Do viruses use vectors to penetrate mucus barriers?

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Abstract

I propose a mechanism by which viruses successfully infect new individuals, despite being immotile particles with no ability for directed movement. Within cells, viral particle movements are directed by motors and elements of the cytoskeleton, but how viruses cross extracellular barriers, like mucus, remains a mystery. I propose that viruses cross these barriers by hitch-hiking on bacteria or sperm cells which can transport themselves across mucosal layers designed to protect the underlying cells from pathogen attack. An important implication of this hypothesis is that agents that block interactions between viruses and bacteria or sperm may be new tools for disease prevention.

Viral-bacterial associations produce disease

A growing body of evidence suggests that many infectious diseases result from close cooperation between viruses, bacteria and fungi [1]. One thoroughly studied example of a mixed viral-bacterial infection is influenza, commonly known as the flu. This disease spreads across the globe each year in seasonal epidemics, killing hundreds of thousands and sometimes millions of people. The initial disease is triggered by the influenza virus; however, most deaths occur due to secondary infections caused by three bacteria, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Hemophilus influenzae*. The effects of bacteria and viruses are so closely associated that the bacterium *Hemophilus influenzae* was initially thought to be the causative agent for influenza [1].

One possible explanation for the association of influenza and bacterial infections is that influenza viral attack weakens the host, and enables the bacteria to infect. However, it is suspicious that certain bacterial strains, but not others, can take advantage of influenza infections. This suggests that the bacteria are more than just opportunistic organisms infecting a weakened host. Rather, it may indicate that viruses and bacteria cooperate to cause disease. Viruses may rely on specific bacteria to take hold of a host, just as much as bacteria may rely on viruses to provide a window of opportunity for host infection. One main obstacle to fighting diseases of this kind is the lack of knowledge about how viruses and bacteria interact to increase the morbidity and mortality of infection.

The influenza virus is transmitted to humans through aerosols that contain the virus. To achieve infection, however, the virus must overcome the body's first line of defense, a thick mucus layer in the nose, throat, and lung, which protects the underlying cells from contact with noxious agents and pathogens. Viruses have no capacity for active movement, but they could potentially spread through mucus by passive diffusion. However, a typical mucus gel is 50–700 μm thick and represents a crowded environment with protein concentrations up to 300 mg/ml [2–4],

which is similar to protein density in the cytoplasm. Assuming that molecular crowding imposes similar constraints on free diffusion in mucus as it does in cytoplasm, the passive diffusion of even relatively small viruses through a typical mucus gel should take several days. This presents a problem for viruses, since mucus is cleared and replenished by the basal epithelium several times each day. It is therefore unlikely that viruses can diffuse through the mucus gel more quickly than mucus bulk flow can sweep them out of the body.

In cytoplasm, viruses employ many mechanisms to travel long distances relative to their own size. Some viruses hitchhike on motor proteins, which mediate transport along microtubules between the periphery and center of cells [5]. Other viruses rely on transport by the actin system, either by newly polymerized actin laments that push a particle, or by myosins that move along actin laments [5]. Viruses also use cells and organisms as vehicles; for example, it has been suggested that Epstein–Barr virus (EBV), an orally transmitted herpesvirus, uses B cells as a transfer vehicle to reach epithelial cells [6]. Other well-studied examples include dengue and yellow fever viruses, which use insects as vectors to bridge travel distances as far as many kilometers [7,8]. I hypothesize that viruses have also evolved strategies to facilitate their dispersal and passage through mucus gels.

A new model of two-way cooperation between viruses and bacteria

In contrast to viruses, numerous bacteria are well adapted to life in mucus and co-exist with a healthy host. Bacteria use various forms of motility, including flagellar propulsion and gliding, to cross mucus barriers at rates much higher than diffusion would allow. By attaching to motile bacteria, viruses may reduce the time it takes them to cross mucus barriers from days to minutes. In addition to efficiently moving through mucus, some bacteria are able to reside within it and resist clearance from the host. For example, bacteria associated with flu attach to mucosal surfaces of the nose and throat [1,9–11] and grow into colonies that protrude into the mucus gel and withstand expulsion. I propose that viruses attach to bacteria that inhabit mucus, and that this initial adsorption to bacteria sets the stage for viral infection (Fig. 1).

