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Chapter 7

Patterns of Infection

Unwanted Guests—Quick Visits and Extended Stays

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The preceding chapters describe essential aspects of viral pathogenesis, including virus–cell interactions; viral spread within a host; and intrinsic, innate, and adaptive immune responses. This chapter extends the theme and addresses diverse patterns of viral infections that are determined by both the virus and the host. Thus, virulence or susceptibility depends upon the specific virus–host combination. This is particularly true in the case of persistent infections, which involve a delicate balance between virus and host. We will focus first on virus virulence and host susceptibility, and then turn to the complex variables that govern persistent infections. Chapters 4–6, on innate, adaptive, and aberrant immunity, and Chapters 11–15, on systems biology approaches, also provide important insights into the patterns of infection.

1. VIRULENCE DEFINED

Viral virulence (or pathogenicity) is the ability of a virus to cause disease in an infected host. Since variants of a single virus can exhibit different levels of severity, viral virulence is an important consideration in studying patterns of disease. Furthermore, understanding the subtleties of viral

virulence has important practical implications, since avirulent or attenuated variants of a virus are often used as live vaccines; examples are those for smallpox, poliomyelitis, measles, and yellow fever.

The virulence phenotype depends upon many variables, including the viral strain; the route of infection; the viral inoculum; and the species, age, and genetic susceptibility of the host. To profile the differences between the virulent and attenuated viruses, it may be necessary to choose an experimental combination of host and route that is somewhere in the spectrum between the most benign and the most severe ends of the disease scale.

1.1 How is Virulence Measured?

The comparative pathogenesis of virulent and avirulent virus strains can be used to elucidate the biological mechanisms underlying their phenotypes. Differences in virulence phenotypes can be qualitative or quantitative. *Qualitative variation* may be manifested in various ways. For instance, viral clones may exhibit differences in their tropism, such that one clone replicates well in the brain while another clone replicates

TABLE 1 Virus Virulence Can Be Measured in Many Ways, Depending Upon the Pathogenesis of Individual Virus Diseases

Measure of Virulence	Requirement 1	Requirement 2	Experimental Animals or Humans?
Quantitative Measures			
Number of infectious units that cause disease or death (infectious units per LD50)	Titration of virus in cell culture (PFU; TCID ₅₀ ; other)	Death or disease endpoint titration in experimental animals	Animals
Ratio of titers to compare different strains of a virus	Titration of virus 1 (PFU per endpoint)	Titration of virus 2 (PFU per endpoint)	Animals
Number of cases of disease per 1000 infections	Count of disease cases in human or animal population	Count of infections in the same population	Humans or animals
Qualitative Measures			
Distribution and severity of pathological lesions	Lesions in different organs and tissues	Semiquantitative measure of pathological lesions	Humans or animals

well in the liver or in the gastrointestinal tract. Alternatively, different clones may spread by different routes, one clone producing viremia whereas another spreads by the neural route. In some instances, viral clones will vary in the innate or acquired immune responses that they induce, or in their susceptibility to antibody or to cellular immune defenses. *Quantitatively*, for any given expression of pathogenicity, it is possible to compare the number of infectious units required to produce a specified outcome such as mortality or other disease parameter for different viral strains (e.g., ID₅₀ or LD₅₀).

Quantitative measurements. Virulence can be measured by various methods that are summarized in Table 1. Death being a binary outcome, it is an ideal measure for experimental models; where the differences in virulence are not as pronounced, a more subtle analysis is useful. For example, virulent and avirulent strains can both be uniformly fatal yet vary in the average days to death of the experimental host. Other common measures of virulence are paralysis (e.g., poliovirus), changes in liver enzymes (any hepatitis virus), decrease in the proportion or number of CD4⁺ lymphocytes (immunodeficiency viruses), or sometimes more distant surrogate measures such as behavioral abnormalities for some neurotropic viruses.

Most comparisons of virulence use a ratio of infectious units to disease outcome. For instance, for a virus that forms plaques in vitro, calculation of the PFU/LD₅₀ makes it possible to distinguish different degrees of virulence. More modern assays of RNA or DNA copies are useful, but they too have potential pitfalls, as they seldom measure complete copies of the viral genome, and could be affected by incomplete genomes that are incapable of replication. To assess the relative virulence of two strains of a given virus that cause similar disease, the infectious unit per 50% disease endpoint for each can be compared (second row in Table 1).

In a clinical setting, virulence can sometimes be measured, but a different approach is required. In some outbreaks, it is possible to estimate the number of infections that have occurred in a population as well as the number of clinical cases. In this instance, the ratio of cases per 1000 infections provides a measure of virulence.

Qualitative measurements. Qualitative comparisons describe differences in the localization of pathological changes or in their nature. For instance, Figure 1 shows a comparison of the central nervous system (CNS) lesions caused by five neurotropic flaviviruses. Some viruses produced severe lesions in the brain, others targeted the spinal cord, and some produced similar lesions throughout the neuraxis, reflecting the multidimensional nature of virulence.

2. HOW CAN VIRULENCE BE ALTERED? CLASSICAL METHODS

Viruses can be manipulated to alter their virulence, either deliberately or inadvertently. Classically, this was accomplished by growing a virus in either animals or cell culture (Table 2). With the advent of molecular genetics, it has become possible to introduce mutations that change virulence. These studies are most informative when the phenotypes differ as much as possible.

2.1 Selection of Viral Clones

One of the simplest methods to obtain virus strains with differing virulence is to select genetic clones of a virus and compare them using one of the measures described above. Preferably, this is done by picking plaques from a culture plate. This method was used by Albert Sabin to identify the attenuated strains of polioviruses that were developed into

