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Pleiotropic mechanisms of virus survival and persistence

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Viruses are enormously efficient infectious agents that have been implicated in causing human disease for centuries. Transmission of these pathogens continues to be from one life form to another in the form of isolated cases, epidemics, and pandemics. Each infection requires entry into a susceptible host, replication, and evasion of the immune system. Viruses are successful pathogens because they target specific cells for their attack, exploit the cellular machinery, and are efficient in circumventing and/or inhibiting key cellular events required of survival. This article reviews some of the advances that have taken place in human virology in the past 50 years, emphasizing mechanisms that contribute to, and are involved with, virus survival and persistence. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:S27-36)**

HISTORY AND PROGRESS

The field of virology was barely half a century old in 1948 when Dr Thomas Francis wrote 2 articles, "Viruses as Agents of Disease" and "The Prevention of Virus Disease," that were published in this journal. Early leadership by Mayer, Ivanovsky, Loeffler, Frosch, Walter Reed and others, allowed progress from "contagium vivum fluidum"[1] and a simple understanding of the existence and predatory nature of viruses to the characterization of viruses with regard to size, resistance to chemical and physical agents, host and tissue selectivity, and pathogenic and immunologic effects. These investigations made it clear that viruses were a very diverse group of pathogens. However, our knowledge of viral-cell interactions and the effect of viruses on the immune system was rudimentary. We held the understanding that a recovered individual is not susceptible to reinfection with the same virus, and that serum contained components that when mixed with virus and injected into a susceptible animal that animal was protected. These basic concepts served as the basis for classic studies of active and passive immunization. But several important advances that occurred between 1948 and 1957 jump-started the field of modern virology. These included the development of cultures of single animal cells,[2,3] Watson and Crick's identification of DNA and the genetic code,[4] the development of optimal medium for growing cells, and the development of the viral plaque assay.[5] By the early 1950s, Max Theiler and Jonas Salk (killed virus) had developed vaccines for yellow fever and polio, respectively, and through the benefits of growing the viruses in cell culture, shortly thereafter Sabin developed the oral (live attenuated virus) vaccine. Introduction of these vaccines into the human masses remains to this day one of the greatest accomplishments of preventive medicine.

In the 1960s, the transition from basic virology to molecular biology began. Viruses and components of viral infections were analyzed using gel electrophoresis, protein-antibody interactions, and biochemical assays to answer basic biologic questions. As a result, greater knowledge of virus replication, viral and cellular receptors, and immunologic interactions was achieved. Specifically, research during this time led to an understanding of the regulation of gene expression including transcription factors, enhancer elements, promoters, aspects of RNA polymerase, and reverse transcriptase, as well as the discovery of proto-oncogenes and tumor suppressor proteins. Scientists tagged viruses to identify intracellular locations of viral proteins, understand nuclear and cytoplasmic shuttling, and map neural circuitry. Complete genomic sequences of viruses have been recorded and entered into public databases. The benefits of databases such as FASTA and BLAST have led to searches in homology between motifs characteristic for specific gene products and the identification of novel viral genes and their functions.

Our knowledge also has advanced from the use of positive and negative selection procedures. Positive selection being the method whereby genomic fragments or single candidate genes are expressed in a suitable cell system and tested for functionality (ie, infection phenotype). In contrast, negative selection is based on the construction of viral mutants that lack specific genes and the implementation of studies that identify a change in phenotype when the viral mutant infects a particular

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cell or animal. Use of these techniques has led to the identification of virulence factors, novel mechanisms of regulation of cell surface receptors, and signal transduction pathways. Within the last decade, the emerging fields of genomics and proteomics have allowed for the functional analysis of a large number of transcripts and protein sequences that are expressed during viral infection that provide new clues as to the regulation of acute, chronic, and persistent viral infections, as well as reactivation and malignant transformation. Excitingly, the last decade has demonstrated that scientists have the knowledge and skill to harness unique features of viruses (eg, adenoviruses, retroviruses, herpesviruses) in implementing gene therapy and targeting processes important in chronic disease and cancer. However, mastery of this field remains to be seen.