What benefits may bacteria reap from such a direct interaction with viruses? Viruses may reciprocate their improved access to epithelial cells by locally inactivating the immune cells that chase the bacteria. A highly efficient immune system continuously contacts and consumes bacteria in order to maintain their density at innocuous levels. Influenza virions can combat the policing activity of a host's immune system by inactivating immune cells; the viral virus NS1 protein, for example, can modulate immune cell activity in many different ways [12]. Bacteria could then exploit this temporary lack of immunological control and expand their populations to densities that result in pathogenic invasion.

According to this model, the onset of flu depends on the physical adsorption of viruses to bacterial cells inhabiting mucus. Once this has occurred, the viruses can invade and thereby pave the way for a virulent bacterial infection. A direct interaction between viruses and bacteria has not been suggested in the context of human disease, but it is a prominent phenomenon in natural waters where viruses concentrate within bacterial communities [13].

Sexually transmitted viruses might derive motility from sperm cells

A different, but conceptually related, strategy may be used by viruses that infect epithelia in the female genital tract, such as the human immunodeficiency virus (HIV), herpes simplex virus (HSV), and human papillomavirus (HPV). All these viruses are primarily transmitted through sexual intercourse, yet the exact mechanism by which sexual contact promotes their infection remains unclear. In the female genital tract, viruses face the same problem as that encountered in the lung: to infect the epithelium, they must penetrate a thick mucus gel, which

is probably difficult to achieve by simple diffusion. Here too, viruses could exploit exogenous sources of motility (Fig. 2).

In contrast to viruses, sperm cells associated with sexual contact are highly motile and well adapted to passage through mucus [14]. Sexually transmitted viruses may have evolved to exploit sperm cells as vehicles for dispersal and mucus penetration within the female genital tract. Consistent with this hypothesis, HPV16 capsids, HIV, and HSV have been found associated with human sperm cells [15–21].

The mechanism of interaction between sperm and virus is not well understood; however, one study suggests that glycosaminoglycans or molecules of similar structure on the surface of sperm enable binding of viruses [22]. Many sexually transmitted viruses, including HPVs, HIV, and HSV, adsorb to epithelial cell-surface glycosaminoglycans (GAGs), particularly heparan sulfate proteoglycans (reviewed in [23]). Thus, the mechanism by which viruses interact with sperm may be similar to the manner in which they adsorb to epithelial cell surface GAGs during early stages of infection. How viruses transfer from their motile vehicles to their target cells is not yet clear. One possibility is that sperm cells have a shorter lifespan than epithelial cells in the female genital tract. Hitch-hiking viruses will likely dissociate from disintegrating sperm and become free to infect the epithelium.

It is noteworthy that a direct interaction with live sperm cells has been documented for the vertically transmitted fish rhabdovirus, which causes infectious hematopoietic necrosis in salmonids [24]. Salmonid fish are oviparous species with external fertilization. The rhabdovirus adsorbs to sperm and transfers with them through the open water toward eggs. Mechanistically similar interactions may occur between sexually transmitted viruses and sperm in humans, and promote virus dispersal and mucosal penetration in the female genital tract [22].

According to this hypothesis, viral infections could be compared to classical vector-borne diseases such as malaria, in which *Plasmodium* spp. use highly motile insect carriers to reach host targets over large distances and bypass their first line of defense, the skin.

I emphasize that the proposed hypothesis does not exclude alternative pathways for viral infection, for example through wounding or otherwise compromised mucosal epithelia. However, these mechanisms are less reliable and will likely not be as efficient as hitchhiking on cells that are optimized for translocation through mucus.