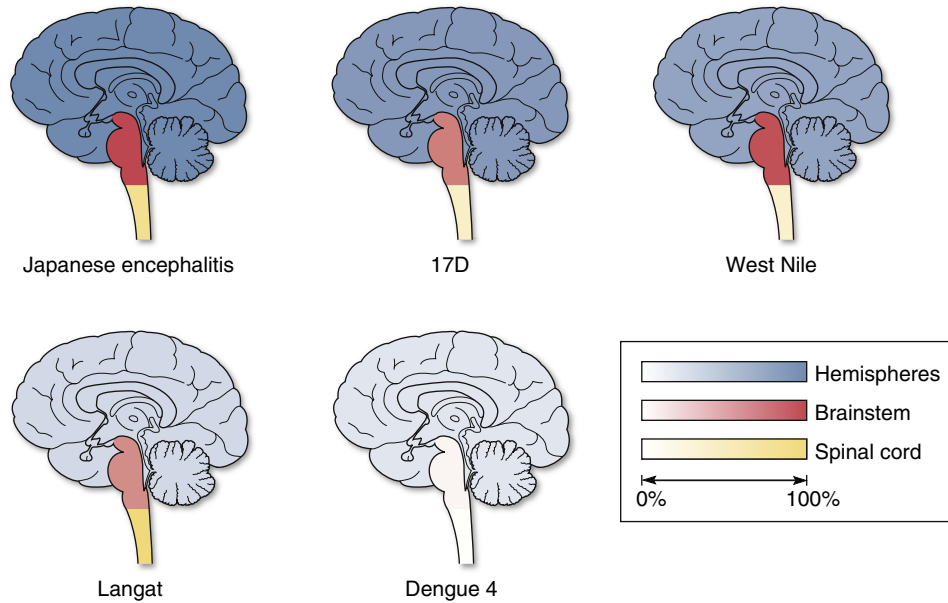


FIGURE 1 Virulence is multidimensional since it may involve different tissue- or organ-specific patterns of infection. In this example, lesion severity is a qualitative as well as a quantitative phenomenon. Nonhuman primates were injected intracerebrally with five different neurotropic viruses and the severity of lesions in different central nervous system regions were graded using a standard scale. *After Nathanson N, Gittlesohn AM, Thind IS, Price WH. Histological studies of the monkey neurovirulence of group B arboviruses. III. Relative virulence of selected viruses. American Journal of Epidemiology 1967, 85: 503–517, with permission.*

Method to Alter virulence	Enhancement or Attenuation of Virulence
Selection of individual viral clones	Either
Passage in animals	Enhancement (usually)
Passage in cell culture at “standard” conditions	Attenuation (usually)
Passage in cell culture under restrictive conditions such as altered temperature	Attenuation (usually)
Passage in selected cell cultures such as neural cells	Enhancement (usually)
Alter viral genome by introducing point mutations that change viral genes or stop codons that inactivate viral genes	Either

oral poliovirus vaccine (OPV). If a virus cannot be plaqued, then endpoint dilution can be used to (hopefully) select individual virus clones.

2.2 Passage in Animals

In the pioneering days of virology, viruses were often maintained by serial passage in animal hosts. It was found that pathogenicity would change during the course of multiple passages, and this adventitious finding was exploited to obtain viruses of different pathogenicity. In general, during repeated animal-to-animal transmission, a virus adapts in order to replicate optimally under the conditions of passage. Yellow fever, a flavivirus, produces fatal hepatitis in monkeys; if passaged intracerebrally in mice, it will become highly neurovirulent

for mice, but will lose its ability to cause hepatitis in monkeys. However in nature, where viruses have adapted to specific hosts, virulence may vary from high (rabies in raccoons or foxes, smallpox in humans) to low (SARS coronavirus in bats, rhinoviruses in humans). Similarly, complex experimental passage of a strain of simian immunodeficiency virus (SIV) led to a neurovirulent strain that is less capable of causing immunodeficiency, but can be used to study neurovirulence when co-inoculated with an immunodeficiency causing virus.

2.3 Passage in Cell Culture

With the advent of cell culture, viruses were usually maintained by in vitro passage. It was soon observed that serial transmission alters the biological phenotype, often reducing

virulence for animals or humans. This observation was exploited in the deliberate search for attenuated variants that could be used as prophylactic vaccines. Experience has demonstrated several underlying principles.

- Apparently identical passage lines can yield virus stocks differing in their virulence.
- An RNA virus stock represents a “swarm” of highly related virus genotypes that may have different phenotypes.
- Passage usually selects for virus clones already present in the population that replicate preferentially and thus alter the phenotype of the virus swarm (see Chapter 17, Viral Evolution).

Historically, the failure to recognize the influence of passage upon the biological phenotype has led to some important errors in virological research. For example, intracerebral passage of poliovirus in monkeys leads to selection of variants that are highly neurotropic but have lost much of their infectivity and pathogenicity when administered by the oral (natural) route. Pathogenesis studies with these neuro-adapted poliovirus resulted in the erroneous conclusion that poliovirus was not an enterovirus but was naturally transmitted by the intranasal route, and this misapprehension led to trials of nasal astringent sprays as a method to protect children against paralysis.

More recently, passage of HIV-1 in T cell lines selected for laboratory variants that differed from wild-type virus in their ability to plaque in MT-2 cells (a T cell line), use of the CXCR4 co-receptor (not the CCR5 receptor), and inability to infect macrophages. When used for serological assays, the adapted viruses were readily neutralized by sera from patients naturally infected with HIV. These findings resulted in the mistaken conclusion that HIV-1 could be readily neutralized and the consequent prediction that it would be relatively easy to develop a prophylactic vaccine. Once it was recognized that viral isolates only maintain their natural phenotype if passaged in primary blood mononuclear cells (PBMC), it became clear that many wild type

HIV isolates are very resistant to neutralization, presenting a daunting challenge for vaccine development.

2.4 Passage in Cell Culture under Restrictive Conditions

There are a number of methods that have been used to enhance the selection of attenuated virus variants from an uncloned virus stock.

Temperature-sensitive mutants. Wild type viruses will replicate well at 37°C and often at temperatures up to 40°C (most cell cultures do not thrive above 40°C). Temperature sensitive (ts) variants, on the other hand, replicate well at 37°C but poorly if at all at 40°C. It is relatively easy to select for temperature sensitive variants and they will often exhibit an attenuated phenotype when tested in animals and a restricted infectivity range in host tissues.

Another method for the selection of attenuated mutants is passage at a low temperature, such as 25°C, about the lowest temperature at which most mammalian cell cultures can be maintained. A cold-adapted influenza virus is temperature sensitive and exhibits restricted pneumotropism in ferrets, an animal that develops severe pneumonia after intranasal infection with wild-type human influenza viruses (Massa et al., 1985). This attenuated strain is used as a live influenza vaccine (see Chapter 19, Viral Vaccines).

3. HOW CAN VIRULENCE BE ALTERED? GENETICS TO THE FORE

3.1 Small Changes in the Genome Can Have Large Effects on Virulence

Determinants of viral virulence may be encoded in any part of the viral genome and changes in only a few nucleotides can have dramatic effects on virulence. Over the last few decades, a large body of information has been assembled regarding these determinants, summarized in [Sidebar 1](#). A few salient examples are described below.

