplex virus have also been described (Vahlne *et al.*, 1979). In these experiments, Herpes simplex type 1 (HSV1) interfered with the adsorption of HSV1 but not of HSV2 to human, monkey, and rabbit permanent cell lines. The adsorption rate was measured by assaying infective virus remaining in the medium or by measuring cell associated [^{3}H]thymidine-labeled HSV. Adsorption profiles demonstrated that the monkey kidney cell line and the rabbit cornea cell line had more HSV1 than HSV2 receptors, while HeLa cells expressed more receptors with affinity for type 2 than for type 1. Human embryonic lung cells and a cell line derived from a human carcinoma of the larynx showed equal amounts of HSV1 and HSV2 receptors. Our experiments demonstrate that HSV1 binds significantly more to murine ependymal cells than HSV2 (Tardieu and Weiner, 1982).

A "viral interference" assay was used to study the specificity of cell surface receptors for retroviruses. Steck and Rubin (1966) first demonstrated retroviral interference by showing that chicken fibroblasts persistently infected by an avian retrovirus were not susceptible to superinfection by the same virus (Rubin, 1960, 1961). Later studies established that the interference resulted from a blockade of viral receptors by endogenously produced viruses (reviewed in Weiss, 1981).

A similar approach has been used to study another group of retroviruses, murine leukemia viruses. Murine leukemia viruses (MuLV) are classified as ecotropic, xenotropic, or amphotropic depending on their ability to infect mouse

cells, cells of other species, or cells of both mice and other species, respectively. Using the "cross-interference" approach, it was shown that cells infected with one ecotropic virus failed to bind a second ecotropic strain (R-MuLV), whereas cells infected with a xenotropic virus bound R-MuLV as well as uninfected cells (Hartley and Rowe, 1976; Besmer and Baltimore, 1977). Thus, murine ecotropic and xenotropic viruses appear to recognize different receptors on the murine cell surface, but various strains of ecotropic MuLV utilize the same receptors, since they are subject to cross-interference (Sarma et al., 1967); DeLarco and Todaro (1976) subsequently showed that infection of murine cells with various ecotropic viruses (S2CL3, AKR, R-MuLV, M-MuLV) prevented the binding of radiolabelled R-MuLV gp71 (the VAP of R-MuLV) to the surface of the infected cells. Interference studies using purified gp71 demonstrated that the ecotropic viruses used the same family of receptors despite marked differences in the antigenic properties of the viruses. It was then confirmed that the murine xenotropic, as well as amphotropic viruses, use a different family of receptors from murine ecotropic viruses, since they did not interfere with viral infectivity or gp71 binding.

Similarly, a class of mouse mammary tumor viruses (MMTV) which share an antigenically similar surface glycoprotein gp52 (the VAP of C3H MMTV and GR MMTV) recognize a common cell surface receptor which is different from the surface receptor recognized by other MMTV which have an antigenically different gp52 (CH3 MMTV and RIII MMTV) (Altrock et al., 1981; Schochetman et al., 1979)). In these experiments, viral binding was studied by measuring binding of ^{125}I-labeled protein A to immune complexes composed of a C3H MMTV gp52 type-specific monoclonal antibody and receptor bound MMTV, or by directly measuring radiolabeled ^{3}H C3H MMTV binding to the cells. Viruses which share class-specific gp52 determinants also share common surface antigen receptors involved in virus adsorption. Finally, the VAPs of type-C and type-D primate retroviruses recognize the same receptors (Moldow et al., 1979), a finding that might reflect a relationship between type-C and type-D VAPs as suggested by their immunological cross-reactivity (Stephenson et al., 1976; Devare et al., 1978).

Most of the above studies were performed using continuous cell lines. It is important, however, to also study viral binding to cells which may be the target of viral infection in vivo. This approach has been most easily implemented using freshly isolated lymphocytes for the study of virus–receptor interaction with lymphocyte subpopulations. For example, Epstein–Barr virus selectively binds to human B lymphocytes, mouse adapted cytomegalovirus binds to murine B lymphocytes, and measles and murine leukemia virus bind to human T lymphocytes (Greaves, 1976). Woodruff and Woodruff (1974) have done a variety of studies on the binding of myxoviruses and paramyxoviruses to murine lymphocytes. They have found that Sendai virus, Newcastle disease virus (NDV),

influenza B virus and influenza A (H2N2) or (H3N2) virus agglutinate T lymphocytes *in vitro,* and, presumably, T lymphocytes have a receptor for these viruses. The receptors on T lymphocytes for the paramyxoviruses they studied (Sendai and NDV) differ from those for the myxoviruses (influenza). This was shown by differences in binding between these group of viruses according to temperature or *in vitro* treatment with fetuin, *N*-acetyl neuraminic acid, or periodate. In addition, after elution of NDV from lymphocytes, the lymphocytes are agglutinable by influenza virus but not by NDV or Sendai virus. The nine serotypes of influenza A virus they studied also demonstrated differences in their ability to bind T and B cells: five strains agglutinated T and B cells whereas four agglutinated only B lymphocytes. Thus lymphocyte receptors can distinguish among various serotypes of influenza A virus.

