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Review on Cells Membrane Invasion by Viruses in Animals

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ABSTRACT

Viruses replicate within living cells and use the cellular machinery for the synthesis of their genome and other components. To gain access, they have evolved a variety of elegant mechanisms to deliver their genes and accessory proteins into the host cell. Many animal viruses take advantage of endocytic pathways and rely on the cell to guide them through a complex entry and uncoating program. These they also do by replicating inside the cell and those bind to a specific receptor and cause conformational changes in the cell membrane thereby causing disease after depleting through the process known as virus pathogenesis. Various viruses use this medium, most recently SARS, HIV and the novel coronavirus which is pandemic now in the global. Various vaccines are on the way and mode of administration will be analyzed.

Keywords: Cell membrane, Coronavirus, SARS, Animals, virus pathogenesis and Invasion

INTRODUCTION

A virus is a submicroscopic infectious agent that replicates only inside the living cells of an organism. Viruses can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea. Viruses are found in almost every ecosystem on Earth and are the most numerous type of biological entity [1]. The study of viruses is known as virology, a subspeciality of microbiology. When not inside an infected cell or in the process of infecting a cell, viruses exist in the form of independent particles, or virions, consisting of the genetic material, i.e. long molecules of DNA or RNA that encode the structure of the proteins by which the virus acts, a protein coat, the capsid, which surrounds and protects the genetic material and in some cases an outside envelope of lipids [2]. The shapes of these virus particles range from simple helical and icosahedral forms to more complex structures. Most virus species have virions too small to be seen with an optical microscope, about one hundredth the size of most bacteria. Viruses spread in many ways. One transmission pathway is through disease-bearing organisms known as vectors: for example, viruses are often transmitted from plant to plant by insects that feed on plant sap, such as aphids; and viruses in animals can be carried by blood-sucking insects [3]. Influenza viruses are spread by coughing and sneezing. Norovirus and rotavirus, common causes of viral gastroenteritis, are transmitted by the faecal–oral route, passed by contact and entering the body in food or water. HIV is one of several viruses transmitted through sexual contact and by exposure to infected blood. The variety of host cells that a virus can infect is called its "host range". This can be narrow, meaning a virus is capable of infecting few species, or broad, meaning it is capable of infecting many [4]. Viral infections in animals provoke an immune response that usually eliminates the infecting virus. Immune responses can also be produced by vaccines, which confer an artificially acquired immunity to the specific viral infection. Some viruses, including those that cause AIDS, HPV infection, and viral hepatitis, evade these immune responses and result in chronic infections. Several antiviral drugs have been developed. The virus replicates and caused damage

thereby causing disease after depleting the host cells through the process known as virus pathogenesis [5].

The cell membrane (plasma membrane) is a thin semi-permeable membrane that surrounds the cytoplasm of a cell. Its function is to protect the integrity of the interior of the cell by allowing certain substances into the cell while keeping other substances out [6]. It also serves as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus the cell membrane also serves to help support the cell and help maintain its shape. The membrane also help to regulate cell growth through the balance of endocytosis and exocytosis. In endocytosis, lipids and proteins are removed from the cell membrane as substances are internalized [7]. In exocytosis, vesicles containing lipids and proteins fuse with the cell membrane increasing cell size. Animal cells, plant cells, prokaryotic cells, and fungal cells have plasma membranes. Internal organelles are also encased by membranes.

