Viral Subversion Mechanisms in Chronic Kidney Disease Pathogenesis

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Viruses cannot autonomously replicate but must rely on the host cellular machinery to support their life cycle. Through natural selection, viruses have evolved strategies to co-opt the host organism to be a better site for their propagation. Some of these strategies are directed at the cellular machinery and involve complicated and ingenious solutions to optimize infection, replication, viral gene expression, and new virion assembly and shedding. Other strategies are directed at the host’s innate and adaptive immune systems that permit the virus to evade clearance mechanisms. The more common pathogenic viral infections in nephrology—cytomegalovirus, HIV-1, hepatitis C virus, polyomavirus BK, and parvovirus B19—all have acquired subversion strategies that benefit the virus but because they interfere with normal cellular and immune processes also have become pathogenic to the host. In addition, the highly prevalent viruses cytomegalovirus, BK, and B19 cause severe disease only in the setting of immunosuppression, revealing the very delicate balance that some viruses have achieved with their host’s immune system. Thus, selective pressure for survival drives both the evolution of more sophisticated viruses and the host immune system as it evolves to combat the environment of adapting and emerging infectious agents. Understanding the molecular mechanisms of these viral subversion strategies may reveal new targets for the development of highly specific antiviral therapies and also aid vaccine development.


All viruses are obligate intracellular parasites and are unable to replicate without the support of the cellular machinery of the host. Because of this dependence, they are at the threshold of what is considered a living organism. For a virus to use a eukaryotic cell as a host, the virus encounters many barriers to completing its life cycle and has evolved mechanisms to modify the host both at the cellular and the organismal level to ensure its survival. This article reviews host–virus interactions, from the perspective of the virus, focusing on the more common pathogenic viruses encountered in nephrology: Cytomegalovirus (CMV), HIV-1, hepatitis C virus (HCV), polyomavirus BK, and parvovirus B19.

Viruses are some of the smallest and simplest living organisms, consisting of only a piece of genetic material, either DNA or RNA, surrounded by a protein coat. All viral genomes contain noncoding sequences that control replication and transcription, as well as coding sequences that specify the viral proteins. How many proteins each virus encodes depends mostly on the physical size of the virion, because there are limitations on the amount of DNA or RNA that can be packaged within the protein coat. Viral proteins can be categorized into three general functions (1). The first function is to provide for the structural proteins of the coat, the second function is to ensure the replication of the genetic material, and the third is to modify the host cellular machinery to facilitate the first two functions. The last function is typically unique to each virus and because it subverts normal cellular activities is the aspect of the virus that often leads to pathogenesis and disease.

All viruses encounter similar barriers when using a eukaryotic cell as a host (1). The first major barrier is entering the cell, and viruses typically exploit either a receptor-mediated mechanism or an endocytic process to cross the plasma membrane (Figure 1). Once inside the cell, the virus encounters a second major barrier: It must cross the nuclear membrane. Some viruses, such as parvovirus B19 (2), have evolved no specific mechanism to cross the nuclear membrane. Subsequently, these viruses can replicate only in actively dividing cells because they must wait for the cell to enter mitosis (where the nuclear envelope breaks down) to gain access to the critical replication and transcriptional machinery of the nucleus. After entering the nucleus, the subsequent steps of replication, transcription, translation of the viral genes, and assembly of new virions require extensive use of the cellular machinery. The final release of new virions can occur by budding, commonly used by the enveloped viruses such as HIV-1, which preserves the host cell. Other viruses have evolved no mechanism to exit the cell and either must wait for the host cell to die or, as in the case of parvovirus B19, induce the cell to die through the expression of the viral nonstructural protein NS1, thereby releasing progeny virions on cell rupture (3). Thus, for a virus to use a cell type as a host successfully, it must accommodate any restrictions that it encounters in the host cell environment or else evolve a strategy to circumvent the barriers.
Subversion Mechanisms

There are several subversion strategies that viruses use to support their replication in the host cell (Figure 1). The strategies are typically deployed through the use of novel, nonstructural “accessory” proteins that either evolve de novo or are co-opted from the host. These accessory genes will be retained in the viral genome if they provide an evolutionary selective advantage to the virus through improved viral fitness or replication capacity. However, because these accessory genes function to modify the host cell, they also frequently interfere with normal cell functions, leading to pathogenesis (Table 1). The more common strategies include molecular mimicry, hijacking host signaling pathways, and oncogenes or transforming proteins to modify the cell cycle.