Despite the exponential growth in virology during the last 30 years, mankind still suffers from transmission and disease when humans serve as hosts to viruses. Moreover, the outcomes are often severe when humans serve as novel hosts to emerging virus infection (eg, avian flu virus, Ebola virus, equine hemorrhagic fever viruses, Hanta virus, human immunodeficiency virus (HIV), Hendra virus, Nipah virus, sudden acute respiratory syndrome (SARS) coronavirus, and West Nile virus).[6] Of great importance is the fact that the oral cavity continues to be the source of transmission of many viruses, the site of replication and asymptomatic shedding of viruses, and a site where persistent viral infections exist, the latter being a prerequisite for virally induced malignant transformation. Clearly, the field of virology has grown to the extent that a "state-of-the-art" paper would be exhaustive in length. Accordingly, this review focuses on specific viral cell interactions that allow the virus to survive the cellular attack and evade the immune system, establish persistent infections, and cause chronic disease. Additional topics will be covered in future reviews.

VIRAL COUNTER DEFENSES

Viruses have developed numerous strategies for subverting the host defenses that are launched during infection. The first innate defense encountered is cellular selectivity during the entry process. Viral attachment proteins bind to specific cell receptors (proteins, carbohydrates, or glycolipids) and coreceptors. The absence of a specific receptor shields the cell from attack. If this level of defense is foiled, upon binding receptors can sense microbial infection and trigger a multitude of antimicrobial and inflammatory responses. The toll-like receptor (TLR) family which consists of 10 to 15 members are well characterized in their ability to detect bacterial components (ie, lipoproteins and lipoteichoic acids, flagellin) as well as unmethylated CpG motif DNA of bacteria and viruses (detected by TLR9), double-stranded RNA (detected by TLR3) and single-stranded viral RNA (detected by TLR7).[7] In particular, TLRs 3, 7, 8, and 9 specialize in viral detection and recognition of nucleic acids within the intracellular compartments which results in defensive signaling.

After receptor binding, entry is modulated by either direct fusion with the plasma membrane or clathrin- or nonclathrin-mediated endocytosis.[8] Viruses that gain entry uncoat and deliver their genetic material and undergo a permissive or nonpermissive infection. A permissive cell permits virus replication and ultimate lysis of the host cell. In contrast, a nonpermissive cell downregulates virus replication and lytic gene expression resulting in little to no viral progeny. Nonpermissive infections can be abortive or persistent, and persistent infections can be active or latent. Latent infections are characterized by silencing of gene transcription, intermittent reactivation, or rarely oncogenic transformation.

At the onset of infection, for a virus to survive within a cell, the virus must balance its own growth with death of the host and circumvention of the immune response. Strategies for survival involve regulating apoptosis, inhibiting interferon production, modulating the major histocompatibility complex (MHC) class I function that ultimately affects the cytotoxic lymphocyte (CTL) and natural killer (NK) response, and limiting cytokine and chemokine production/function. Long-term survival (ie, latency) requires downregulation of lytic gene expression, inhibition of apoptosis, and minimizing the inflammatory response.

APOPTOSIS

Apoptosis, or programmed cell death, is a highly regulated and conserved series of sequential cellular events that results from receptor- or mitochondrial-mediated pathways in response to a variety of stimuli, including viral infection and the appearance of double-stranded RNA. The process is regulated (Fig 1) by a family of aspartate-specific cysteinyl proteases, or caspases, that converge at a number of downstream points resulting in proteolytic cleavage and enzyme activation.[9] Caspases are segregated into 2 distinct subfamilies. The "apoptotic" caspases (2, 3, 6, 7, 8, 9, and 10) are involved in the cascade that results in protease production, chromatin condensation, and cellular degradation. The "inflammatory" caspases (1, 4, and 5) provide a second round of defense against viral infection.[10] The inflammatory caspases are involved in the proteolytic maturation of key cytokines (ie, interleukin (IL)-1β and IL-18). Cytokine IL-18, also known as interferon (IFN)-inducing factor, directs the production of IFN-γ.[11] In turn, IFN-γ induces