Using an indirect immunofluorescence technique, we have found a receptor for reovirus type 3 on murine and human lymphocytes whereas only minimal binding was visualized for reovirus type 1 (Weiner *et al.,* 1980a). These studies have recently been extended by quantitative studies using ^{125}I-labeled virions which show saturable binding of reovirus type 3 to lymphocytes and only minimal binding of reovirus type 1 with no saturable component (Epstein *et al.,* in preparation). Thus, lymphocytes have different receptors for reovirus serotypes and only the receptor for reovirus 3 has a sufficiently high affinity for the binding to be characterized.

More recently, the *in vitro* affinity of ^{3}H-labeled mouse hepatitis virus 3 for macrophages and lymphocytes from both naturally resistant and susceptible mice was shown to be identical (Krystyniak and Dupuy, 1981). In addition, ^{3}H-labeled encephalomyocarditis virus bound to resident peritoneal macrophages. In contrast, unstimulated splenic lymphocytes did not have detectable numbers of EMC virus receptors, but these receptors could be induced on both T and B lymphocytes by mitogenic stimulation (Morishima *et al.,* 1982).

In addition to lymphocytes and macrophages, other cells of biological interest can be studied. We have a particular interest in viral receptors on nervous system tissue and techniques exist to obtain freshly isolated cells from the central nervous system, such as oligodendrocytes (Snyder *et al.,* 1980), astrocytes (Farooq and Norton, 1978), ependymal cells (Manthorpe *et al.,* 1977), or in some instances neurons (Farooq and Norton, 1978). Freshly isolated cells can then be used to identify viruses which have an affinity for them. We have initiated this approach to study viral receptors on freshly isolated human and murine ependymal cells (Tardieu and Weiner, 1982). In these experiments, viral binding to the ependymal cells was demonstrated by indirect immunofluorescence using specific antiviral antiserum. Reovirus type 1 (which induces hydrocephalus in mice) bound to the surface of isolated human and murine ciliated ependymal cells whereas reovirus type 3 (which does not induce hydrocephalus *in vivo*) did not. The binding property of reovirus type 1 to ependymal cells was then mapped to

the viral hemagglutinin (the VAP) with the use of single-segment recombinant clones between reovirus 1 and 3 (Weiner *et al.*, 1980c). Clone 3.HA1, which contains nine genes from reovirus type 3 and one, the S1 gene, which encodes for the viral hemagglutinin, from type 1, bound to ependymal cells, whereas the reciprocal clone 1.HA3 did not. In addition, mumps virus, measles virus, parainfluenza type 3, and Herpes simplex virus type 1 bound to murine ependymal cells, whereas Herpes simplex virus type 2 and poliovirus type 1 did not. (Further work on CNS viral receptors by McLaren and Holland is described later in this article.)

Thus, it can be demonstrated that unrelated viruses may share a common receptor on the cell surface, and that viruses with the same VAP usually bind to the same receptor even if other parts of the virus are different (retroviruses, recombinant clones of reoviruses). On the other hand, different serotypes of a virus may (polioviruses, coxsackie A viruses) or may not share (reoviruses, Herpes simplex viruses, influenza viruses) a common cell surface receptor.

Does a virus bind to different cells using identical or different cell receptor sites? This issue is particularly relevant since most studies of viral binding have utilized permanent cell lines. It is not known to what extent there is homology between surface receptors on the different cell lines to which a virus binds, or, more importantly, whether results obtained from binding studies using transformed cells can be generalized to *in vivo* virus–surface receptor interactions. Early investigations suggested that different structures served as viral receptors on different cell lines (Kodza and Junglebut, 1958; Sabin, 1959; Holland and McLarren, 1961). One experimental approach to address this issue is to compare two different permissive cell lines for such variables as number of receptors per cell, affinity of these receptors for virus, or susceptibility of receptors to inactivation by agents such as proteolytic enzymes. For example, Sindbis virus replicates in both mammalian and mosquito cell lines. Smith and Tignor (1980) studied the attachment of two Sindbis virus strains (avirulent or neurovirulent) to these cell lines both before and after enzyme treatment of the cells. Mammalian cellular receptors for the avirulent strain were sensitive to proteolytic cleavage while mosquito cells were insensitive to protease, phospholipase, and neuraminidase. The difference was less striking but still present for the neurovirulent strain.

Reovirus type 3 binds both to freshly isolated lymphocytes and to L cells, a murine fibroblast cell line. Although binding studies using ^{125}I-labeled virus suggested similarities between receptors on these two types of cells, xenogeneic antiidiotypic antibodies raised against hemagglutinin-specific antireovirus type 3 antibodies (see Section III,C) bind to the same lymphocyte subpopulations as reovirus type 3 but do not bind to L cells (Nepom *et al.*, in preparation; Tardieu *et al.*, 1982). Assuming that this antiidiotypic antibody recognizes the CRS for reovirus type 3 on lymphocytes and neurons, it would appear that the receptor sites on lymphocytes and neurons express an antigenic determinant which is absent from the receptor site on L cells.

In an analogous approach, the attachment kinetics of radiolabeled encephalomyocarditis virus were compared on established murine and human cell lines (McClintock *et al.*, 1980). The receptor for this virus on human cells had a higher affinity for virus than that on murine cells. In addition, the attachment of the virus to HeLa cells was temperature-independent over the range 0 to 40°C whereas attachment to murine cells progressively decreased with increasing temperature (associated with an increased rate of dissociation of virus).

