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VIRUS LIFE CYCLE

Viruses contain a genome consisting of either DNA or RNA, which is surrounded by a protein coat that is usually assembled in either an icosahedral or helical configuration. In many viruses the core is enclosed in a lipid-containing membrane called the viral envelope, which consists of a lipid bilayer containing one or more virus-coded surface glycoproteins. The structural properties of virus particles (virions) provide the basis for their classification into families. Important features that distinguish each family are the size and type of nucleic acid, the size and symmetry of the nucleocapsid, and the presence or absence of an envelope. Based on these and a few other characteristics, animal viruses have been classified into families, each of which has a characteristic virion structure and a common replication strategy.

The viral replication cycle is initiated by adsorption of the virion to host cell receptors, which are described in more detail in the next section. Following adsorption, enveloped viruses enter cells by a process of membrane fusion, which may be either low pH–dependent or pH-independent (White, 1992), and is mediated by specific viral glycoproteins. The pH-independent fusion occurs directly with the cellular plasma membrane; such fusion has been well documented for paramyxoviruses and certain retroviruses. In contrast, many other enveloped viruses enter cells by endocytosis. They subsequently are localized in endosomes, and the low pH of the endosome triggers a conformational change in the viral glycoprotein that activates their membrane fusion activity. The viral envelope then fuses with the surrounding endosomal membrane. The best-studied example of this fusion mechanism is with influenza virus, for which the molecular structure of the fusion protein (hemagglutinin) has been determined in both its neutral pH form (Wilson et al., 1981) and its low pH form (Bullough et al., 1994), demonstrating that exposure to low pH triggers a dramatic conformational change that activates fusion activity. Most nonenveloped viruses also enter cells by endocytosis, but the exact process by which their genomes subsequently are released into cells is not well understood.

All viruses share two processes that are fundamental to their replication: synthesis of proteins encoded by the viral genome and replication of the viral nucleic acid. The replication of most RNA viruses is restricted to the cytoplasm, whereas the replication of DNA viruses usually takes place in the nucleus. During replication, the biosynthetic machinery of the infected cell is diverted to synthesis of viral components. Icosahedral viruses form by a process of self-assembly; the protein subunits form a symmetrical shell (capsid) containing the viral nucleic acid. The assembly and release of enveloped viruses occurs by budding at a cellular membrane (Table 41.1). Viral membrane glycoproteins are synthesized in the rough endoplasmic reticulum and are transported through the exocytic pathway. For many enveloped viruses, assembly occurs by budding at the cell surface, and the completed virions are released immediately from the cell. However, some families of enveloped viruses are assembled by budding at intracellular membranes, as described in more detail below.

Many viruses initiate their infection processes by interaction with epithelial cells at mucosal surfaces. The cell biology of this virus–cell interaction is one of the important factors that play a role as a determinant of viral pathogenesis. In this chapter, we discuss the alternative routes of entry of viruses into mucosal epithelial cells, which are primarily determined by the distribution of viral receptors on cell surfaces. We also describe the process of viral release, which typically occurs in a polarized fashion in epithelial cells and tissues.
**VIRAL RECEPTORS**

**Definition of virus receptors**
The initial event in the viral life cycle is the interaction of one or more viral surface proteins with specific components present on the cell surface. The macromolecules on the plasma membrane that are essential for the virus to initiate the infectious cycle are designated as virus receptors. Either cell surface proteins or surface lipids may serve as receptors for specific viruses. The presence or absence of suitable receptors is an important factor for determining whether a cell is sensitive or resistant to infection by a specific virus. Therefore, the tropism of a virus, e.g., for neural, respiratory, or intestinal cells, is often determined by the level of expression of virus receptors in such tissues. Multiple noncovalent interactions between viral proteins and cell surface components are required to mediate the specific binding of a virus to a cell. The residues of the viral proteins that are involved in this interaction are designated as the receptor binding site. In the case of enveloped viruses, they usually are part of an individual protein, e.g., in the influenza hemagglutinin, where they are arranged in the form of a pocket at the tip of the glycoprotein molecule. The residues of the receptor binding site may, however, belong to more than one viral polypeptide, as in the case of the nonenveloped polioviruses, where they are part of a canyon-like depression in the capsid structure. The portion of the receptor that is actually interacting with the virus, the receptor determinant, may be composed of a number of amino acids, as in the case of CD4, the receptor for human immunodeficiency virus (HIV), or intercellular adhesion molecule 1 (ICAM-1), the receptor for most strains of rhinoviruses.

Influenza viruses and several other viruses specifically recognize sialic acid residues. For these viruses, sialic acid functions as a receptor determinant. It should be noted that it is not appropriate to designate sialic acid as the receptor, because free sialic acid is bound very weakly by virions and, more important, is unable to mediate the infection of cells. It is functional only when present as a constituent of a glycoprotein or glycolipid. Therefore, cell surface sialoglycoconjugates are the receptors for the viruses that recognize sialic acids.

**Cellular receptors for virus attachment**
To identify a specific surface protein as a virus receptor, an approach involving the following two criteria has been most successful: a monoclonal antibody directed against this protein prevents virus infection; and receptor-negative cells become sensitive to virus infection after transfection with the receptor-encoding gene from receptor-positive cells. By these approaches, receptors for a number of viruses have been identified and their functions have been verified by additional evidence such as tissue distribution and in vitro binding studies (Table 41.2). However, the initiation of virus attachment involving the fusion of the virus envelope with the plasma membrane in the case of enveloped viruses is often an essential step for the productive infection of the cell.