Molecular Mimicry Strategies

Molecular mimicry is a strategy to imitate host proteins, and, in many instances, the viral homologues are genes that are directly pirated from the host genome through recombination events (4,5). As part of the viral life cycle, many viruses will integrate within the host genome, and it is during these recombination events that host genes may be incorporated within the viral genome. As is typical of many of the DNA viruses, targets for molecular mimicry are frequently immunomodulatory proteins directed at neutralizing the host's antiviral measures or proteins that preserve infected cells from death by blocking apoptosis or cell-mediated killing activities.

The large β-herpes virus CMV is a classic example of a virus that uses molecular mimicry to modify the innate, adaptive, and inflammatory immune responses of the host. CMV produces approximately 160 proteins, and of these, only 50 to 60 are necessary for viral replication (6). The remaining 100 viral proteins have evolved to manipulate the host's immune system through mimicking host cytokines and chemokines (virokines), cytokine and chemokines receptors (viroreceptors), and soluble cytokine-binding proteins that function as cytokine scavengers (Table 1). These viral homologues have broad-ranging effects on lymphocyte recruitment, cytokine activation, apoptosis, and T and B cell functions, ultimately resulting in the persistence of a lifelong infection with periodic reactivations.

A key immune evasion strategy of CMV is to modulate the MHC I and II (6). The CMV glycoproteins US2, US3, US6, and US11 function to downregulate posttranslationally MHC I cell surface expression. US3, which is expressed in the endoplasmic reticulum early in infection, also prevents the loading of peptides into MHC I and disrupts intracellular MHC II invariant chain interactions. These viral proteins effectively function to elude effector and cytotoxic T cell responses and thus prevent the normal host response to eliminate infected cells. CMV also produces many chemokines and chemokine receptor homologues that can function as either agonists or antagonists to cytokine and chemokine activity. For example, CMV produces an IL-10 homologue (vIL-10) that functions to suppress the production of other proinflammatory cytokines and the activity of macrophages (4). Alternatively, some virokines function to support virus dissemination, rather than immune evasion, by recruiting immune cells to the site of infection, thereby providing additional new target cells for the virus to infect.

RNA viruses, such as the flavivirus HCV, also have evolved intricate immune evasion strategies (Table 1). After infection, the appearance of the HCV RNA in the cytoplasm triggers the release of IFN, the key effector molecules of the immediate early response to viral infection (7). The HCV nonstructural (NS) protein complex NS3/4a inactivates components of the Toll-like receptor (TLR) signaling complex, blocking the initiating mechanism of the IFN antiviral mechanism. This blockade of a key innate immune response can result in the persistence of HCV beyond the acute infection.

Figure 1. Eukaryotic cell barriers to the viral life cycle and types of subversion mechanisms that are used by the virus. Viruses require the use of the host cellular machinery to replicate their nucleic acid and produce viral proteins for the assembly of new virion. In addition, the virus, whether intracellular and extracellular, must escape the antiviral defense mechanisms of the host's immune system. For the virus to survive, it must evolve complex strategies to circumvent any cellular blocks to completing its life cycle and to evade the host immune system.
phase, especially in individuals with a weakened or suppressed immune system (8).

Hijacking Strategies

As already discussed, viruses can modify the functions of cytokines and chemokines by producing viral homologues of these key immunomodulatory host proteins. However, another strategy for viruses to accomplish the same effect is to modulate the expression of the host cytokine and chemokine genes by altering the activity of host transcription factors. This “hijacking” of signal transduction or transcription factor activity is a second major subversion strategy that is used frequently by RNA viruses to manipulate the host cell (5,9). Typically, viruses achieve these novel functions not by directly mimicking host protein function but through the evolution of unique accessory proteins to interact with important intracellular signaling cascades.