Thus, from these three models, it appears that there may be structural differences between receptor sites for a given virus on various cells. A definitive answer to this issue requires the isolation and characterization of the CRU for a particular virus and the determination of which component of the receptor the virus binds.

E. *In Vitro* Manipulation of Cell Receptor Sites

The attachment of a virus to a cellular receptor site is only the first step in a series of events (internalization, uncoating, replication, and assembly) that ultimately results in viral replication. The presence of a specific receptor on the cell surface is a necessary but not sufficient condition for viral replication. Thus, for nonpermissive cells, an important question is whether restriction occurs at the receptor or intracellular level.

1. *Transfer of Epstein–Barr Virus (EBV) Receptors to Receptor-Negative cells*

In vitro, EBV infection occurs only in human and some primate lymphocytes, and EBV receptors are present only on B lymphocytes (Jondal and Klein, 1973; Greaves 1976). To determine if the host-range restriction of EBV growth was receptor-mediated, EBV receptors from purified Raji cell membranes were transferred into the membranes of murine lymphocytes and cells from a human T cell line both of which were nonpermissive for the virus (Volsky *et al.*, 1980). Transfer was accomplished using vesicles reconstituted from a mixture of purified Raji membranes and Sendai virus envelope proteins. Successful implantation of receptor-rich membranes into the membrane of the nonpermissive cells was demonstrated by monitoring the fate of radioiodinated donor membrane, and was confirmed by the detection of surface EBV receptors and complement C3 receptors (which are closely associated with EBV receptor) (Yefenof *et al.*, 1976) on implanted cells. EBV receptors could be detected for 36 hours after implantation and radiolabeled EBV bound specifically to receptor-implanted cells. Furthermore, the implanted receptors were biologically functional, since virus penetration and replication were demonstrated in the normally resistant cells as measured by the expresssion of EBV early nuclear antigen and EBV capsid antigens.

2. Bypassing Receptor-Mediated Barriers to Infection

Replication of a virus can occur in a cell lacking appropriate viral receptors if the barrier to infection at the cell surface is circumvented. This has been achieved with poliovirus by two methods: (1) direct inoculation of viral nucleic acid into the cytoplasm of a cell lacking poliovirus receptors and (2) physical entrapment of the virus into the cell by fusion of the cellular membrane with Sendai virus (which incorporate polioviruses bound nonspecifically to the cell surface) or with virus-containing liposomes (Enders et al., 1967; Wilson et al., 1977).

3. Binding of Polyoma and Sendai Virus to Specific Gangliosides

Many studies have demonstrated the importance of sialic acids in the binding of Polyoma or Sendai virus (and other myxo- and paramyxoviruses) to the cell surface, and sialidase treatment of host cells can prevent viral infection with these viruses (Hirst, 1942; Klenk et al., 1955). These cellular receptors have been identified as glycoproteins with N-acetyl neuraminic acid as the terminal sugar in the carbohydrate side chains (Gottshalk, 1957). To further elucidate the role of sialic acid in cell permissiveness, binding of virus to isolated, highly purified gangliosides of defined structure was studied (Svennerholm and Fredman, 1980). Initially, binding to polystyrene Petri dishes coated with different gangliosides was studied (Holmgren et al., 1980), then host cells were made resistant to Sendai virus by removal of endogenous viral receptor with Vibrio cholerae sialidase (Markwell and Paulson, 1980). These receptor-negative cells were then incubated with individual purified gangliosides. Incubation of cells with gangliosides containing the sequence NeuAcα2, 3 Galβ1, 3GalNAc fully restored susceptibility to Sendai virus infection. Furthermore, incubation with gangliosides with a sequence ending with two sialic acids in a NeuAcα2, 8Neu-Ac linkage, rather than a single sialic acid, was 100 times more effective (Markwell et al., 1981). In an analogous way, susceptibility to Polyoma virus infection was restored by implantation of the sequence NeuAcα2, 3Galβ1, 3GalNAc but not the sequence NeuAcα2, 6Galβ1, 4GlcNAc even though the latter sequence contained a comparable amount of sialic acid (Fried et al., 1981). Thus, Sendai and Polyoma virus interact with specific ganglioside sequences and cell susceptibility to infection can be modified by implantation of different gangliosides into the cell membrane.

4. Inhibition of Receptor Binding Using Antireceptor Antiserum

The attachment of enteroviruses to HeLa cells can be inhibited by heterologous antiserum raised against HeLa cells, suggesting that these antibodies in some way affect cell surface viral receptors (Quersin-Thiry, 1958; Axler and Crowell, 1968; Much and Zajac, 1973). This approach is limited, however, by the lack of fine specificity of the antiserum. Monoclonal antibodies directed

against the cell surface offer a more specific avenue for the generation of anti-receptor antibody and recent work showing the blocking of Coxsackie virus binding to HeLa cells by monoclonal antibodies demonstrates that this should be a feasible approach (Campell and Cords, 1982). A similar approach has recently been described for monoclonal antibodies made against the thyrotropin receptor. These monoclonal antibodies competitively block binding of thyroid stimulating hormone (TSH) but are unable to stimulate adenyl cyclase activity as TSH does. This result suggests the existence of a second domain on the receptor which is associated with the stimulating activity (Yavin et al., 1981).