**Table 41.1. Cellular Sites of Replication and Assembly of Selected Virus Families**

| Virus Family | Envelope | Site of Replication and Neocapsid Assembly | Site of Budding (for enveloped viruses) |
|--------------|----------|------------------------------------------|----------------------------------------|
| RNA viruses  |          |                                          |                                        |
| Arenavirus   | +        | Cytoplasm                                | Plasma membrane                        |
| Bunyavirus   | +        | Cytoplasm                                | Golgi complex                          |
| Coronavirus  | +        | Cytoplasm                                | Rough endoplasmic reticulum            |
| Orthomyxovirus | +      | Nucleus and cytoplasm                    | Plasma membrane                        |
| Paramyxovirus| +        | Cytoplasm                                | Plasma membrane                        |
| Picornavirus | −        | Cytoplasm                                |                                        |
| Calicivirus  | −        | Cytoplasm                                |                                        |
| Astrovirus   | −        | Cytoplasm                                |                                        |
| Reovirus     | −        | Cytoplasm                                |                                        |
| Retrovirus   | +        | Nucleus and cytoplasm                    | Plasma membrane                        |
| Rhabdovirus  | +        | Cytoplasm                                | Plasma membrane                        |
| Filovirus    | +        | Cytoplasm                                | Plasma membrane                        |
| Togavirus    | +        | Cytoplasm                                | Plasma membrane                        |
| Flavivirus   | +        | Cytoplasm                                | Endoplasmic reticulum                  |
| DNA viruses  |          |                                          |                                        |
| Adenovirus   | −        | Nucleus                                  |                                        |
| Herpesvirus  | +        | Nucleus                                  | Nuclear envelope                       |
| Hepadnavirus | +        | Nucleus                                  | Endoplasmic reticulum                  |
| Papillomavirus| –       | Nucleus                                  |                                        |
| Parvovirus   | –        | Nucleus                                  |                                        |
| Polyomavirus | –        | Nucleus                                  |                                        |
| Poxvirus     | +        | Cytoplasm/plasma membrane                | Cytoplasm                              |
Table 41.2. Viruses that Recognize Defined Proteins as Virus Receptors

| Virus                                      | Receptor                              | Reference                        |
|--------------------------------------------|---------------------------------------|----------------------------------|
| **Retroviruses**                           |                                       |                                  |
| HIV                                        | CD4                                   | Maddon et al., 1996              |
| *Murine leukemia viruses*                  |                                       |                                  |
| ecotropic (murine cells)                   | Cationic amino acid                   | Albritton et al., 1989           |
| amphotropic (murine and other cells)       | Phosphate transporter (GLVR2)         | Miller et al., 1994; Zeijl et al., 1994 |
| Gibbon ape leukemia virus                  | Phosphate transporter (GLVR1)         | O’Hara et al., 1990              |
| Feline leukemia virus, avian leukosis      | Phosphate transporter (GLVR1)         | Takeuchi et al., 1992            |
| sarcoma virus                              |                                       |                                  |
| Subgroup A                                 | ALSV-A receptor                       | Bates et al., 1993               |
| Subgroups B and D                          | Cytopathic ALSV receptor (CAR1)       | Brojatsch et al., 1996           |
| Bovine leukemia virus                      | BLV receptor                          | Ban et al., 1993                 |
| **Picornaviruses**                         |                                       |                                  |
| Poliovirus                                 | Poliovirus receptor                   | Mendelsohn et al., 1989          |
| Rhinovirus, major serogroup                | Intercellular adhesion molecule (ICAM-1) | Greve et al., 1989; Staunton et al., 1989 |
| Rhinovirus, minor serogroup                | Low-density lipoprotein receptor      | Hofer et al., 1994               |
| Echoviruses 1 and 8                        | Integrin (VLA-2)                      | Bergelson et al., 1992           |
| Echovirus 7                                | Decay-accelerating factor (DAF; CD55) | Bergelson et al., 1992; Ward et al., 1994 |
| Coxsackievirus B                           | Coxsackievirus-adenovirus receptor (CAR) | Bergelson et al., 1997         |
| Hepatitis A virus                          | HAVcr-1                               | Kaplan et al., 1996; Huber et al., 1994 |
| Encephalomyocarditis virus                 | Vascular cell adhesion molecule (VACM-1) |                                  |
| **Togaviruses**                            |                                       |                                  |
| Sindbis virus                              | Laminin receptor                      | Wang et al., 1992                |
| **Coronaviruses**                          |                                       |                                  |
| TGEV, FIPV, CCoV, HCoV-229E                | Aminopeptidase N                      | Delmas et al., 1992; Yeager et al., 1992 |
| Mouse hepatitis virus                      | Biliary glycoprotein (MHVR)           | Dveksler et al., 1991            |
| SARS-CoV                                   | Angiotensin-converting enzyme 2       | Li et al., 2003                   |
| **Paramyxovirus**                          |                                       |                                  |
| Measles virus                              | Membrane cofactor protein (MCP, CD46), signaling leukocyte activation molecule (SLAM) | Naniche et al., 1993; Dong et al., 1993; Tatsuo et al., 2000 |
| **Adenoviruses**                           |                                       |                                  |
| Adenovirus 2                               | Coxsackievirus-adenovirus receptor (CAR) | Bergelson et al., 1997         |
| **Herpesviruses**                          |                                       |                                  |
| Herpes simplex virus 1 and 2               | Nectin-1, nectin-2                    | Geraghty et al., 1998            |
| Pseudorabies virus                         | Tumor necrosis factor receptor family | Montgomery et al., 1996         |
| Epstein-Barr virus                         | Complement receptor 2 (CD21)          | Frade et al., 1985; Nemerow et al., 1985 |
|                                                                                   |                                      |                                  |
infection may be more complex than a simple interaction of the virus with a single receptor. For example, viruses may use alternative surface constituents for attachment to cells. HIV has been reported to attach not only to CD4 (Dalgleish et al., 1984) but also to galactosyl ceramide (Bhat et al., 1991), although with lower affinity. It also requires an interaction with specific coreceptors, CXCR4 or CCR5, to initiate infection (see the following section).

The criteria for receptor identification mentioned previously apply only to defined protein receptors. A large number of viruses recognize determinants that may be present on multiple surface components (Table 41.3). Herpes simplex viruses use glycosaminoglycan chains of surface proteoglycans for attachment to cells (WuDunn and Spear, 1989). Influenza viruses and several members of the paramyxoviruses and coronavirus are known to recognize sialic acids (a designation for all derivatives of neuraminic acid). The importance of this acidic sugar for virus binding can be demonstrated by the inactivation of receptors after treatment with sialidase (neuraminidase) and by the regeneration of receptors by enzymatic resialylation of cells. In this way it has been shown that these viruses may have a strict preference for a certain type of sialic acid. While influenza A and B viruses have a preference for N-acetyleneuraminic acid (Rogers and Paulson, 1983), influenza C virus recognizes only N-acetyl-9-O-acetylneuraminic acid as a receptor determinant (Herrler et al., 1985). Furthermore, virus binding may be restricted to sialic acids that are connected to the adjacent sugar in a defined linkage type.