Structure of the Cell membrane

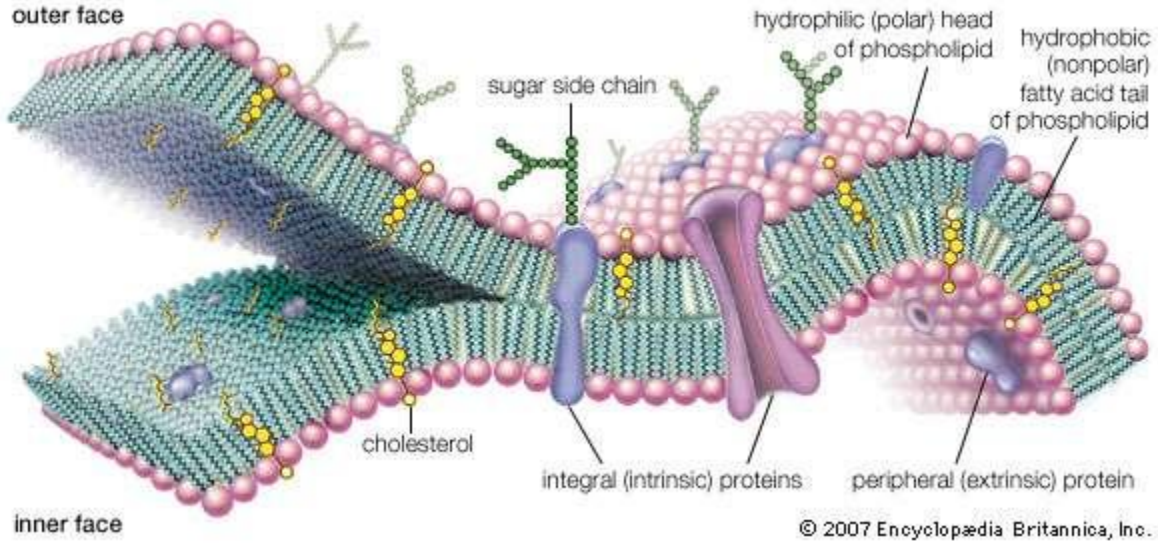

Fig 1: shows the structure of the membrane and its components [8].

Fundamental aspects of viral pathogenesis

Viral pathogenesis involves several steps that must occur for the virus to infect and cause disease in the host which are virus entry into the host, primary virus replication, virus spread within the host, infection of cells with special affinities for the virus (cell tropism), cellular injury, host immune response, viral clearance or persistence, and viral shedding and transmission [9]. Virus entry occurs either through the mucosa of the respiratory, gastrointestinal tract, or urogenital tract, by transcutaneous inoculation into the blood stream through blood transfusion or insect vector bites, and by maternal–fetal transmission across the placenta. Virus replication usually occurs at the portal of entry and can cause disease there; for example, influenza virus and respiratory disease, and rotaviruses and gastrointestinal disease. Some viruses produce diseases at sites distant from their portal of entry [10]. The mechanism of spread varies; viruses may reach their target cells via nerves or through the blood stream or lymphatics. Viruses also have affinity for certain organ and cell types. Such cell tropism is usually mediated by specific cell surface receptors on the host cell with which the virus envelope or capsid can interact to initiate infection. Viruses produce cellular injury by either direct destruction of the infected cell or by alteration in cell physiology [11]. Inactivation of host cellular protein synthesis is a hallmark of

many virus infections. Cellular damage may ultimately result in clinical illness, though this depends on several factors, including the host immune response to viral infection. Some viruses can persist in the host for a prolonged period of time. In chronic infections, the virus can be continuously detected in the host (e.g., infections with hepatitis viruses B or C). During virus latency, the virus persists in a dormant (non-replicating) form, but can reactivate intermittently to an infectious form (e.g., HSV-1 and HSV-2) [12].

Virus Mode of entry into the cell membrane.