5. Modification of the Receptor Associated with Viral Binding

It has recently been shown that binding of insulin can alter the conformation of insulin receptors on fat cells (Pilch and Czech, 1980). A similar question can be raised for viral receptors: can viral binding to the cell surface modify its own receptor? There are only a few investigations related to this issue.

Following the incubation of human lymphoblastoid cells with Epstein–Barr virus (Hinuma et al., 1975), or of Ehrlich ascites tumor cells with mengovirus (Geschwender and Traub, 1979), modulation of cell-surface viral receptors (capping) was observed. This occurred after binding of virus alone. In our investigations using reovirus, capping of reovirus type 3 receptors on the surface of murine lymphocytes required cross-linking by antiviral antibody (Epstein et al., 1981). Levanon et al. (1977) have demonstrated that adsorption of infective encephalomyocarditis virus enhances fluidity of the plasma membrane.

Binding of paramyxo- and orthomyxoviruses to the cell surface can result in destruction of the receptor itself. The hemagglutinin of paramyxoviruses binds to neuraminic acid-containing cell surface receptors and has neuraminidase activity which eliminates natural neuraminic acid-containing receptors from infected cells (reviewed by Choppin and Scheid, 1980). These two functions reside on two separate proteins in orthomyxoviruses. The role of neuraminidase activity, which is paradoxically present on the same viral protein that determines viral binding, is unclear. It has recently been shown that these two opposing activities can be regulated by environmental conditions such as chloride concentration and pH: high concentrations of halide ion enhance hemagglutinating activity and decrease elution from erythrocytes, while they inhibit neuraminidase activity (Merz et al., 1981). Studies with a specific chemical inhibitor of neuraminidase (Palese et al., 1974a; Palese and Compans, 1976) and temperature-sensitive mutants (Palese et al., 1974b) suggest a role for neuraminidase activity during the release of newly synthesized virus. Because of enhancement by low chloride ion concentration and an acidic pH, the neuraminidase activity is most prominently expressed intracellularly. In contrast, the ionic environment in the extracellular fluids favors virus attachment over receptor-destroying activity.

F. Age Dependency of Viral Permissiveness

The age dependency of susceptibility to viral infection is relevant to the question of the role of receptors in the nonpermissiveness of a cell. In several instances, cells from adult animals are not as permissive for viral replication as cells from newborn animals, even though they appear to have the appropriate viral receptors. For example, allantoic sac cells from young chicken embryos are more permissive for influenza or vesicular stomatitis viruses than cells from older embryos. This age-dependent difference in permissiveness is due to a late intracellular event since viral attachment and penetration are the same in cells from young and old chicken embryos (White, 1959; Morahan and Grossberg, 1970). In our investigations, we have found that both isolated newborn and adult ependymal cells have receptors for reovirus type 1 even though ependymitis is observed more prominently in newborns (Tardieu and Weiner, 1982). In contrast, in studies of Coxsackie B5 infection of fibroblasts, Kunin (1962) reported that a slightly decreased ability to absorb the virus occurs in adult as compared to newborn cells, correlating with a decreased permissiveness of adult cells for viral replication. In this instance, the age-dependent reduction in permissiveness may be related to a change in receptor affinity for virus in older cells.

G. Genetic Control of Cell Receptor Site Expression

In a few cases, a genetic basis for the expression of viral receptors on different cell types can be demonstrated. For example, cells from different mammalian species differ in their susceptibility to poliovirus, i.e., human cells are susceptible while murine cells are not. Somatic hybrids made between permissive (human) and resistant (rodent) cells (Belehradek and Barski, 1969; Wang et al., 1970) demonstrated that hybrids could be infected by poliovirus only when human chromosome 19 was present (Miller et al., 1974). Since viral replication does not require the presence of human genes once the viral nucleic acid has entered the cell (Holland et al., 1959; Wang et al., 1970), these experiments demonstrated that chromosome 19 carries the structural gene for the poliovirus receptor. On the other hand, permissiveness of cells for echo-7 and rhino-1A viruses could not be linked to the presence of a specific human chromosome (Miller et al., 1974).

The genetic basis for the specificity of retrovirus cell surface receptors has recently been reviewed (Weiss, 1981). Utilizing interspecies somatic cell hybridization techniques, the gene encoding for the CRS for ecotropic MuLV on murine cells has been assigned to chromosome 5 (Oie et al., 1978; Ruddle et al., 1978; Marshall and Rapp, 1979) and the gene encoding the CRS for endogenous feline C-type virus (RD114) on human cells assigned to human chromosome 19 (Schnitzer et al., 1980). It should be noted, however, that, for MuLV, the gene

expressed on chromosome 5 does not solely regulate leukemia virus replication. Another gene (Fv-1), located on murine chromosome 4, regulates the replication of the virus at a postpenetration step and may influence integration of proviral DNA into the host chromosome (Rowe and Sato, 1973; Gazdar *et al.*, 1977). As noted by Ruddle *et al.* (1978), additional host control is exerted at the level of differentiation, since bone marrow-derived but not thymus-derived lymphocytes are able to support replication of exogenous MuLV. The genetic regulation of receptor expression is, therefore, only one part of the genetic regulation of cell permissiveness for the virus.