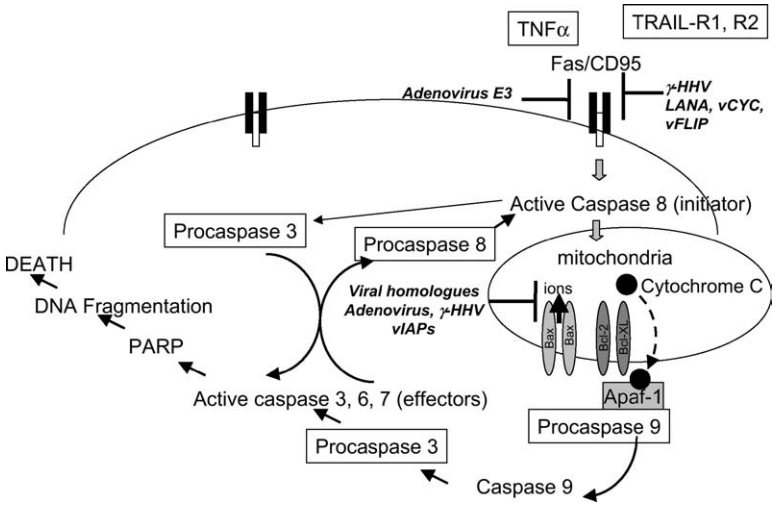

Fig 1. Viral mechanisms involved in subverting the apoptotic pathway. *PARP*, Poly (ADP-ribose) polymerase.

expression of proteolytic active subunits that lead to proteolysis and antigenic processing by TAP proteins. TAP proteins are critical for displaying viral antigens on the cell surface (see Cellular Immunity, below).[12]

Clearly, apoptosis is an important target of virus defense, because early destruction of an infected cell could greatly reduce replication and the number of viral progeny produced. Interestingly, viruses have evolved several methods for suppressing or delaying apoptosis as well as encoding proteins that function as inducers of apoptosis. This apparent yin-yang relationship with apoptosis is important to prolong the life of the cell yet facilitate the release and spread of viral progeny at the appropriate time.[13,14]

Viruses regulate apoptosis by several mechanisms including the targeting of the tumor suppressor gene product p53, the Fas death receptor, and by producing caspase inhibitors and viral Bcl-2 homologs.[15] Adenovirus, for example, encodes several gene products that influence apoptosis. The E1A gene product stabilizes p53 and induces p53-dependent apoptosis.[14,16] In contrast, the adenovirus E3 gene product promotes degradation of Fas, and the adenovirus E1B proteins antagonize p53 function. Viral homologs of Bcl-2, an apoptosis suppressor that binds with Bax, are produced by adenovirus, Epstein-barr virus (EBV, BHRF1 protein), and other viruses.[17,18]

There are several classes of caspase inhibitors encoded by viruses. These include the serine proteinase inhibitors (serpins: CrmA/SPI-2), viral inhibitors of apoptosis (vIAPs), p35, and inhibitors of procaspase 8 protease (also known as FLICE).[19] CrmA and p35 block caspase 1, previously termed IL-1β−converting enzyme (ICE).[20] Caspase 1 functions primarily as an activator of proinflammatory cytokines, but also has apoptosis-inducing ability in select mammalian cells, such as neurons. The vIAPs appear to inhibit Bax-mediated apoptosis in human cells rather than directly inhibiting caspases.[21]

Several human herpesviruses encode FLICE-inhibitory proteins (FLIPs) that block TRAIL-mediated cell death by interfering with procaspase 8 protease (FLICE) activation.