Testing the hypothesis

Influenza is an ideal model to test whether viruses cooperate with bacteria to overcome mucus barriers. This hypothesis makes two testable predictions: 1. The influenza virus physically binds to bacteria associated with flu morbidity and mortality. 2. The influenza virus and the bacteria that it binds will not cause disease on their own, but only in combination with each other.

Two parallel approaches can be taken to test these predictions. First, one may ask if the influenza virus directly associates with bacteria *in vitro* or *in vivo*. Protocols to produce and isolate influenza particles are established, and the cultivation of *S. pneumoniae*, *S. aureus*, and *H. influenzae* is routine in many laboratories. These protocols could be the basis for a test of the proposed model's first prediction. If viral particles do attach to bacteria, one may ask if a combination of influenza virus and bacteria is necessary to cause infection. Mice are well-established models for studying human disease, and germfree mice would be especially useful in testing the second prediction of the proposed model because they are natively devoid of microbes and hence can be infected with defined combinations and quantities of viruses and

bacteria. One would infect germfree mice with the influenza virus, individual bacteria, or combinations of both, and test whether viruses and bacteria indeed depend on each other to establish infection.

If we find that viruses and bacteria indeed cooperate directly to manifest the flu, it is possible that cooperative interactions of this kind are also the basis of other polymicrobial infectious diseases such as AIDS, chickenpox, or measles. With germfree mice and the proposed biochemistry experiments, we have a good stage on which to dissect the underlying microbial interactions of these diseases as well.

Implications: new strategies for viral disease prevention and treatment

The potential importance of virus-vehicle association for virus dispersal and mucosal penetration suggests that blocking viruses from binding to bacteria or sperm may be a new strategy to prevent viral diseases [22]. Good candidates to achieve this are peptides derived from the respective virus or benign intact viruses that shield binding sites on the surface of bacteria or sperm. Such tools may offer a critical advantage over vaccination with antigens, as is undertaken for Influenza or HPV: Viral populations can rapidly escape neutralization by specific antibodies by evolving new surface properties. But to bypass inhibitors of virus-vehicle attachment, the viral populations need to evolve new mechanisms that exploit novel binding sites on their vehicles, which are presumably limited. Hence, blocking viral attachment to its vehicle may be a more sustainable method of disease prevention than conventional vaccination.

It has become standard practice to treat certain viral infections, for example of the upper respiratory tract, with antibiotics. The rationale is that these infections might be of bacterial origin, or that the antibiotics will prevent secondary infections. In light of the hypothesis presented here, a specific antibiotic would slow or stop a viral infection by killing bacteria within the mucosa and thereby eliminating the foundation for successful viral entry into our bodies.