Sidebar 1 Genetic determinants of viral virulence and attenuation

- The use of mutant viral clones has made it possible to identify the role of specific genes and proteins as determinants of virulence.
 - There are no “master” genes or proteins that determine virulence, and attenuation may be associated with changes in any of the viral proteins as well as in untranslated genomic sequences.
 - Virulence phenotypes can be altered dramatically by a change that leads to an alteration in a single amino acid, or by a single nucleotide change in a noncoding region. Variants with mutations in several critical sites may be more attenuated than those with a single point mutation.
- The frequency of reversion to virulence is inversely proportional to the number of discrete attenuating mutations.
- Reversion to virulence of an attenuated variant can involve back mutation at the genetic site of attenuation, but can also be produced by compensatory mutations at a different site in the same protein or even in another viral protein.
 - Attenuating mutations are often host range alterations that affect replication in some cells or tissues but not others.
 - Although many attenuated viral variants have been identified, only in a relatively few instances has the mechanism been identified at a biochemical or structural level.

OPV is comprised of attenuated clones of each of the three poliovirus serotypes that—in comparison with virulent wild-type polioviruses—have markedly reduced neurovirulence after direct intrathalamic or intraspinal injection in macaques. OPV viruses that have reverted to virulence after feeding to humans—such strains are routinely isolated from the stool of vaccinated children—have a small number of nucleotide differences in comparison with the attenuated parent vaccine strains. Recombinants between parent and revertant OPV viruses can therefore be used to identify the influence of individual critical nucleotides in neuropathogenesis (Minor, 1992). In type-3 OPV, there are only four important nucleotide determinants, which are located both in the nontranslated region of the genome and in the genes encoding the structural proteins.

In the case of influenza virus, changes in virulence may also be due to specific combinations of viral genes. There has long been a mystery how the 1918 influenza epidemic caused such a high mortality with an estimated 50 million deaths. An analysis using recombinant viruses—constructed through gene reassortment between the reconstructed 1918 virus and a less-pathogenic contemporary strain—indicated that virulence is multigenic (Table 3). Pathogenesis analysis showed that the 1918 virus triggers an outpouring of cells and cytokines in the lung, reflected in the profiles of gene expression. This is

an instance of a host response that is a deleterious determinant of virus virulence.

3.2 Manipulating the Viral Genome to Alter Virulence

With the introduction of molecular genetics into virology, it has become relatively easy to alter the viral genome in a controlled manner. Genetic changes that can be deliberately introduced include point mutations that alter function of individual proteins, or inactivation of nonessential viral genes by introduction of stop codons or deletions of substantial gene segments. Relevant methods are described in Chapters 11–13.

For instance, HIV has four accessory genes—Nef, Vif, Vpu, Vpr—that are not essential for replication in most cell cultures. SIV strains lacking one or more of these genes are attenuated when used to infect rhesus macaques, compared to their parental counterparts. In fact, it was hoped that an HIV strain lacking the Nef gene might be used as an attenuated viral vaccine in humans. A group of patients infected with a naturally Nef-deleted strain of HIV remained healthy, absent antiretroviral treatment for many years but eventually developed AIDS, ending this approach to a vaccine; the Nef-deleted HIV strain was attenuated but still too dangerous to use in humans.

TABLE 3 Multigenic Determination of Virulence. The 1918 Influenza Virus (H1N1 Antigenic Type) Was Reconstructed and Used to Make Reassortants with a Contemporary Human H1N1 Isolate (Tx/91). Parent and Reassortant Viruses Were Tested for Virulence after Intranasal Inoculation of Adult Mice. Reassortants Encoding Many of the Genes of the Virulent 1918 Virus Showed Considerable Virulence but Were Less Lethal than the Parent 1918 Virus, Indicating That Virulence was Associated with the Full Spectrum of Viral Genes Acting in Concert

Viral Gene	Genetic Composition of Parent and Reassortant Viruses			
	1918	Tx/91	1918	Tx/91
HA	1918	Tx/91	1918	Tx/91
NA	1918	1918	1918	Tx/91
M	1918	1918	1918	Tx/91
NP	1918	1918	1918	Tx/91
NS	1918	1918	1918	Tx/91
P1	1918	1918	Tx/91	Tx/91
P2	1918	1918	Tx/91	Tx/91
P3	1918	1918	Tx/91	Tx/91
Titer in lung (Log ₁₀ EID ₅₀ /per ml)	7.5	5.2	6.0	3.0
Mortality in mice (10 ⁶ PFU intranasal)	100%	0%	100%	0%
Survival (days)	3 days		6.2 days	

This is from Fornek, Korth, and Katze, *Advances in Viral Research*, 70:81–100, 2007.

After Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solorzano A, Swayne DE, Cox NJ, Katz JM, Taubenberger JK, Palese P, Garcia-Sastre A. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 2005, 310: 77–80, with permission.

Another pertinent example is the H5N1 strain of influenza virus that had produced a high mortality among a few poultry farmers in China. To investigate the potential transmissibility (and therefore the virulence for the human population) of H5N1 virus, two research groups independently conducted controversial “gain-of-function” experiments. As a surrogate for human subjects, both investigative teams used ferrets. Although ferrets could be infected intranasally with wild-type H5N1 virus, they did not transmit infection to other ferrets housed in close proximity. However, when the researchers introduced a few mutations and performed animal-to-animal passages, they obtained virus mutants that were transmissible by aerosols. Recent genetic analyses have suggested that there are at least three transmissibility determinants in this H5N1 virus: basic amino acids adjacent to the cleavage site in the viral hemagglutinin; amino acid 627 in PB2, one of the viral polymerase molecules; and a short sequence in the NS1 protein that may influence interferon or other host responses.

4. ENTER THE HOST: HOW VIRULENT AND ATTENUATED VIRUSES DIFFER IN THEIR PATHOGENESIS

The sequential steps in viral pathogenesis are described in Chapter 3, Basic Concepts. The ability of a virus to move through each of these steps can impact virulence. There are examples of viruses that differ in their infectivity at the portal of entry, in their ability to disseminate, or in their replicative capacity in target organs or tissues. In many instances, the pathogenicity of a virus strain is determined by its ability to evade host defenses such as the intrinsic, innate, or adaptive immune response. Once again, it is the virus–host interaction that determines virus virulence.

4.1 Sequential Steps in Infection

Portal of entry. Respiratory viruses such as influenza, that cause severe disease, replicate well in the lower respiratory

tract where the temperature is close to 37°C. However, the rhinoviruses, which do not grow well at 37°C, cannot infect the lung proper, and are confined to the upper respiratory tract since they are adapted to replicate at 33°C and only cause mild illness (the common cold). As mentioned above, a cold-adapted influenza virus is sufficiently attenuated to be used as a vaccine. In this instance, attenuation is associated with the ability to replicate well at 33°C but not at 37°C.