Both non enveloped and enveloped viruses share the same main steps and routes of virus entry, which begin with attachment to cell-surface receptors and end with the delivery of the viral genome to the cell cytoplasm (Fig.2) [13]. After binding to receptors which are proteins, carbohydrates or lipids. Viruses use two main routes to enter the cell which is the endocytic and non-endocytic routes. The endocytic route is usually by transport in clathrin-coated vesicles or pits, while non-clathrin-coated pits, macropinocytosis or caveolae are also used. Some viruses can induce internalization by endocytosis for example, simian virus 40 (SV40), which induces local actin polymerization and dynamin recruitment at the site of entry3 (Fig.2). The non-endocytic route of entry involves directly crossing the plasma membrane at neutral pH (Fig.2). Viruses that use the non-endocytic route can also enter cells by the endocytic pathway for example, human immunodeficiency virus type 1 (HIV-1). Membrane fusion a basic cellular process that is essential for phagocytosis, pinocytosis and vesicular trafficking is a basic mode of entry by enveloped viruses that use the endocytic or non-endocytic routes [14]. The process is regulated and is mediated by membrane proteins once the membranes are in close proximity to each other. For both enveloped and non-enveloped viruses, entry into cells involves important conformational changes of the viral ENTRY PROTEINS or the host-cell receptors, which are induced by low endosomal pH. This can occur either by penetration (for non-enveloped viruses) or fusion (for enveloped viruses). After entry into the host cell, many viruses, including HIV-1 and SV40, are transported through the cytoplasm as nucleoprotein complexes [15]. Surface-exposed nuclear localization signals on the nucleoprotein complex allow targeting to and entry into the nucleus, and infection of non-dividing cells.

