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VIEWPOINT

Evolution of Cell Recognition by Viruses

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Evolution of receptor specificity by viruses has several implications for viral pathogenesis, host range, virus-mediated gene targeting, and viral adaptation after organ transplantation and xenotransplantation, as well as for the emergence of viral diseases. Recent evidence suggests that minimal changes in viral genomes may trigger a shift in receptor usage for virus entry, even into the same cell type. A capacity to exploit alternative entry pathways may reflect the ancient evolutionary origins of viruses and a possible role as agents of horizontal gene transfers among cells.

Although viral entry into cells is not the only determinant of cell tropism, ever since the first evidence that animal viruses (*1*) and bacterial viruses (*2*) enter cells through specific receptors, considerable effort has been put into the identification of those structures that mediate cell recognition by viruses and the transfer of their genetic material into cells. The picture of how viruses exploit surface cellular macromolecules to initiate their infectious cycles has become increasingly complex (*3*, *4*). Receptors used by viruses belong to widely different families of proteins, carbohydrates, or lipids, often in complex cell surface matrix structures (*4*, *5*) (Table 1). Some of them are involved in immune modulation, signaling pathways, or cell adhesion or have no known function.

A Receptor for Several Viruses, a Virus for Several Receptors

A survey of different virus groups illustrates that receptor usage does not generally show any obvious correlation with virus phylogeny (Table 1). It is often not possible to anticipate its use of one type of receptor molecule or another (*3*–*5*). For example, at least two receptors have been proposed to mediate entry of human hepatitis C virus (HCV) into hepatocytes: CD81, a member of the tetraspanin superfamily of proteins (*6*), and the low-density lipoprotein receptor (LDLR) (*7*). Comparison of these proposed receptors for HCV with the receptor for hepatitis A virus (a mucine-like class I integral membrane glycoprotein) and for duck hepatitis B virus (the C-domain of carboxypeptidase D, pg180) (*8*) indicates that despite their specificity for the same target organ, hepatitis viruses use disparate molecules for entry into hepatocytes. The picornaviruses, which encompass several important human and animal pathogens and share structural features in their capsids, may use several macromolecules as receptors (*9*). Likewise, some receptors are shared by coronaviruses associated with different pathologies (*5*) (Table 1).

Perhaps the most emblematic example of cross-phyla sharing of a receptor is coxsackievirus adenovirus receptor (CAR) (*10*). CAR is used by adenoviruses 2 and 5, which are agents of respiratory disease in children, as well as by coxsackieviruses B1 to B6, which are associated with febrile illness, meningitis, and some cardiopathies. Of the many examples, the interaction of the human influenza A virus hemagglutinin with *N*-acetylneuraminic acid, and the ensuing conformational alterations involved in pH-dependent membrane fusion, are one of the best characterized at the structural and functional levels (*11*) (Table 1).

Thus, the susceptibility of different cell types to a virus, in the absence of a characterized receptor indicates the existence of alternative receptors. Herpes simplex viruses interact with one of at least three virus entry-mediator proteins (Hve A is a member of the tumor necrosis factor receptor protein family and Hev B and Hev C are two members of the immunoglobulin superfamily), yet cells lacking these receptors may still allow efficient penetration of the virus. The related tumor-causing Epstein-Barr virus (EBV) shows a marked B lymphotropism owing to expression of a specific receptor, CD21 (or CR2). Again, EBV can replicate in differentiated epithelial cells that do not express CD21, implying the participation of some other unidentified receptor (*5*). Furthermore, receptor expression alone may not be sufficient for a productive viral infection. Mice made transgenic for the functional form of the poliovirus receptor (PVR) become susceptible to poliovirus and develop limb paralysis. Yet, the distribution of PVR mRNA in human and mice tissues does not match the replication sites of the virus (*12*, *13*).