Viremia. Most systemic viruses spread via the bloodstream. If viral strains differ in the duration and titer of the induced viremia, this may alter their ability to reach critical target organs and thereby influence virulence. Wild-type isolates of poliovirus vary in the degree of viremia that they produce and this correlates with their paralytogenicity after extraneural infection.

Neural spread. Some viruses spread along neural pathways rather than by viremia, and neurally spreading viruses can also be experimentally attenuated. Rabies virus is a good example of an “obligatory” neurotrope. Attenuated vaccine strains of rabies virus, obtained by passage of wild-type virus in nonneural cells, show a marked reduction in their virulence when tested by intracerebral injection in mice. The attenuated phenotype is maintained if the virus is passaged in BHK-21 cells, which come from kidney tissue, but reverts to greater virulence when the virus is passaged in cultured neural cells (usually astrocytic) or in the brains of suckling mice.

Neuroinvasiveness. West Nile virus isolates are grouped into two major lineages, based upon genetic sequence. When tested in mice, all isolates have high neurovirulence, but there are major variations in neuroinvasiveness (Table 4). West Nile virus was introduced into the United States in 1999 (see Chapter 16, Emerging Viral Diseases) and spread from New York state across the country to the West coast, with outbreaks of encephalitis in humans. Recent isolates from humans and birds in the United States are among the most neuroinvasive strains of West Nile virus, consistent with the severity of this still emerging viral disease.

TABLE 4 Different Wild-type Isolates Show Distinct Qualitative and Quantitative Patterns of Virulence. Although All Isolates of West Nile Virus are Equally Neurovirulent, They Differ in Neuroinvasiveness. Virus Strains Were Titrated in 4-Week-old NIH Swiss Mice by Intraperitoneal (ip) or Intracerebral (ic) Routes of Inoculation

Lineage	Virus Strain	Neuroinvasiveness (PFU per ip LD50)	Neurovirulence (PFU per ic LD50)
1	USA99b	0.5	0.1
	EGY50	50	0.7
	AUS91	>10,000	3.2
2	SA58	3.2	0.3
	CYP68	>10,000	0.5

After Beasley DWC, Li L, Suderman MT, Barrett ADT. Mouse neuroinvasive phenotype of West Nile virus strains varies depending upon virus genotype. *Virology* 2002, 296: 17–23, with permission.

4.2 Tissue Tropism

Variants of a single virus can differ in their relative pathogenicity for different tissues or organs, which confers a multi-dimensional character upon virulence. As mentioned above, in the search for attenuated poliovirus strains, Sabin used passage in cell culture to obtain the virus strains used to formulate OPV. When fed to susceptible subjects, these strains replicated as well as wild-type isolates, but (compared to wild-type isolates) had only minimal neurovirulence in monkeys.

HIV-1 is another example. All HIV-1 strains replicate well in primary cultures of peripheral blood mononuclear cells (consisting mainly of T lymphocytes). Some wild-type strains also replicate in primary cultures of monocyte-derived macrophages but not in transformed lines of T lymphocytes. What is the explanation for these differences in tropism? Macrophage-tropic strains of HIV-1 use the CD4 primary receptor very efficiently; an ability required to infect macrophages as they express CD4 at much lower levels than lymphocytes. Most primary isolates of HIV-1 use only the CCR5 co-receptor and therefore cannot infect T cell lines that express only the CXCR4 co-receptor. However, primary cultures of T lymphocytes express high levels of CD4, and both co-receptors, CCR5 and CXCR4, and can be used by all strains of HIV-1.

4.3 Host Intrinsic Response

Animal hosts have evolved a large set of constitutive non-immune defenses against viral invaders, many of which

have been discovered only recently. In turn, viruses have evolved counter measures to overcome these host defenses, and these counter measures are one determinant of virulence. The best studied example is HIV-1. A comparison of HIV-1 with the enzootic SIV viruses of African nonhuman primates has identified a number of host cellular “antiviral” genes and proteins, such as APOBEC3C, tetherin, and trim5 α , which constitute host intrinsic defenses. In some instances, there are cognate viral proteins that counteract these intrinsic host defenses (see Chapter 9, HIV and AIDS and Chapter 16, Emerging Viral Diseases). For example, SIVcpz of chimpanzees was acquired from African monkeys, but underwent key genetic changes to circumvent the intrinsic antiviral proteins encoded in the chimpanzee genome. In turn, these mutations permitted SIVcpz to cross the species barrier into humans, who share similar antiviral proteins with chimpanzees.

4.4 Host Innate Response

The pattern and dynamics of the innate response to a specific virus strain can play an important role in its virulence. A variety of studies have been done using “omics” approaches to understand the innate response to virus infections.

When virulent and avirulent strains of H1N1 influenza virus were compared in mice, the virulent virus initiated a faster, more robust, and sustained inflammatory response in the lungs (Figure 2), resulting in severe lesions (Korth et al., 2013). It is speculated that a similar phenomenon accounted