Human influenza viruses, for example, have a clear preference for N-acetylneuraminic acid attached to galactose in an α-2,6-linkage (Rogers and Paulson, 1983). This example already shows that it is not appropriate to designate sialic acid as the receptor, because only those surface glycoconjugates that contain the correct type of sialic acid in the correct linkage may serve as receptors for these viruses. Another restriction in the recognition of sialic acid-containing receptors is imposed by the fact that the interaction between individual sialic acid residues and the influenza hemagglutinin is rather weak. Therefore, an efficient binding of influenza viruses to the cell surface requires a multivalent interaction between the virus and its cellular receptors, and the number of sialic acid residues and their spatial orientation are important factors for a receptor for influenza viruses. Mucin-type glycoproteins that are highly glycosylated and contain clusters of O-linked oligosaccharides are expected to be suitable receptors. Among the surface proteins of Madin-Darby canine kidney (MDCK) cells, influenza C virus was found to bind primarily to a mucin-type glycoprotein designated gp40 (Zimmer et al., 1995). Therefore, this sialoglycoprotein is a potential receptor for the infection of MDCK cells by influenza C virus. Sialic acid may serve as a receptor determinant not only on glycoproteins but also on glycolipids. The potential role of specific gangliosides as receptors has been demonstrated for Sendai virus, a member of the paramyxovirus family (Markwell et al.,

Table 41.3. Viruses that Recognize the Carbohydrate Portion of Glycoproteins as Receptor Determinants

| Viruses                                      | Receptors                                      | Receptor Determinant | Reference            |
|----------------------------------------------|-----------------------------------------------|----------------------|----------------------|
| Influenza A and B                            | Sialoglycoconjugates                           | Neu5Ac               | Carrol and Paulson, 1983 |
| Influenza C                                  | Sialoglycoconjugates (gp40)                    | Neu5,9Ac₂           | Herrler and Klenk, 1987 |
| Paramyxoviruses                              | Sialoglycoconjugates (gangliosides)            | Neu5Ac               | Markwell et al., 1981 |
| Coronaviruses BCoV, HCoV-OC43                | Sialoglycoconjugates (gp40)                    | Neu5,9Ac₂           | Shultzte et al., 1996 |
| Reoviruses                                   | Sialoglycoconjugates                           | Sialic acid          | Paul et al., 1989    |
| Rotaviruses                                  | Sialoglycoconjugates                           | Sialic acid          | Yolken et al., 1987  |
| Polyomaviruses                               | Sialoglycoconjugates (gp40–42)                 | Sialic acid          | Fried et al., 1981   |
| Canine parvovirus                            | —                                             | Sialic acid          | Basak et al., 1994   |
| Herpes simplex virus                         | Proteoglycans                                  | Glycosaminoglycans   | WuDunn and Spear, 1989 |
| Pseudorabies virus                           | Proteoglycans                                  | Glycosaminoglycans   | Mettenleiter et al., 1990 |
| Respiratory syncytial virus                  | Proteoglycans                                  | Glycosaminoglycans   | Krasat and Strecker, 1997 |
| Theiler's murine encephalomyelitis virus      | Sialoglycoconjugates                           | Sialic acid          | Zhou et al., 1997    |
| Porcine reproductive and respiratory syndrome virus | Proteoglycans                                  | Glycosaminoglycans   | Reddi and Lipton, 2002 |
| Adeno-associated virus-2                     | Proteoglycans                                  | Glycosaminoglycans   | Summerford and Samulski, 1998 |
| HIV                                          | Galactosyl ceramide                            |                      | Bhat et al., 1991    |
| Parovirus B19                                 | P antigen (globoside)                         |                      | Brown et al., 1993   |
1981). Irrespective of the binding to a glycoprotein or to a glycolipid, for the reasons given earlier, only a subset of the surface sialglycoconjugates are expected to be suitable receptors for viruses that use sialic acid as a receptor determinant.

Viruses may also attach to the cell surface and initiate infection without involving their own receptor-binding activity. HIV has been shown to incorporate cell surface components such as ICAM-1 and cyclophilin A into the viral membrane (Fortin et al., 1997; Saphire et al., 1999); the binding activities of these molecules allow HIV to attach to cells by interacting with lymphocyte function-associated molecule 1 (LFA-1) and surface-bound heparin sulfate structures, respectively. Virus attachment may also be mediated by cellular C-type lectin receptors that recognize the mannosereich oligosaccharide side chains on the surface protein gp120 of HIV (Curtis et al., 1992). Some of these binding activities may be used in a concerted action and enable the virus to attach to cells that express only a low level of CD4. Cells containing receptors for immunoglobulins may be infected by viruses that are complexed with nonneutralizing antibodies (Porterfield, 1986). This mechanism of virus entry has been described for several viruses, e.g., HIV and dengue virus. Overall, the availability of alternative binding strategies may broaden the spectrum of cells that are susceptible to infection.

**Cellular receptors for virus entry**

Virus entry is best understood in the case of viruses with a lipid envelope (see first section of text), which introduce their genome into the cell by a fusion event between the viral and the cellular membrane. Whether fusion occurs by a low-pH-dependent or a pH-independent process, it usually requires a conformational change of the viral fusion protein just prior to the fusion reaction. In this process a fusion-active domain is exposed and thus is able to interact with the target membrane. With paramyxoviruses, in which the receptor-binding activity and the fusion activity are located on different surface proteins, fusion occurs at the plasma membrane. The receptor binding protein (HN) interacts with the fusion protein (F). Binding to sialic acid residues of cell surface receptors is thought to trigger a conformational change in the HN protein that induces the F protein to adapt the fusion-active form (Lamb, 1993).

Cellular proteins may also be involved in the virus-induced fusion reaction. In the case of HIV, binding to CD4 results in attachment to cells but not in virus entry. Chemokine receptors have been shown to be required for the fusion of the viral membrane with the host cell membrane (Weiss and Clapham, 1996). It has been suggested that binding of the viral protein gp120 to CD4 results in a conformational change in gp120, and this in turn results in the creation of a new recognition site for a chemokine receptor. The interaction of gp120 with the chemokine receptor is thought to trigger the fusion activity of the transmembrane protein gp41 and is an important determinant of the viral tropism.

Macrophage-tropic strains that predominate in the early stage of an HIV infection use a different type of receptor (CC-CCR5) than do T-cell tropic strains that are more prominent in later stages of infection, which use the receptor CXCR-4. This finding shows that there is a high degree of flexibility in the interaction of the viral surface proteins with cellular receptors. Some strains of HIV have been reported to use chemokine receptors even to infect CD4-negative cells. A role for cellular proteins in mediating the fusion reaction after virus attachment has also been reported for herpes simplex virus. Following primary attachment via the surface glycoproteins gC and gB to glycosaminoglycans on surface proteoglycans (WuDunn and Spear, 1989), another surface protein, gD, binds to a specific receptor of the nectin family of surface proteins (Krummenacher et al., 1998). This interaction enables HSV—in a reaction that also involves the additional viral glycoproteins gB, gH, and gL—to initiate the fusion between the viral and the cellular membrane.