Modulation, Expansion, and Shifts in Receptor Usage

The reasons why structures implicated in immune responses, cell signaling, cell-cell recognition, recruitment, and inflammation abound among viral receptors (*5*, *9*) are not obvious. Possibly, these structures are subsets of the most abundant type of molecules found on cell surfaces capable of triggering the uptake of virus particles and the irreversible conformational changes that must precede uncoating and genome replication. Given the population structure of RNA viruses (*14*), key issues for understanding changes in host cell specificity are the genetic distances that a viral genome must bridge and the selective forces involved.

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Across viral groups, single amino acid replacements in capsid or surface proteins have been identified that affect receptor recognition, cellular tropism, and pathogenesis. A residue of influenza virus hemagglutinin confers specificity for sialic acid linked to galactose by either an α2,6 or an α2,3 linkage (*11*, *15*). A single mutation in the capsid allows the primate restricted P1/Mahoney strain of poliovirus to paralyze mice (*16*). Variant D of encephalomyocarditis virus, a picornavirus usually asymptomatic for rodents, can induce diabetes in mice through destruction of pancreatic β cells. The diabetogenic variant includes an amino acid replacement along the capsid pit, likely affecting receptor interaction and cell tropism (*17*). A mutant in the capsid of Theiler's murine encephalomyelitis virus results in altered tropism and suppression of the chronic demyelinating disease that usually follows infection with wild-type virus (*18*). A single amino acid substitution in the glycoprotein of lymphocytic choriomeningitis virus alters its affinity for the α-dystroglycan receptor; high-affinity binding is associated with immunosuppression and viral persistence in mice, whereas low-affinity binding results in clearance of the infection (*19*, *20*). Research on the Edmonston vaccine strain of measles virus (MV) led to the identification of CD46 as a receptor for this virus and, indeed, transgenic mice expressing CD46 may show typical pathogenic manifestations of the virus (*21*). Unexpectedly, marmosets lacking CD46 were still susceptible to several isolates of MV, but not to the Edmonston vaccine strain. Some natural isolates of MV do not enter cells through CD46; instead, they use as a receptor the signaling lymphocyte-activation molecule (SLAM or CDW 150) (*22*), a glycoprotein expressed on some types of B and T lymphocytes. A single amino acid replacement in the surface hemagglutinin of the MV envelope is sufficient to allow the virus to bind to CD46 (*23*). Thus, virus variants with different host cell specificities may differ very slightly. Given the genetic heterogeneity and dynamics of viral populations, substantial leaps are within reach of replicating viral quasi-species (the mutant swarms composing RNA virus populations) (*14*).

In some cases, a selective force allowing multiplication in a given type of cell can be identified as the likely trigger of a tropism alteration. MV isolated in marmoset B cell lines can infect some primate B and T cell lines and retain pathogenicity for monkeys. This is not the case for MV isolated on Vero cells, because such isolates manifest a different tropism and host range (*22*), implying that the type of cell used for MV isolation exerts a selective force on the virus. Positive selection in the modification of host cell tropism has also been documented for human immunodeficiency virus–1 (HIV-1) (*24*). Primary isolates of HIV-1 generally use the transmembrane chemokine receptor CCR5, but isolates adapted to grow in T cell lines shift their preference to CXCR4 (*24*). Furthermore, a modified form of RANTES, which is a natural ligand for CCR5, selects mutants that use CXCR4 in an in vivo mouse model (*25*). Likewise, an avian retrovirus variant with an expanded host range has been selected that recognizes a receptor on chicken cells and a distinct receptor on quail cells (*26*). The introduction of avian influenza viruses into humans has resulted in pandemic outbreaks of influenza in the human population (*14*). The receptor-binding specificity of the avian influenza hemagglutinin was altered early after transmission to humans and pigs (*27*), constituting a case of positive selection by the recipient host. In other cases, the selective constraint is not obvious, although the result may be a

Table 1. Examples of cell surface components involved in entry of more than one virus into cells. In this manuscript, receptor is defined as any cell surface macromolecule involved in virus entry into the cell. Assignments are based on (*4*, *5*, *9*, *11*, *19*, *24*, *31–35*) and references therein.