Acknowledgments

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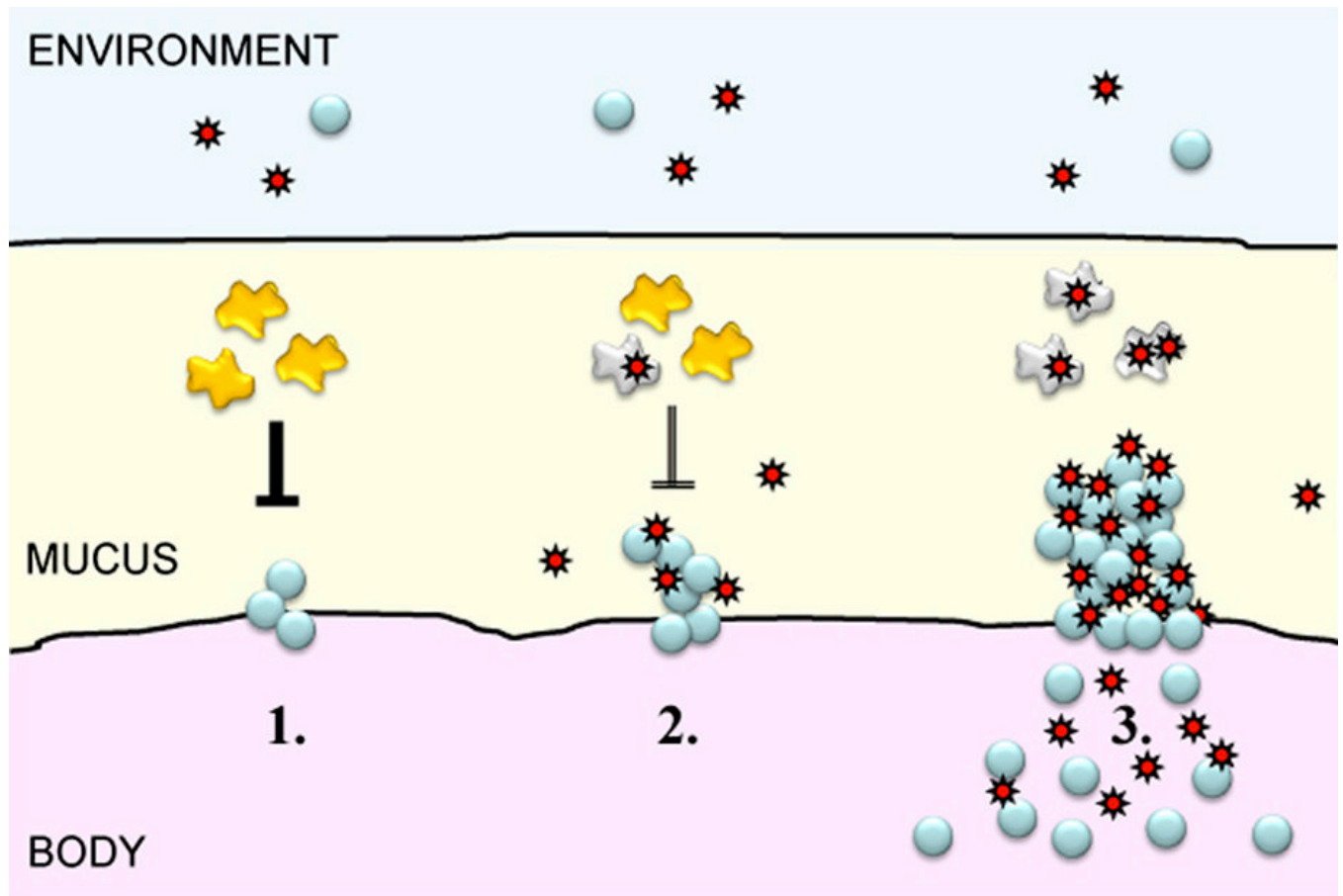
Figure 1. Two-way cooperation of viruses and bacteria to produce disease
1. Bacteria (blue) associated with the flu attach to mucosal cell surfaces of nose and throat and grow colonies. Immune cells (yellow) keep the density of bacteria at innocuous levels. 2. Viruses (red) adsorb to the sessile bacteria, setting the stage for viral infection. Viruses reciprocate the improved access to the host by inactivating the immune cells (grey) that chase the bacteria. 3. Bacteria exploit this temporary lack of immunological control to increase their populations to levels that result in pathogenic invasion.