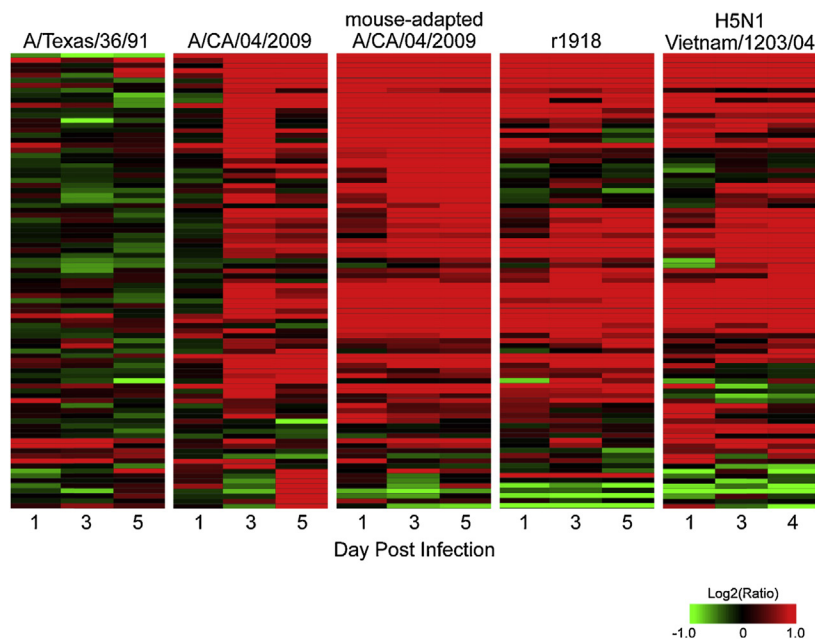


FIGURE 2 Differential induction of inflammatory gene expression by influenza viruses causing mild or severe respiratory disease. Shown are changes in inflammatory gene expression profiles over time in the lungs of mice infected with A/Texas/36/91 (a nonpathogenic seasonal isolate), A/CA/04/2009 (a mildly pathogenic 2009H1N1 pandemic isolate), or highly pathogenic mouse-adapted 2009H1N1, r1918, or avian H5N1 viruses. Expression values are represented as the average of the log₂ratio of infected to respective mock-infected samples for three biological replicates per condition. Red and green indicate that gene expression is increased or decreased relative to mock, respectively. From Korth MJ, Tchitchek N, Benecke AG, Katze MG. *Systems approaches to influenza virus host interactions and the pathogenesis of highly virulent and pandemic viruses*. *Seminars in Immunology*. 2013; 25(3):228–39, with permission.

for the virulence of the 1918 influenza virus in humans. Another study found that the dynamics of the inflammatory response to H5N1 and H1N1 viruses in mice plays a key role in the outcome of infection. A rapid response that quickly resolves is associated with survival and recovery. If the infection is not resolved quickly, the response may cause so much inflammation that it enhances pulmonary dysfunction and endangers the host.

Fatal Ebola infections are associated with an excess outpouring of proinflammatory cytokines (a cytokine “storm”) and a disseminated intravascular coagulopathy (McElroy et al., 2014). The terminal events appear to be a shock syndrome accompanied by multiple organ failure. To construct a mouse model of Ebola disease, it was necessary to use a mouse-adapted strain of the virus (Rasmussen et al., 2014). A screen of inbred lines of mice showed that only a few selected lines were susceptible to full-blown lethal hemorrhagic shock syndrome, while other lines could be infected but recovered. Lethal outcomes were associated with inflammatory signaling and vascular permeability, markers of a cytokine storm. In this mouse model, differences in host susceptibility appear to be associated with different alleles of the endothelial tyrosine kinases *Tie1* and *Tek* (*Tie2*). How these genes influence the likelihood of a cytokine storm remains to be clarified.

4.5 Host Adaptive Immune Response

Altered virulence and pathogenicity of variant viruses may be mediated through the host adaptive immune response (see Chapter 5 Adaptive Immunity). For instance, clone 13 of lymphocytic choriomeningitis virus (LCMV) differs from the Armstrong strain by virtue of its ability to replicate more rapidly in dendritic cells and macrophages (professional antigen-presenting cells). The rapid destruction of dendritic cells by clone 13 interferes with antigen presentation, thereby suppressing the immune response which, in turn, permits the virus to escape clearance. As a result, clone 13 initiates a persistent infection without acute illness. In contrast, the Armstrong strain, which does not interfere with induction of adaptive immunity, causes a benign immunizing infection with rapid virus clearance. These differences have been mapped to just two amino acids in the surface protein of LCMV. This example illustrates the delicate balance between acute and persistent infections caused by different variants of a single virus.

4.6 Viroceptors and Virokines

DNA viruses with a large genome, particularly the herpesviruses and the poxviruses, encode a number of proteins that counter host defenses. Virokines are viral proteins that mimic host cytokines stimulating cell proliferation and increasing the number of virus targets. Viroceptors are viral proteins that mimic receptors for host defensive cytokines,

“decoying” them away from their intended cellular receptors. For example, vaccinia virus encodes a complement control protein that blocks the complement cascade and a tumor necrosis factor viroceptor that binds this host defense molecule. Herpes simplex virus (HSV) encodes two glycoproteins, gE and gI, that act as an Fc receptor; the receptor binds and inactivates antiviral antibodies.

5. HOW DO VIRUSES PERSIST?

The prototypical viral infections are acute, and induce host-defensive responses that clear the virus and leave the host with long-lasting virus-specific immunity. However, many viruses are capable of persisting, often for the lifetime of the host. In order to persist, a delicate balance must be achieved so that, on the one hand, the host is not killed by the destructive effects of the virus while, on the other hand, the virus is able to evade the multitude of immune defenses that act to eliminate it. How this happens is the theme of this section.

The mechanisms of persistence range along a spectrum (Sidebar 2). At one extreme are viruses that continue to replicate at high titers over long periods of time, while at the other extreme are viruses that become latent, emerging at rare intervals to replicate for short periods of time. Between these ends of the spectrum are examples of smoldering infections that share characteristics of both replication and latency. Viruses employ a variety of strategies to escape immune surveillance, and these tend to be specific for different styles of persistence. Thus, immune tolerance often characterizes high titer persistent infections, whereas active immune responses are seen in many latent infections. Some selected examples of each style of persistence are listed in Table 5.

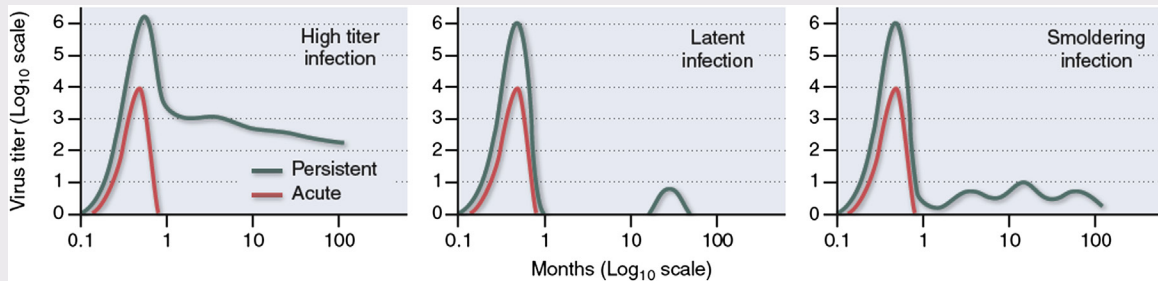
5.1 Immune Clearance of Acute Viral Infection

As a prelude to consideration of persistence, it is useful to briefly recapitulate the mechanisms by which the immune response controls and eliminates an acute virus infection. During the innate response, effector T lymphocytes can destroy virus-infected cells, produce antiviral cytokines, and recruit mononuclear cells to sites of viral replication and destruction (see Chapter 4, Innate Immunity). During the adaptive response, antibody neutralizes and opsonizes free infectious virions (see Chapter 5, Adaptive Immunity). In some instances, both antibody and virus-specific effector lymphocytes can purge virus-infected cells without destroying them. It is these mechanisms that a virus must evade in order to persist.