For example, the β-herpesviruses (cytomegalovirus (CMV)) encode a viral inhibitor of caspase activation (vICA) which inhibits caspase 8 (FLICE) activation,[22] and γ-herpesviruses encode vFLIPs (eg, K13) which inhibit activation of caspases by molecular mimicry.[23] CMV also encodes a viral mitochondrial inhibitor of apoptosis (vMIA) (encoded by the U_L37 gene) which inhibits activation of mitochondrial pores in a manner similar to members of the antiapoptotic Bcl family.[24,25] The alpha herpesvirus HSV-1 encodes several anti-apoptotic gene products (ie, ICP4, ICP27, γ34.5, U_s3, gJ)[26-30] that modulate apoptosis at several levels, including antagonism of double-stranded RNA-activated protein kinase (PKR), a downstream induction molecule of the interferon signaling pathway[31,32] Of note, all γ-herpesviruses express viral homologues of cellular antiapoptotic genes, including 1 or 2 Bcl-2 homologues.[33]

INTERFERON

Interferon, discovered in the late 1950s when scientists observed that virus-infected cells secrete a factor that mediates the transfer of a viral-resistant state,[34] is a family of regulatory glycoprotein cytokines that modulate both innate and adaptive antimicrobial immunity. They are products of an infected cell genome and one of the key factors in the host response against

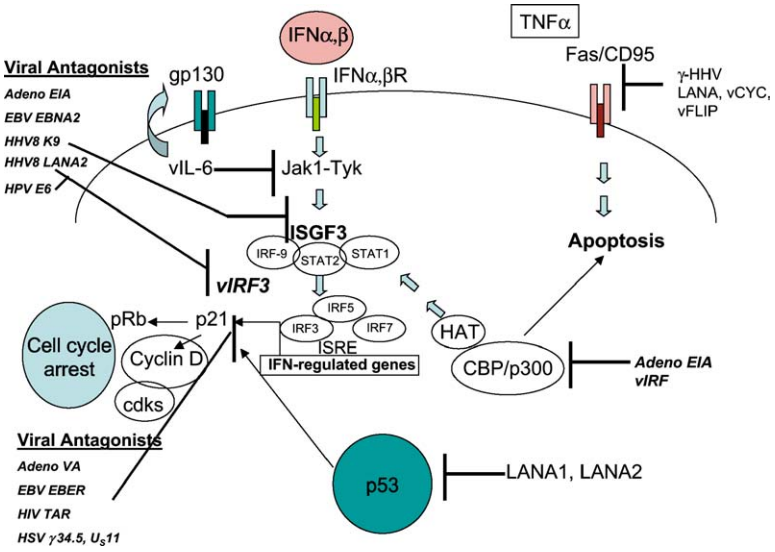

Fig 2. Viral mechanisms involved in subverting the interferon response. *IFN*, Interferon; *IFNR*, IFN receptor; *IRF*, INF regulatory factor; *ISRE*, IFN stimulable response element; *HAT*, histone acetyl transferase.

viral infection. IFNs serve as an early defense system that precedes the onset of the immune response and are triggered by envelope glycoproteins, CpG DNA, or double-stranded RNA. In recombinant formulations, they have been used in medicine and dentistry to combat various viral infections.[35,36]

Human IFNs are classified based on the sequence of amino acids into 3 main groups – α, β, and γ – and 3 that are less extensively studied (ω, κ, and τ, not discussed further in this review). IFN-α and -β are produced rapidly when viral factors interact with cellular pattern-recognition receptors such as TLRs and cytosolic receptors. Historically, synthesis of IFN-α has been attributed to macrophages and B cells, and IFN-β has been considered to be produced by fibroblasts. More recently, plasmacytoid dendritic cells have been shown to produce IFN-α preferentially to IFN-β. Both IFN-α and -β prevent the replication of viruses by inducing formation of secondary messengers which include IFN regulatory factor (IRF) 3, IRF-5, IRF-7, c-Jun/ATF-2, and NF-κB.[37] IFN-γ is synthesized by activated T lymphocytes and natural killer (NK) cells following receptor-mediated stimulation or in response to cytokines produced by macrophages or antigen-presenting cells (ie, primarily IL-12, IL-18, and IFN-α/β) or by stimulation through T cell receptors (TCRs) or NK cell receptors. It is a powerful activator of mononuclear phagocytes, thus enhancing their ability to destroy intracellular microorganisms and tumor cells. IFNs mediate their antiviral action through IFN-stimulated genes (ISG), which number in hundreds. IFNs also regulate the cell cycle and have antiproliferative effects.

