Redefining Chronic Viral Infection

Herbert W. Virgin,[1,*] E. John Wherry,[2,*] and Rafi Ahmed[3,*]
[1]Department of Pathology and Immunology, Department of Molecular Microbiology, and the Midwest Regional Center for Excellence in Biodefense and Emerging Infectious Diseases, Washington University School of Medicine, St. Louis, MO 63110, USA
[2]Immunology Program, The Wistar Institute, Philadelphia, PA 19104, USA
[3]Emory Vaccine Center and Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA
*Correspondence: virgin@wustl.edu (H.W.V.), jwherry@wistar.org (E.J.W.), ra@microbio.emory.edu (R.A.)
DOI 10.1016/j.cell.2009.06.036

Viruses that cause chronic infection constitute a stable but little-recognized part of our metagenome: our virome. Ongoing immune responses hold these chronic viruses at bay while avoiding immunopathologic damage to persistently infected tissues. The immunologic imprint generated by these responses to our virome defines the normal immune system. The resulting dynamic but metastable equilibrium between the virome and the host can be dangerous, benign, or even symbiotic. These concepts require that we reformulate how we assign etiologies for diseases, especially those with a chronic inflammatory component, as well as how we design and interpret genome-wide association studies, and how we vaccinate to limit or control our virome.

Our metagenome includes all organisms that live on us or in us. The bacteria that colonize us can vary from being symbionts to opportunists to pathogens, with the balance between extreme outcomes of infection determined by host immunity and bacterial virulence. Commensal bacteria are known to regulate the host immune response and systemic physiology in fundamentally important ways. Less recognized are the contributions to our metagenome from viruses that establish chronic infection and retroviral elements that infest our chromosomes. How the dynamic nature of the interactions between this internal virome and the host imprints our immune system, and perhaps our physiology more generally, has been given little consideration. Some chronic infections are so common that any immunologic imprint of these agents or potential contribution of these viruses to disease are obscured by the fact that they are part of the normal flora of most humans. Thus, a redefinition of chronic virus infection that sets the stage for new and interesting experiments is needed to analyze this fundamental aspect of our biology. We all carry, for good or ill, many lifelong viral passengers.

Comparing Acute and Chronic Viral Infection

Acute viral infection is a nonequilibrium process, whereas chronic viral infection is a process in dynamic and metastable equilibrium. During acute infection, both the host and virus change continuously until infection is resolved, kills the host, or becomes chronic (Figure 1). Certain genes in a virus or in the immune system are niche specific in that they function during acute but not chronic infection. Failure of these immune system genes to function effectively or too-effective evasion of immunity by viral genes can have devastating consequences. In contrast, during chronic infection, viral and host genes balance each other. How some viruses manage to persist despite the impressive immune armamentarium of the host, without causing overt disease, is a great unsolved mystery in immunobiology.

Our understanding of host immunity and viral virulence comes largely from studies of resolving acute infection (Figure 1). Clearance of viral antigen, effector cells, and cytokines allows the immune system to reset to an uninfected but memory-armed state that includes quiescent "hair-triggered" memory B and T cells, as well as plasma cells that continuously produce antibody. Studies of acute infection have been extremely valuable for defining viral virulence and host immunity. However, the "viral" and "host" rules that govern acute versus chronic viral infection differ markedly. For example, whereas CD8 T cells that recognize peptides presented by classical major histocompatibility complex (MHC) molecules are important during both acute and chronic infection, CD8 T cell responses restricted by nonclassical MHC molecules play an important part in the control of some chronic viral infections (Braaten et al., 2006; Swanson et al., 2008; Moretta et al., 2003).

Two events are fundamental to establishment of chronic viral infection (Figure 1). First, the virus must evade sterilizing immunity (the complete elimination of a virus). Second, the immune system must adjust to the continuous presence of viral antigen-driven inflammatory responses in order to limit viral replication to an acceptable level without untoward damage to permanently infected tissues. If the immune system cannot eliminate the virus, unrestrained immune attack on virus antigen-bearing cells causes immunopathology. Thus, downregulation of inflammation during chronic viral infections can result in decreased tissue damage, at least for noncytopathic viruses. Immunopathology can be severe in animal models and has been implicated in human chronic infections caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) (Guidotti and Chisari, 2006). It is important to realize that viruses that rely on a living but chronically infected host for their own survival must carefully avoid mechanisms that overwhelm immunity and kill their hosts. Indeed, given the rapid pace of viral

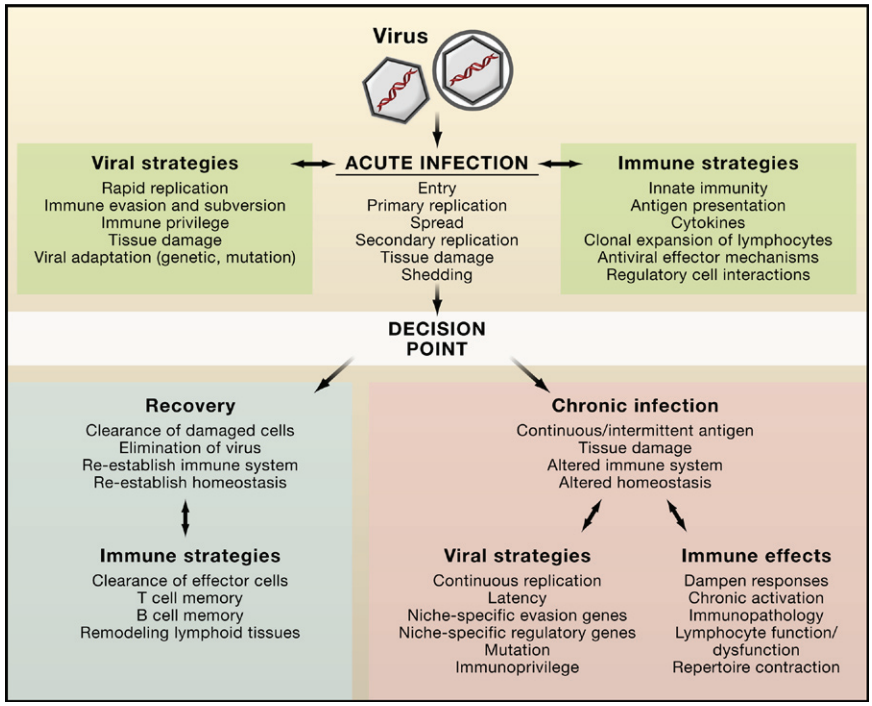

Figure 1. The Course of Viral Infection

When a virus enters the host, there is an initial nonequilibrium phase of acute infection. During this phase, viral and immune strategies compete for dominance. Assuming the host survives, a decision point is reached at which the infection is either cleared or becomes chronic. This decision point may be reached very early in infection for viruses that can establish a latent infection, in which case the infection is permanent regardless of the course of acute infection. If recovery occurs, the immune system must reset by clearing the antigen and re-establishing immune homeostasis. If the balance shifts toward chronic infection, a new set of viral and host strategies interact to define a metastable equilibrium in which viral replication is held in check, but the virus is not cleared.

evolution, it stands to reason that viruses that are relatively benign clinically, despite establishing chronic infection, would have evolved to greater virulence if this was advantageous.

The balances between the vigor of the antiviral immune response, immunopathology, and viral strategies for maintaining chronic infection without killing the host are poorly understood. Importantly, even when the host and virus are in a metastable equilibrium, chronic infection is dynamic, with host and viral processes balanced on a knife's edge such that small changes can disrupt the equilibrium with unfortunate consequences. This dynamism has fundamentally important effects on the host, including setting a level of cytokines in tissues and serum that may alter the function of innate immunity, antigen-presenting cells (APC), and lymphocytes. An example of the timeframe involved in establishing such relationships comes from herpesviruses, which derive from an ancestral virus shared by birds, reptiles, and mammals (McGeoch et al., 2006) and have therefore shared more than 100 million years of coevolution with their current hosts. Given the length of this genetic relationship, it is highly likely that adaptations in both host and viral genomes enhance our capacity to coexist with these age-old passengers. These complex interactions between virus and host form an intricate network of interdependent genes and processes that must be understood in molecular detail if we seek to intervene without causing harm.

Persistent Viruses: Old Enemies and New Passengers

It would be most appropriate to categorize viruses that establish chronic infection by the mechanisms used to establish persistence, but these are ill defined. We categorized them here by the estimated prevalence of chronic human infection to emphasize the contribution of our virome to our metagenome (Table 1, Figure 2). The numbers in Figure 2 are extrapolations to the entire earth's population from the estimated prevalence of antiviral antibodies in serum (seroprevalence) (Table 1). Because seroprevalence data are often obtained only from developed countries, the impact of chronic infections on humanity as a whole is poorly characterized. This is an important deficiency in our current understanding of the human metagenome. However, a rough calculation reveals that there are many billions of chronic virus infections in humans, with each of the ~6.75 billion of us harboring ~8–12 chronic infections (Figure 2, Table 1). Some of these viruses are so common that one would be hard put to find uninfected control patients for studies to identify viral associations with disease or effects on the "normal" immune system. The fact that most of us do not show obvious symptoms of these infections marks these viruses as part of the metagenome of normal individuals. This formulation is not meant to detract from the obvious point that some of these viruses cause human disease. Rather, it calls attention to the need to seriously consider what these infections mean for humans without overt disease and the need to understand chronic diseases that are considered "nonviral" based on our inability to compare infected to noninfected individuals.

Little is known about some of the viruses that inhabit us. This is due to the focus of research on viruses that cause disease in a high proportion of infected individuals. Many of the most prevalent chronic virus infections only rarely cause disease, are not associated with any disease at all, or cause disease primarily in immunocompromised individuals (Figure 2, Table 1). In contrast, some of the best understood viruses such as HCV, HBV, and human immunodeficiency virus (HIV) are of great medical importance but infect a lower proportion of the human population in comparison to their less virulent counterparts (Figure 2, Table 1). Other viruses rarely establish chronic infection but when they do, they are highly virulent. For example, measles virus can cause relentlessly progressing neurogenerative disease (Table 1).