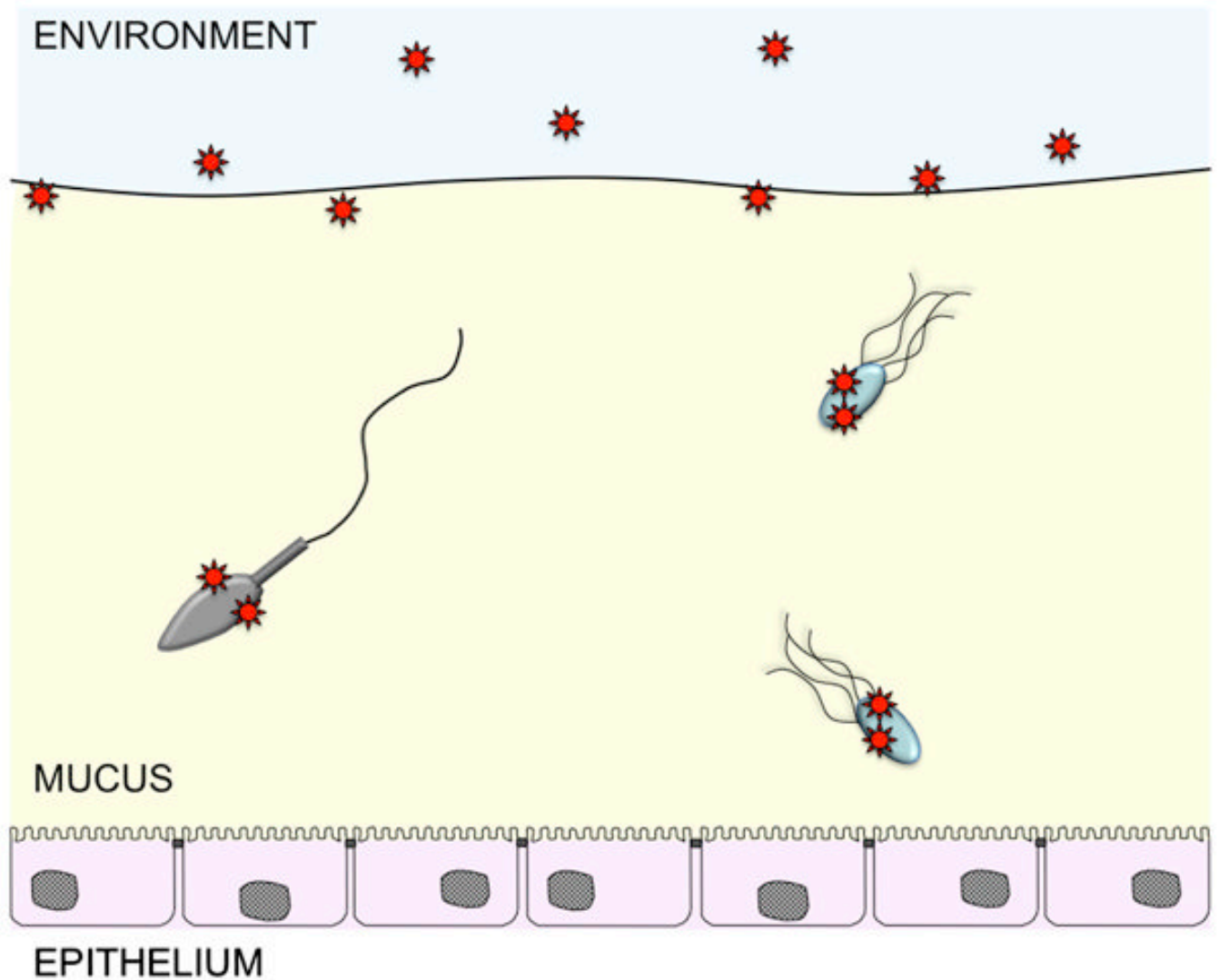
Figure 2. Viruses may exploit exogenous motility to penetrate mucus barriers
Numerous bacteria (blue) are well adapted to life in mucus and co-exist with a healthy host.
Bacteria can cross mucus barriers at rates much higher than diffusion by using various forms
of motility such as flagellar propulsion and gliding. In contrast, viruses (red) are immotile
particles with no ability for directed movement. By attaching to motile bacteria, viruses would
be able to cross mucus barriers much faster than diffusion would allow. Similarly, viruses that
infect epithelia in the female genital tract may have evolved to exploit sperm cells (grey) as
vehicles for dispersal and mucus penetration.