Viral evasion of IFN occurs by several strategies. In the majority of infections, viruses encode products that antagonize either the IFN signal transduction pathway or cellular proteins induced by IFN that are responsible for inhibiting virus replication (Fig 2).[38] Adenovirus, EBV, papillomavirus, and members of the Paramyxovirinae subfamily encode proteins that inhibit the JAK-STAT (Janus kinase−signal transducer and activator of transcription) signaling pathways that are required for IFN production. Specifically, adenovirus encodes the oncoprotein E1A which inhibits the activation of ISG factor 3 (ISGF3).[39,40] Paramyxovirinae reduce the effectiveness of the IFN response by targeting STAT1 for degradation or by interference with STAT phosphorylation or stability.[41,42] Kaposi's sarcoma−associated herpesvirus (KSHV) encodes the pleiotropic gene product latency-associated nuclear antigen (LANA) that acts downstream of ISGF3 and inhibits p53.[43,44] In an alternate approach, HPV encodes 2 proteins, E6 and E7, that bind to IRF-3 and IRF-1, respectively, both of which inhibit the transactivation functions of the bound IRF.[45,46]

Viruses also encode proteins that mimic cellular components of the IFN signal transduction pathway, including homologs of the IFN receptors, a viral ISRE-like promoter element, and viral homolog of IRF (vIRF). For example, Poxviruses antagonize IFN signals by encoding soluble IFN receptor homologs.[47,48] EBV encodes a viral ISRE and HHV8 encodes vIRF from the K9 ORF that functions as a repressor of transcriptional activation induced by IFN-α, -β, and -γ.[49] In addition, several viruses have developed strategies to inhibit

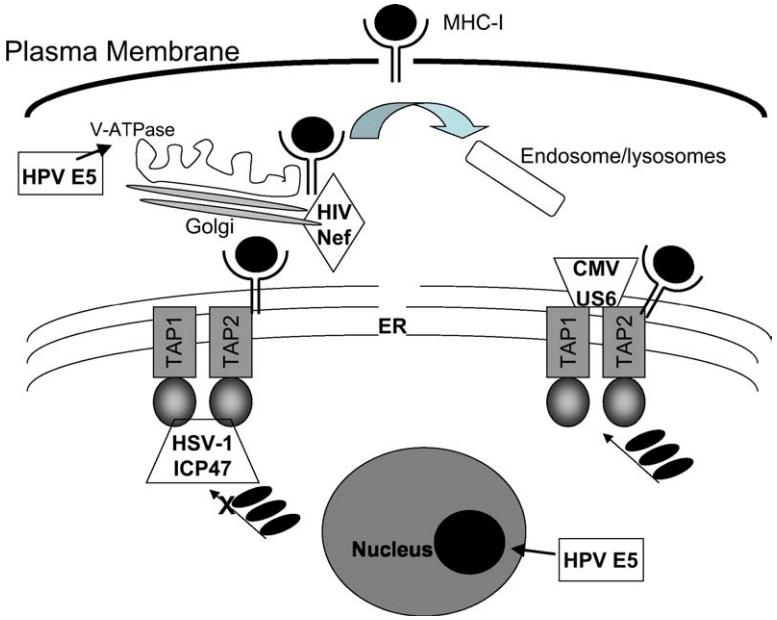

Fig 3. Viral mechanisms involved in subverting the MHC-1 response.