IV. Viral Components Which Recognize Cellular Receptors

Identification of the subviral components which are responsible for binding of viruses to cell surfaces has preceded structural understanding of the cellular receptors themselves. This section briefly summarizes current data concerning the viral attachment protein (VAP) of selected viruses.

A. PICORNAVIRUSES

Picornaviruses are small nonenveloped viruses with icosahedral symmetry. The 22- to 30-nm capsid contains 60 copies of a "structural unit" consisting of four separate polypetides, VP1, 2, 3, and 4. Some picornaviruses contain a few copies of a precursor protein (VP0) which contains uncleaved VP2:VP4. It has been known for many years that shortly after poliovirus binds to cells, a fraction of the attached virus elutes (Halperen *et al.*, 1964). This eluted virus has lost the polypeptide VP4 and is no longer infectious (Lonberg-Holm and Philipson, 1974). These experiments suggested that VP4 was the attachment protein. Further studies, however, provided contradictory evidence: (1) VP4 could not be labeled by techniques which label surface proteins such as ^{125}I-labeling using lactoperoxidase (e.g., Lonberg-Holm and Butterworth, 1976). (2) Naturally occuring empty capsids (top component) of some picornaviruses demonstrated identical binding characteristics as native virus, but were shown to lack VP4. (3) Antibodies to VP4 do not recognize the surface of native virions (Talbot *et al.*, 1973). Other evidence suggests that VP1 is the binding protein. VP1 is expressed on the surface of the capsid, and trypsin treatment of virions (which renders them incapable of binding to cells) appears to primarily cleave VP1, although some studies also have shown cleavage of VP3 (Boulanger, 1975; Boulanger and Lonberg-Holm, 1981). For Coxsackie virus B3 there is also evidence that VP2 is present at the capsid surface (Philipson *et al.*, 1973). Some investigations have suggested that no single protein functions as the viral attachment site, but that cooperative interactions among the viral proteins result in a unique conforma-

tional state which allows binding. This view is supported by the demonstration of differences in antigenicity between native virions ("D antigenic") and inactive subviral particles ("C antigenic") for several enteroviruses and rhinoviruses. Native particles carry few of the antigenic determinants of inactive particles (Lonberg-Holm and Yin, 1973). Since the ability to attach to cells is irreversibly lost during the conversion from D- to C-antigenicity (Lonberg-Holm and Yin, 1973), this suggests that the conformation of capsid polypeptides may play a key role in the ability of the virion to attach to host cells, perhaps by regulating exposure of a polypeptide sequence carrying the determinants required for binding activity.

B. ADENOVIRUSES

The adenoviruses (mammalian and avian) are larger and more complex than picornaviruses, and (for mammalian viruses) are classified according to species-specific hemagglutination properties. Adenovirus capsids are icosahedral and contain 252 capsomers. Most of these are called hexons because each has 6 neighbors. The 12 apical capsomers are surrounded by only 5, and are therefore called pentons. Each of these consists of a penton base and a 10- to 30-nm projection called a fiber. The fiber consists of three polypeptide chains. It has been shown that adenovirus binds to cells via determinants located in the terminal knob of the fiber. The fiber is also the hemagglutinin (Norrby et al., 1969). The ability to solubilize and purify the adenovirus fiber protein has led to the demonstration of serotypic differences in hemagglutinin among subgroups of adenoviruses.

C. REOVIRUSES

Reoviruses are nonenveloped viruses consisting of two concentric icosahedral capsid shells that surround a segmented double-stranded RNA genome. The outer capsid is composed of three polypeptides (μ1C, σ3, and σ1) which are individually coded by three different viral genes. The σ1 polypeptide makes up 1–2% of the outer capsid (24 copies per virion) and is located at the vertices of the icosahedral structure. The σ1 polypeptide is the major determinant of reovirus interactions with cells. It is the viral hemagglutinin, elicits the formation of neutralizing antibody, and is responsible for development of delayed type hypersensitivity, generation of suppressor T cells, and generation of cytolytic T lymphocytes (Weiner and Fields, 1977; Weiner et al., 1980b; Greene and Weiner, 1980; Fontana and Weiner, 1980; Finberg et al., 1979). As described previously, it also determines the serotype specificity of viral tropism for different cells in the nervous system and the ultimate pattern of CNS virulence (Weiner et al., 1977, 1980c; Tardieu and Weiner, 1982). Tryptic peptide analysis of the

σ1 polypeptide from reovirus serotypes 1, 2, and 3 has demonstrated both unique and common methionine- and tyrosine-containing peptides. This suggests that certain regions of the hemagglutinin have been conserved, while others have "drifted" with resultant serotypic changes (Gentsch and Fields, 1981). Monoclonal antibodies prepared against the viral hemagglutinin of reovirus type 3 have defined at least four antigenically different domains. One class of antibodies had neutralizing activity; a second class only hemagglutination inhibition (HI) activity. One monoclonal antibody had neutralizing and HI activity and a fourth class of monoclonal antibodies had no detectable neutralization or HI activity. These results suggest that marked functional specialization exists within regions of the reovirus type 3 hemagglutinin (Burstin *et al.*, 1982). This separation of regions for hemagglutination and neutralization raises the possibility that, for other viruses as well, there might be separate viral determinants which bind to either red cells (hemagglutination) or host cells (infection). This suggests that RBC receptors and receptors on other cell types may not be homologous.