In HIV-1 infection, the virus has evolved six NS accessory proteins (Table 1). The function of the accessory proteins in the viral life cycle is fairly well known (10), but their role in contributing to host pathogenesis is just beginning to emerge. In HIV-associated nephropathy (HIVAN), it is now accepted that a part of the pathologic process is the direct infection of renal epithelia, which results in the expression of these accessory proteins within kidney cells (reviewed in references [11,12]). Extensive work with recombinant viruses and cell culture systems (13–17) and also studies with transgenic rodents (18–25) have shown that the expression of these accessory genes, independent of infection per se, is directly responsible for many aspects of HIVAN pathogenesis. In both rodent models and infected patients, HIVAN is characterized by phenotypic changes in epithelial cell behavior, including apoptosis, cellular dedifferentiation, and, most significant, excessive proliferation (26).

As an example of a hijacking strategy in HIVAN, HIV-1 is widely known to interfere with the signal transduction pathway that activates the transcription factor NF-κB (27,28). NF-κB is the central regulator of most immune and inflammatory processes and controls the transcription of many host genes (29). As with all viruses, the expression of the viral genes depends on the available host transcription factors. HIV-1 has evolved to exploit NF-κB because it is a very potent transcriptional activator in T cells and macrophages, the primary targets for HIV-1 infection. NF-κB activation is controlled by phosphorylation and degradation of its inhibitor, IκB, which results in the removal of the inhibitor from NF-κB, allowing it to translocate to the nucleus and activate transcription. HIV-1

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. of Viral Proteins</th>
<th>Role in Host Pathogenesis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B19</td>
<td>4 (9c)</td>
<td>Total Nonstructural, Accessoryb</td>
<td>Cytotoxic; induces IL-6 expression</td>
</tr>
<tr>
<td></td>
<td>2 (4c)</td>
<td></td>
<td>None identified</td>
</tr>
<tr>
<td>NS1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BK</td>
<td>6</td>
<td></td>
<td>Blocks apoptosis, increases cell division</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>None identified</td>
</tr>
<tr>
<td>CMV</td>
<td>160</td>
<td></td>
<td>Immunomodulatory</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1</td>
<td>15</td>
<td></td>
<td>Mechanism unknown, possible contribution to disease in transgenic mice</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nef</td>
<td></td>
<td></td>
<td>Hijacks Src-signaling (Stat3 and MAPK1,2 targets); hijacks the cell cycle (cyclin D1 expression and retinoblastoma phosphorylation)</td>
</tr>
<tr>
<td>Vpr</td>
<td></td>
<td></td>
<td>Mechanism unknown, contributes to disease in transgenic mice</td>
</tr>
<tr>
<td>Rev, Vif, Vpu</td>
<td></td>
<td></td>
<td>None identified</td>
</tr>
</tbody>
</table>

aCMV, cytomegalovirus; dsRNA, double-stranded RNA; HCV, hepatitis C virus; MAPK, mitogen-activated protein kinase.
bNonstructural proteins with enzymatic functions are not included.
cNine unique mRNA transcripts have been identified; expression and/or function has not been determined for all putative proteins.
infection of both immune cells (30,31) and kidney cells (32) results in the hijacking of the NF-κB activation process at the level of IκB phosphorylation, inducing a persistent degradation of IκB and subsequent persistent activation of NF-κB. This hijacking strategy ensures that the viral genes are transcribed at a high rate; however, the disrupted NF-κB activation also alters the expression of many NF-κB–dependent host genes. In HIVAN, some of these host target genes include Fas and FasL (33) and cyclin D1 (34). The HIV-1–induced dysregulation of these host genes leads to increased apoptosis and increased proliferation, respectively, in renal parenchymal cells, both contributing processes in HIVAN pathogenesis.