5.2 High Titer Infections

For a persistent virus to replicate at high titer, it must avoid catastrophic pathogenic effects, either because it is

Sidebar 2 Mechanisms of persistence



- *High titer replication* requires that virus either be noncytotoxic or that there is rapid replacement of target cells by cellular proliferation. Immune surveillance is unable to eliminate the virus, due to tolerance, immune complex formation, viral mutation, or other mechanisms.
- *Latency* usually requires that the viral genome persist in a nonreplicating mode, either integrated into the genome of the host cell or as an episome, although intermittent active replication may occur. Immune surveillance may be competent to eliminate replicating virus but not latent viral genomes.
- *Smoldering infections* involve continuous productive infection and cell-to-cell transmission at a low level. Potentially effective immune surveillance is circumvented by mechanisms such as antigenic variation, infectious immune complexes, or intercellular bridges.

Modified after Johnson R. Neurotropic Virus Diseases. Raven Press, 1985, with permission.

not acutely cytotoxic or because it attacks target cells that can be replenished regularly and effectively. Many viruses can replicate productively without causing cell death, and a number of them can cause persistent infections. In such instances, the initial dynamics resemble those of an acute infection, following which the virus titer decreases somewhat but then reaches a set point that may be maintained indefinitely or gradually decline. Examples of this pattern are hepatitis B virus (HBV), HIV, and LCMV of mice.

Immune tolerance and persistent infection with nonlytic viruses. A virus that persists at a high level has to escape an effective immune response that would control the infection. Therefore, high-titer virus persistence is often accompanied by immune “tolerance,” an apparent absence of a virus-specific adaptive immunity. The mechanisms by which tolerance can be induced include deletion of “forbidden” clones of naïve T lymphocytes in the thymus, or exhaustion of peripheral virus-specific T lymphocytes in the presence of excess antigen (see Chapter 6, Aberrant Immunity). Tolerance may be limited to specific components of the effector limb of the immune response. For instance, hepatitis B persistence is characterized by absence of antibody against HbsAg but not against HBcAg. LCMV persistence is characterized by absence of cellular immune responses while virus-specific antibody is produced.

Virus-specific exhaustion of effector T lymphocytes has best been analyzed in the LCMV model of persistent infection. There is a marked difference in the properties of LCMV-specific CD8⁺ virus-specific memory T cells that are generated during acute versus persistent infections. Acute infections induce memory T cells that exhibit the

cardinal properties of self-renewal in the absence of antigen (and high levels of receptors for IL-7 and IL-15), whereas the memory T cells from animals with persistent infections express low levels of these two interleukin receptors, and gradually disappear in the absence of antigen. In this model, the memory cells associated with persistent infection fail to differentiate into effector CD8⁺ T cells capable of eradicating persistent infection.

Evidence in the LCMV model for the role of immune tolerance in maintaining viral persistence is provided by the experimental termination of persistence by intravenous injection of virus-specific CD8⁺ T cells, obtained from animals undergoing acute infection. Similar results have been obtained with HBV, where cytolytic T lymphocytes (CTLs) specific for HBs epitopes clear virus from hepatocytes.

Lytic viruses. It is unusual for high titer persistence to be produced by a cytotoxic virus, but the primate lentiviruses represent an important exception. The main target cells for these lentiviruses are CD4 lymphocytes that often undergo lytic infection. It has been calculated that the continuous destruction of CD4 cells results in a reduction of the average half-life of these cells from 75 to 25 days. However, early in the course of infection the bone marrow is able to respond to the abnormal rate of destruction by increasing the production of naïve CD4 cells at a rate sufficient to maintain a reasonable concentration of circulating CD4 cells. This permits a lytic virus to persist at a high titer for an extended period of time in the relative absence of clinical illness; eventually, the bone marrow is unable to compensate and CD4 levels drop, leading to functional immunodeficiency.

TABLE 5 A Selected List of Human and Animal Viruses that Cause Persistent Infections through Different Mechanisms

Virus family Virus example Disease	Host(s)	Site of persistence	Cytocidal in permissive cells	Immune response
High Titer Replication				
Hepadnaviruses Hepatitis B virus Cirrhosis	Human (newborn)	Hepatocytes	No	Split tolerance
Flaviviruses Hepatitis C virus Cirrhosis	Human	Hepatocytes	No	Variable
Arenaviruses Lymphocytic choriomeningitis virus Glomerulonephritis	Mouse (newborn)	Macrophages, other cells	No	Split tolerance
Latent Infection				
Herpesviruses Herpes simplex virus Cold sores, encephalitis	Human	Sensory neurons	Yes	Yes
Cytomegalovirus Pneumonitis, retinitis, hepatitis	Human	Lymphocytes	Yes	Yes
Epstein–Barr virus Mononucleosis	Human	B cells	Yes	Yes
Varicella-zoster virus Herpes zoster	Human	Sensory neurons	Yes	Yes
Smoldering Infection				
Paramyxoviruses Measles Subacute sclerosing panencephalitis	Human	Neurons, glia	Yes	Super normal
Lentiviruses HIV AIDS	Human	CD4 lymphocytes	Yes	Variable
Polyomavirus JC virus Progressive multifocal leucoencephalopathy	Human	Oligodendrocytes	Yes	Yes
Lentiviruses SIV AIDS	Nonhuman primates	CD4 lymphocytes	Yes	Variable
Lentiviruses Visna-maedi virus Pneumonitis, encephalitis	Sheep	Monocytes	Yes	Yes
Equine infectious anemia virus Anemia	Horses	Monocytes	Yes	Yes
Oncogenic Infection				
Polyomaviruses Human papillomavirus Carcinoma cervix, other sites	Human	Epidermal cells	Yes	Yes
Hepadnaviruses Hepatitis B virus Hepatocellular carcinoma	Human (newborn)	Hepatocytes	No	Split tolerance

TABLE 5 A Selected List of Human and Animal Viruses that Cause Persistent Infections through Different Mechanisms—cont'd

Virus family Virus example Disease	Host(s)	Site of persistence	Cytocidal in permissive cells	Immune response
Flaviviruses Hepatitis C virus Hepatocellular carcinoma	Human	Hepatocyte	No	Variable
Herpesviruses Epstein–Barr virus Burkitt's lymphoma	Human	B cells	Yes	Yes
Herpesvirus Human herpesvirus 8 Kaposi's sarcoma	Human	Lymphocytes	Yes	Yes
Lentivirus Human lymphotropic virus-1 Adult T cell leukemia	Human	CD4+ lymphocytes	No	Yes