Importantly, the number of recognized persistent viruses has increased with the advent of new molecular technologies. For example, there are five human polyomaviruses, three of which

Table 1. Chronic Virus Infections in Humans

			Disease during Chronic Infection		
Virus, Primary Nucleic Acid, Estimated Percent of Humans Infected	Major Site of Persistence (Organ or Cell)	Acute Infection Examples	Within Normal Hosts	Within Immunocompromised Hosts	References
Endogenous retroviruses (ERV), DNA, 100%	All	Not applicable	Unknown	Unknown	Seifarth et al., 2005; Virgin, 2007b
Anellovirus/Circovirus, DNA, 90%–100%	Many tissues	Unknown	Unknown	Unknown	Davidson and Shulman, 2008; Ninomiya et al., 2008; Hino and Miyata, 2007
Human herpesvirus 6 (HHV-6), DNA, >90%	Lymphocytes?	Roseola	Unknown	Meningoencephalitis, secondary infections, immunomodulatory?	Straus, 2000; Yamanishi et al., 2007
Human herpesvirus 7 (HHV-7), DNA, >90%	Lymphocytes?	Roseola	Unknown	Unknown	Straus, 2000; Yamanishi et al., 2007
Varicella zoster virus (VZV), DNA, >90%	Sensory ganglia neurons and/or satellite cells, lymphocytes	Chicken pox	Herpes zoster	Disseminated disease, hepatitis, pneumonitis	Zerboni and Arvin, 2008; Straus, 2000
Cytomegalovirus (CMV), DNA, 80%–90%	Myelomonocytic cells	Mononucleosis	Rare	Disseminated disease, vasculitis, pneumonitis, retinitis, hepatitis, gastroenteritis, meningoencephalitis	Mocarski et al., 2007
Epstein-Barr virus (EBV), DNA, 80%–90%	Pharyngeal epithelial cells, B cells	Mononucleosis	Burkitt's lymphoma, nasopharyngeal carcinoma, non-Hodgkin's lymphoma	CNS lymphomas, oral hairy leukoplakia, lymphoproliferative disease	Rickinson and Kieff, 2007; Straus, 2000; Kieff and Rickinson, 2007
Polyomavirus BK, DNA, 72%–98%	Kidney	Unknown	Unknown	Hemorrhagic cystitis (post bone marrow transplantation), nephropathy (post kidney transplantation)	Zur, 2008
Polyomavirus JC, DNA, 72%–98%	Kidney, CNS	Unknown	Unknown	Progressive multifocal leukoencephalopathy	Zur, 2008
Adeno-associated virus (AAV), DNA, 60%–90%	Many tissues	Unknown	Unknown	Unknown	Gao et al., 2004; Berns and Parrish, 2008; Schnepp et al., 2005a, 2005b; Chen et al., 2005; Erles et al., 1999; Blacklow et al., 1968
Herpes simplex type 1 (HSV-1), DNA, 50%–70%	Sensory ganglia neurons	Pharyngitis, encephalitis, keratitis,	Cold sores, encephalitis, keratitis	Increased severity of same diseases, pneumonitis, hepatitis	Straus, 2000
Adenovirus, DNA, up to 80%	Adenoids, tonsils, lymphocytes	Upper respiratory infection, gastroenteritis	Unknown	Enteritis, hemorrhagic cystitis, pneumonitis, hepatitis, others	Garnett et al., 2002; Wold and Horwitz, 2008
Herpes simplex type 2 (HSV-2), DNA, 20%–50%	Sensory ganglia neurons	Genital herpes	Genital herpes, encephalitis	Increased severity of same diseases	Straus, 2000
Kaposi's sarcoma herpesvirus (KSHV) or human herpesvirus 8, DNA, 2%–60%	Endothelial cells, B cells	Unknown	Castleman's disease, Kaposi's sarcoma	Kaposi's sarcoma, primary effusion lymphoma	Ganem, 2006
Hepatitis B virus (HBV), DNA, 350 million, ~5%	Hepatocytes	Hepatitis	Cirrhosis, hepatocellular carcinoma	Same diseases	McGovern, 2007; Rehermann and Nascimbeni, 2005
GB virus C, RNA, 1%–4%	Lymphocytes	Unknown	Unknown	Unknown	Stapleton et al., 2004; Berzsenyi et al., 2005
Papilloma virus, DNA, <5%	Epithelial skin cells	Unknown	Papilloma, cervical and other mucosal carcinomas	Increased severity and incidence of same diseases	Leggatt and Frazer, 2007; Howley and Lowy, 2007

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Table 1. *Continued*

Virus, Primary Nucleic Acid, Estimated Percent of Humans Infected	Major Site of Persistence (Organ or Cell)	Acute Infection Examples	Disease during Chronic Infection: Within Normal Hosts	Disease during Chronic Infection: Within Immunocompromised Hosts	References
Hepatitis C virus (HCV), RNA, 170 million,~2.5%	Hepatocytes	Hepatitis	Cirrhosis, hepatocellular carcinoma	Same diseases	Rehermann and Nascimbeni, 2005; Lemon et al., 2007
Human immunodeficiency virus (HIV-1 and HIV-2), RNA, 33 million, ~0.5%	$CD4^+$ T cells, monocyte/macrophages	Acute febrile illness	AIDS	AIDS	UNAIDS, 2008; Kuritzkes and Walter, 2007
Hepatitis D virus (HDV), RNA, 15 million, ~0.2%	Hepatocytes	Unknown	Exacerbation of chronic HBV infection	Unknown	Taylor et al., 2007
Human T cell leukemia virus type 1 (HTLV1), RNA, 10–20 million, ~0.2%	T cells	Unknown	Adult T cell leukemia (2%–6% of carriers), tropical spastic paraparesis, myelopathy, uveitis, dermatitis	Unknown	Matsuoka and Jeang, 2007; Lairmore and Franchini, 2007
Xenotropic murine leukemia virus-related virus (XMLV), RNA, unknown	Prostate	Unknown	Prostate Cancer?	Unknown	Urisman et al., 2006; Dong et al., 2007
HTLV II, III, IV, RNA, unknown	T cells	Unknown	Unknown	Unknown	Matsuoka and Jeang, 2007; Lairmore and Franchini, 2007
Polyomavirus MC, DNA, unknown	Merkel cell carcinoma	Unknown	Merkel cell carcinoma?	Unknown	Zur, 2008
Polyomavirus KI, DNA, unknown	Lung	Unknown	Unknown	Unknown	Zur, 2008
Polyomavirus WU, DNA, unknown	Lung	Unknown	Unknown	Unknown	Zur, 2008
Rubella virus, German measles, RNA, rare	CNS	Rubella, arthritis	Progressive rubella panencephalitis	Unknown	Hobman and Chantler, 2007
Parvovirus B19, DNA, rare	Bone marrow erythroid progenitors	Fifth disease, arthritis	Aplastic crisis in hemolytic anemia, hydrops fetalis, chronic bone marrow deficiency	Red cell aplasia	Berns and Parrish, 2008; Norja et al., 2008
Measles virus, RNA, rare	Neurons and supporting cells in CNS	Measles	Subacute sclerosing panencephalitis, measles inclusion body encephalitis	Unknown	Griffin, 2007
Coxsackie, RNA, rare	Myocardial cells	Hand foot and mouth disease, herpangina	Myocarditis	Unknown	Chapman and Kim, 2008; Whitton and Feuer, 2004

Shown in the first column are the viruses known to chronically infect humans, their primary nucleic acid type, and an estimate of the prevalence of infection in humans. Additional columns list known sites of persistent infection and the diseases associated with infection. When incidence numbers are not clearly available worldwide, estimates from the United States are substituted. For many viruses, the specific sites of persistence are incompletely defined. The associated clinical syndromes presented are not an exhaustive list. CNS, central nervous system.

were discovered quite recently (Zur, 2008). The initially discovered JC and BK polyomaviruses infect between 72% and 98% of humans. It is likely that most humans carry many or all of these five viruses and thus harbor chronic polyomavirus infections in the nervous system, kidney, lung, and skin (Table 1). There are many animal polyomaviruses (Zur, 2008), and polyomavirus infection is highly species specific. This suggests prolonged coevolution of humans with at least five different polyomaviruses. Similarly, the eight known human herpesviruses are extremely efficient at establishing chronic infections; most of us carry many herpesviruses for our entire lives (Table 1). New polyomaviruses are not our only recently recognized passengers. Anelloviruses and related viruses, as well as adeno-associated virus (AAV), are now recognized to infect most humans by the end of childhood (Davidson and Shulman, 2008; Ninomiya et al., 2008; Hino and Miyata, 2007) (Table 1). Many human tissues harbor AAV genomes either as integrated sequences in genomic DNA or as circular episomes. We predict that the advent of high-throughput genome sequencing will significantly increase the number of known human viruses and the number of viruses that contribute to our metagenome via the establishment of chronic infection.

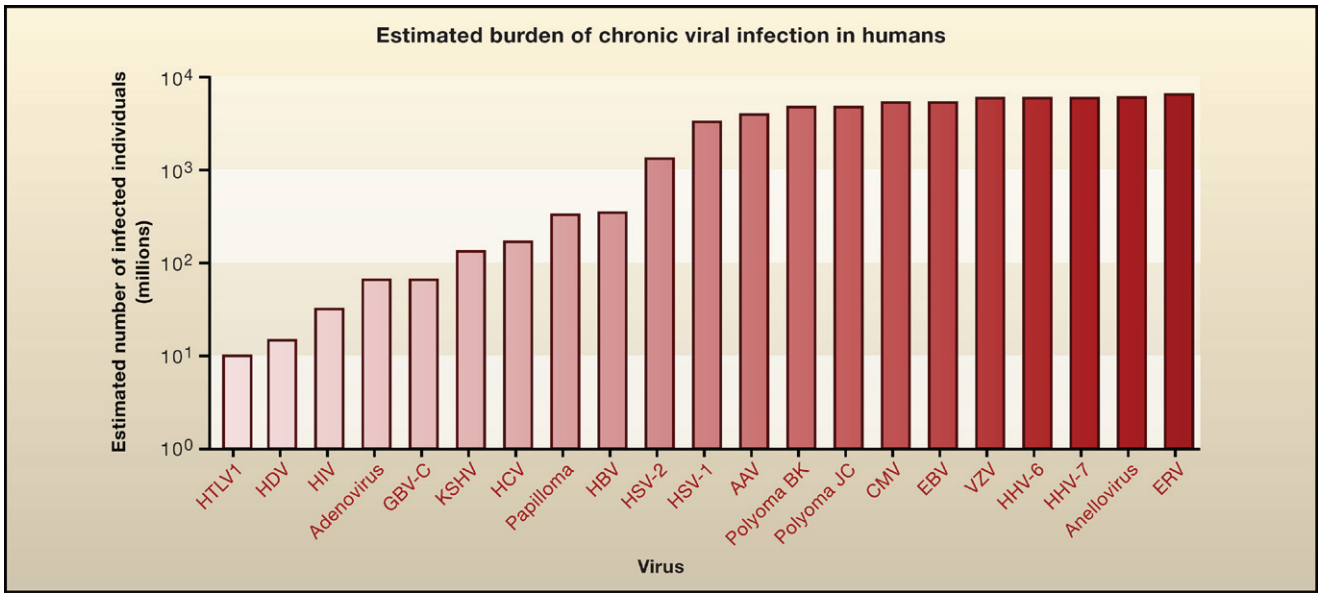

Figure 2. Chronic Viral Infections in Humans

The number of humans infected with different chronic viruses is estimated from the percentage of humans carrying a given virus (Table 1), assuming that the world population is 6.75 billion. Where available, the lower reasonable estimate of infection prevalence from Table 1 is used. For adenovirus, the situation is unclear and the prevalence of chronic infection is arbitrarily set at 1%. For some viruses in Table 1 that contribute to our virome, the prevalence of infection is not sufficiently well defined for inclusion in the graph. These include xenotropic murine leukemia virus-related virus (XMLV), human T cell leukemia virus (HTLV II, III, IV), and polyomaviruses MC, KI, and WU. The estimates in the graph are approximate as they apply data on prevalence in the limited populations studied to date to the global human population.