Oncogenes and Transformation Strategies
The increased proliferation of epithelial cells in HIVAN represents a significant difference in the pathogenic mechanism from the other, more common forms of FSGS, which are associated with overall loss of renal cell mass (35). This nonmalignant transformation of renal epithelial cells underlies both glomerular pseudocrescent and tubular cyst formation, each a significant pathologic component of HIVAN. Using cell culture studies, this transformation includes both increased proliferation and loss of contact inhibition and seems to be caused by the function of the HIV accessory protein Nef (13) through its hijacking of the Src-dependent signal transduction pathways (15). Pharmaceutical suppression of this proliferative phenotype in a transgenic mouse model of HIVAN resulted in a significant improvement in kidney function and pathology (36,37). Although Nef seems to be a critical disease determinant in HIVAN pathogenesis, the exact causal mechanism is not known. Nonetheless, future therapies directed at inhibiting Nef function may be useful in preventing or slowing the progression of HIVAN. Studies in transgenic models have shown that limiting the effect of HIV on the cell cycle alone through the use of cyclin kinase inhibitors can improve renal function and histopathologic changes, demonstrating that this may be a useful target for drug development (36,37).

Modifying the host cell cycle and oncogenic transformation is an important survival strategy for viruses that can replicate only in actively dividing cells, such as the polyomaviruses BK, JC, and SV40. Some of the most potent transforming proteins are the polyomavirus tumor (T) antigens. T antigens are accessory proteins that are required for replication of the viral DNA and also hijack the cell cycle by blocking the function of p53 and the retinoblastoma family members (38). The potency of T antigens to transform cells into a state of neoplastic proliferation is best characterized for the SV40 T antigen, which is frequently used in research applications to immortalize cell lines for their unlimited propagation in vitro. The polyomavirus BK, which naturally infects cells of the uroepithelium, produces a small t and large T antigen (Table 1), and a possible link between BK virus infection and cancer has been proposed (39). Although it is has been shown that BK viral sequences are present in cancers of the kidney and urinary tract, a role for the virus in oncogenesis is still debated (40–42). BK infection alone may not induce cancer, because BK virus infects up to 90% of the population (39) and most infected individuals do not develop cancer. Alternatively, it remains a possibility that the BK T antigen may be oncogenic, and BK infection could be a contributing factor to urogenital and other cancers either in combination with another mutagenic event (either in the host cell or in the virus) or possibly in the setting of co-infection with another virus, such as HIV-1 (43) or SV40 (44).

Symbiosis of Host–Virus Interactions
In the best possible scenario, the interaction of a virus with its host is commensal, in which the virus benefits from exploiting the host to ensure its own propagation but the host remains unharmed. The only evolutionary driving force for the virus is to replicate: To generate as many progeny virus as possible and to infect as many hosts as possible. Whereas some viruses establish a lifelong infection of the host while others move quickly from one host to the next, causing disease in the host is counterproductive to the virus’s biologic success, because a sick or dead host will only impede its ability to replicate.

Adaptation and Attenuation
An example of a virulence-defeating host–virus interaction is Ebola infection in humans (45). Although Ebola is highly transmissible between humans, it is also highly pathogenic, resulting in almost immediate death of the host. Because Ebola is a recent infectious agent to appear in humans, the immune system has not had the evolutionary time needed to establish the symbiotic balance between virus and host, in which both the virus adapts to the host and the human immune system devises a way to control the new infectious agent. Over time, the biologic success of the virus (dependent on natural selection) improves in the host by both attenuation, a process whereby the virus becomes less pathogenic, and by an increased viral replication capacity.

Another emerging infectious disease, HIV-1, has similarly not had the evolutionary time to adapt and attenuate to humans (46). Simian immunodeficiency virus (SIV), now accepted to be the ancestor to HIV-1, has been infecting chimpanzees for likely 10,000 yr or more (47). Within the past 100 yr, SIV made a species jump to humans (48). A significant difference between SIV infection in chimps and HIV infection in humans is that chimps do not develop AIDS and die from their infections. A recent study showed that the viral accessory protein Nef may play a significant role in this difference (49). Versions of the Nef protein are found in HIV-1, HIV-2, and the various SIV strains, and it functions to remove or prevent the plasma membrane presentation of receptors on the cell surface. One cell surface receptor that is efficiently downregulated by the SIV Nefs is CD3, which through association with the T cell receptor initiates activation-induced cell death, a normal and self-limiting process in the homeostasis of the immune system. The HIV-1 Nefs in human cells do not effectively downmodulate CD3. In the setting of a chronic infection, this failure to downmodulate CD3 results in robust activation-induced cell death, causing a depletion of effector T cell populations, which subsequently causes immunodeficiency and AIDS. SIV has adapted to the chimpanzee immune system during the past 10,000 yr to be less pathogenic, in part by protecting the chimp’s immune system through CD3 downmodulation.
Balance of Host–Virus Interactions and Immunosuppression