There is no generally accepted designation for cellular proteins involved in the process of virus entry following the attachment to the plasma membrane, but designations such as entry mediators, coreceptors, or entry cofactors have been used to distinguish them from attachment receptors. However, in some cases it may be difficult or impossible to differentiate between the proteins involved in attachment and those involved in virus entry, because some variants or strains of viruses may have evolved to use the latter cellular proteins also for virus attachment.

**Virus receptors on epithelial cells**

The presence or absence of suitable surface receptors is a critical determinant for the sensitivity or resistance, respectively, of cells to virus infection. The plasma membrane of epithelial cells is divided into an apical domain and a basolateral domain that differ from each other in their composition. As a consequence, virus receptors may be present on one domain of the cell surface and absent from the other. A polarized distribution of virus receptors is expected to have important implications for virus infections. If a virus receptor is restricted to the apical surface, virus infection is possible only through this membrane domain and, in the context of an organism, only via the lumen of the body cavity that is lined by the respective epithelium (Table 41.4). An example of an apical virus receptor is aminopeptidase N, which serves as a receptor for porcine transmissible gastroenteritis virus (TGEV) and related human, canine, and feline coronaviruses (Delmas et al., 1992). These viruses enter the organism via the respiratory or gastrointestinal tract, causing localized infections of the respective epithelium, and therefore require virus receptors only on the apical surfaces of epithelial cells. The surface distribution of aminopeptidase N is consistent with the role of this protein as a coronavirus receptor. Apical surface receptors are also required for infections by human influenza viruses.

Sialic acid, the receptor determinant recognized by these viruses, is abundantly present on both the apical and...
basolateral surfaces of most epithelial cells. As mentioned in the preceding section, there are several restrictions in the recognition of sialic acids. Therefore, only a limited number of surface sialoglycoconjugates are expected to fulfill the requirements for an influenza virus receptor. Suitable receptors appear not to be present on all epithelial cells of the respiratory tract. Within the tracheal epithelium, human influenza A viruses were found to bind to ciliated cells but not to nonciliated cells, suggesting that functional receptors are expressed on the surface of the former cells (Couceiro et al., 1993). A further limitation in the use of sialoglycoconjugates as receptors is imposed by the requirement of influenza viruses for endocytotic uptake. In this context it is interesting to note that glycoprotein gp40, a potential receptor for influenza C virus, is subject to endocytosis at a rate that is similar to the kinetics of virus internalization (Zimmer et al., 1995). Localization on the apical surface of epithelial cells is also characteristic of proteins that are membrane-anchored by glycosol phosphatidyl inositol residues. Decay accelerating factor, which belongs to this group of proteins, is a receptor for some members of the picornavirus family (Ward et al., 1994).

Virus receptors on the basolateral plasma membrane domain are required for viruses that approach epithelial cells from the serosal side, e.g., after spread via the blood stream. Both CD4 and galactosyl ceramide, an alternative attachment receptor for HIV, have been reported to be localized on the basolateral side of epithelial cells (Yahi et al., 1992), and this may explain the intestinal tropism of HIV. Canine parvovirus has also been shown to infect epithelial cells via the basolateral domain (Basak and Compans, 1989). This entry site is consistent with the basolateral location of the transferrin receptor, which has been identified as a receptor for canine and feline parvoviruses (Parker et al., 2001). The viruses for which receptor expression is restricted to the basolateral domain may nevertheless enter through the lumen of the intestine and be transported to the basolateral surface by binding to M cells, followed by transcytosis. Several viruses, including reoviruses, poliovirus, and HIV, have been reported to be transported efficiently across the M cells by transcytosis (Amerongen et al., 1991), after which they could initiate a retrograde infection of the epithelial cell layer.

Membrane cofactor protein (CD46), a regulatory protein of the complement system, has been identified as a receptor for measles virus (Naniche et al., 1993). With several epithelial cells, CD46 has been shown in vivo and in vitro to be a basolateral protein (Maisner et al., 1996). This distribution is consistent with the infection of epithelial cells in the late

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Table 41.4. Sites of Entry and Release of Selected Viruses in Polarized Epithelial Cells

| Virus (family, species) | Cell Type | Site of Entry | Site of Release | References |
|------------------------|-----------|---------------|-----------------|------------|
| **Bunyavirus**          |           |               |                 |            |
| Punta Toro             | Vero C1008| ND            | Basolateral     | Chen et al., 1991 |
| Black Creek Canal      | Vero C1008| Apical        | Apical          | Ravkov et al., 1997 |
| **Coronavirus**        |           |               |                 |            |
| Transmissible gastroenteritis | LLCPK1 | Apical        | Apical          | Rossen et al., 1994 |
| Bovine coronavirus      | MDCK      | Apical        | Apical          | Schultz et al., 1996 |
| Mouse hepatitis        | Murine kidney | Apical    | Basolateral     | Rossen et al., 1995 |
| **Orthomyxovirus**     |           |               |                 |            |
| Influenza              | MDCK      | Nonpolar      | Apical          | Rodriguez-Boulan and Sabatini, 1978; Fuller et al., 1984 |
| **Paramyxovirus**      |           |               |                 |            |
| Measles                | Caco-2, Vero C1008 | Apical         | Apical          | Blau and Compans, 1995 |
| Sendai wild type       | MDCK      | ND            | Apical          | Rodriguez-Boulan and Sabatini, 1978 |
| Sendai F1-R mutant     | MDCK      | Bidirectional |                 | Tashiro et al., 1990 |
| **Retrovirus**         |           |               |                 |            |
| HIV-1                  | Vero C1008| ND            | Basolateral     | Owens et al., 1991 |
| **Rhabdovirus**        |           |               |                 |            |
| Vesicular stomatitis   | MDCK      | Basolateral   | Basolateral     | Rodriguez-Boulan and Sabatini, 1978; Fuller et al., 1984 |

ND, not determined
stage of a measles virus infection, when the virus spreads from the bloodstream to different epithelial tissues. In CaCo-2 cells, CD46 has been shown to be localized predominantly on the apical surface (Blau and Compans, 1995). An apical localization of CD46 would be consistent with the initial stage of the measles virus infection, when the virus enters the organism via the respiratory tract. However, CD46 serves as a receptor only for vaccine strains that are applied by injection and do not enter the organism via respiratory infection.

Signaling leukocyte activation molecule (SLAM) has been shown to function as a high-affinity receptor for wild-type measles virus (Tatsuo et al., 2000). This protein is present on various types of lymphocytes and activated macrophages but absent from epithelial, endothelial, and neuronal cells. SLAM-negative cells are infected with low efficiency, possibly by using a so far unidentified low-affinity-receptor (Hashimoto et al., 2002). Such a receptor may also be involved in the apical infection of the respiratory epithelium in the initial stage of the measles virus infection. Alternatively, measles virus may cross the epithelial barrier through sites where the integrity of the epithelial sheath is damaged or by intercellular passage of infected macrophages.