Types of Chronic Viral Infection

Viruses have evolved highly effective strategies for establishing chronic infection despite the presence of an active host antiviral immune response. Thus, they should be viewed as highly sophisticated molecular machines that have been "studying" us far longer than we have been studying them. There are three general strategies for chronic viral infection: continuous replication, latency and reactivation, and invasion of the genome followed by vertical spread from generation to generation. Individual viruses usually rely mostly on one strategy, but viruses can utilize more than one mechanism. For example, HIV effectively utilizes both continuous replication and establishment of latency (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997), a dangerous combination. The differences between these strategies have profound implications for the immune system and for virologists and immunologists designing ways to prevent or control harmful chronic viral infections. These differing viral strategies expose viral genomes to distinct evolutionary forces, resulting in significant molecular differences between viruses that persist through different mechanisms.

Continuous Productive Replication

Continuous replication depends on the generation of infectious virions despite ongoing antiviral immunity (Figure 3). Viruses in this category include HIV, HBV, and HCV in humans, simian immunodeficiency virus (SIV) in nonhuman primates, and lymphocytic choriomeningitis virus (LCMV) in mice. Continuous replication can generate up to 10^{12} particles per day for HBV and HCV (Rehermann and Nascimbeni, 2005). There is enormous opportunity for evolution of the virus genome during such infections. Viruses that persist via continuous replication express potentially antigenic viral proteins that are required for viral assembly and release, resulting in continuous antigenic stimulation of lymphocytes (Figure 3). Continuous replication has other effects on the immune system—low levels of tissue damage and stimulation of inflammatory cytokines and costimulatory molecules may alter the normal immune system. Some viruses, however, are very efficient at avoiding the generation of such inflammatory signals. For example, HBV infection can proceed for weeks without any significant induction of either innate or adaptive immunity in a "stealth" approach to establishing chronic infection (Wieland and Chisari, 2005).

Latent Infection and Reactivation

Cellular latency (herein termed latency) is neither slow viral replication nor the presence of viral nucleic acid without the capacity to reactivate. Rather, latency is a unique transcriptional and translational state of a virus in which the productive replication cycle, and thus the expression of most or all antigens, is silent but can reinitiate (Figure 3). Cellular latency should not be confused with clinical latency, a term used alternately to describe either the time between infection and signs of illness or a host carrying a latent virus. The nature of latency is influenced by the virus, the cell infected, and the immunologic milieu of the host. There can be several different transcriptional forms of latency for a given virus, reflecting different patterns of gene and antigen expression. For example, Epstein-Barr virus (EBV) utilizes three distinct latency gene expression programs (Rickinson and Kieff, 2007; Kieff and Rickinson, 2007). The differentiation state of the latently infected cell is an essential variable in latency and reactivation. For example, papillomaviruses latently infect epithelial stem cells and initiate different viral

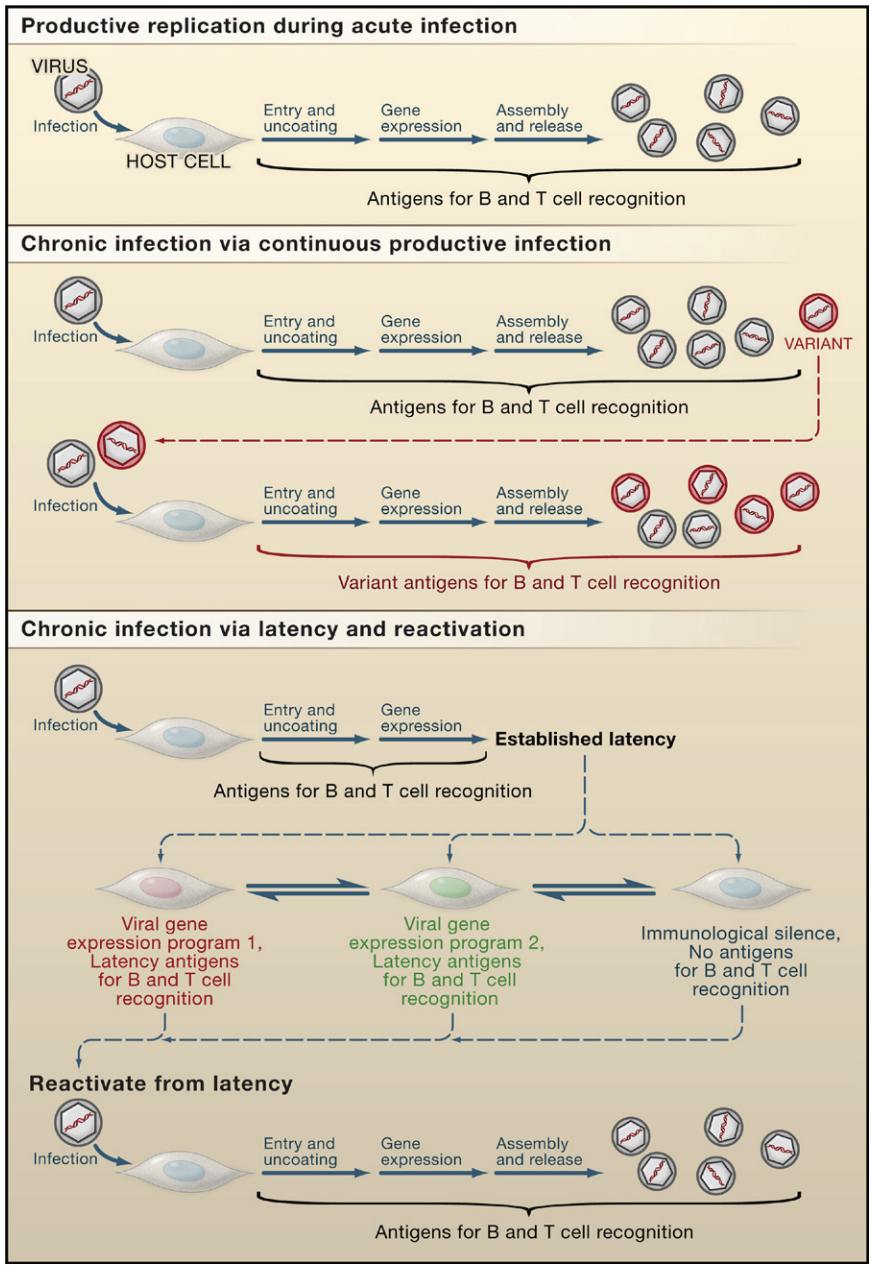

Figure 3. Processes of Acute and Chronic Viral Infection

Viruses use a range of strategies for acute and chronic infection. (Top) The strategy used during acute infection results in the expression of antigens associated with the production of new viruses. (Middle) When a virus persists via continuous productive replication, the same antigens expressed during acute infection are produced, but the virus has the opportunity to evolve under immune selection to produce viruses that express neo-antigens and have altered pathogenetic capacities. (Bottom) In contrast to the previous two processes, viruses that persist via latency and reactivation can generate antigens associated with productive replication but also can enter into transcriptional states in which antigens associated with latent infection are expressed. In the most extreme case, no antigens are expressed, resulting in immunological silence. There may be more than one latency-associated gene program, and it is likely that latency gene programs are cell type specific. When these viruses become reactivated, they reinitiate a productive replication program and once again express viral antigens associated with the production of new viruses.

transcriptional programs as skin cells differentiate and move toward the body surface, eventually resulting in shedding of a metabolically dead cell-husk filled with infectious virus (Longworth and Laimins, 2004).

In contrast to continuously replicating viruses, latent viruses retreat from adaptive immunity into a transcriptionally and antigenically quiescent state. Perhaps the most frightening example of this is HIV, which can survive in memory CD4 T cells in the proviral state without expressing any proteins that can be recognized by the immune system. Similarly, EBV can establish latency in memory B cells with undetectable expression of protein-coding RNAs, at least until the latently infected cell divides (Hochberg et al., 2004). There is no known immune mechanism to recognize such latent virus-infected cells; these cells cannot be cleared unless the virus can be induced to express antigen. This explains the exceptional success of viruses that target long-lived cells for latency. Examples of these cells include neurons (HSV, varicella zoster virus or VZV), hematopoietic stem cells (cytomegalovirus or CMV), epithelial stem cells (papillomaviruses), and memory lymphocytes (HIV, EBV, KSHV or Kaposi's sarcoma associated herpesvirus) (Table 1).

The capacity to establish an immunologically silent infection has obvious implications for the feasibility of preventative or therapeutic vaccines. Preventing these infections would require either immune blockade of infection before establishment of latency or the induction of an immune response that can both trigger viral emergence from latency and eliminate the now-visible infected cells (Figure 3). The kinetics of the establishment of immunologically silent latency are poorly understood due to the complexity of studies of the natural history of infection in humans and the difficulty of quantifying latently infected cells even using advanced molecular technologies. Rapid establishment of immunologically silent latency, as may occur during HIV infection, could make sterilizing immunity impossible to attain. The mechanisms responsible for the choice between latent versus productive infection and the process of reactivation are poorly understood for many viruses. This restricts the consideration of how one might eliminate latently infected cells. A better understanding of the viral mechanisms of latent infection might provide novel approaches to immune system control of such infections.

Viruses that establish latency must intermittently reactivate to generate new infectious virus for spread within and between hosts, thereby generating a wave of antigen. Abortive reactivation occurs frequently for herpesviruses such as EBV and murine CMV (Kurz and Reddehase, 1999; Laichalk and Thorley-Lawson, 2005), and perhaps other viruses. In this process, viruses express part of the productive replication gene program encoding antigens that may activate the immune system, but reactivation is aborted before generation of infectious virus. Frequent full or abortive reactivation events result in repetitive immune stimulation, a situation that may be similar to what is observed during continuous replication.

Chronic Infection of the Germline

Mammalian genomes are chronically "infected" by a huge number of endogenous retroviral elements (ERVs) that spread vertically from one host generation to the next as integrated viral genomes or partial genomes in host chromosomes. Some ERVs are replication competent, but many are replication defective. And yet even such defective ERVs can express proteins. Human ERVs from over 30 lineages constitute perhaps 8%–9% of the human genome (Virgin, 2007b). ERVs are transcribed in a variety of normal and diseased tissues, and they can proliferate within the genome over evolutionary time either by production of viruses that reinfect the genome or by retrotransposition (Seifarth et al., 2005; Virgin, 2007b).

ERVs can have two effects on the immune system. First, they can encode B or T cell antigens (Miyazawa et al., 1987; Wang-Johanning et al., 2008; Levisetti et al., 2003). Antibodies to such an ERV-encoded antigen contribute to spontaneous arteritis in the SL/Ni strain of laboratory mice (Miyazawa et al., 1987). An antigenic ERV protein is also expressed in non-obese diabetic mice concurrent with the development of diabetes (Levisetti et al., 2003). Second, ERV-encoded superantigens can shape the T cell repertoire (Meylan et al., 2005; Sutkowski et al., 2001; Stauffer et al., 2001). These superantigens can directly stimulate T cells bearing specific forms of the T cell receptor, resulting in deletion of the stimulated T cells. Human ERV-K18 encodes a superantigen that negatively selects specific human T cells (Stauffer et al., 2001; Sutkowski et al., 2001; Meylan et al., 2005) and whose expression is increased by EBV infection or interferon-α (IFN-α) (Sutkowski et al., 2001; Stauffer et al., 2001). This is a very important point when considering the role of chronic virus infection in shaping the human immune system. Interactions between chronic viruses, as in the example of EBV inducing the expression of an immunoregulatory ERV protein, may define the antigen-sensing repertoire of T lymphocytes.