It is of interest to note that the more common viral infections related to chronic kidney disease include both viruses such as HIV-1 and HCV, which have symptomatic infections and a low prevalence (2 and 0.4% respectively in the United States), and other viruses such as B19, BK, and CMV, which have a very high prevalence (80 to 90% of the US population seroconvert in childhood), and individuals typically remain asymptomatic throughout life (50). Why does a virus that infects everyone cause disease in only a few and typically only in the setting of immunosuppression? The obvious answer centers on the immunocompromised hosts and underscores the delicate balance that exists between the host antiviral mechanisms and the ability of the virus to replicate.

CMV is an example of a virus that has achieved a successful but delicate balance between the immune system and viral escape. CMV infection causes severe disease only in the fetus or newborn infants or in an immunocompromised adult, situations in which the immune system is not fully functional (6). Immunosuppression upsets this balance, permitting the virus to escape the clearance mechanisms of the host. In both acute (51) and likely chronic graft dysfunction (52), CMV disease primarily involves the activation of cell-mediated immunity and is associated with inflammation and fibrosis in the graft.

As another example, B19 reactivations in immunocompromised patients (53) cause epo-resistant anemia from the direct infection and lysis of erythroid progenitor cells, because the B19 life cycle requires the death of the host cell to release new virions (54). In addition, patient morbidity and graft injury result from a general, multiorgan inflammation (myocarditis, pneumonitis, hepatitis) and subsequent organ damage from scarring (55). Thus, in transplantation, the reactivations of CMV, BK, and B19 generally result in a constant state of immune activation, with end organ damage occurring as a result of uncontrolled immune and inflammatory processes. Current therapies to control these reactivations have been most successful with the use of specific antivirals that exploit a vulnerable point in the viral life cycle, such as the use ganciclovir to manage CMV infection and disease. A better understanding of the basic biology of these different viruses and how they escape latency will aid the future development of therapeutic agents to limit their replication in immunocompromised hosts.

In the setting of transplantation, several mechanisms have been proposed for the reactivation of both BK and CMV infections (56). Polyomaviruses have evolved no specific mechanism to cross the nuclear membrane and therefore can infect only terminally differentiated cells. In the setting of transplantation, the frequently associated ischemic injury results in cell death of primarily tubular epithelia, a known target cell for BK infection. The normal repair response from ischemic injury includes the remaining tubular epithelial cells to proliferate to replace the lost cells. This reparative proliferation becomes an ideal environment for replication and propagation of BK virus in the kidney epithelium. Similarly, there are widely known cellular responses to allogeneic transplantation, such as the production of inflammatory cytokines TNF and IFN-γ, which are known to activate the host transcription factors NF-κB and activator protein-1. In CMV reactivation, the immediate-early (IE) gene set must be expressed to institute a new round of viral replication. Interestingly, the CMV major immediate early promoter has evolved to bind NF-κB and activator protein-1. The normal host responses to an allogeneic graft induce transcription factors that control the expression of the CMV immediate early genes, subsequently providing an environment to initiate a burst of new virus production.

Conclusions

Viruses are incapable of autonomous replication and must use the available cellular machinery of the host to support their life cycle. Because of this, viruses have evolved or borrowed from the host unique survival mechanisms to permit completion of their life cycle and include molecular mimicry, hijacking, and oncogenic transformation. Host pathogenesis develops from both the host’s immune response to the infection and the disruption of the normal cell cycle and cellular functions as a result of hijacking strategies that are used by the virus. It is survival-driven selective pressure and co-evolution of the virus with the host that shape not only the virus but also the host and its immune system. In this co-evolution, the processes of adaptation and attenuation improve viral fitness as well as establish a more commensal symbiosis by making the virus less pathogenic to the host. Studying the adaptation and subversion strategies of “old” viral infections in humans may provide insight into how to treat “new” infections that are highly virulent and result in severe disease and mortality.

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Disclosures

None.

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