Receptor class	Cellular structure*	Virus (*Family*)*
Cell adhesion and cell-cell contact proteins	CXCR4 (TM7 family)	HIV, SIV, FIV (*Retroviridae*)
	CD4 (Ig superfamily)	HIV, SIV (*Retroviridae*); HHV-7 (*Herpesviridae*)
	α-Dystroglycan	LCMV, Lassa fever virus (*Arenaviridae*)
	Integrins	Adenovirus 2, 3, 12 (*Adenoviridae*); FMDV, coxsackievirus (A9, B1, B3, B5), echovirus (1, 8, 9 Barty); human parechovirus 1 (*Picornaviridae*); hantavirus (*Bunyaviridae*); human papillomaviruses (*Papovaviridae*); rotavirus SA11 (*Reoviridae*)
	ICAM-1 (Ig superfamily)	Major group HRV, coxsackievirus (A13, A18, A21) (*Picornaviridae*)
	MHC I (Ig superfamily)	SV40 (*Papovaviridae*); adenovirus 5 (*Adenoviridae*); coxsackievirus A9, echovirus 7 (*Picornaviridae*)
Complement control protein superfamily	CD46	Measles virus (*Paramyxoviridae*); HHV-6 (*Herpesviridae*)Coxsackievirus (A21, B1, B3, B5), echovirus (3, 6, 7, 11-13, 20, 21, 24, 29, 33), enterovirus 70 (*Picornaviridae*)
	DAF (CD55)	
Other proteins	Aminopeptidase-N	Human coronavirus 229E, TGEV, FIPV, CCV (*Coronaviridae*)
	CAR (Ig superfamily)	Coxsackievirus (B1-B6) (*Picornaviridae*); adenovirus 2, 5 (*Adenoviridae*)
	LDLR protein family	Minor group HRV (*Picornaviridae*); HCV, BVDV (*Flaviviridae*); subgroup A avian leukosis and sarcoma virus (*Retroviridae*)
	PVR and related proteins HveB and HveC (Ig superfamily)	Poliovirus (*Picornaviridae*); HSV (*Herpesviridae*)
Extracellular matrix components and sugar derivatives	Heparan sulfate glycoaminoglycan	HSV, human cytomegalovirus, BHV, PRV (*Herpesviridae*); HIV (*Retroviridae*); vaccinia virus (*Poxviridae*); adenovirus 2, 5 (*Adenoviridae*); AAV2 (*Parvoviridae*); Dengue virus, CSFV (*Flaviviridae*); FMDV (*Picornaviridae*); Sindbis virus (*Togaviridae*); HRSV (*Paramyxoviridae*); human papillomavirus (*Papovaviridae*)
	Sialic acid (*N*-acetylneuraminic acid, *N*-acetyl-9-*O*-acetylneuraminic acid, *N*-glycolylneura-minic acid)	Influenza virus (*Orthomyxoviridae*); reovirus type 3, group A porcine rotavirus (*Reoviridae*); human coronavirus OC43, BCV, TGEV (*Coronaviridae*); adenovirus 8, 19a, 37 (*Adenoviridae*); Sendai virus, human parainfluenza virus 3, NDV (*Paramyxoviridae*); bovine enterovirus, TMEV strain DA, HRV 87 (*Picornaviridae*)