Some viruses make use of the asialoglycoprotein receptor or the immunoglobulin receptor to initiate infection. Both proteins are localized on the basolateral surface of epithelial cells. The asialoglycoprotein receptor has been reported to be a potential receptor for Marburg virus (Becker et al., 1995), and its presence on hepatocytes may explain the hepatotropism of this virus, although different receptors may be required to infect other tissues. The receptor for polymeric IgA has been reported to mediate the entry of antibody-complexed Epstein-Barr virus into an established human epithelial cell line (Sixbey and Yao, 1992). While CD21 is used by this virus to infect B lymphocytes and several epithelial cells, the antibody-dependent route is an alternative way to enter epithelial cells. To what extent this mechanism contributes to the infection of the nasopharyngeal epithelium remains to be established.

Polarity of virus entry

The examples presented in the preceding section show how the surface distribution of receptors affects the way in which a virus enters an epithelial cell. At present, our knowledge about this aspect of virology is very limited because the identity of the majority of virus receptors has not yet been elucidated. In addition, several cellular proteins, acting either as alternative receptors or in a consecutive way, may be involved in the initial stage of virus infection. The polarized distribution of any of these cell surface constituents, whether it is required for the attachment or for the entry step, may restrict the uptake of a virus to a specific domain of the plasma membrane. For some viruses, polarity of virus entry (see Table 41.4) has been demonstrated, although their receptors have not yet been identified. In the case of SV40, a nonenveloped virus, attachment to the cell surface and infection have been detected only via the apical membrane domain of epithelial cells (Clayson and Compans, 1988). On the other hand, vesicular stomatitis virus and vaccinia virus, two enveloped viruses, infect epithelial cells predominantly via the basolateral domain (Fuller et al., 1984; Rodriguez et al., 1991). The cellular receptors for these viruses appear to be restricted to the respective segment of the plasma membrane.

Virus entry is not polarized for all viruses. The receptor for poliovirus, which is a structural homolog of the nectin family of adhesion molecules, is present mainly on the basolateral surface of epithelial cells (Tucker et al., 1993a). Nevertheless, a significant fraction of it was detected on the apical membrane domain. Recognition of the 65-kD protein by poliovirus is so efficient that virus infection is possible from both the serosal and the luminal side (Tucker et al., 1993a). However, the poliovirus receptor is present also on M cells and germinal centers within Peyer's patches (Iwasaki et al., 2002). Therefore, poliovirus enters the organism not necessarily via the apical surface of the intestinal epithelium. In contrast to the poliovirus receptor, the mucin-like glycoprotein gp40 is localized predominantly on the apical surface of the MDCK-1 cells, with only a minor fraction detectable on the basolateral side (Zimmer et al., 1995). This glycoprotein is the major surface protein of MDCK-1 cells recognized by influenza C virus and bovine coronavirus. Despite this similarity, bidirectional entry was found for the influenza C virus, as has been shown also for influenza A viruses (Fuller et al., 1984; Schultzze et al., 1996). On the other hand, coronavirus was able to infect only via the apical surface (Schultzze et al. 1996). It remains to be shown whether bovine coronavirus is unable to recognize the low amount of gp40 present on the basolateral surface or whether it requires an additional cellular surface protein that is present only on the apical membrane domain.

A complex picture has been described for the entry of herpes simplex virus into MDCK cells. While wild-type virus can initiate infection via both domains of the plasma membrane, mutants lacking either of the viral surface glycoproteins gC or gG are restricted in virus entry to the basolateral side (Tran et al., 2000). The exact function of gG is unclear, but it appears to be involved in a postattachment step of virus entry. The viral glycoprotein gC mediates the binding to glycosaminoglycans on surface proteoglycans (see later discussion). Such glycan structures are present on both surfaces of MDCK cells, consistent with the bidirectional entry of wild-type HSV. As mentioned earlier in this chapter, entry of herpes simplex viruses or pseudorabies virus requires the interaction of glycoprotein gD with a member of the nectin family of adherence proteins. This cellular receptor for herpesvirus colocalizes with adherence junctions and may explain infection via the basolateral membrane by viruses spreading from neurons to epithelial cells. However, the cellular localization of nectins does not provide a straightforward explanation of infection via the apical membrane. Disruption of tight junctions results in redistribution of nectins from the junction sites to the whole cell surface, with a concomitant increase in susceptibility to infection by herpes simplex virus (Yoon and Spear, 2002). Thus, virus approaching the epithelium from the apical side may enter the organism by infection of cells that have lost their polarized organization or by
using a receptor different from nectin in the adherence junction, possibly with the help of glycoprotein gG.

Colocalization with cellular junction complexes is not unique to herpesvirus receptors. Both the receptor for several coxsackieviruses and adenoviruses, CAR (Bergelson et al., 1997), and the junction adhesion molecule, a reovirus receptor (Barton et al., 2001), are integral tight-junction proteins. This location is consistent with the inefficiency of these viruses to infect epithelial cells via the apical plasma membrane (Rubin, 1987; Walters et al., 1999). As far as adenoviruses are concerned, major histocompatibility complex (MHC)-I-α-2 protein, a potential alternative attachment receptor, and integrins αVβ3, which facilitate virus entry, are also basolateral proteins and not available for apical infection. Therefore, these viruses are efficient in entering epithelial cells from the basolateral side. For initial infection of an organism, they depend on other entry strategies: they may infect the epithelium at sites where the polarized organization of the cells is disturbed; they may use receptors that have not yet been identified; they may be taken up by a nonspecific mechanism (pinocytosis); and they may be transcytosed by M cells, as has been reported for reoviruses. These examples not only illustrate the complexity of virus–receptor interactions in the infection of epithelial cells but also demonstrate the importance of the identification of additional virus receptors to understand this process.

**Accessory factors affecting virus entry into epithelial cells**

The fusion activity of many viral surface glycoproteins depends on a posttranslational proteolytic cleavage event. At the cleavage site, most of the fusion proteins contain a motif of several basic amino acids that is recognized by furin-like proteases. Such proteases are encountered by the viral glycoproteins during passage through the secretory pathway of most cells. The hemagglutinin of human influenza viruses, however, contains a single arginine at the cleavage site, requiring a trypsin-like protease for the activation process. Such a protease, trypsin/CLARA, is secreted by Clara cells that are part of the respiratory epithelium (Kido et al., 1992). Influenza viruses that are released into the lumen of the respiratory tract can be converted by this protease into the infectious, fusogenic form. The availability of an enzyme for proteolytic activation is one factor that restricts the infection by human influenza viruses to the respiratory epithelium. Proteolytic activation events also are known to be important for the initiation of infection by rotaviruses and reoviruses, which encounter the appropriate enzymes in the lumen of the intestinal tract. Thus, epithelial cells may provide conditions that favor the infection by certain viruses.