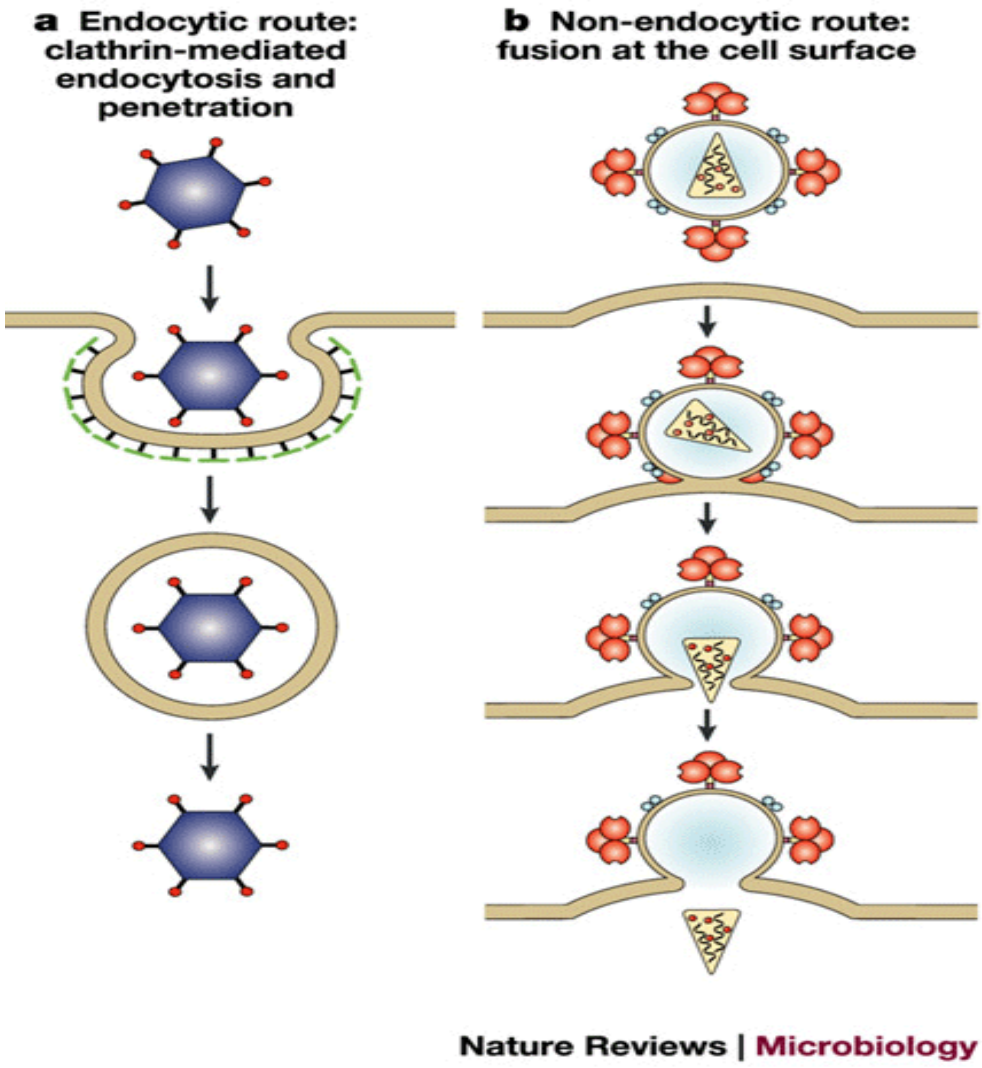

Figure 2: Two main virus entry pathways.

Entry of viruses such as SV40, echovirus 1 (EV1), HIV-1, measles virus, Ebola virus and Epstein–Barr virus (EBV), can be enhanced by lipid microdomains, known as LIPID RAFTS4,5. Other viruses, including influenza, can use lipid rafts as a platform on which to concentrate a sufficient number of viral molecules into virions for efficient exit from one cell and entry into another cell6. However, fusion of Semliki Forest virus (SFV) or Sindbis virus (SIN) with liposomes does not require rafts, and the presence of rafts can be inhibitory to membrane fusion7. It remains to be established whether rafts are involved in the infectious cell entry of SFV and SIN8 [16]. Some viruses enter cells through direct cell-to-cell contacts, using structures that are formed by the polarized cytoskeleton, adhesion molecules and viral proteins at the infected cell junction, which is known as the 'virological synapse'9. Direct cell-to-cell transmission of viruses by this process, for example, retroviruses (such as human T-cell lymphoma-leukaemia virus type 1 (HTLV-1) and HIV-1), herpesviruses (such as herpes simplex virus (HSV) and varicella-zoster virus) and poxviruses (such as vaccinia virus) is poorly understood. In many cases it is not clear whether cell-to-cell transmission by this route involves membrane fusion or penetration, or direct transfer of the virus

through cell junctions [17]. Efficient and rapid cell-to-cell transmission of some viruses, such as HIV-1, could alternatively be mediated by virions that are budding or have just been released into the space between the closely apposed interacting cells or by cell-to-cell fusion. Cell-to-cell transmission might protect viruses from the actions of the immune system and could be an important route of transmission in vivo. Viruses such as HIV-1 and poliovirus can enter and exit cells without crossing membranes by a process known as transcytosis. Transcytosis as vesicular transport from one side of a cell to the other is used by multicellular organisms to selectively move material (usually macromolecules) through cells between two environments without modifying it. Viruses have usurped this mechanism to cross the epithelial cell barrier and infect the underlying cells [18]. The kinetics and efficiency of entry vary greatly between viruses from different families, between viruses within a family, between viruses within a genus and even between isolates of the same species. Some viruses, such as adeno-associated virus serotype 2 (AAV-2), SFV and influenza can cross the endosomal membranes very rapidly (within seconds), and the efficiency of entry can be as much, or more than, 50% (which means that 50% of attached viruses enter cells). Single AAV-2 virions can cross membranes in less than a second11 and individual influenza virions cross membranes in as little as one or more seconds. Other viruses, such as HIV-1, take one or more minutes to enter cells, and the efficiency of entry is poor compared with AAV-2 often as low as 0.1% [19]. The kinetics and efficiency of virus entry might be related to the virus structure and it seems that the best kinetics and efficiency of entry are observed for viruses that use low pH as an entry trigger and have flattened structures such as SFV. Cell-bound SFV can fuse in seconds with an efficiency of 80% [19]. Membrane lipid composition and structure also affect the kinetics and efficiency of virus entry. Both non-enveloped and enveloped viruses can use the energy of metastable states in viral entry proteins to expose hydrophobic sequences that can destabilize host-cell membranes. However, after this, the formation of different intermediates leads to the formation of membrane pores (in the case of non-enveloped viruses) or membrane fusion pores (in the case of enveloped viruses) [20]. Often, conformational changes in a single virus protein can mediate membrane fusion. Enveloped viruses can fuse with the plasma membrane or from inside an endosome. The penetration and entry of non-enveloped viruses might resemble the entry of toxins, such as anthrax toxin [20]. Entry of enveloped viruses has similarities with intracellular fusion processes, such as exocytosis.