IFN-inducible, RNA-dependent protein kinase (PKR). PKR, when antagonized, leads to phosphorylation of eIF-2α which results in inhibition of the IFN-induced antiviral response of the host.[50] Adenovirus, herpesviruses, influenza, and SV40 antagonize PKR by different mechanisms involving degradation of PKR, prevention of PKR activation, and resistance to downstream kinase activation.[51-53]

CELLULAR IMMUNITY

The MHC class I–restricted T cell response can result in a lethal hit before virus replication. Thus, many viruses have developed strategies for interfering with antigen presentation to MHC class I molecules and intracellular trafficking of MHC molecules. Viruses target the MHC-I at almost all steps of its trafficking: in the endoplasmic reticulum (ER), in the cytoplasm on its way to the surface, and after the MHC reaches the cell surface (Fig 3).

One key target in the viral defense against the cellular arm of the immune system is attack of the transporter protein associated with antigen processing (TAP). TAP loads short antigenic peptides to the MHC which stabilize the class I complexes and allows their migration to the cell surface. Without the peptide cargo, MHC class I molecules are unstable and dissociate. HSV-1 and HSV-2 encode infected cell polypeptide (ICP)-47, an immediate early gene product, that interacts with the TAP protein in the cytosol to prevent peptide binding to TAP.[54] Human cytomegalovirus (HCMV) encodes U_S6, a 183–amino acid glycoprotein, that blocks peptide transport by binding to TAP in the endoplasmic reticulum.[55] Although efficient at both retaining MHC-I molecules and preventing CTL recognition, HCMV also uses additional viral proteins (U_S2, U_S3, U_S6, and U_S11) to evade the immune system.[56] A different approach is taken by EBV. This human γ-herpesvirus encodes a glycine-alanine repeat (GAr) domain on EBV-encoded nuclear antigen (EBNA) 1 that inhibits ubiquitin/proteasome-dependent proteolysis of EBV antigens.[57] Thus, processing (ie, degradation) of viral proteins into antigenic peptides is restricted.[58] KSHV, a lymphotropic γ-herpesvirus, interferes with MHC-I antigen presentation by ubiquitinating the cytosolic domain of the MHC-I.[59] Herpesviruses also produce proteins that target MHC class I molecules for degradation in lysosomal compartments[60] and downregulate expression of major histocompatibility complex molecules by shutting off host cell protein synthesis by the gene known as virion host shutoff (VHS, U_L41).[61]

In contrast, HIV with its simple genome encodes fewer proteins but accomplishes similar immune evasion by pluripotent accessory proteins. For example, Nef, 1 of the 6 regulatory proteins encoded by HIV, has multiple functions. In addition to enhancing virion infectivity, Nef binds to and inhibits the surface expression of the major MHC-I,[62] downregulates the cell surface expression of CD4 (the main HIV receptor),[63] and facilitates CD4 receptor endocytosis.[64] Through the function of Nef in redirecting the trafficking of immune receptors, infected T lymphocytes are able to hide from the immune system allowing for viral spread.

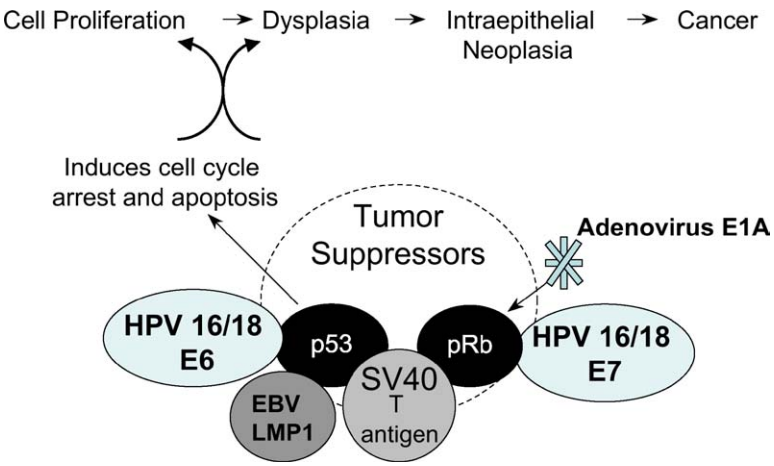

Fig 4. Viral mechanisms involved in dysregulation of apoptosis and increased risk for progression to neoplasia.