Viral Strategies for Chronic Infection

Viruses have evolved a multitude of strategies for evading the immune system and persisting in the host. Some rely on the role of individual viral genes that have targeted effects on infected cells or the immune system itself. Others rely on specific mechanisms of tropism and pathogenesis that render the immune system ineffective. The location, timing, and magnitude of the immune response relative to the speed of virus replication and spread is a major determinant of the eventual outcome of viral infection (clearance versus establishment of chronic infection) (Figure 1). This was demonstrated in a recent study visualizing both infected cells and antigen-specific T cells in situ during the early stages of LCMV and SIV infections (Li et al., 2009).

Viral Tropism and Immune Privilege

Viruses persist in specific cell types during chronic infection, a fact with important implications for understanding the failure of sterilizing immunity. The viral gene expression program, and thus the antigens expressed, may differ from one cell type to the next, particularly for ERVs or viruses that persist via latency/reactivation (Figure 3). For example, the genes expressed by EBV in B cells and epithelial cells during chronic infection can differ (Rickinson and Kieff, 2007; Kieff and Rickinson, 2007). Importantly, surrogate target cells studied in vitro to assess immunity or vaccination may not reflect the antigenic dominance hierarchy of primary cells infected in vivo. In addition, immune effector mechanisms can be more or less effective depending on the specific cell type. Cell lines used in vitro may not reflect these tissue-specific immune interactions. For example, murine γ-herpesvirus 68 (MHV-68, γHV68) establishes latency in both B cells and macrophages. The cytokine interferon-γ (IFN-γ) regulates γHV68 latency and inhibits reactivation in macrophages, but it has minimal effects on latent infection of B cells (Steed et al., 2007). Furthermore, proteins contained in the "lytic" granules of cytotoxic T cells can participate in inhibition of HSV reactivation from neurons without causing lysis of the target cell (Knickelbein et al., 2008). Together, these factors complicate extrapolation of in vitro immunologic studies to the real situation in tissues during chronic infection.

The cell type specificity of viral and host gene functions contributes to increased efficiency of the immune system in some tissues in comparison to others (immunoprivilege). This is an important contributor to maintenance of chronic viral infection. For example, the immune system fails to clear LCMV from the kidney or murine γHV68 from the elastic media of the great arteries at times when other tissues are efficiently cleared (Oldstone et al., 1986; Dal Canto et al., 2001). Immunoprivilege likely contributes to HSV persistence in neurons and papillomavirus persistence in epithelial cells. The genitourinary tract is a particularly difficult place for the immune system to police and can be persistently infected with human T cell leukemia virus (HTLV), human cytomegalovirus (HCMV), KSHV, EBV, HIV, LCMV, HSV, and polyomaviruses (Virgin, 2007b).

Cell tropism may have other important effects in vivo. For example, LCMV clone 13 efficiently establishes persistent infection in immunocompetent mice, partially due to its tropism for fibroblastic reticular cells and dendritic cells (Sevilla et al., 2004; Mueller et al., 2007). For HIV, different reservoirs of latency are cleared with different kinetics by antiviral drugs and perhaps the immune system (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997). Chronic viruses commonly target more than one cell type (Zerboni and Arvin, 2008; Virgin, 2007b). For example, VZV targets neurons and T lymphocytes, EBV targets B lymphocytes and epithelial cells, and HIV targets T cells and macrophages. Immune processes that deal with HSV reactivation in neurons may be distinct from those that control HCV replication in hepatocytes. Therefore a "one size fits all" immunologic approach may not be effective in eliminating or controlling a given persistent infection.

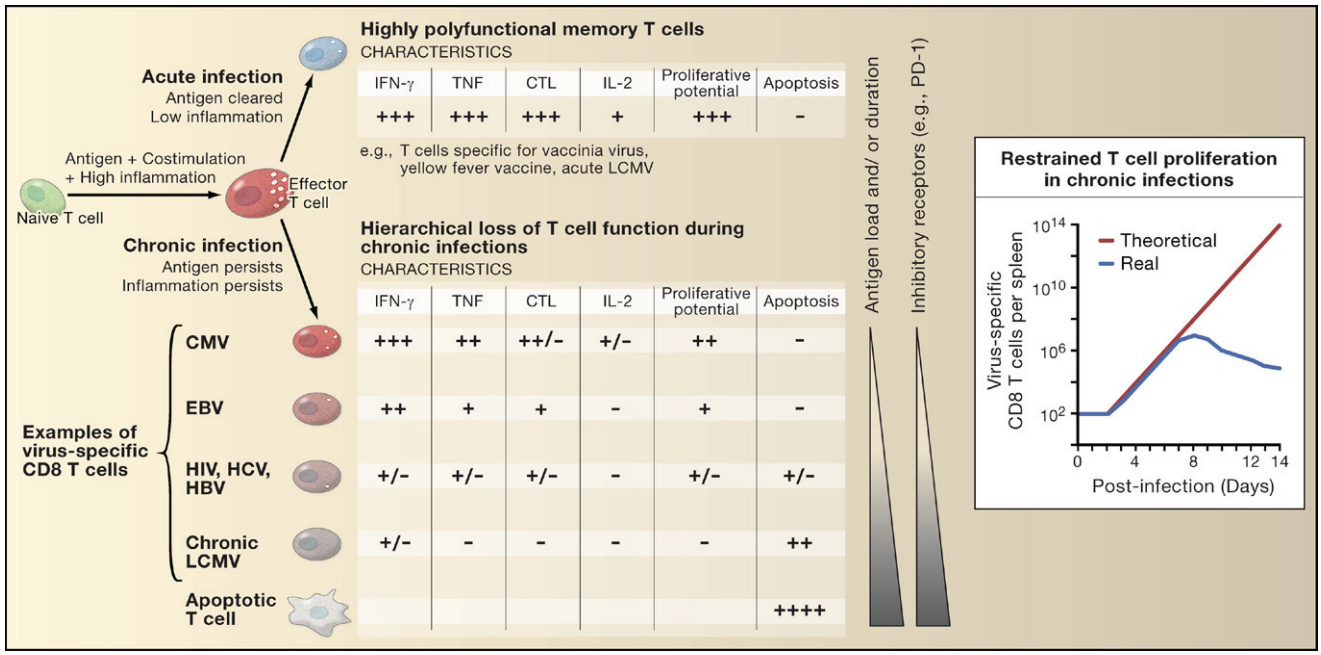

Figure 4. T Cell Functionality during Acute versus Chronic Viral Infection

During viral infection, naive T cells are primed by antigen, costimulation, and inflammation to differentiate into effector T cells. If the infection is cleared and antigen and inflammation are eliminated or substantially reduced, some of these functional effector T cells can further differentiate into highly polyfunctional memory T cells. These memory T cells are capable of coproducing multiple cytokines (for example, IFN-γ, TNF, IL-2), becoming cytolytic, and proliferating vigorously. These cells also have high survival capacity and are maintained for long periods in the absence of antigen. In humans, T cells specific for vaccinia virus or the live attenuated yellow fever virus vaccine have these properties. In contrast, during a chronic infection, antigen and inflammation persist following the effector phase and these factors can influence T cell function. During some persisting infections, antigen levels may be low and virus-specific CD8 T cells that possess multiple functions can develop. These cells might also express low levels of inhibitory molecules such as PD-1. In humans, CMV-specific CD8 T cells can sometimes exist in such a state of functionality. As antigen or viral load increases, T cells become less polyfunctional, losing effector functions in a hierarchical manner. Expression of inhibitory receptors can increase in this setting. EBV-specific CD8 T cells in humans could be an example of this type of T cell. As viral load and antigen increase, T cells become increasingly less functional, occasionally lacking many if not most effector functions. Inhibitory receptor expression increases and the susceptibility to apoptosis becomes greater. HIV-, HCV-, and HBV-specific CD8 T cells have been described in these stages of dysfunction. Ultimately, if the severity or duration of the infection is high or prolonged, virus-specific T cells can be completely eliminated, leading to the loss of virus-specific T cell responses. T cell dysfunction can become progressively worse even during the same infection as the viral load and/or inhibitory signals increase. Whereas the dysfunction can be reversed at early stages of exhaustion, it becomes more permanent as exhaustion progresses and the cells become more terminally differentiated. (Inset) It is critical to restrain T cell proliferation during chronic infections. In the typical kinetics of a CD8 T cell response to viral infection (blue line), the number of antigen-specific T cells peak at approximately one week post-infection before declining. If antigen-driven proliferation is not downregulated during persisting infections and T cells continue to divide every ~4–6 hr, the number of antigen-specific T cells would rapidly reach catastrophic levels (red line).

Viral Evasion and Subversion of Immunity

Because there is a survival advantage for viruses that are shed for prolonged periods, it is no surprise that many viruses have genes that play a niche-specific role during chronic infection. The existence of niche-specific genes that have specialized roles during chronic infection is well demonstrated by the latency genes of EBV (Rickinson and Kieff, 2007; Kieff and Rickinson, 2007), which are specialized to prolong the infection of memory B cells and to respond to and regulate B cell activation and differentiation. These genes perform multiple functions, including maintenance of the viral episome, regulation of B cell antigen receptor signaling, and regulation of cell cycle and apoptosis. Genes such as the v-cyclin and v-Bcl-2 of murine γHV68 are also niche specific for chronic infection, playing a role in reactivation from latency but being less important for acute infection (Van Dyk et al., 2003; Loh et al., 2005). The γ-herpesvirus v-cyclins have evolved the capacity to interact with a broader range of cyclin-dependent kinases than their closest host homologs (Upton et al., 2005). Similarly, γ-herpesvirus Bcl-2 proteins have evolved to have highly effective interactions with the host cell death and autophagy pathways that are not subject to normal host cell regulatory mechanisms (Loh et al., 2005; Wei et al., 2008). These are examples of the type of niche-specific adaptation fostered by prolonged coevolution of virus and host.