In contrast to most high titer persistent infections, lentiviruses induce immune responses rather than tolerance. The immune response to lentiviruses is quite effective, as judged by its ability to rapidly contain the acute phase of infection, resulting in a reduction from peak viremia at about 6 weeks to a set point about 1000-fold lower at about 3–6 months. Once this set point is reached, a dynamic equilibrium is established between virus production and clearance. The plasma half-life of individual SIV virions is <30 min in the absence of immunity and about 10 min in infected animals with an established immune response. It has been calculated that to maintain virus titers of 10^2 – 10^4 infectious virions per ml of plasma requires the production of 10^{10} – 10^{12} new infectious virions daily. In this instance, high titer persistence is maintained by an extraordinary rate of virus production that exceeds the rate at which a potent cellular immune response can clear virus-infected cells. See Chapter 15, Mathematical Approaches, for a discussion of how differential equations are used to model viral production and clearance.

5.3 Latent Infections

Latent infections are produced by a considerable number of human herpesviruses, including HSVs, varicella-zoster virus (VZV), Epstein–Barr virus (EBV), and cytomegalovirus (CMV). There is a characteristic sequence of events following primary infection. Initially, the virus replicates in permissive cells at the portal of entry. The virus is lytic and destroys these permissive target cells. Once immune induction has occurred, the virus is cleared and appears to be eliminated.

However, the viral genome persists in a latent form. Latency occurs in one or more cell types—such as neurons for HSV and VZV—that are distinct from the permissive cell types that support productive lytic infection. Neurons

are restrictive or permissive, depending upon their physiological state. Under conditions of restriction, the virus undergoes the early steps of entry and uncoating, but further steps in replication are blocked. In some instances, the double-stranded DNA genome integrates into the host genome, while in other examples the genome persists as a nonintegrated episome, in the nucleus or cytoplasm.

If latency occurs in cell types that—like neurons—do not divide, then there is no need to replicate the latent genome. If the viral genome is integrated into the host genome, as with retroviruses, then it will be automatically replicated during the cell cycle. Episomal DNA can also be replicated by the enzymes involved in copying cellular genomes. However, there are no parallel mechanisms for RNA, so RNA viruses cannot assume a latent state unless they undergo reverse transcription to DNA intermediates.

Latency maintains the viral genome for the lifetime of the infected host. Activation of latent infections may occur at irregular intervals, or it may never occur in some infected individuals. Activation of latent genomes can be initiated by a number of stimuli, characteristic for each virus. For instance, HSV can be activated by fever, sunburn, and trigeminal nerve injury. Most of these stimuli appear to act upon the primary sensory neurons in which latent HSV genomes are maintained. Waning of the immune response can enhance the risk of activation of some herpesviruses, such as VZV.

Following reactivation of HSV, the viral genome may spread by axoplasmic transport in both centripetal and centrifugal directions. Centrifugal spread conducts the virus to the skin where it may replicate and spread, causing herpes labialis (“fever blister” or “cold sore”). After spreading for a few days, host defenses prevent further spread, and the skin lesion heals. Centripetal spread from the trigeminal ganglion conducts the HSV genome to the CNS, where, in relatively few instances, it can cause a devastating encephalitis.

Typically, viruses that cause latent infections induce innate and acquired immune responses, brisk and potent immune response that clears the initial infection. When the latent infection is activated, immune surveillance limits its spread, but virus produced during activation may be spread to another host. For instance, activation of latent VZV produces characteristic skin lesions in older adults; seronegative children exposed to virus aerosolized from these lesions can develop chicken pox, the primary form of VZV infection.

5.4 Smoldering Infections

“Smoldering” infections fall between the extremes of high titer persistence and latency. Infectious virus is produced, but at minimal levels that may require special methods for detection and isolation. Virus continues to spread from infected to uninfected cells but often at an indolent tempo. If the virus is pathogenic, it may produce a gradually progressive chronic disease. There is a detectable immune response to the virus. The ability of a virus to spread in the presence of a potentially effective immune response is a paradoxical phenomenon, and involves a variety of strategies, several of which are described below.

Immunologically privileged sites. There are a few organs and tissues that appear to favor virus persistence, particularly the brain and kidney. The brain has classically been considered an immunologically “privileged” site because immunological effector mechanisms may spare “foreign” cells in the brain (in contrast to foreign cells in other sites). There are at least two factors that account for virus persistence in the brain. First, the blood–brain barrier limits the trafficking of lymphocytes through the brain and, second, neurons express little if any MHC Class I molecules, rendering them relatively poor targets for virus-specific CTLs.

The kidney is the other major tissue that frequently harbors persistent viruses, such as JC and BK polyomaviruses, and cytomegalovirus. There is no clear explanation why virus in the kidney should be able to evade immunological surveillance, although it has been speculated that lymphocytes may not readily cross the subendothelial basement membrane to access infected glomerular epithelial cells.

Infectious immune complexes. In some instances where a virus persists in the presence of an active immune response, infectivity in the blood circulates in the form of immune complexes that are composed of infectious virions coated by virus-specific antibodies. Immune complexes can be demonstrated by the addition of anti-IgG antisera that will “neutralize” the infectivity. The molecular mechanism through which an antibody-coated virion can retain its infectivity has never been well elucidated. One possibility is that the complex is bound to Fc receptors on macrophages and internalized in vacuoles in which the complex dissociates, followed by infection of the macrophage.

Antigenic variation. During the course of persistent infection, there may be a selection for viral variants that are able to escape neutralization. Such resistant virus variants usually represent point mutations, often in the viral attachment protein. This phenomenon has been observed with several persistent lentiviruses such visna/maedi virus of sheep, equine infectious anemia virus, and very prominently HIV (discussed in Chapter 9, HIV and AIDS).

Intercellular bridges. In some instances, the process of entry of viruses into cells can be short circuited, so that a transient intercellular bridge is formed. The bridge permits the viral genome to pass from cell to cell without having to survive in the extracellular environment, thus providing a means of avoiding neutralizing antibody. This phenomenon is probably operative in subacute sclerosing panencephalitis (SSPE), a progressive fatal neurological disease (discussed in more detail below). In SSPE, a defective variant of measles or rubella virus spreads gradually from neuron to neuron in spite of extraordinarily high titers of neutralizing antibody in the extracellular fluid of the brain parenchyma.