Virus structure and receptor recognition

Virus evolution has resulted in several receptor-recognizing surface structures, which frequently have protrusions (spikes) about 10 nm or longer that are formed by the entry proteins for example, coronaviruses and poliovirus. Non-enveloped viruses are often small and stable, and can form crystals that diffract to good resolution [21].

Recognition of Virus Receptors on Host Cells.

Although virus receptors have diverse sequences, structures and cellular functions, there is a preference for molecules that are involved in cell adhesion and recognition by reversible, multivalent avidity determined interactions [22]. Viruses might have evolved to bind to abundant cellular receptors, or to bind to cellular receptors that have relatively low affinity for their natural ligands with high affinity or to bind to receptors that have both of these characteristics. The receptor-recognition interactions of different viruses and even different isolates of the same virus can vary significantly. Typically, low-affinity (μM–mM) binding interactions between viral attachment proteins and their cognate receptors involve a small area of interaction between the viral and cellular receptors and do not lead to conformational changes in the entry

proteins [23]. For example, binding of influenza HA and SV40 to sialic acid. High-affinity (nM–pM) interactions with virus receptors involve a large area of interaction (about 10 nm2) between the viral and cellular receptors, and often involve large conformational changes. For example, the binding of HIV-1 gp120 to CD4 and one of the CO-RECEPTORS CCR5 or CXCR4, and poliovirus to CD155, both result in conformational changes. One exception is the high-affinity binding of the adenovirus fibre to the coxsackievirus-adenovirus receptor (CAR), which does not involve significant conformational changes. The high-affinity receptor-binding site can be located in a deep crevice (or canyon) on the viral protein, such as in the picornaviruses, or can contain loops, cavities and channels, such as the adenovirus knob and HIV gp120. Rather unusually, the HveA receptor-binding site on HSV-1 glycoprotein D is situated on an amino-terminal extension at one edge of the glycoprotein D molecule rather than being assembled from many parts of the glycoprotein D sequence, as for a typical binding surface or binding pocket. It undergoes conformational changes on binding to the receptor. There is no correlation between the structure of the viral entry protein structure and the structure of the cellular receptor. Viruses from the same family, such as retroviruses, can bind to different cellular receptors, and the same cellular molecule, for example, sialic acid, can serve as a receptor for several different viruses [24]. The conformational changes that are induced by interactions with one receptor can be required to expose the binding site for another receptor, for example the interaction between CD4 and gp120 induces the exposure of a high-affinity binding site for a co-receptor (typically CCR5 or CXCR4) on HIV-1 gp120. In this case, CD4 serves as an 'attachment' receptor that ensures specific binding to CD4-expressing cells and the co-receptor serves as a 'fusion' receptor that induces conformational changes that lead to exposure of fusogenic sequences. In some strains of HIV-1, co-receptors can mediate both attachment and fusion in the absence of CD4 [24]. Entry can also be initiated by binding to a low-affinity receptor, such as heparin sulphate, followed by higher affinity interactions. The role of many receptors in entry remains unresolved or controversial. Entry into cells through interactions with more than one receptor seems to be widely used by viruses, especially for the infection of specific types of cells in vivo. In the case that a specific cell receptor is absent, alternative virus receptors have also been identified for some viruses. For example, galactosyl ceramide and its sulphated derivative (sulphatide) can support low level HIV-1 infections in some CD4-negative cell lines, although the roles of such alternative receptors in vivo remain unknown [25]. Although the number of identified receptors for human viruses has increased rapidly during the past two decades, most virus receptors remain uncharacterized, and there are only a few X-ray crystal or cryoelectron microscopy structures of entry proteins in complex with receptors. Identification of new receptors is important for understanding virus tropism, pathogenicity and the mechanisms of entry. Recently, the receptor for the severe acute respiratory syndrome coronavirus (SARS-CoV), the angiotensin-converting enzyme 2 (ACE2) was identified, only months after the virus was discovered, and the receptor-binding domain has been localized to amino-acid residues 303–537 of the SARS-CoV entry protein [26]. Virus-receptor function is affected by membrane organization. Lipid rafts have been intensively studied to determine any possible role in virus entry5. Although rafts are well characterized, their role in virus entry is controversial. Studies on HIV-1 entry illustrate the controversies that still exist with regard to the role of rafts in virus entry. Together, depletion of cholesterol and inhibition of glycosphingolipid synthesis decrease the efficiency of HIV-1 Env-mediated membrane fusion typically by about two-fold which could indicate effects on membrane fusion owing to the disruption of raft integrity. The raft