On the other hand, the environmental conditions of some epithelia may exclude the infection by certain viruses. In order to infect an organism in the intestinal epithelium via the oral route, viruses have to survive the harsh conditions encountered within the gastrointestinal tract: low pH, proteolytic enzymes, and bile salts. The detergent-like action of bile salts is expected to be especially detrimental for enveloped viruses and explains why intestinal infections are caused mainly by nonenveloped viruses, e.g., rotaviruses, caliciviruses, and enteroviruses.

Coronaviruses are exceptional among viruses with an intestinal tropism, because they contain a lipid envelope. Porcine TGEV is an enteropathogenic coronavirus. As mentioned earlier, TGEV uses aminopeptidase N as a receptor to infect cells of the intestinal epithelium. In addition, it has a sialic acid binding activity. This binding activity is dispensable for infection of cultured cells. However, loss of the sialic acid binding activity, e.g., by a point mutation, results in the loss of enteropathogenicity (Krempl et al., 1997). It has been proposed that the ability of TGEV to recognize sialic acid results in the binding of sialoglycoconjugates to the viral surface. Sialylated cellular components such as intestinal mucus that are bound to the viral surface glycoprotein may increase virus stability and help it to survive the detrimental action of bile salts. In addition, binding to sialoglycoproteins may enable TGEV to attach to and penetrate through the glyocalyx covering the apical membrane of intestinal cells (Schwegmann-Wessels et al., 2002). The glyocalyx has been shown to prevent apical infection of epithelial cells by adenovirus, even when the CAR receptor was redirected to the apical membrane (Pickles et al., 2000), and treatment of cells with neuraminidase abolished this inhibitory effect. Therefore, the viral receptor–destroying enzymes of influenza viruses, paramyxoviruses, and coronaviruses (neuraminidases or acetylenes) may be required not only for release from the infected cell but also for penetration through the glyocalyx. A hydrolytic enzyme activity that acts on mucus has also been ascribed to the gamma1 protein of reoviruses (Bisaillon et al., 1999). This enzyme facilitates the penetration through the protective barrier of the mucus layer covering the intestinal epithelium. Thus, infection of mucosal surfaces may require viruses to evolve protective mechanisms to survive under specific environmental conditions.

**Polarized release of viruses from epithelial cells**

**Virus assembly at the plasma membrane**

Early studies of the release of influenza virus from polarized cells by Murphy and Bang (1952) reported that influenza virus is assembled and released by budding from the surface of the chorioallantoic membrane of embryonated eggs. They also observed that the release of the virus was polarized, occurring exclusively at the free apical surface. Rodriguez-Boulan and Sabatini (1978) reported the directional budding of enveloped viruses from polarized MDCK cells; vesicular stomatitis virus (VSV) was released predominantly from the basolateral plasma membrane, whereas influenza and Sendai virions were released from the apical domain. Subsequent studies with many viruses have revealed that viruses which assemble by budding at the plasma membrane are usually released from epithelial cells in a polarized
fashion and that such directional release also occurs with some nonenveloped viruses.

The glycoproteins of enveloped viruses accumulate at the site of virus assembly, and association of viral core proteins with the viral glycoproteins leads to virus release by a process of budding, or outfolding, of the membrane. The glycoproteins of a number of enveloped viruses that assemble at the plasma membrane were found to be directionally transported to the same surface from which virus buds, even when expressed from recombinant vectors in the absence of other virus-specific proteins (Roth et al., 1983; Jones et al., 1985; Stephens et al., 1986). These observations led to the hypothesis that the site of plasma membrane accumulation of the envelope glycoprotein(s) determines the site of viral assembly. Studies of the assembly and release of HIV particles in polarized epithelial cells supported this hypothesis (Owens et al., 1991). Expression of the HIV core (Gag) protein in epithelial cells in the absence of the envelope glycoprotein resulted in the assembly and release of HIV-like particles in approximately equivalent amounts from both the apical and basolateral surfaces. In contrast to this nondirectional pattern of release, coexpression of the Gag and envelope proteins resulted in directional release of viral particles at the basolateral domain. The HIV envelope glycoprotein, when expressed from a recombinant vector, is almost exclusively found at the basolateral surface (Owens and Compans, 1989), indicating that its interaction with the core proteins determines the site of viral assembly and release.

In contrast to these observations, recent studies have provided evidence that factors other than the glycoproteins of some enveloped viruses can play a key role in determining the site of virus release. Measles virus was released from the apical surface of polarized epithelial cells, although its surface glycoproteins H and F were expressed at high levels on the basolateral membranes (Maisner et al., 1998). The glycoprotein (GP) of Marburg virus, a filovirus, was transported preferentially to the apical surfaces of polarized MDCK cells, whereas the release of infectious monkey virus occurs at the basolateral surface (Sanger et al., 2001). A VSV mutant was also constructed in which the glycoprotein lacked a basolateral targeting signal and was expressed in a nonpolarized fashion; however, release of the virus still occurred mainly at the basolateral surface (Zimmer et al., 2001). All of these viruses possess a matrix protein that is believed to play a major role in virus assembly and could be responsible for determining the site of viral assembly and release. Such a role for the matrix protein has been demonstrated in the case of measles virus (Naim et al., 2000).

Virus assembly at intracellular membranes

Bunyaviruses and coronaviruses are two families of enveloped RNA viruses that are assembled by budding at intracellular membranes. Assembly of most bunyaviruses occurs by budding at smooth-surfaced membranes in the Golgi complex (Murphy et al., 1973; Smith and Pifat, 1982). Punta Toro virus, a member of the sandfly fever group of bunyaviruses, was assembled in the Golgi complex and subsequently released almost exclusively from the basolateral surfaces of polarized epithelial cells (Chen et al., 1991). Immunoelectron microscopic analysis of hepatocytes infected with another bunyavirus, Rift Valley fever virus, also indicated preferential release from the basolateral domain (Anderson and Smith, 1987). However, Rift Valley fever virus budding at the basolateral plasma membrane was also sometimes observed, indicating that at least one of the viral components contains the appropriate signals to direct vectorial transport of the viral proteins to the basolateral surface. The available evidence indicates that the viruses which assemble at intracellular membranes are transported to the cell surface by vesicular transport, thus resembling secretory proteins. The polarized release of enveloped virions may therefore be analogous to the directional release of endogenous secretory proteins from polarized cells. A possible mechanism for such polarized secretion involves a specific interaction with a membrane-bound receptor, which is targeted to a specific plasma membrane domain. In the case of some viruses, it is possible that the polarized transport of the viral receptor itself could play a role in such targeting. However, in other viruses, entry and release occur at opposite sides of epithelial cell layers, and other mechanisms must be involved in the release process. Release of certain viruses could occur by a default pathway for secretion, which may be directionally or nondirectionally, depending on the cell type examined.