Viruses encode a broad array of genes that enable them to evade immunity during acute or chronic infection (Virgin, 2007b). These genes may contribute to the establishment of chronic infection by preventing the clearance of acute or chronic infection (Figure 1). Viral immune evasion genes can act by (1) decreasing expression of molecules required for T cell or natural killer (NK) cell recognition, (2) inhibiting antigen presentation, (3) acting as agonists or antagonists of cytokines and chemokines, and (4) blocking intracellular antiviral or proinflammatory effects of interferons or other cytokines. NK cells and IFN-αβ, usually thought of as important during acute infection, can influence latent and persistent infection by herpesviruses (Barton et al., 2005; Steed et al., 2007; Biron et

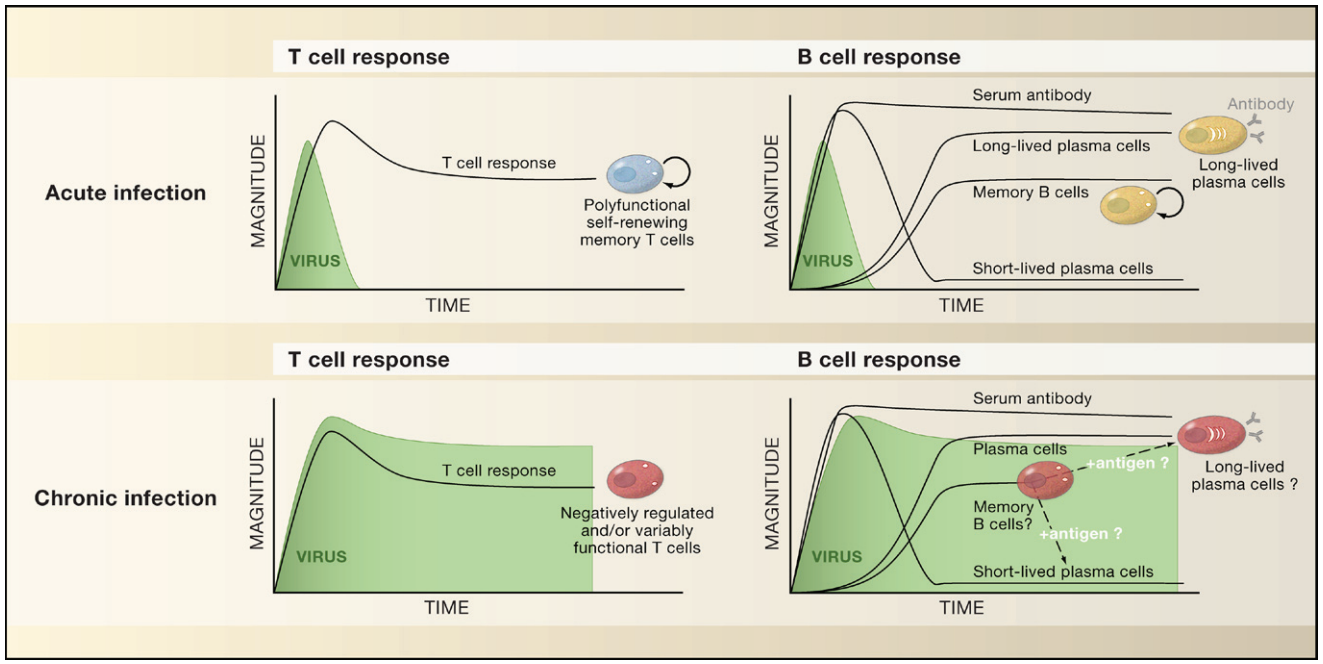

Figure 5. T and B Cell Responses during Acute and Chronic Infection

Following acute viral infection, T cell responses are generated and play a role in the control of initial viral replication. (Top left) After the clearance of infection, T cells form a pool of stable, highly polyfunctional memory T cells that can persist long-term via self-renewal in the absence of antigen. (Top right) The B cell response following an acute viral infection is characterized by the initial generation of short-lived plasma cells that contribute to an early phase of serum antibody production. After clearance of the virus, memory B cells and long-lived plasma cells from the germinal center are generated. Long-lived plasma cells home to the bone marrow and constitutively produce immunoglobulins that maintain circulating serum antibody levels for long periods. Memory B cells are maintained and can give rise to plasma cells upon reactivation. (Bottom left) During chronic viral infection, T cell responses are also generated and can persist over time. However, these virus-specific T cells generated during chronic infection are subject to negative regulation, lose polyfunctionality, and become antigen dependent rather than developing the ability to persist long-term via antigen-independent self-renewal. (Bottom right) Although less is known about the dynamics of B cell responses during chronic viral infection than about T cell responses, serum antibody and plasma cells are clearly generated during persisting infections. It is not clear whether the plasma cell populations generated during chronic viral infections have the same characteristics as those formed following clearance of acute infection. In addition, memory B cells might be formed during chronic viral infection, but the continuous presence of antigen could drive these memory B cells toward becoming short- or long-lived plasma cells.

al., 1989). It will be important to determine which viral immune evasion genes are critical for persistence in vivo so that these genes can be specifically targeted for pharmacologic intervention or vaccination.

Mutation and Selection of Viral Variants

The error-prone RNA polymerases of viruses such as HCV and HIV are major contributors to chronic infection via the generation of quasispecies—swarms of viruses with different sequences—that can evade the immune system or develop new pathogenetic properties (for example, see Pfeiffer and Kirkegaard, 2005). In addition to point mutations, duplications, deletions, recombination events, and even genomic acquisition of host mRNAs have occurred in different viruses. Perhaps best studied of these are immune escape virus mutants, which have a well-established role in evading adaptive immune responses (for examples, see Pircher et al., 1990; Bowen and Walker, 2005). During HCV, HIV, or SIV infection, these mutations accumulate in CD8 T cell epitopes, a process associated with disease progression and viral escape from vaccine and T cell-mediated control (Bailey et al., 2004; Bowen and Walker, 2005). Immune escape mutations in viral proteins have been found that inhibit multiple steps in antigen presentation, including proteasomal generation of peptide, binding of peptides to MHC class I molecules, and recognition of peptide:MHC complexes by the T cell receptor (Bowen and Walker, 2005).

Immune Responses during Chronic Viral Infection

The viral strategies described above for the maintenance of chronic infection are balanced by the effects of the virus-specific immune response, thereby establishing a metastable equilibrium between host and pathogen. It is well established that there is a complex regulatory network that actively inhibits immune responses during chronic viral infections. This regulation of the immune response is essential for two main reasons. The first reason is to reduce excessive immunopathology. This is particularly important for the CD8 T cell response that can cause massive tissue damage by killing infected cells and releasing inflammatory cytokines. Thus, it is not surprising that cytotoxicity and the production of cytokines such as tumor necrosis factor (TNF) are tightly regulated during chronic infections, and that these functions are often reduced or lost in CD8 T cells responding to persistent viruses (Figure 4). The second reason is to regulate the extraordinary proliferative potential of virus-specific T cells. During the acute phase of infection, virus-specific T cells can double in number every 4–6 hr and

undergo 3–4 doublings per day (Murali-Krishna et al., 1998; Miller et al., 2008). This translates into a 10-fold increase per day. If this level of proliferation were to continue unchecked, one would have astronomical (and potentially catastrophic) numbers of virus-specific T cells within a very short time (Figure 4, inset). It is essential to put brakes on this remarkable proliferative capacity. In fact, a hallmark of virus-specific T cells during chronic infections is a greatly reduced ability to proliferate in response to antigen.

It is important to emphasize that despite this diminished proliferative capacity and a decrease in some effector functions (described below), virus-specific T cells play a crucial role in controlling viral replication and thereby keeping our virome in check. For example, this is true for EBV and HCMV infections, as well as for HIV infection in "elite" controllers. Elite controllers are a unique subset of HIV-infected individuals who are able to limit HIV infection to a very low level for long periods. However, during progressive HIV, HCV, or HBV infection, T cell dysfunction can become so profound that the T cells are unable to effectively control viral replication. Thus, there are varying degrees of function and dysfunction in T cells during persistent infections (Figures 1 and 4). A better understanding of the mechanisms responsible for progressive T cell dysfunction during chronic infection may allow us to develop immunological and vaccine approaches that reduce the disease burden of some of these intractable chronic infections in humans.

T Cell Responses

In considering the nature of T cell responses during chronic infection, it is useful to start with understanding the responses to acute infection. Memory CD8 and CD4 T cell differentiation following acute viral infections results in the formation of high-quality, long-lived memory cells (Kaech and Wherry, 2007). These T cells have several cardinal properties that allow them to confer protective immunity. Chief among these properties is the ability to persist long-term in the absence of antigen and to respond rapidly upon re-exposure to the pathogen. This recall response is characterized by rapid elaboration of effector functions such as cytotoxicity and cytokine/chemokine production, proliferation accompanied with substantial increases in T cell numbers, and migration of effector T cells to sites of infection.

The differentiation of robust memory T cells after acute infection follows a general program, with the critical memory properties developing gradually over time. Both the strength of antigen stimulation and inflammation can impact the kinetics or pattern of this differentiation. For example, inflammation is essential for effective activation of naive T cells and generation of effector and memory T cells, but higher levels of inflammation can foster the generation of more terminally differentiated effector T cells (Mescher et al., 2006; Joshi et al., 2007). The tissue microenvironment can also, perhaps via virus-dependent local inflammatory signals, affect the capacity of T cells to produce the cytokine interleukin-2 (IL-2), alter homing properties (Mora et al., 2003), change memory T cell phenotype (Marzo et al., 2007), and define the overall pattern of memory T cell differentiation (Masopust et al., 2006). If the primary immune response clears the infection (Figure 1), some T cells will differentiate into long-lived memory T cells (Figure 5). In fact, the ability to respond efficiently to homeostatic signals such as IL-7 and IL-15 and undergo homeostatic self-renewal is a property that is only acquired by memory T cells that develop in the absence of antigen.

Many of the key properties of effective memory T cells develop after the clearance of antigen and inflammation following acute infection. During chronic viral infections, where antigen and/or inflammation persist, antiviral T cell responses can differ dramatically. In addition, virus-specific CD4 and CD8 T cells often exhibit various stages of exhaustion (Figures 4 and 5) (Shin and Wherry, 2007; Klenerman and Hill, 2005). Exhaustion-associated T cell defects range from loss of effector functions to failure to exhibit antigen-independent homeostatic proliferation. CD8 T cell exhaustion was first described during LCMV infection of mice where virus-specific CD8 T cells initially developed effector functions such as cytokine production and cytotoxicity but lost these properties as the chronic infection progressed (Zajac et al., 1998; Wherry et al., 2003, 2007). This loss of function is hierarchical (Figure 4), with some functions exhausted early or in the presence of low viral load (for example, IL-2 production, cytotoxicity, proliferation) and others (e.g., IFN-γ production) persisting longer (Zajac et al., 1998; Wherry et al., 2003). Eventually, especially if viral load is high or if help from CD4 T cells is lacking, virus-specific CD8 T cells deficient for most effector functions (for example, ex vivo cytotoxic activity, production of IL-2, TNF, and IFN-γ) are found (Zajac et al., 1998; Wherry et al., 2003). In the extreme situation, exhausted CD8 T cells can be physically deleted (Zajac et al., 1998; Moskophidis et al., 1993; Wherry et al., 2003). The severity of T cell dysfunction during chronic infection correlates directly with the level of infection and expression of inhibitory receptors by virus-specific T cells and correlates inversely with help from CD4 T cells. Similar types of T cells have been described in many experimental models, and dysfunctional virus-specific CD8 T cells have also been described during human chronic infections (Shin and Wherry, 2007; Klenerman and Hill, 2005).

Defects in memory T cell differentiation during chronic viral infections are not just confined to effector functions. They also include a failure to develop into self-renewing antigen-independent memory T cells (Shin and Wherry, 2007). Reduced expression of IL-7 and IL-15 receptors, and an inability to effectively respond to these cytokines, results in defective antigen-independent homeostatic self-renewal (Shin and Wherry, 2007). These virus-specific CD8 T cells require cognate antigen, instead of IL-7 and IL-15, for maintenance (Shin et al., 2007). The failure to undergo IL-7- and IL-15-driven antigen-independent maintenance leads to a loss of virus-specific CD8 T cells if the antigen is removed. In this situation, changes in antigen expression due to antiviral therapy or generation of neoepitopes by viral mutants may result in the loss of these "antigen-addicted" CD8 T cells with important consequences for viral re-emergence or reinfection.