HPV utilizes 2 early proteins (E5 and E7) to persist undetected within epithelial cells. The early gene product E7 of the oncogenic strains HPV-16 and -18 downregulates MHC-I expression at the transcriptional level by inhibiting the promoters of the MHC-I heavy chain, TAP-1, and LMP-2.[65] E5 decreases MHC-I expression at the transcriptional level and causes retention of MHC-I in the Golgi apparatus. Within the Golgi, HPV E5 inactivates the ATPase proton pump system. As a result, acidification is blocked, local pH rises, and MHC-I trafficking is perturbed.[66] Thus, it is clear that viruses have achieved ingenious methods for interference with MHC-I antigen presentation and inhibition of the cellular immune response.

VIRAL PERSISTENCE

Viruses persist in cells because they are able to downregulate key processes that if left unattended would result in cell death. Originally attributed in part to the immune response, increasing evidence suggests regulation of key genes plays an important role in the process. Specifically, regulation of viral transcription and genomic replication allows for long-term viral stability and survival.

Many viruses, including those that cause persistent infections and chronic disease (ie, hepatitis C virus, hepatitis B virus, HIV, human herpesviruses, HPV, and JC virus), are successful because of their cell tropism and ability to autoregulate their replication efficiently within specific cells. Common features of autoregulation include sensors to the external environment, negative feedback loops, transcriptional enhancers specific for cells that host the persistent infection, and transcriptional silencers. In some cases autoregulation results in steady-state levels of virus replication; in other infections, the virus enters latency only to reactivate intermittently.

The importance of autoregulation is apparent from both in vivo and in vitro studies.[67] For example, during lentivirus (HIV, simian immunodeficiency virus, and feline immunodeficiency virus) infection, viremia peaks early after infection then declines to a steady-state level. The effect is not altered by the presence of steroid-induced immunosuppression, and clearance of infected white blood cells is not associated with an earlier presence of antibody, cytokine response, or cytotoxic lymphocyte activity.[68] In another common clinical example, successful antiviral therapy results in dramatic drops in viremia, often to undetectable levels.[69] However, replication of virus often rebounds rapidly to pretreatment levels upon drug withdrawal, and in vitro studies indicate that cellular and immune functions are not contributory to the observed outcome. Even when antiviral therapy achieves a sustained virologic response (ie, absence of viremia 6 months after the end of treatment), highly sensitive assays (ie, polymerase chain reaction) detect residual viral genomes in most patients, indicating the ability of viruses to persist and autoregulate based on their environment.[70,71]

Herpesviruses are well known to establish latency in a variety of cell types, and this family of viruses has the ability to autoregulate. This is illustrated by HSV-1 and HSV-2, which can undergo dichotomous life cycles: a lytic infection in epithelium and a latent infection in neurons. In fact, when neuronal cells are infected with HSV-1 or HSV-2 at low multiplicity of infection in vitro, the majority of cells can survive for many days even in the absence of immune cells and without the addition of antiviral drugs to the culture medium.[72] Similarly, if the rare neuronal cells supporting replication are eliminated using acycloguanosine in the above mentioned system,

over 95% of the remaining population harbors a quiescent infection for weeks after the antiviral drug is removed, again in the absence of immune cells.[73,74]