*Abbreviations of cellular structures: CAR, coxsackievirus-adenovirus receptor; DAF, decay-accelerating factor; Hve, herpesvirus entry protein; ICAM-1, intracellular adhesion molecule type 1; Ig, immunoglobulin; LDLR, low-density lipoprotein receptor; PVR, poliovirus receptor; TM7, transmembrane seven. Abbreviations of virus names: AAV2, adeno-associated virus type 2; BCV, bovine coronavirus; BHV, bovine herpesvirus; BVDV, bovine viral diarrhea virus; CCV, canine coronavirus; CSFV, classical swine fever virus; ECMV, encephalomyocarditis virus; FIPV, feline infectious peritonitis virus; FIV, feline immunodeficiency virus; FMDV, foot-and-mouth disease virus; HCV, hepatitis C virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HRSV, human respiratory syncytial virus; HRV, human rhinovirus; HSV, herpes simplex virus; LCMV, lymphocytic choriomeningitis virus; NDV, Newcastle disease virus; PRV, pseudorabies virus; SIV, simian immunodeficiency virus; SV40, simian virus 40; TMEV, Theiler's encephalomyelitis virus; TGEV, transmissible gastroenteritis virus.

remarkable expansion of host cell tropism. The important animal pathogen foot-and-mouth disease virus (FMDV) provides an example.

Alternative Receptors to Enter the Same Cell Type

FMDV may use several receptors. The first to be identified was integrin $\alpha_v\beta_3$ (*28*). The interaction occurs through an Arg-Gly-Asp (RGD) triplet, a signature sequence for recognition of some integrins, located on a mobile loop protruding from the capsid (*29*, *30*). Integrin $\alpha_v\beta_6$, and perhaps $\alpha_5\beta_1$, may also be involved in entry of FMDV into cells (*31*). FMDV, adapted to cell culture, makes use of heparan sulfate (HS) as an alternative receptor (*32*–*35*). Following this seminal observation with FMDV, several other viruses have been shown to evolve in cell culture to use HS. Another entry pathway for immune complexes of FMDV involves immunoglobulin Fc receptors (*36*), which mediate transport of the acid-labile particles into endosomal vesicles for uncoating. This entry pathway may also be responsible for the severe pathological manifestations accompanying reinfection by Dengue virus, which is an example of a virus exploiting a prior immune response for its own benefit.

Remarkable changes in receptor specificity during the course of passage in cell culture have been documented for FMDV (*35*, *37*) (Fig. 1). Although the parental virus enters cells through an RGD-dependent integrin, multiply passaged virus acquires several amino acid substitutions in its capsid surface, which dispense with integrin-recognition RGD (*38*). The modified FMDV acquires the ability to infect human K-562 cells, which do not express integrin $\alpha_v\beta_3$, and several human and animal cell lines that were nonpermissive for the parental virus (*35*, *37*, *39*). The multiply passaged virus acquires the capacity to bind heparin, as expected, yet the RGD-independent pathway of cell entry does not require binding to heparin (*35*). Thus, a third, RGD- and HS-independent, and an as yet unidentified mechanism of penetration of FMDV into cells must be operating. Receptor blockage experiments with synthetic peptides (*35*) provide evidence that when HS binding is impaired, the RGD-dependent entry pathway was again used by the virus.

Could expansions of host range for FMDV occur in the field? Current evidence suggests that RGD-dependent integrins are the receptors used by FMDV in cattle (*37*), and variants adapted to bind HS in cell culture are attenuated for this host (*33*). Yet, FMDVs with replacements within the RGD (or at neighboring positions thought to be critical for integrin binding) were isolated from cattle challenged with virulent virus, when the animals were partially protected by immunization with synthetic peptides (*40*). However, critical, unanswered questions remain. Could these variant viruses still use integrins? Did they maintain the capacity to initiate infection in cattle, or were they merely dead-end products of evolution driven by the immune response evoked by the synthetic peptides? Chance contacts of variant viruses with potential new hosts have been considered a plausible scenario for disease emergence (*41*). In the case of FMDV, the possibility of host range shifts acquires a particular relevance in view of the difficulties tradi-