B Cell Responses

The fundamental nature of the B cell response is also altered by chronic infection. Antiviral B cell responses following acute viral infection occur in a series of stages (Figure 5). Initial B cell activation results in the generation of a population of short-lived antibody-producing cells and a population of activated

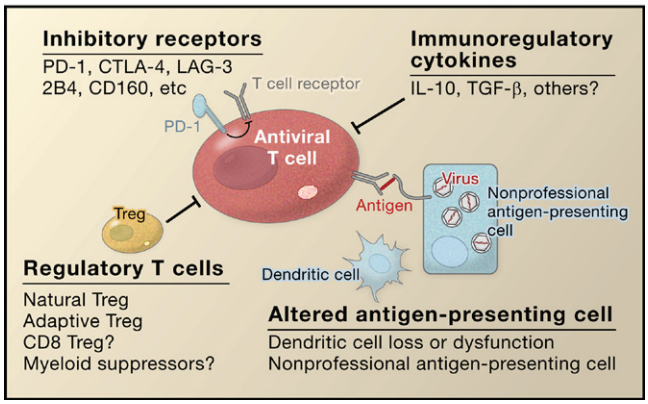

Figure 6. Immunoregulatory Pathways Inhibit Antiviral T Cell Function during Chronic Viral Infection

Four distinct classes of events could limit T cell responses to persisting viruses. First, T cell intrinsic expression of inhibitory receptors such as PD-1, CTLA-4, LAG-3, and others can negatively regulate T cell function and limit the effectiveness of antiviral activity. Second, immunoregulatory cytokines such as IL-10, TGF-β, and possibly additional factors produced by other cells in the environment can modulate and suppress vigorous antiviral T cell responses. Third, regulatory cells can modulate antiviral effector T cells. These regulatory cells could include traditional FoxP3$^+$ natural or adaptive CD4$^+$ T regulatory cells (Tregs), regulatory CD8 T cells, or myeloid suppressor cells. Finally, changes in antigen-presenting cell (APC) usage or function could impact virus-specific CD8 T cells. The quality of T cell stimulation can also be impacted by changes in dendritic cell function or number, as well as differences in antigen presentation by professional versus nonprofessional APC during persisting viral infection.

B cells that enter the germinal center reaction. The short-lived antibody-producing cells provide an initial burst of usually low-affinity antibody (Hangartner et al., 2006; Ahmed and Gray, 1996) that can limit the spread of virus and blunt the infection until higher-affinity antibody-producing cells emerge from the germinal center (Hangartner et al., 2006). In the germinal center, in addition to affinity maturation and isotype switching, B cells begin differentiation along one of two possible lineages (Calame et al., 2003). Some B cells emerge as memory B cells that are capable of long-term persistence and endowed with the capacity to respond vigorously upon reinfection. Other germinal center B cells differentiate into long-lived plasma cells that home to the bone marrow and some mucosal sites where they constitutively produce copious amounts of antibody. These cells can persist for months to years in the absence of antigen (Slifka et al., 1998; Manz et al., 1997) and are responsible for the prolonged maintenance of antiviral antibodies observed in humans for decades in the absence of viral reinfection (Hammarlund et al., 2003; Crotty et al., 2003)

Although there is considerably more known about exhaustion and dysfunction of T cells during chronic viral infection, several recent studies indicate that B cells may also be negatively impacted in these settings. A recent study has defined B cell exhaustion during HIV infection (Moir et al., 2008). This study shows that HIV-specific B cells, but not B cells specific for other antigens, have a low proliferative capacity and express Fc-receptor-like-4 (FCRL4) that can generate signals that inhibit B cell function. In addition, there is also evidence that the inhibitory coreceptor programmed death 1 (PD-1) influences B cell responses during chronic SIV infection as in vivo blockade of this pathway enhances the SIV-specific antibody response in primates (Velu et al., 2008). Further, a high ratio of antigen to antigen-specific B cells can lead to terminal differentiation of B cells and ultimately loss of effective IgG responses (Zellweger et al., 2006). It has also long been known that alterations in B cell responses, B cell tolerance, and the accumulation of antibody-antigen immune complexes are associated with chronic viral infections and can contribute to disease (Casali and Oldstone, 1983). These observations are consistent with the data on T cell exhaustion described above and suggest that B cells are also likely to be negatively influenced by persisting viral infection. Future studies addressing the differentiation pattern of virus-specific B cells during chronic viral infections, the mechanisms of B cell dysfunction, and the role of CD4 T cell exhaustion in this process should be informative.

Mechanisms Regulating Immunity during Chronic Infections

Several mechanisms regulate immune responses during chronic viral infection (Figure 6). These include inhibitory receptors on T cells, altered APC, immunoregulatory cytokines, and regulatory T cells (Tregs). These mechanisms can work in concert to limit immunopathology. However, this dampening of T cell function may also prevent the immune system from clearing the infection.

Inhibitory Receptors on T Cells

Upregulation of inhibitory receptors by exhausted CD8 T cells is an important mechanism of T cell dysfunction during chronic viral infections. The inhibitory receptor PD-1, a CD28 family costimulatory/coinhibitory molecule, regulates CD8 T cell exhaustion during chronic LCMV infection in mice (Barber et al., 2006; Wherry et al., 2007). PD-1 is highly overexpressed by virus-specific exhausted CD8 T cells in comparison to functional memory CD8 T cells. Notably, blockade of the PD-1:PD-1 ligand (PD-L) pathway during chronic LCMV infection leads to the recovery of T cell function and reduced viral load (Barber et al., 2006). This is a fundamentally important observation showing that some changes in CD8 T cells during chronic infection are reversible. These results have been extended to humans where the PD-1:PD-L pathway regulates HIV-, HBV-, and HCV-specific T cells in vitro (Sharpe et al., 2007). In vivo studies in the primate SIV model also demonstrate the importance of the PD-1 pathway, as PD-1 blockade improves T cell (and B cell) responses, lowers viral load, and extends life span in chronically infected animals (Velu et al., 2008).

A key question is whether PD-1:PD-L blockade reprograms the entire T cell population or only a subset of dysfunctional T cells. Recent work suggests that only a subset of exhausted CD8 T cells responds effectively to PD-1:PD-L blockade in vivo (Blackburn et al., 2008). Surprisingly, the responsive subset expresses intermediate levels of PD-1 (PD-1^{Int}), whereas the subset that could not be rescued expresses the highest levels of PD-1 (PD-1^{Hi}) (Blackburn et al., 2008). Furthermore, these two subsets of exhausted CD8 T cells are found in different anatomical locations in mice (Blackburn et al., 2008). Similarly, circulating HCV-specific CD8 T cells in humans are PD-1$^{Lo/Int}$, whereas those in the liver are PD-1^{Hi} (Nakamoto et

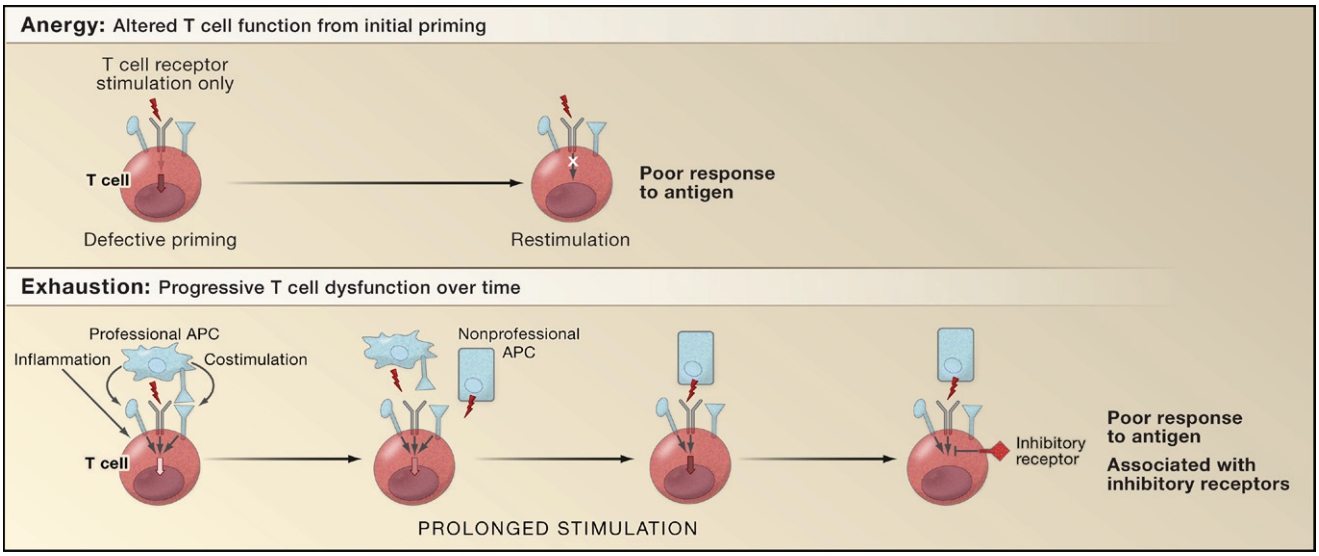

Figure 7. Differences between T Cell Anergy and T Cell Exhaustion

Anergy is a type of T cell dysfunction in which T cells are initially primed improperly by signaling (red lightning bolt) through the T cell receptor (TCR) in the absence of costimulatory or inflammatory signals. Such anergized T cells fail to develop proper function from the outset and are refractory to subsequent TCR stimulation. In contrast, exhausted T cells are primed by antigen (red lightning bolt), costimulation, and inflammation. These T cells initially develop effector functions, but prolonged (and perhaps excessive) stimulation leads to progressive loss of function over time. They eventually exhibit a poor responsiveness to antigen. In agreement with these distinct modes of induction of T cell anergy versus exhaustion, there are also key molecular differences between T cells exhibiting these two distinct types of dysfunction.

al., 2008). Only circulating PD-1$^{Lo/Int}$ HCV-specific CD8 T cells respond to PD-1 pathway blockade in vitro (Nakamoto et al., 2008). The issue of how to manipulate the immune system in different tissue environments has not been studied in sufficient detail, emphasizing the importance of correctly selecting relevant clinical samples for assessment of the efficacy of immune interventions during chronic viral infection.

One possible reason for the poor responses of PD-1^{Hi} exhausted CD8 T cells is the coexpression of additional inhibitory receptors such as LAG-3, CTLA-4, 2B4/CD244, CD160, GP49, and PirB on the cells (Figure 6) (Blackburn et al., 2009). In fact, simultaneous blockade of multiple inhibitory receptors substantially improves reversal of exhaustion in comparison to PD-1 blockade alone (Blackburn et al., 2009). Recent studies in humans also demonstrate PD-1 and CTLA-4 coexpression on some HIV-specific CD4 T cells (Kaufmann et al., 2007). These observations indicate that multiple inhibitory receptor pathways can cooperate to restrain optimal T cell responses during chronic infections. It will be important to define patterns of inhibitory receptor coexpression during different chronic infections and to delineate the precise T cell properties and pathways regulated by diverse inhibitory receptors. Using this knowledge, it might be possible to augment beneficial T cell functions without enhancing immunopathology.