component glycosphingolipids Gb3 and GM3 seem to interact with gp120 in the presence of CD4, which could also indicate that rafts are involved in HIV-1 entry [27]. However, recent data indicate that HIV-1 infection does not depend on the presence of CD4 and CCR5 in rafts and it has been proposed that cholesterol modulates HIV-1 entry by an independent mechanism, perhaps related to membrane merging or modulation of co-receptor binding. Although the exact role of rafts in receptor-expressing host cells is controversial, recent experiments have clearly shown the importance of rafts for membrane fusion by clustering sufficient numbers of influenza HA molecules in rafts 200–280nm in diameter. So, it appears that in both receptor expressing and Env-expressing cells the function of rafts is mainly to increase the local concentrations of molecules that are involved in entry. Glycosphingolipids might not only provide the structural basis for raft formation, but could also interact directly with viral entry proteins and cellular receptor molecules.

Virus Incubation Period

During most virus infections, no signs or symptoms of disease occur through the stage of virus dissemination. Thus, the incubation period (the time between exposure to virus and onset of disease) extends from the time of implantation through the phase of dissemination, ending when virus replication in the target organs causes disease. Occasionally, mild fever and malaise occur during viremia, but they often are transient and have little diagnostic value [28]. The incubation period tends to be brief (1 to 3 days) in infections in which virus travels only a short distance to reach the target organ (i.e., in infections in which disease is due to virus replication at the portal of entry). Conversely, incubation periods in generalized infections are longer because of the stepwise fashion by which the virus moves through the body before reaching the target organs. Other factors also may influence the incubation period. Generalized infections produced by togaviruses may have an unexpectedly short incubation period because of direct intravascular injection (insect bite) of a rapidly multiplying virus. The mechanisms governing the long incubation period (months to years) of persistent infections are poorly understood [29]. The persistently infected cell is often not lysed, or lysis is delayed. In addition, disease may result from a late immune reaction to viral antigen (e.g., arenaviruses in rodents), from unknown mechanisms in slow viral infections during which no immune response has been detected (as in the scrapie-kuru group), or mutation in the host genetic material resulting in cellular transformation and cancer.