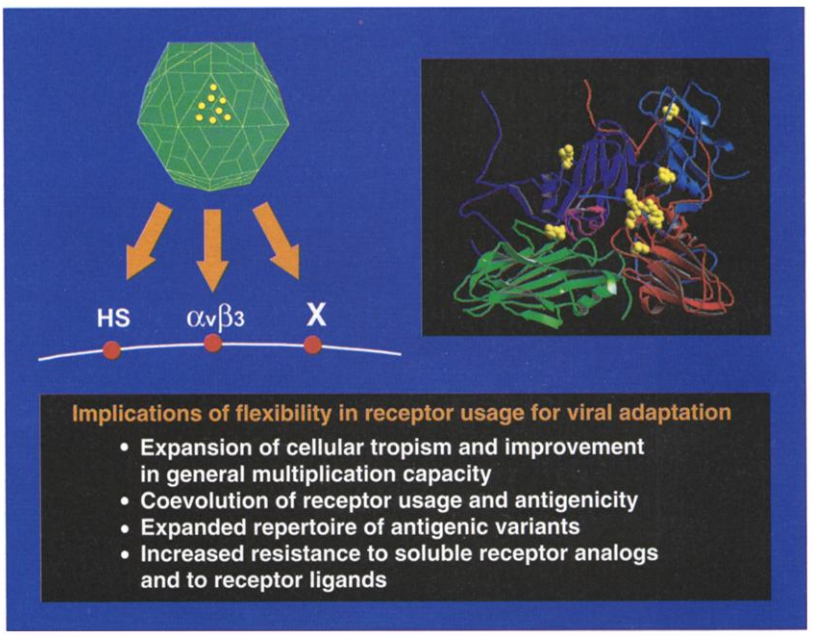

Fig. 1. Flexibility in receptor usage by FMDV. Two hundred and thirteen serial passages of FMDV in BHK-21 cells resulted in eight amino acid replacements in the virus capsid that expanded receptor usage (HS, heparan sulfate; integrin $\alpha_v\beta_3$; X, an unidentified receptor). Replacements are depicted in van der Waals spheres (yellow) on a ribbon protein diagram of a crystallographic protomer of FMDV C-S8c1 (VP1, blue; VP2, green; VP3, red). VP1 from a neighboring protomer is shown at the upper right (light blue). Structure based on (*53*, *58*). At the center of the protomer, the mobile antigenic G-H loop of VP1 (*29*, *30*, *53*, *58*) is depicted (magenta) in a position corresponding to that found in the complex with neutralizing monoclonal antibody SD6 (*53*). None of the sites of the eight amino acid replacements found in the multiply passaged virus could interact directly with residues of the loop (*35*, *53*, *58*). However, it cannot be excluded that the mobility of the G-H loop could be influenced by the replacements (*29*, *30*, *35*).

Table 2. Evidence of coevolution of cell recognition and antigenicity in viruses.

Viral system	Main observations
Influenza virus	Amino acid residues within the sialic acid–binding pocket of virus hemagglutinin are accessible to neutralizing antibodies (*45*).
	Antigenic and hemagglutinin variants selected upon egg adaptation (*50*).
Poliovirus	Receptor recognition influenced by residues of antigenic sites (*51*, *52*).
Foot-and-mouth disease virus	Overlap of integrin- and antibody-binding sites (*53*).
	Monoclonal antibodies selected variants with altered integrin recognition (*35*, *38*, *39*).
	Adaptation to cell culture may result in antigenic variation (*33*, *35*, *54*).
	Some amino acid residues involved in heparin binding map at antigenic sites (*33*–*35*).
	Antigenic variants with altered receptor specificity can be selected in vivo (*40*).
Theiler's murine encephalomyelitis virus	Neutralization epitopes map close to the putative receptor binding region (*55*).
	Mutations associated with adaptation to some culture cells map in antigenic sites (*56*).
Yellow fever virus	Amino acid residues critical for virus neurotropism are involved in antibody binding (*57*).

tionally encountered for effective global FMD control and the recent expansion of the disease to areas such as Japan, Taiwan, and Europe that were previously free of FMD (*42*, *43*).