5.5 Clinical Examples of Smoldering Infections

In its typical course, measles spreads primarily to children by the respiratory route and causes a systemic febrile infection with a rash that usually resolves in 1–2 weeks with no serious consequences. However as indicated previously, SSPE is a rare complication of measles (~1 case per 100,000 primary infections) that occurs unpredictably in apparently normal children following uneventful recovery from acute measles. Several years after measles, these children develop a progressive encephalitis that is invariably fatal in 6–12 months. Measles antigens can be detected in either biopsy or postmortem brain, and electron microscopy reveals measles nucleocapsids in neurons and glial cells in the brain.

SSPE variant viruses exhibit underexpression or defects in one or more viral proteins; these mutations inhibit budding and the production of free-infectious virions. In this example, escape from immune surveillance is associated with the selection of variant viruses that lose the ability to mature and bud properly while maintaining the gene functions for replication of the viral nucleocapsid. The incomplete virus core can still be transmitted from cell to cell, and is slowly cytotoxic, leading to the clinical progressive and ultimately fatal encephalitis.

HBV infection follows different courses, depending upon age at the time of infection. Infection of adults is often a self-limited acute process, with clearance of the virus usually accompanied by hepatitis. Neonatal infection (transmission from a woman who is a chronic virus carrier to her newborn infant) usually leads to persistence (Figure 3). The infected infant has high levels of circulating HBsAg and

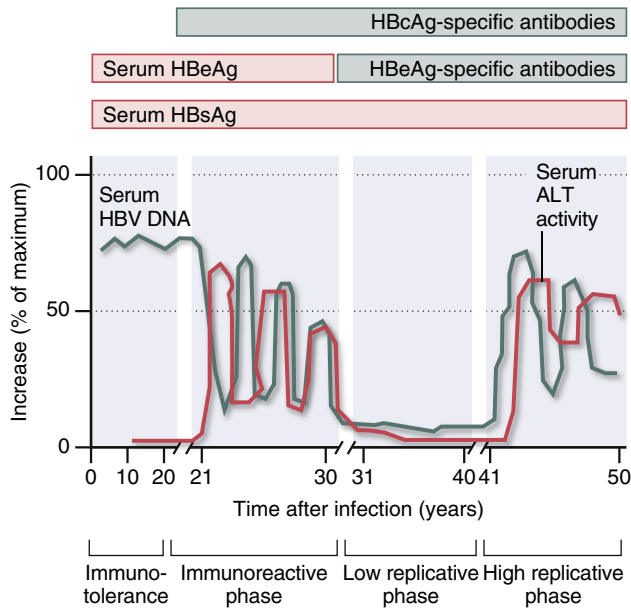


FIGURE 3 Complexities of a smoldering virus infection. The course of persistent hepatitis B virus following infection of a newborn infant. Most such HBV infections begin as an immunotolerant process with high virus titers and a minimal cellular immune response, which may last for decades to a lifetime. However, for reasons that are not known, the infection may shift into one of three other modes, a low replicative phase, an immunoactive phase, or a high replicative phase. In the low replicative phase, patients may control or clear the infection with the development of anti-HBe antibody and recovery of cellular immunity. In the immunoactive or high replicative phases, the immune response attempts to clear the virus but is only partially successful, with resultant necroinflammatory hepatitis that may progress to cirrhosis (permanent liver damage). ALT, alanine transaminase; a liver enzyme whose level in the serum reflects liver function. After Rehermann B, Nascimbeni M. *Immunology of hepatitis B virus and hepatitis C virus infection*. *Nature Reviews Immunology* 2005, 5: 215–229, with permission.

serum HBV DNA. Anti-HBs (viral surface antigen) or anti-HBe (precore antigen) antibodies cannot be detected in the serum of infected infants but anti-HBc (viral core antigen) antibodies are present, a state sometimes called “split tolerance.” Persistence is associated with high dose cellular tolerance against HBsAg, with only minimal levels of HBs-specific CD8+ T cells due to the deletion or exhaustion of antigen-specific T lymphocytes. Since CD8+ T cells play a major role in control or clearance of HBV, this is a major factor in persistence.

Persistent HBV high titer replication may last for decades to a lifetime. However, through an unknown mechanism, the infection may shift into one of three other modes, a low replicative phase, an immunoactive phase or a high replicative phase. In the low replicative phase, patients may control or clear the infection with the development of anti-HBe antibody and recovery of cellular immunity. In the immunoactive or high replicative phases, the immune response attempts to clear the virus but is only partially successful, with resultant necroinflammatory hepatitis that

may progress to cirrhosis (permanent liver damage) and/or hepatocellular carcinoma.

6. REPRISE

Virus virulence and host susceptibility are interdependent properties that are determined by the virus–host combination and that together result in the manifestations of infection. A virus that is virulent in one setting may be innocuous in another, and a host may be susceptible or resistant depending upon age, route of infection, or properties of the virus.

Virus virulence can be measured in a variety of ways, based on mortality, illness, or pathological lesions, each of which can be quantified. The virulence phenotype also may be qualitative, involving differences in the tropism of different viral variants. Wild-type isolates of a virus may vary in virulence, and virulence variants can also be selected by experimental manipulation. Attenuated variants often exhibit host range or temperature-sensitive phenotypes, and may provide candidates for live virus vaccines. The attenuated phenotype can be manifested at any step during the course of infection, from invasion, to spread, involvement of target organs, or shedding. Virulence or attenuation can be mapped to specific viral genes and individual nucleotides, and may be associated with one or many viral genes, including noncoding sequences. For viruses with large and complex genomes, virulence may be conferred by accessory genes that act as virokines or viroceptors.

A number of viruses from a wide variety of virus families, can persist for months, years, or even lifelong. In persistent infections there is a delicate balance, so that the host is not killed by the destructive effects of the virus and the virus manages to evade immune surveillance. Most persistent infections fall into one of three distinct patterns. (1) Persistence of the virus—usually a noncytotoxic agent—at high titer in various tissues, often associated with immune tolerance due to deletion or exhaustion of antigen-specific T lymphocytes. (2) Latent infections, in which the virus persists as an untranslated genome that eludes recognition by the host immune response; Latent infections may reactivate periodically and reactivations are usually terminated by host immune surveillance. (3) Smoldering infections fall between the other two patterns, with the virus continuing to replicate but at a low level in the face of a brisk immune response. Evasion of immune surveillance is achieved by a number of mechanisms, such as immunologically privileged tissue sites, intercellular bridges, and antigenic variation. Persistent infections may be asymptomatic but often are associated with a wide variety of chronic diseases. As we will see in the following chapter, some persistent viral infections can also lead to cancer.

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