Entry inhibitors, antibodies and vaccines

Entry is an attractive target for inhibition because the entry machinery is extracellular and it is therefore easier for drug molecules to reach than intracellular targets. Any step(s) of the entry process can be targeted by an entry inhibitor. Various types of molecules, such as proteins, peptides, carbohydrates, small organic molecules, nucleic acids and supramolecular structures, including liposomes and phage, have been found to inhibit entry. Yet, out of more than 30 antiviral drugs, there are only two entry inhibitors Synagis and T-20 that have been approved by the US Food and Drug Administration (FDA) for clinical use (excluding human immune globulin for use against hepatitis A and measles, and virus-specific polyclonal human immune globulins for use against cytomegalovirus, hepatitis B, rabies, RSV, vaccinia and varicella-zoster). Only T-20 (which is marketed as enfuvirtide) is used for the treatment of ongoing viral (HIV-1) infection [30]. The humanized monoclonal antibody Synagis (which is also known as palivizumab) is used for the prevention of RSV infections in neonates and immunocompromised individuals. T-20 is not a small molecule regarded as the 'gold standard' for a drug but is a peptide that cannot be taken orally [31]. A small organic molecule entry inhibitor (pleconaril) showed promising results for the treatment of infections

caused by the picornaviruses that cause the common cold, but was not approved by the FDA owing to concerns about potential interactions with other drugs although different formulations are presently being evaluated for use in life-threatening disease. At present, a number of compounds are in clinical trials, including small organic molecules that bind to the HIV-1 co-receptor CCR5, and entry inhibitors are also being tested for efficacy as microbicides [31].

CONCLUSION

Viral pathogenesis is a key field of investigation in a One Health approach to zoonotic and emerging diseases. This field encompasses the evolution of viruses, including genetic changes through immune pressure, mutation, recombination, and reassortment that may change transmission, receptor usage and host range, vector competence, and virulence. Examples like influenza viruses, SARS coronavirus, and novel coronal virus known as covid-19 is prevalent today. Minor changes in viral genes encoding ligands for cell receptors may result in a shift in cell tropism and host range from an animal reservoir to humans, or a shift in vector competence, causing increased virus transmission to humans. Elucidating the factors underlying such changes requires collaborative efforts of molecular virologists and cell biologists, as well as experts on the responses to infection of individual organisms like National Center for Disease Control (NCDC). The same conclusions concerning a multidisciplinary approach apply to understanding disease expression through systems biology. Signal transduction pathways cause pro-inflammatory changes following viral infections, as well as the innate and adaptive immune responses to viral proteins can also be clarified by some filed of biochemist.

REFERENCES

1. Baranowski, E., Ruiz-Jarabo, C. M. & Domingo, E. (2011). Evolution of cell recognition by viruses. *Science* **292**, 1102–1105.
2. Barnard, R. J. & Young, J. A. (2013). α-retrovirus envelope-receptor interactions. *Curr. Top. Microbiol. Immunol.* **281**, 107–136.
3. Blumenthal, R., Clague, M. J., Durell, S. R. & Epand, R. M. (2003). Membrane fusion. *Chem. Rev.* **103**, 53–69.
4. Bomsel, M. & Alfsen, A. (2016). Entry of viruses through the epithelial barrier: pathogenic trickery. *Nature Rev. Mol. Cell Biol.* **4**, 57–68.
5. Carfi, A. (2014). Herpes simplex virus glycoprotein D bound to the human receptor HveA. *Mol. Cell* **8**, 169–179.
6. Chen, Y. A. & Scheller, R. H. (2016). SNARE-mediated membrane fusion. *Nature Rev. Mol. Cell Biol.* **2**, 98–106.
7. Colman, P. M. & Lawrence, M. C. (2016).The structural biology of type I viral membrane fusion. *Nature Rev. Mol. Cell Biol.* **4**, 309–319.
8. Dimitrov, D. S. (2011). Cell biology of virus entry. *Cell* **101**, 697–702.
9. Dimitrov, D. S. (2018). Quantitation of HIV-1 infection kinetics. *J. Virol.* **67**, 2182–2190.
10. Dimitrov, D. S., Willey, R., Martin, M. & Blumenthal, R. (2012). Kinetics of HIV-1 interactions with sCD4 and $CD4^+$ cells: implications for inhibition of virus infection and initial steps of virus entry into cells. *Virology* **187**, 398–406.
11. Fass, D. (2019). Structure of a murine leukemia virus receptor-binding glycoprotein at 2.0 angstrom resolution. *Science* **277**, 1662–1666.
12. Hogle, J. M. (2014). Poliovirus cell entry: common structural themes in viral cell entry pathways. *Annu. Rev. Microbiol.* **56**, 677–702.
13. Hogle, J. M., Chow, M. & Filman, D. J. (2017). Three-dimensional structure of poliovirus at 2.9 A resolution. *Science* **229**, 1358–1365.