D. Myxoviruses and Paramyxoviruses

For paramyxoviruses, the two glycoproteins which project from the viral surface have been isolated and purified (see Scheid, 1981, for review). The HN glycoprotein, which possesses both hemagglutinating and enzyme (neuraminidase) activity, is the viral receptor-binding protein and exists on the surface in a dimer configuration. It has been suggested that a single active site serves both functions, but this remains to be clarified (Scheid *et al.*, 1972). The F glycoprotein is responsible for fusion activity (and thus hemolysis) and is involved in virus penetration into the cell (to be discussed in the next section). Morbilii viruses (measles, canine distemper virus) lack neuraminidase and their binding glycoproteins are designated H, rather then HN.

The three serotypes of influenza virus comprise the myxovirus group and, unlike paramyxoviruses, influenza virions contain separate spikes for the hemagglutinin (HA) (present as a trimer) and the neuraminidase (NA) (present as a tetramer) (Schild, 1979). Influenza C virions differ as they have no neuraminidase. HA is the glycoprotein responsible for hemagglutination or adsorption to host cells and antigenic variations in this protein are largely responsible for periodic epidemics of influenza. Monoclonal antibodies raised against the HA of influenza A have identified three or four nonoverlapping antigenic domains on the protein (Wiley *et al.*, 1981; Wilson *et al.*, 1981). Direct correlation of these domains with functional differences have yet to be defined, but the structural definition of the HA has provided initial answers. The host–receptor binding site and antigenic determinants are located on a globular region which lies on top of a long fibrous coiled coil; the fusion activation peptide is located

near the virus membrane end of the molecule (Laver and Valentine, 1969; Gerhard *et al.*, 1981; Wiley *et al.*, 1981; Wilson *et al.*, 1981).

E. RETROVIRUSES

The viral attachment proteins of several retroviruses have been isolated and used in two types of experiments: (1) cross-interference experiments as discussed in Section III,D (reviewed by Weiss, 1981) and (2) experiments to define antigenic domains on this molecule with monoclonal antibodies. These latter experiments were performed using the major external glycoprotein (gp52) of mouse mammary tumor virus (MMTV). Two topographically distinct sites have been identified on gp52. One site functions as a target for neutralization antibody and was defined by the observation that all monoclonal antibodies (MAb) which neutralized virus infectivity also competed for binding of a neutralizing MAb (used as a standard). The second site bound antibody but this binding had no effect on neutralization. This site was topographically distinct and its MAb could not compete for binding of a second neutralizing MAb (Massey and Schochetman, 1981a). It was further shown that the neutralizing site described above was not the receptor binding site but was adjacent to it as monoclonal antibodies were found which competed for the binding of the first neutralizing MAb but did not neutralize the virus. These antibodies functioned as blocking antibodies and protected virus particles from neutralization (Massey and Schochetman, 1981b).

F. CORONAVIRUSES

Two glycoproteins are associated with the envelope of the A59 strain of mouse hepatitis virus (MHV): the E2 glycoprotein which makes up the peplomers of the virus and the E1 glycoprotein which is deeply embedded in the viral membrane (the portion of the E1 glycoprotein which protrudes from the viral membrane contains a small glycosilated portion) (Holmes *et al.*, 1981). Monospecific antibodies directed against E2 glycoprotein prevent viral attachment. Virions lacking E2 (either because of growth in the presence of tunicamycin or treatment with bromelain) do not attach to the cell membrane. In addition, isolated E2 binds to the same receptor as intact virus since pretreatment of cells with unlabeled, concentrated MHV blocks the binding of raidolabeled E2. Thus, the E2 glycoprotein appears to be the virus attachment protein for the A59 strain of MHV (K. Holmes, personal communication; Holmes *et al.*, 1981).

V. Virus–Receptor Interactions and Pathogenicity

A major feature of certain viral infections is selective damage to specific tissues, and in some instances to specific cells within a tissue. The classic

example is poliovirus infection of anterior horn cells in the spinal cord. Other examples include selective infection of the limbic system by rabies virus, infection of ependymal cells by mumps virus (reviewed in Johnson, 1980), and infection of pancreatic beta cells by encephalomyocarditis virus or Coxsackie B4 virus (Craighead and McLane 1968; Boucher and Notkins, 1973; Yoon *et al.*, 1980).

It has long been postulated that the *in vivo* tropism of viruses is mediated in part by the presence or absence of specific receptor sites for viruses on the surface of the target cells (Holland, 1961).