Viruses regulate replication of their genome in a complex manner, but achieve the outcome through use of viral sensors, repressors, and effectors. Viral sensors "sense" perturbations in the viral equilibrium within the cell and signal change at the appropriate time. With many viruses (ie, HIV, hepatitis C virus, hepatitis B virus), envelope proteins play the role of sensors, because envelope proteins can influence virus replication in both a positive and a negative manner.[75-78] For HPV, regulation is through cellular factors that bind to the promoters of E1 and E2.[79] For HSV-1, transcription of the immediate-early (IE) genes during the lytic infection is regulated by the binding of a tegument protein (VP16) with the cellular protein host cell factor (HCF) and Oct-1.[80] Thus, VP16 would seem to be a logical choice for a sensor. However, latency can be established in the presence of VP16[81] and reactivation occurs without the transactivating domain of VP16.[82] Thus, downstream factors of VP16 (eg, ICP0, ICP4, or other unknown factors) serve as sensors of the environment and regulate the balance between latency and reactivation.

The "effectors" (transactivators and replicative enzymes such as RNA polymerase) modulate virus replication and are the targets of the sensors. Effectors are tightly regulated (ie, repressed at certain times) but dynamically modifiable, typically by proteins bound to critical regions of the genome. These proteins afford protection by limiting changes in conformation of, or enzymatic action on, the restricted gene. Histones are the most notable guardians of the effectors. Histones permit access of DNA to specific activators or repressors, general transcription factors, and RNA polymerase by posttranslational modification (acetylation, methylation and phosphorylation) of their amino terminal tails.[83,84] For example, hyperacetylation of histones is associated with an "open chromatin" conformation and transcriptional activation, whereas hypoacetylation of the histone complex is associated with condensed (hetero-) chromatin and gene silencing. Several human herpesviruses[85-87] utilize these mechanisms for regulating latency and reactivation. In addition, the active regions of the latent α-herpesvirus genome appear to be segregated from the repressed gene regions by boundary or insulator elements, similar to that found on cellular chromosomes[88] (D Bloom, personal communication). These chromatin insulators appear to be able to protect genes in one region from the regulatory influence of adjacent regions through conserved CTCF motifs.[89] Herpesviruses also encode proteins such as LANA and latency-associated transcripts (LAT) that appear to regulate viral transcription during latency.[44,90]

Integration, and the site of integration, into the host chromatin is another mechanism that can regulate viral gene transcription. For example, viruses that integrate (ie, HIV) preferentially select chromosomal sites where high-level transcription of key transactivators is maintained.[91] This is accomplished by viruses preferentially integrating in chromatin regions characterized by an open structure (a hallmark of actively transcribed genes). The process by which this is regulated is not completely clear, but it has been suggested that cellular proteins may interact with integrase, the viral protein that catalyzes the integration reaction, in a manner that is site specific.

Viral persistence increases the likelihood of chronic infection and replication, but under certain circumstances also contributes to increased risk of oncogenic transformation. This can occur through chromosomal instability and virus integration,[92] and the ability of several specific viral proteins to bind and inactivate p53 or less frequently pRb (Fig 4).[93] p53 is a checkpoint protein that interacts with CDK/cyclin inhibitors and p16, p27, and p21 to arrest the cell cycle in the G1 phase and can send signals for apoptosis through the regulatory proteins Bax, Bcl-2, and c-myc.[94] The clinical importance of p53 inactivation is exemplified in that persistent HPV infection is associated with an increased risk of developing cervical cancer in young women, and recent findings suggest that the persistence of HPV DNA in treated tissue after cancer therapy is highly predictive of local recurrence.[95]

CONCLUSION

In this brief review, examples of mechanisms that contribute to survival and persistence of viruses within their host were presented. Emphasis was placed on mechanisms that permit survival of host defenses, evasion of the immune system, and establishment of chronic infections. Detailed knowledge of these processes has led to many therapeutic successes. However, additional knowledge is required for us to make strides in eliminating human suffering caused by these intracellular pathogens. It is hoped that 50 years from now when another review may appear in this journal on this topic, we will have a better understanding of how to eliminate persistent viral infections and identify patients at risk for virally induced complications of acute and chronic infections and will have harnessed the power of viruses to undergo selective lytic replication in tumor cells and modulate chronic disease.

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