14. Igakura, T. (2013). Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science* **299**, 1713–1716.
15. Kielian, M., Chatterjee, P. K., Gibbons, D. L. & Lu, Y. E. (2018). Specific roles for lipids in virus fusion and exit. Examples from the α-viruses. *Subcell. Biochem.* **34**, 409–455.
16. Lakadamyali, M., Rust, M. J., Babcock, H. P. & Zhuang, X. (2016). Visualizing infection of individual influenza viruses. *Proc. Natl Acad. Sci. USA* **100**, 9280–9285.
17. Lowy, R. J., Sarkar, D. P., Chen, Y. & Blumenthal, R. (2017). Observation of single influenza virus-cell fusion and measurement by fluorescence video microscopy. *Proc. Natl Acad. Sci. USA* **87**, 1850–1854.
18. Mancini, E. J., Clarke, M., Gowen, B. E., Rutten, T. & Fuller, S. D. (2018). Cryo-electron microscopy reveals the functional organization of an enveloped virus, Semliki Forest virus. *Mol. Cell* **5**, 255–266.
19. Mothes, W., Boerger, A. L., Narayan, S., Cunningham, J. M. & Young, J. A. (2017). Retroviral entry mediated by receptor priming and low pH triggering of an envelope glycoprotein. *Cell* **103**, 679–689.
20. Pelkmans, L., Puntener, D. & Helenius, A. (2014). Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. *Science* **296**, 535–539.
21. Rawat, S. S. (2013). Modulation of entry of enveloped viruses by cholesterol and sphingolipids. *Mol. Membr. Biol.* **20**, 243–254
22. Remeta, D. P. (2018). Acid-induced changes in thermal stability and fusion activity of influenza hemagglutinin. *Biochemistry* **41**, 2044–2054.
23. Rossmann, M. G. (2010). Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature* **317**, 145–153.
24. Seisenberger, G. (2015). Real-time single-molecule imaging of the infection pathway of an adeno-associated virus. *Science* **294**, 1929–1932.
25. Sieczkarski, S. B. & Whittaker, G. R. (2012). Dissecting virus entry via endocytosis. *J. Gen. Virol.* **83**, 1535–1545.
26. Skehel, J. J. & Wiley, D. C. (2016). Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu. Rev. Biochem.* **69**, 531–569.
27. Stubbs, M. T. (2011)Anthrax X-rayed: new opportunities for biodefence. *Trends Pharmacol. Sci.* **23**, 539–541.
28. Takeda, M., Leser, G. P., Russell, C. J. & Lamb, R. A. (2016). Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. *Proc. Natl Acad. Sci. USA* **100**, 14610–14617.
29. Waarts, B. L., Bittman, R. & Wilschut, J. (2017). Sphingolipid and cholesterol dependence of α-virus membrane fusion. Lack of correlation with lipid raft formation in target liposomes. *J. Biol. Chem.* **277**, 38141–38147
30. Wang, J. (2015). Protein recognition by cell surface receptors: physiological receptors versus virus interactions. *Trends Biochem. Sci.* **27**, 122–126.
31. White, J., Kartenbeck, J. & Helenius, A. (2011). Fusion of Semliki forest virus with the plasma membrane can be induced by low pH. *J. Cell. Biol.* **87**, 264–272.