T Cell Anergy versus Exhaustion

T cell dysfunction that occurs during chronic viral infections is distinct from the previously described state of T cell anergy (Figure 7). One fundamental difference between anergy and exhaustion is the differing mechanisms of induction of these types of T cell dysfunction. Anergy is induced when T cells do not receive all the necessary signals for T cell activation (for example, the cells receive TCR stimulation but no costimulation) and therefore fail to develop into functional effector cells. In contrast, T cell exhaustion during chronic viral infection is associated with initially normal effector differentiation followed by a progressive loss of function over time. The molecular signature of T cell exhaustion reveals pervasive changes in transcription in comparison to functional T cells (Wherry et al., 2007), including overexpression of inhibitory receptors, altered expression of transcription factors, changes in signal transduction, and downregulation of key metabolic genes (Table 2). These transcriptional changes are not the same as those observed in anergic T cells, confirming that T cell exhaustion and anergy are indeed distinct processes (Wherry et al., 2007). Whereas exhaustion is the major type of T cell dysfunction during chronic viral infection, anergy may play a role when viruses utilize genetic strategies that directly inhibit costimulatory pathways in APC.

Regulatory T Cells

There is emerging evidence for a role of regulatory T cells (Tregs) (Belkaid and Rouse, 2005) in the equilibrium established during chronic viral infection. Foxp3^{+}CD4^{+} Tregs can negatively regulate effective immunity during acute herpesvirus infections (Fernandez et al., 2008; Suvas et al., 2003). Paradoxically, Tregs can also facilitate effective immunity during the early stages of acute infection by modulating effector T cell recruitment to infected sites (Lund et al., 2008). However, Tregs are more often associated with ineffective immune responses during chronic infections, including those by Friend leukemia virus (Zelinskyy et al., 2006), HIV (Kinter et al., 2007; Nilsson et al., 2006), HCV (Boettler et al., 2005; Ebinuma et al., 2008; MacDonald et al., 2002), and HBV (Franzese et al., 2005; Xu et

Table 2. Cell-Intrinsic Mechanisms of T Cell Exhaustion during Chronic Viral Infection

Type of Defect	Examples	Potential Consequence
Overexpressed inhibitory receptors	Overexpression of PD-1, LAG-3	e.g., Inhibition of T cell responses, reduced TCR signaling, limited effector functions and proliferation
Signaling	Altered expression of LCK, NFATc, IL-7R	e.g., altered TCR and cytokine receptor signal transduction
Altered chemotaxis, homing, and adhesion	Increased expression of CCR5, CCL5, CCL3, decreased expression of several integrins	e.g., enhanced inflammatory cell recruitment, altered adhesion
Changes in transcription factor expression	PBX3, Blimp-1, Eomes	e.g., altered differentiation state
Metabolic deficiencies	Reduced expression of mRNAs encoding ribosomal subunits, citric acid cycle enzymes	e.g., reduced energy metabolism, defects in protein translation

Different types of cell-intrinsic changes occur in lymphocytes during exhaustion. These are listed here with specific examples of the defects and their potential consequences. PD-1, programmed death 1; LAG-3, lymphocyte activation gene 3; LCK, leukocyte-specific protein tyrosine kinase; NFATc, nuclear factor of activated T cell c; CCR, chemokine (C-C motif) receptor; CCL, chemokine (C-C motif) ligand; PBX3, pre-B cell leukemia homeobox 3; Blimp-1, B lymphocyte induced maturation protein 1; Eomes, Eomesodermin. This table is adapted from Wherry et al., 2007.

al., 2006). Precisely how Treg cells influence viral infection is not well understood. These cells could act through cell-to-cell contact, inhibition of APC maturation, production of immunoregulatory cytokines, or direct inhibition of CD8 T cell effector function. Treg cells that limit effective viral control could lead to prolonged antigen expression and secondary T cell exhaustion, indicating the potential for synergy between mechanisms that inhibit T cell effectiveness during chronic viral infection. Enhancing immune control of chronic viral infection likely will require that multiple pathways are targeted at the same time.

Immunoregulatory Cytokines

Immunoregulatory cytokines modulate immune responses to viral infection and may be interrelated with Treg function. For example, when the IL-10 pathway was blocked, virus-specific CD8 T cell responses to chronic infection with LCMV were enhanced (Ejrnaes et al., 2006; Brooks et al., 2006). The cellular source of this IL-10 remains under debate, and it will be important to identify the source and mechanism of the regulation of IL-10 production during chronic infection. In addition to IL-10, other suppressive or regulatory cytokines such as transforming growth factor β (TGF-β) can impact immune responses during persisting infections (Alatrakchi et al., 2007). Although these, and likely other, immunomodulatory cytokines impact immunity during chronic infections, the mechanisms responsible for these effects are largely unknown. IL-10 and TGF-β both can alter the functions of APC and innate immune effector cells such as macrophages. These effects could provide a significant imprint of chronic viral infection on the immune system in general with consequences for responses to self or foreign antigens (see below).

Immunologic Imprint of Chronic Viral Infection

What are the consequences to the host of the dynamic but metastable equilibrium that is established between virome and host during chronic infection? One obvious consequence is the development of severe disease during progressive chronic infection by viruses such as HIV and HCV, and the less frequent development of severe disease during chronic EBV or HSV infection. Aside from directly causing disease, are there additional fundamental consequences of chronic viral infection? As a first step to addressing this question, the nature and the level of activity of the immune system during chronic viral infection must be considered. All chronic virus infections are under continuous immune surveillance. Viruses such as CMV, polyomaviruses, and EBV cause more and different diseases in immunosuppressed patients in comparison to individuals with a functioning immune system, indicating that immunity in normal individuals contains these viruses (Table 1). This concept is supported by studies showing that herpesvirus infection is associated with ongoing B and T cell activation at mucosal surfaces where reactivation occurs (Zhu et al., 2007; Hislop et al., 2005). These responses are present in the absence of overt disease and thus impact the immune system in apparently normal hosts.

The scope and impact of continuous immune surveillance of chronic viral infection should not be underestimated. Reactivation of HSV-2 in the genitourinary system is extremely frequent even in asymptomatic persons (Wald et al., 2000), and thus the attendant chronic T cell response in this tissue (Zhu et al., 2007) might reasonably be expected to have significant effects on mucosal immunity at this site. Even asymptomatic viruses such as anelloviruses are under continuous immune surveillance (Davidson and Shulman, 2008; Ninomiya et al., 2008; Hino and Miyata, 2007) (Table 1). It has been estimated that over 90% of anelloviruses in serum are replaced daily with over a billion new virions, suggesting that significant antigen expression may occur for years after the acquisition of infection in the first years of life. This immune system dynamism might be neutral for the host, but we argue that it has a critically important role—through changes in tissue cytokines and in B and T cells—in defining the "normal" human immune system. This "immunologic imprint" of chronic viral infection may fundamentally alter the response of the host to new infections, vaccines, or neo-epitopes that emerge during immune selection of viral variants.

Virome Effects on Innate Immunity

Ongoing immune responses to chronic viral infection strongly affect innate immunity through the actions of cytokines released during chronic stimulation of T cells and possibly NK cells. This may be particularly important for viruses harbored in lymphoid tissues during chronic infection (Table 1) where the bystander effects of cytokines might be expected to have special importance. Stimulation of freshly explanted CD4 or CD8 T cells with CMV peptides induces rapid secretion of cytokines

IFN-γ, TNF-α, and MIP1-β, indicating that cells primed to rapidly respond to viral antigen continuously circulate through the body (Sester et al., 2002; Casazza et al., 2006). Similar findings have been reported for EBV-specific CD4 and CD8 T cells (Hislop et al., 2005). Importantly EBV-specific T cells represent up to 20% of the total CD8 T cells in the noninflamed tonsils of long-term EBV carriers (Hislop et al., 2005). This is an anatomic site at which prolonged virus shedding occurs and stimulates these T cells (Hislop et al., 2005). Given the high level of chronic herpesvirus infection in the population (Table 1), and evidence for continuous shedding of herpesviruses at mucosal sites with attendant antigen expression and T cell activation, it is reasonable to conclude that mucosal production of powerful cytokines is frequent in most normal humans. This has obvious potential importance for regulating mucosal barrier function or in the case of HIV, which replicates in activated lymphocytes, for enhancing infection.

New evidence in animals indicates that chronic virus infection can fundamentally alter innate immunity to nonviral pathogens. IFN-γ expression during herpesvirus latency can symbiotically protect the host from infection by the bacteria *Listeria monocytogenes* and *Yersinia pestis*, the causative agent of plague (Barton et al., 2007). Thus, the détente developed between herpesviruses and their hosts over tens of millions of years of coevolution may offer benefits to the host. This protection may come at the cost of enhanced autoimmunity (Peacock et al., 2003). In addition, the prolonged presence of Sendai virus viral nucleic acids in mice is associated with IL-13-dependent NKT cell activation that can, in turn, contribute to reactive airway disease (Kim et al., 2008). Further, abnormal interferon secretion by plasmacytoid dendritic cells predisposes to secondary infection during chronic LCMV infection (Zuniga et al., 2008). It has been proposed that chronic activation of innate immune responses contributes to immune dysfunction in HIV and SIV infection (Mandl et al., 2008). Together, these examples provide a convincing case for a significant immunologic imprint of chronic viral infection on the nature of innate immune responses. Much remains to be done to define the balance between immunologic benefit and immunologic harm for chronic infection of humans by viruses that seldom cause overt disease.

Because responses to our virome imprint the immune system, studies in mice that lack chronic infections may sacrifice relevance for ease of experimentation. Most experimental animals are not chronically infected with relevant viruses, but humans carry many chronic viral infections (Table 1, Figure 2). Thus, it is possible that differences between human and murine immune responses reflect the immunologic imprint of our virome rather than inherent differences in immunity. This has implications for how data from animals raised in virus-free barrier facilities are used to define mechanisms of immunity and to validate vaccine approaches to be applied in hosts with multiple chronic infections. Studies of immunologic mechanisms are clearly easier when confounding effects of concurrent infection are removed, but the immune system does not exist in a vacuum. Studies in multiply infected animals whose immune systems are subject to chronic activation and the consequent imprint of chronic infection should be compared with studies in standard laboratory animals.

Virome Effects on Adaptive Immunity

There is significant crosstalk between chronic viral infections, as well as between viral infections and other types of infection. For example, HIV and HCV coinfection worsens the prognosis of both infections (De et al., 2002; Greub et al., 2000). HCV-dependent worsening of HIV disease progression (De et al., 2002; Greub et al., 2000), though controversial (Sulkowski et al., 2002), is interesting because HCV does not cause overt immunosuppression. The impact of persisting infections is not always negative. For example, infection by herpesviruses (HHV-6 or HHV-7) or GB virus C may inhibit HIV progression in some settings (Grivel et al., 2001; Lisco et al., 2007; Xiang et al., 2001; Tillmann et al., 2001). The mechanisms responsible for such complex interactions remain poorly defined.