The overlap between the integrin recognition site and a major antibody-binding site of FMDV has prompted the isolation of viable mutants with profoundly altered antigenicity that contain RED, RGG, or even GGG instead of RGD (*38*, *44*). This is not only for FMDV; for several viruses, there is some overlap between receptor binding sites and antigenic sites (*45*), facilitating coevolution of antigenicity and host cell tropism (Table 2). Thus, evolution of tropism may be an indirect outcome of antibody-directed selection. Alternatively, evolving viral quasi-species can drift to produce ample variant repertoires, some of which may be endowed with the potential to manifest a new tropism when confronted with a matching receptor molecule.

Ancestral Coadaptations and the Problem of Viral Disease Emergence

The analyses of complete cellular and viral genomes suggest that viruses have deep evolutionary roots in the cellular world, as shown by the shared functional motifs between cells and viruses (particularly similarities between viral and cellular proteins involved in genome replication), discernible sequence identities between some plant and animal RNA viruses, and the convergent phylogenies of viruses with multispecies host ranges and their hosts [(*46*), and reviewed in several chapters of (*47*)]. Horizontal gene transfers among cells of different ancestry [those lineages that led to present-day eukaryotes, bacteria, and archaeobacteria (*48*)], achieved by several mechanisms, including virus-mediated gene delivery, could have been a crucial element in coevolutionary adaptation (*47*, *48*). The binding of two isoforms of a cellular protein to two receptors is not foreign to differentiated organisms: A two–amino acid insertion into ectodysplasin, a member of the tumor necrosis binding family, changes its receptor specificity, and the differential expression of the two forms plays a role in epidermial morphogenesis (*49*). The capability to use alternative receptors may thus represent a modern adaptation of viruses to cope with highly differentiated organisms. Conversely, the uptake of cellular genes by viruses has been amply documented in transducing bacteriophages, RNA and DNA tumor viruses, cytopathic variants of the flavivirus bovine viral diarrhea virus, and several defective viruses that have acquired host sequences by nonhomologous recombination (*47*).

Whether or not it originates from ancient gene transfers, the documented flexibility in receptor usage by viruses has many implications for human and animal disease that need to be addressed. In a positive sense, a deeper understanding of the nuances of receptor specificity could be exploited for the engineering of precise virus-mediated gene delivery systems. Specific targeting would help in gene therapy requiring cell, tissue, or organ specificity. The other side of the coin is that the dynamics of receptor usage may render ineffective antiviral therapies based on the administration of receptor ligands or receptor analogs. It may also have unpredictable consequences in organ transplantation or xenotransplantation. Virus encounters with cells that express distinct receptors or different isoforms of the same receptor may result in the selective amplification in the recipient of minority variants present in the donor. This makes the concept of the species barrier rather fluid. For example, swine vesicular disease is closely related to human coxsackievirus B5, and cross-species transmission is known to occur among parvoviruses (*47*). In the parvoviruses, sequence differences between capsid protein genes of canine parvovirus and isolates infecting other hosts were associated with changes in antigenicity and in binding to sialic acid receptors (*47*).

The emergence of human and animal diseases must be viewed in a broad context of environmental, ecological, technological, and even sociological factors (*41*) whose effects are primarily demographic alterations of infected and susceptible hosts, as well as of virus vectors. This complex set of influences interweaves with the genetic lottery of virus mutations that are unavoidably and unpredictably generated during replication. A close survey of viruses that infect animals asymptomatically or that cause acute or chronic disease, with an assessment of their capacity to modify receptor recognition and host cell tropism, should be considered one of the priorities for prevention of human disease emergence. The evidence also cautions against the deliberate release of virulent viruses as pest control agents. Such practices must be at best regarded as premature, given our still rudimentary knowledge of factors mediating changes in receptor recognition, cell tropism, and the host range of viruses.

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