A. ROLE OF CELL SURFACE RECEPTORS IN PATHOGENICITY

The role of specific cellular receptors as determinants of cell tropism has been extensively studied (recently reviewed by Crowell and Landau, 1979; and Crowell *et al.*, 1981). Initial studies with picornaviruses using organ minces and homogenates demonstrated a correlation between the presence of receptors on cells and the known *in vivo* tropism of poliovirus. Human and monkey CNS tissue and intestine were able to adsorb polioviruses whereas tissues from human lung, heart, and skin were not. The correlation was not absolute, however, since receptors were also detected on human liver, monkey heart, and skeletal muscles. Furthermore, poliovirus vaccine strains which did not induce cell damage were shown to bind to brain tissues (McLaren *et al.*, 1959; Holland, 1961; Kunin and Jordan, 1961; LaPlaca, 1963; Harter and Choppin, 1965). A second line of evidence demonstrating a relationship between cell surface receptors and pathogenicity was the presence of a correlation between the grouping of viruses by receptor specificities and their classification according to subgroups which were derived from patterns of pathogenesis (see Section III,C and Lonberg-Holm *et al.*, 1976). Finally, specific organ cultures have been used to show different growth specificities for picornaviruses. For example, some rhinoviruses multiply only in differentiated organ cultures of trachea (Hoorn and Tyrell, 1966); Coxsackie viruses A1 and A5 grow in differentiating primary fetal mouse muscles cultures but do not grow in nondifferentiating mouse cultures (Came and Crowell, 1964; Landau *et al.*, 1972). In contrast, receptors for human enteroviruses exist on tissues which are not involved in their pathogenesis and in species other than their natural hosts (Holland, 1961; Kunin and Jordan, 1961; LaPlaca, 1963; Campbell, 1965).

A genetic approach has been used to define the molecular basis for the different patterns of virulence and central nervous system cell tropism exhibited by reovirus serotypes 1 and 3. Using recombinant clones derived from crosses between reovirus types 1 and 3, it has been shown that the hemagglutinin of reovirus (encoded by the S1 gene) determines the central nervous system cell tropism of the reovirus serotypes (Weiner *et al.*, 1977, 1980c). Reovirus type 3

and clone 1.HA3 (containing nine genes from type 1 and the gene encoding the hemagglutinin from type 3) cause a fatal encephalitis in newborn mice with neuronal destruction but no ependymal cell damage whereas reovirus type 1 and clone 3.HA1 (the reciprocal clone to clone 1.HA3) cause ependymal infection without neuronal damage. The affinity of the two serotypes for two different cell types in the nervous system appears to be due to the specific interaction of the viral hemagglutinin with the receptors on the surface of either ependymal cells or neuronal cells. These results have been confirmed *in vitro* by demonstrating that reovirus type 1 and clone 3.HA1 (but not reovirus type 3 and clone 1.HA3) bound to isolated human and murine ependymal cells (Fig. 2A and B) (Tardieu and Weiner, 1982). The reciprocal results have been shown on neural cells in culture (Dichter and Weiner, unpublished data; see Fig. 2E and F).

The M variant of encephalomyocarditis virus (EMC) produces a diabetes-like syndrome in certain strains of mice by infecting and destroying pancreatic beta cells. Cultured pancreatic beta cells from mice resistant to EMC-induced diabetes are less able to absorb infectious EMC virus than beta cells from susceptible strains, suggesting that genetically determined differences in surface viral receptors on these cells may be one of the factors controlling susceptibility to the disease (Chairez *et al.*, 1978).

The presence of virus receptors on lymphocytes may correlate with the specific effect that some viruses may have on the immune response. T lymphocytes have a receptor for measles virus and measles infection is associated with a depression of tuberculin skin hypersensitivity, and a suppression of helper cell activity (McFarland, 1974). Reovirus type 3 binds primarily to the Ly2,3 subset of murine T lymphocytes (the suppressor/cytotoxic subset) as well as to the human counterpart (T8^{+} cells). This binding is a property of the viral hemagglutinin (Epstein *et al.*, 1982). Furthermore, *in vitro*, reovirus type 3 induces suppressor T cells capable of suppressing Con A proliferation (Fontana and Weiner, 1980). This, the generation of functionally active suppressor T cells *in vitro* by reovirus type 3 appears to be secondary to the interaction of the viral hemagglutinin with a specific receptor on the Ly2,3 subset of murine lymphocytes.

B. ROLE OF VIRUS ATTACHMENT PROTEINS IN PATHOGENICITY

The specificity of myxo- and paramyxoviruses for particular cell types depends both on the structural and functional activity of the viral surface glycoproteins and on the ability of the cells to cleave these proteins (reviewed by Choppin and Scheid, 1980). The interaction between a paramyxovirus and the cell surface is mediated by two glycoproteins projecting from the external surface of the virion: the hemagglutinin–neuraminidase (HN) and the fusion (F) glycoprotein. Binding to cellular neuraminic acid-containing receptors is a property of the HN

glycoprotein. Although this activity differs considerably according to the amount and position of neuraminic acid in the molecule (Markwell *et al.*, 1981), the abundance of sialic acid in many biologic membranes limits the importance of the binding step as a determinant of cell specificity and tissue tropism. An active fusion (F) glycoprotein, however, is a requisite for infectivity and cell-to-cell spread of infection. The fusion glycoprotein acts during viral penetration, a step beyond adsorption. Virus infectivity requires cleavage of a precursor F0 glycoprotein into two subunits (F1, F2) and the host must provide the enzyme responsible for this cleavage (reviewed in Choppin and Scheid, 1980). Thus, host-dependent cleavage of F0 is required for infectivity and therefore host range and tissue tropism of virus is determined by availability of the appropriate protease (Scheid and Choppin, 1975, 1976).