Nonenveloped viruses

The assembly and release of nonenveloped viruses in epithelial cells has only been investigated to a limited extent, and the mechanism of release of such viruses is not well understood. However, studies with SV40 and poliovirus have indicated that nonenveloped viruses may also be targeted for release at a particular plasma membrane domain. SV40 is a nonenveloped DNA virus that is assembled in the nucleus of infected cells, and virions were found to be almost exclusively released from the apical surfaces of polarized monkey kidney epithelial cells (Clayson et al., 1989). It was also found that treatment of infected cells with the sodium ionophore monensin, which is known to be an effective inhibitor of vesicular transport, resulted in the inhibition of SV40 release but had no inhibitory effect upon viral protein synthesis or the intracellular assembly of infectious virus. High levels of SV40 release were observed prior to detectable cell lysis, and numerous virions were found to be enclosed within membrane-bound cytoplasmic vesicles during the period of maximal viral release (Clayson et al., 1989). These results suggested that the vectorial transport and release of SV40 may be mediated by a vesicular transport mechanism. Since the SV40 receptor is expressed on apical surfaces (Clayson and Compans, 1988), targeting of progeny virions to this domain might be mediated by their association with membrane-bound viral receptor molecules within transport vesicles.

Poliovirus is a nonenveloped RNA virus that replicates and is assembled in the cytoplasm, and progeny virions are
observed free within the cytoplasm or within membrane-enclosed vesicular bodies (Dales et al., 1965; Suhy et al., 2000). Poliovirus was found to be released predominantly from the apical surfaces of infected human Caco-2 intestinal cells (Tucker et al., 1993a). Although the mechanism of vectorial release is unclear, the targeting of poliovirus-containing vesicles or cytoplasmic aggregates to the apical plasma membrane may be involved.

Adenovirus was found to be released preferentially from basolateral surfaces of human airway epithelial cells (Walter et al., 2002). Subsequent to release, the integrity of the epithelial cell layers was disrupted in a process mediated by binding of the viral fiber protein to its receptor, CAR, allowing the virus to be released by paracellular passage to the apical surface. Further work is needed to unravel the process by which such nonenveloped viruses are directionally released from cells.

**POLARIZED ENTRY AND RELEASE AS DETERMINANTS OF VIRAL PATHOGENESIS**

The finding of polarized entry and release of viruses in epithelial cells has led to increasing interest in the importance of such processes within the infected organism. There are several alternative mechanisms by which viruses may traverse epithelial cell layers, and the cell biology of virus infection could play a role in this process. If a viral receptor molecule is localized exclusively on basolateral surfaces, the barrier to virus entry at epithelial tissues is more substantial than if the receptor is expressed on the apical surface or is nonpolarized. Free virus or infected cells could traverse the epithelial or endothelial barrier by paracellular passage through junctional complexes. Alternatively, virus could penetrate epithelial cell layers by transcytosis, a process that has been observed following interaction of several viruses (including HIV) with M cells, which cover mucosal lymphoid tissues (Wolf et al., 1981; Amerongen et al., 1991). Another mechanism could involve infection via the apical surface and subsequent release of progeny virions at the opposite surface. Finally, the epithelial cell layers could be disrupted as a result of the infection process, enabling the virus to traverse the barrier.

The release of a virus from the apical surface of an epithelial cell results in shedding into the lumen and away from underlying tissues, and such infections may have an increased likelihood of remaining localized at the epithelial surface. Conversely, basolateral release might be expected to favor the establishment of a systemic infection. Although these simple generalizations are probably not applicable to many virus infections, in some cases correlations of these types have been observed. Sendai virus, a murine parainfluenza virus, is found to be exclusively pneumotropic, whereas a Sendai mutant designated F1-R results in a systemic infection (Tashiro et al., 1990). The wild-type virus was released by budding at the apical surfaces of the bronchial epithelium, whereas the F1-R mutant virions were observed to be released by budding in a bipolar manner at both the apical and basolateral surfaces. The bidirectional budding of the F1-R mutant was shown to be correlated with the distribution of viral glycoproteins on both plasma membrane domains (Tashiro et al., 1990). Based on these observations, it was concluded that the site of budding of Sendai virus from the bronchial epithelium is a primary determinant of organ tropism in mice.

Viruses in the family Coronaviridae exhibit interesting differences in their patterns of entry and release in epithelial cells. TGEV causes a localized infection in the epithelial cells of the intestinal tract of pigs, and viral entry as well as virus release occur preferentially at the apical plasma membrane (Rossen et al., 1994). The infectious process is therefore likely to involve shedding of virus into the gut lumen, with spread to adjacent epithelial cells. In contrast to TGEV, murine hepatitis virus (MHV) initially infects nasal epithelial cells but subsequently establishes a systemic infection. In epithelial cell cultures, MHV was found to infect cells at the apical surface, but progeny virions were preferentially released at the basolateral surface (Rossen et al., 1995), suggesting that differences in the sites of release between the two viruses could play a role in the different disease patterns that they induce.

The Bunyaviridae represent another virus family in which interesting differences have been observed in the pattern of virus entry and release. Most members of this family are transmitted to humans by an insect vector and result in a systemic infection. They are assembled intracellularly by budding into the lumen of the Golgi cisternae. Their glycoproteins are localized in the Golgi complex, presumably because of specific Golgi retention signals. After budding intracellularly, virions are transported by vesicular transport to the basolateral plasma membrane, where virus release occurs (Chen and Compans, 1991). Recently, a novel group of New World hantaviruses has been discovered that are transmitted by aerosol and cause an acute pulmonary syndrome with high mortality. Unlike other members of the Bunyaviridae, the New World hantaviruses were found to be released exclusively at the apical plasma membrane, and virus entry was also restricted to the apical surface (Ravkov et al., 1997). The glycoproteins of New World hantaviruses were found to be expressed at high levels on the apical cell surfaces, and assembly of virions by budding was observed at the apical plasma membranes of infected cells (Goldsmith et al., 1995; Ravkov et al., 1997). This site of entry and release is consistent with the tropism of these viruses for the respiratory tract. Hantaviruses are excreted in the urine of their rodent hosts, and the apical release of virus from kidney cells may also be relevant to this process.