The effect of chronic virus infection extends beyond alterations in the biology of antigen-specific T cells responding to viral antigens. There is considerable compression of the T cell repertoire in chronically infected animals. The ongoing response to HSV and CMV is so robust that the proportion of T cells specific for these viruses increases over time to the point that diminished responses to new antigens may contribute to the progressive decrease in immune system responsiveness as we age (Nikolich-Zugich, 2008). Further, studies of sequential virus infection reveal potentially relevant crossreaction between viruses such as EBV and influenza A virus (Selin et al., 2006). During chronic viral infection, crossreactivity could be a double-edged sword if crossreactive T cells become dysfunctional due to exhaustion or inhibition by Tregs. In addition, prior virus infection can alter the T cell cytokine response to subsequent infections by different viruses; prior immunity to one virus can markedly alter vaccine responses to a second virus (Selin et al., 2006). Together, these observations provide a strong basis for considering that chronic virus infection may strongly imprint the adaptive immune response.

The imprint of the cytokine environment created by chronic viral infection on adaptive responses to new pathogens is more speculative. However, T cells require inflammatory signals for full activation and differentiation into effector and memory T cells (Curtsinger et al., 1999). In vivo studies have confirmed the important role of inflammatory cytokines (including IL-12 and IFN-αβ) in T cell activation and differentiation (Kolumam et al., 2005). IFN-γ has also been implicated in modulating the differentiation of memory CD8 and CD4 T cells (Whitmire et al., 2005). In addition to influencing CD8 T cell activation and expansion (Mescher et al., 2006), IL-12, IFN-αβ, and other inflammatory signals shape T cell memory. Priming CD8 T cells in vivo in the absence of inflammation results in blunted effector T cell responses but more rapid generation of memory T cells (Pearce and Shen, 2007; Badovinac et al., 2005). In addition, increased levels of inflammation can lead to the generation of terminally differentiated effector T cells (Joshi et al., 2007; Sarkar et al., 2008). Thus, inflammation has a critical role in adaptive immune T cell activation and differentiation. It is plausible that prolonged inflammation, secondary to the immunologic imprint of our virome, can skew T cell responses toward more effector T cells and fewer memory precursors, generating a long-lasting impact on the quality of the memory T cell population.

Redefining Chronic Viral Infection

Recognizing that chronic viruses are contributors to our normal metagenome has two highly interrelated and fundamentally important consequences. First, we need to reformulate how we attribute disease to specific viruses and how genome-wide association studies are performed in humans. Second, we need to recognize that our normal immune system is subject to the imprint of the dynamic equilibrium between our genome and the endogenous virome.

Virome as Part of the Metagenome: The Implications

What implications does the presence of an internal virome that is in a dynamic equilibrium with our immune system have for human disease? Classically, a virus is considered to a potential cause of disease when it is present in persons with disease and absent from persons without disease. Recent findings suggest that this simple approach does not capture the entire role of viruses in disease, indicating the need to reformulate our criteria for disease causality during chronic viral infection. Even viruses of low virulence occasionally cause severe illness in apparently normal people—a phenomenon that has been attributed to stochastic events, human genetics, viral dose, viral variants, or transient immunosuppression. Although each of these explanations may apply, a sea-change in how we define viral infection is being driven by a combination of classical genetics and the genomic revolution (Bustamante et al., 2008). In this emerging view, the well-known role of host genetic variation comes to the fore because highly specific allelic polymorphisms or mutations predispose a limited number of individuals to diseases caused by viruses that otherwise only rarely cause overt disease. In a real sense, the genes in the host "cause" the disease, as the viruses can infect many but cause disease in only a few.

The key factors in this emerging formulation are (1) new estimates of the number of genes that confer susceptibility to a given virus and (2) the exquisite specificity of some alleles for certain viruses. Random mutagenesis of the mouse genome suggests that there are about 300 genes that determine resistance to murine CMV (Beutler et al., 2006). Furthermore, sets of genes can act in a combinatorial fashion to confer viral resistance. For example, interactions between killer inhibitory (KIR) alleles encoding NK cell receptors and MHC class I alleles contribute to susceptibility to HIV, HCV, and EBV infection (Khakoo et al., 2004). There are many allelic variations that influence chronic viral infection (Virgin, 2007b). These include CCR5 variations that affect infection by HIV, HLA variations that affect development of cervical cancer and infection by papillomavirus, HIV, HBV, and HCV, CXCR4 variations and the development of warts, *EVR1* and *EVR2* variations and the development of papillomavirus-induced epidermodysplasia verruciformis, and complement gene variations that affect HBV vaccine responsiveness. Furthermore, the effects of allelic variation in genes, alone or in combination, can be highly specific for a given infection. For example, deficiency in the innate immune-signaling molecules MyD88 or IRAK-4 is associated with severe pyogenic bacterial infection, but patients appear to have normal resistance to viral infections (von Bernuth et al., 2008; Ku et al., 2007). In contrast, mutations in UNC93B1 and TLR3, two proteins involved in innate immune responses to virus infection, selectively predispose the host to herpes simplex encephalitis (Casrouge et al., 2006; Zhang et al., 2007).

These new data suggest that we should consider that many or all humans are genetically "immunocompromised" to a greater or lesser extent for specific infections, including infection by viruses that constitute our virome. Thus, a virus present in all of us may cause disease only in those unfortunate enough to have a specific constellation of genes. Therefore, attributing a disease to a "viral" cause based on the presence or absence of the virus falls by the wayside, especially for viruses that infect most or all humans (Table 1). In this view, we have only identified the easily detected "viral" diseases—those caused by viruses that are virulent in a high enough proportion of people to allow informative comparison between infected and uninfected persons. Further viral diseases may be awaiting either the identification of new viruses or the assessment of relationships between individual human genes, the virome, and specific diseases. A provocative example of this has recently been discovered. The new human virus xenotropic murine leukemia virus-related virus (XMLV) (Table 1), recently detected in prostate cancer tissues (Urisman et al., 2006; Dong et al., 2007), is not present in all prostate cancer patients. However, it is possible that XMRV causes prostate cancer in individuals with a specific immunologic abnormality. Chronic XMRV infection is strongly associated with homozygous mutations in the interferon-regulated antiviral molecule RNaseL, and RNaseL mutations predispose the host to prostate cancer (Urisman et al., 2006; Dong et al., 2007). Thus, the causal role for a virus may be clear only in the light of a patient's total genomic complement of focal immune abnormalities.

Given the breadth of chronic viral infection (Figure 2, Table 1), these changes in our understanding of the relationship between human genetics and virus-induced disease requires that we be open to new ways to look at disease causality. The immunologic imprint of chronic virus infection provides one potential mechanism by which the virome may contribute to a range of inflammatory diseases. Even diseases not generally considered as inflammatory in nature may be influenced by our virome. For example, mice chronically infected with murine CMV exhibit hypertension due to alterations in the renin-angiotensin system that is responsible for regulating systemic blood pressure (Cheng et al., 2009). Thus, the existence of a viral contribution to our metagenome may exact a large price in those of us with specific genetic predispositions to disease. The bottom line is that many major diseases that have been associated with inflammation—including cardiovascular disease, neurodegeneration, allergic diseases, cancer, and autoimmunity—occur in persons that are chronically infected by viruses. However, the potential of persisting viruses to contribute to disease in specific genetic settings has not been definitively examined. Therefore, genome-wide association studies should be extended to include variations in the virome as part of normal human genetic variation.

The Viral Metagenome Immunologic Imprint

Redefining the normal immune system as bearing the imprint of ongoing responses to chronic viruses with very different genetic strategies for avoiding sterilizing immunity has important implications for how we consider immune interventions

to prevent or treat infections. The imprint of responses to our virome may influence preventative or therapeutic vaccination against the chronic viruses themselves but may also change how responses occur to unrelated pathogens, viral variants that arise during chronic infections with viruses such as HIV or HCV, or self-antigens. Our emerging understanding of the differences between acute and chronic infection provides hope that specific interventions such as targeting inhibitory receptors or cytokines may, in concert with vaccination, offer effective control of these pernicious pathogens. However, viral strategies such as rapid mutation to diverse quasispecies or the capacity to retreat into an immunologically silent latent reservoir will still present profound challenges, even if mechanisms regulating T and B cell dysfunction during chronic infection can be defined and effectively targeted. For example, elimination of HIV might, if the virus establishes immunologically silent latency immediately after initial infection, require enforced reactivation of the virus and simultaneous elimination of a rapidly mutating virus. Treating or eliminating hepatitis C must similarly deal with the genetic variation of the virus. It must also deal with the fact that the role of inhibitory receptors in T cell dysfunction may be different between the relevant hepatic T cells and the more easily obtained circulating T cells.

The immunologic imprint of responses to our virome needs to be taken into account for the development of therapeutic as well as preventative vaccines. The recent failure of the adenovirus-based HIV vaccine, despite generation of detectable immune responses to target antigens, calls to mind the challenges to be met (Fauci et al., 2008; Walker and Burton, 2008). One important issue is whether responses present in persistently infected persons, such as those with persistent adenovirus, may alter the response to antigens in viral vectors derived from a persistent virus. For example, antibodies to an adenovirus vaccine vector particle can alter dendritic cell-dependent T cell responses to vector-encoded antigen (Perreau et al., 2008). Another issue is whether the imprint of chronic infection by viruses unrelated to either the virus being targeted by the vaccine or the vaccine vector itself can alter responses. Given the breadth of our virome, the imprint of chronic virus infection may be a confounding variable in human vaccine responses. Yet another issue is the fundamental one of selecting the best model systems for analyzing immune responses relevant to humans (Virgin, 2007a). As experimental mouse or primate systems are further and further removed from the chronic infection-imprinted natural state, will their responses be less and less relevant to responses in humans?

Understanding Chronic Virus Infections

Given that chronic virus infections may be harmful in only a limited number of genetically predisposed persons or may even be symbiotic, the costs versus benefits of eliminating chronic viruses must be carefully considered. We argue that infections that are generally considered harmless or unimportant play a role in shaping the normal immune response, at the cost of inducing disease in rare individuals with highly specific alterations in immune system genes. No one would doubt the essential importance of vaccinating against HCV or HIV. But what about the other chronic viruses that may be part of our normal metagenome and either do not cause disease or cause disease in only a few individuals? Can interventions be targeted to those whose genetic constitution renders them specifically vulnerable to a given viral infection so that we can eliminate disease without eliminating infection in all people? Reformatting the epidemiologic examination of viruses and disease to take into account the full genetic background of the host, including the virome, is a possibility, but genomic platforms are not yet sufficiently robust to make this a reality. The era of personalized vaccination and immunotherapy may be before us, but there is much to learn about viral immunity. Future studies need to specifically focus on defining the mechanistic details of the disease and immunologic imprint of responses to our virome, so that we can distinguish friend from foe and intervene appropriately.

ACKNOWLEDGMENTS

H.W.V., E.J.W., and R.A. are supported by the NIH. H.W.V. is supported by the Broad Medical Foundation. E.J.W. is supported by the Commonwealth of Pennsylvania and the Ellison Medical Foundation, and R.A. is supported by the Bill and Melinda Gates Foundation. We thank A. Arvin, E. Barto, B. Beutler, J.-L. Casanova., F. Chisari, L. Corey, I. Davidson, E. Dubberke, V. Fraser, D. Ganem, L. Gooding, J. Gordon, A. Grakoui, E. Kieff, D. Knipe, L. Lenaerts, K. Murphy, C. Parish, L. Ratner, C. Rice, B. Roizman, A. Shaw, D. Thorley-Lawson, E. Unanue, B. Walker, C. Walker, D. Wang, D. White, J. Wilson, and L. Zerboni for sharing comments and manuscripts with us.

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