It has recently been shown that neuraminidase (NANase) activity of the HN glycoprotein of mumps virus contributes to cytopathology. Although the HN glycoproteins of the six studied strains of mumps virus are similar in size and antigenic composition, each strain possesses a neuraminidase with distinct enzymatic properties. Strains with active NANase cause little cytopathology and no cell fusion on African green kidney cell lines, whereas infection with strains having less active NANase cause extensive cell fusion. Thus, viral NANase appears to contribute to full expression of the activity of the F protein and ultimately to cytopathology (Merz and Wolinsky, 1981). This extends the previous observation that influenza virions with less active NANase cause more cytopathology in tissue culture and were more pathogenic *in vivo* than virions containing active NANase (Smith and Cohen, 1956; Choppin, 1963; Choppin and Tamm, 1964). Moreover, only strains of mumps virus with less active NANase were both neuroinvasive and neurovirulent (Wolinsky and Stroop, 1978; McCarthy *et al.*, 1980).

Thus, the pathogenicity of myxo- and paramyxovirus depends upon an interaction of viral glycoproteins and the cell surface at a step beyond viral adsorption. Cell specificity is determined by the availability of a protease on the surface of the cell to cleave one of the viral glycoproteins and allow viral penetration into the cell. There are few data concerning the role of viral receptor interactions in the pathogenicity of other enveloped viruses.

C. INDUCTION OF CELL-SPECIFIC AUTOIMMUNITY FOLLOWING VIRAL INFECTION

Autoimmune reactions against host tissue have been reported after certain viral infections. These include the production of autoantibodies against a variety of host antigens in experimental animals and man (DNA, lymphocytes, myelin) (reviewed in Onodera *et al.*, 1981), and in man, the well-documented immune-mediated damage to peripheral nerve myelin in infectious polyneuritis (Guillain-

Barre Syndrome) which can occur 2–3 weeks after viral infections or following swine flu immunization (Schonberger *et al.*, 1981). The mechanisms by which a viral infection can lead to an autoimmune response are not well understood, however, two possible mechanisms are relevant to the present review: (1) auto-antibodies which appear following viral infection may recognize shared antigens between a viral protein and a determinant on the surface of the target cell; and (2) through the idiotypic–antiidiotypic network (described in Section II,C) anti-idiotypic antibodies could be produced which recognize the viral receptor on the cell surface. Thus, the affinity of these autoantibodies for a particular cell would be identical to the tropism of the virus itself for the cell. To test these two hypotheses, we recently performed the following experiment: splenic lympho-cytes from adult mice infected with purified reovirus (type 1 or 3) particles were fused with NS-1 myeloma cells. The resultant clones were then screened by radioimmunoassay for their ability to bind virus, T lymphocytes, brain, liver, and lung tissues. We found that (1) during the course of the normal immune response to reovirus, autoantibodies were generated which reacted with normal tissue, (2) monoclonal antibodies were generated which identified shared anti-genic structures between viral determinants and normal tissue, and (3) some monoclonal antibodies appeared to have the same affinity for cells as the virus (putative antiidiotypic antibodies which recognize viral receptors) (Tardieu *et al.*, 1982).

In another group of experiments, performed by Onodera *et al.* (1981), it was shown that mice infected with reovirus type 1 developed transient diabetes and a runting syndrome. Sera of infected mice contained autoantibodies that, by immu-nofluorescence, reacted with cytoplasmic antigens of the islets of Langerhans and anterior pituitary of uninfected mice, both target structures of the virus. The autoantibodies appeared to be directed against insulin or growth hormone. Since reovirus type 3 did not induce autoantibodies to growth hormone, using recombi-nant clones, it was possible to show that the ability to induce autoantibodies to growth hormone was a property of the viral hemagglutinin (Onodera *et al.*, 1981).

VI. Conclusion

Further progress in the study of virus–receptor interactions should occur in the following three areas: (1) the development of more sophisticated approaches for both quantitative (e.g., rigorous binding studies using radiolabeled virus with high specific activity) and qualitative (e.g., cell sorting techniques) measure-ments of viral interactions with the cell surface; (2) the production of monoclonal antibodies against cell receptors and against viral components. These reagents will lead to the isolation and biologic characterization of both the CRU and the

functional domains on the virus attachment protein; and (3) techniques for the isolation of single cell suspensions from organs such as brain. This approach will allow direct study of viral interactions with biologically relevant cells. The comparison of viral–receptor studies on permanent cell lines with studies utilizing freshly isolated cells is important since there is increasing evidence that different structures might serve as receptor sites for a virus on cells of different origins.

The role of receptors in determining the *in vivo* affinity of certain nonenveloped viruses for specific cell types and thus determining viral pathogenicity is well established. It must be emphasized, however, that a cell is not permissive for a virus merely because it has a cell surface receptor to which the virus binds. Finally, although additional studies are needed, receptors may play a less important role in the pathogenicity of enveloped viruses than for nonenveloped viruses.

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