**EXAMPLES OF VIRAL INFECTION OF EPITHELIAL TISSUES**

**Gastrointestinal tract**

The infectious process of reoviruses provides a model for the pathogenesis of viral infections associated with the gastroin-
testinal tract (Sharpe and Fields, 1985). Following ingestion, the reovirus particle undergoes proteolytic cleavage mediated by a host protease in the lumen of the gastrointestinal tract (Bodkin et al., 1989). The infection is subsequently established in epithelial cells, predominantly in the ileum in the case of reovirus type 1, or throughout the small intestine and colon in the case of reovirus type 3 (Rubin et al., 1986). Following oral inoculation of mice, binding of virus to the apical surface of M cells was observed, followed by transcytosis to the basolateral surface (Wolf et al., 1981). Once released from the basolateral surface, the virus establishes an infection in the adjacent epithelial cells and subsequently spreads to other sites, probably via the lymphatic system and bloodstream (Kauffman et al., 1983). Infection of the enteroocytes adjacent to the M cells is thought to be mediated by virus binding to their basolateral surface; preferential binding to the basolateral surfaces of intestinal epithelial cells has been demonstrated in a cell culture system (Rubin, 1987).

Poliovirus is one of the most important enteroviruses that infects humans. Shortly after ingestion, virus can be recovered from lymphoid tissues, suggesting that these are the sites of primary replication. Within 4 days of ingestion, the highest titers of virus were found to be associated with the tonsils and Peyer’s patches (Bodian, 1959). Occasionally, infection with poliovirus results in invasion of the central nervous system, probably via the blood (Bodian, 1959). Infection of neurons leads to transport to the anterior horn of the spinal cord and is associated with significant pathological lesions. In the later stages of infection, virus may be recovered from the feces, which is the predominant means by which dissemination occurs. Poliovirus infection of the gastrointestinal epithelium results in lesions of the Peyer’s patches, and evidence suggests that poliovirus is endocytosed by M cells of human Peyer’s patches (Sicinski et al., 1990). A likely scenario for the infection process is as follows: ingested poliovirus binds to the surface of M cells, which are subsequently infected and/or transport the virus by transcytosis to the underlying lymphoid tissue. A localized infection of cells in the Peyer’s patch is initiated, followed by a viremia leading to infection of other target organs and tissues such as the central nervous system, brown fat, and somatic lymph nodes (Bodian, 1959). Release of virus into the feces may be mediated by the movement of infected lymphocytes from lymphoid tissues into the lumen of the gut (Bodian, 1959) and/or infection of nonlymphoid gut epithelial cells (Sabin, 1956), resulting in vectorial transport and preferential release of virions from their apical surface into the gut lumen (Tucker et al., 1993b).

**Respiratory tract**

Influenza virus is one of the most important causes of morbidity and mortality in humans. Influenza A viruses preferentially establish an infection in the ciliated epithelial cells of the respiratory tract. The infected cells are destroyed, leaving a layer of basal cells overlying the basement membrane, with gaps between cells that allow passage of fluids into the lumen (Small, 1990). Following this early destruction phase, the remaining epithelial cells of the basal layer begin to divide and regenerate the epithelia. Although damage is generally confined to the epithelium lining the upper respiratory tract, in some cases severe pathological changes can also occur in the epithelia of the lower respiratory tract, resulting in viral pneumonia. Influenza infections of humans do not usually result in extensive viremia (Louria et al., 1959). Influenza virus entry and release are likely to be largely restricted to the apical surfaces of epithelial cells, as observed in epithelial cell culture systems. Such a restriction is consistent with the establishment of the type of localized infection observed during influenza infection of humans.

The primary site of replication of rhinoviruses is the epithelial surface of the nasal mucosa (Douglas, 1975). Immunolocalization studies have demonstrated a tropism for columnar epithelial cells in this region (Turner et al., 1982). The cellular receptor for rhinoviruses has been identified as the ICAM-1 (Greve et al., 1989), which is restricted to the luminal surface of the lung epithelium (Albelda, 1991). Since ICAM-1 is widely distributed in other tissues, it is unlikely to have an important role as a determinant of tissue tropism. The receptor is expressed on the apical surface, so transepithelial transport is not required for infection, suggesting there is little selective pressure for the virus to further invade the mucosal surface. Virus is shed into nasal secretions, and the titers of progeny virus in secretions are correlated with the extent of mucosal infection and the severity of illness. There are several potential reasons for restriction of the infection to the upper respiratory tract (Couch, 1996). Because of the sensitivity of rhinoviruses to reduced pH, they are unable to survive passage through the gastrointestinal tract. However, direct inoculation of rhinoviruses into the small intestine of volunteers also failed to result in the establishment of infection, indicating that the low pH sensitivity of the virus is not the sole cause of the failure of these viruses to infect the intestinal tract. The optimal growth temperature of these viruses is 33°C, as found in the upper respiratory tract, and this may play a major role in restricting the site of infection.

**IMPLICATIONS FOR EFFECTIVE VACCINES**

Viruses exhibit a diverse pattern of interactions with epithelial cells. The site of virus entry is determined by the distribution of specific cellular receptors, whereas the site of release is determined primarily by sorting signals present in the viral proteins and their interaction with cellular transport pathways. The restriction of entry and release of some types of viruses to apical surfaces is consistent with a pattern of localized infection of epithelial cell surfaces by these agents. Other types of viruses are released at basolateral plasma membrane domains after infection of epithelial cells or are transported across epithelia by transcytosis and are thus more readily able to spread to other tissues. In both types of infection, immune responses in mucosal secretions represent
the first line of defense against the initial interaction of a virus with apical cell surfaces. Therefore, development of vaccines that induce such responses is an important objective for prevention of virus infection at mucosal surfaces. For viruses that cause localized infections at epithelial surfaces, mucosal immune responses are also likely to be the most important mechanism for preventing spread of the infection process. Viruses that are able to traverse epithelial cell layers are subsequently also accessible to systemic immune responses, which play a role in preventing the spread of infection to other sites. In addition, following the initial infection of epithelial cells, the infected cells also can serve as targets for cytotoxic T lymphocytes by presentation of processed viral antigens in association with MHC molecules on their basolateral surfaces. It is believed that such responses play an important role in the clearance of virus from infected tissues.

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