The Host Control of Lytic and Latent Infection with Human Herpesvirus–8

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(See the article by Brown et al., on pages 1054–62.)

For pathogens to win the molecular arms race against their human foes and establish chronic and latent infection, they require (1) adequate exposure with a certain infectious load, (2) the fitness and mechanisms to subvert innate and adaptive immune surveillance machinery, and (3) according to this issue of the *Journal of Infectious Diseases* [1], a compliant host with favorable cytokine gene haplotypes that might function to alter innate and adaptive immune responses. Once latency has been established, subsequent lytic reactivation is characteristically associated with clinical disease in the host—including, in the case of the γ-herpesviridae, a variety of tumors.

There is no better demonstration of the role and importance of innate immunity, even despite the lack of memory and clonal expansion, than the low risk of establishing viral infection after needlestick injuries [2, 3]. After initial infection has been established, adaptive, rather than innate, immunity is probably more important. This is supported, for example, by a lack of influence of NK cell counts during the evolution of AIDS-defining cancers [4, 5].

Kaposi sarcoma (KS) remains the most common tumor in HIV-infected individuals. The lower incidence and regression of KS observed in those individuals fortunate enough to receive highly active antiretroviral therapy (HAART) [6, 7] may result from a variety of effects. The first of these effects concerns immune reconstitution, as is demonstrated in organ transplant recipients, in whom a reduction in the immunosuppressive therapy prescribed to prevent organ rejection may lead to the regression of KS [8–10]. Furthermore, early during the AIDS epidemic, it was recognized through anecdotal reports that regression of KS occasionally occurred during monotherapy with the nucleoside analogue zidovudine [11]. Paradoxically, the disease worsens in some individuals with KS who continue receiving HAART, despite the controlling of virologic and immunologic parameters. This clinical response, known as "immune reconstitution inflammatory syndrome," occurs secondary to an immune response against latent antigens in previously diagnosed pathogens [12].

HAART may also have a direct effect on HIV-1 Tat, a transactivating protein that induces the growth of KS spindle cells in vitro [13], protects KS herpesvirus (KSHV)–positive cell lines against chemotherapy-induced apoptosis [14], is angiogenic in transgenic mice [15], and contains arginine-lysine–rich sequences with homology to human angiogenic factors [16]. This is a controversial area, because baboons infected with HIV-2, whose Tat lacks these "angiogenic" amino acids, can develop "KS-like lesions" [17], and Tat is unable to directly activate lytic KSHV replication [18], despite initial reports [19]. Importantly, cases of KS in HIV-negative persons are well known, and we now observe KS developing and progressing in HIV-1–seropositive individuals with fully suppressed HIV-1 loads.

This issue of the *Journal of Infectious Diseases* describes, for the first time (to our knowledge), host immunogenetic factors that influence susceptibility to lytic infection with human herpesvirus (HHV)–8, the best-known predictive harbinger of clinical KS, as well as the control of latent HHV-8 infection. The authors studied 28 single-nucleotide polymorphisms (SNPs) in 14 cytokine, growth factor, and chemotactic genes in 172 HHV-8–infected individuals without KS with a median age of 75 years. An important hallmark of this investigation is that all participants were HIV seronegative, but all had evidence of HHV-8 infection, as demonstrated by 3 distinct assays. In addition, the authors chose to examine genetic susceptibility factors in the absence of KS, to minimize any influence of disease, or of retroviral-induced immunodeficiency, on the reported relationships.

Specifically, poorly controlled and lytic HHV-8 infection, as assessed by the detection of antibodies to the lytic K8.1 antigen, and latent HHV-8 infection, as assessed by the detection of antibodies to latent nuclear antigen, were associated with

an overrepresentation of a 3 locus haplotype, interleukin (IL)–4 (−1098G/−588C/−168C), and the functional promoter variant of IL-6 (−236C), as well as IL-12A (−798T/277A), respectively. If these relationships can be substantiated and the relationship between cytokine genes and HHV-8 and HIV-1 coinfection can be established, this may have significance for the commonly observed cases of AIDS-associated KS and suggests a third possibility for the mechanism of action of HAART— that of downstream cytokine effects leading to a resolution of KS.

Evidence for the interaction between HAART and cytokines has come from work in which the systemic administration of the protease inhibitors saquinavir and indinavir to nude mice leads to the regression of angioproliferative KS-like lesions [20]. These drugs were also found to block basic fibroblast growth factor or vascular endothelial growth factor (VEGF)–induced angiogenesis in a chorio-allantoic membrane assay with a potency similar to that of paclitaxel, which is a highly efficacious treatment for relapsed KS [21]. Because these drugs inhibit the in vivo growth and invasion of an angiogenic tumor cell line, it has been suggested that protease inhibitors are potent antiangiogenic and antitumor molecules that might also be used in the treatment of non–HIV-associated KS and in other HIV- and non–HIV-associated tumors.

The connection among haplotypes of IL-4, IL-12A, and a single IL-6 promoter variant is not immediately apparent. The collective influence of the SNPs at the haplotype level on the transcriptional activity of these genes is unknown, although a host that overproduces IL-4 and IL-6 with decreased amounts of IL-12 is likely a preferred environment for lytic replication. It is conceivable that the haplotypes may influence these pleiotropic molecules functionally, as opposed to quantitatively, and it is very likely that other SNPs exist in other genes, with odds ratios associated with lytic infection similar to those observed here, despite the very wide confidence limits. In this study, certain poly-

morphisms in the genes for IL-1A, IL-13, and VEGF approached but did not reach significance. Thus, combinations of haplotypes from genes in like pathways (Th1 and Th2) may ultimately contribute together to influence overall susceptibility and control of viral infection.

Markers of viral control, here by proxy, of a susceptibility gene that might alter host cytokine levels during viral infection are unlikely to be borne out in simple reverse-transcription polymerase chain reaction or plasma ELISAs that detect levels of the transcribed mRNA or translated proteins with a high degree of intra- and intersample heterogeneity. Similarly, multiplexed protein arrays may not detect them adequately, because detection problems with inherently diverse and heterogeneous molecules are enormously complex [22]. Preservation conditions that are optimal for some cytokines will not be optimal for others, and some cytokines or growth factors will bind to and saturate their array targets in a linear manner, whereas others demonstrate nonlinear dynamics. Such problems will ultimately be resolved, perhaps by performing multiple sample reactions for each test, to maintain optimal conditions for certain groups of cytokines. However, this is an intrinsic problem of working with proteins that display far more chemical and structural heterogeneity than DNA sequences (e.g., the fact that blood-group antigens are not even proteins). A further level of biological complexity that may need to be considered is that cytokines are produced locally by almost all cell types and that they are transient; their detection, if not at a local viral microenvironment, may not reflect a true relationship.

The discovery of an environment that favors lytic replication is, however, unlikely to represent a chronic advantage to the virus. Brown et al. [1] have begun the process of elucidating a host genetic fingerprint with susceptibility to lytic viral infection— a situation that probably represents a lack of immunogenetic control. The continuous interactions between host and pathogens

during their coevolution have shaped the immune system; in turn, viruses have learned to manipulate host immune control mechanisms to facilitate their propagation. Perfect viral infections, according to the most basic of the selfish gene theories, establish an equilibrium by not killing their hosts—a situation that favors host survival, which ultimately propagates the virus as long as there is a certain low level of lytic replication occurring. Thus, hosts with immunogenetic features that favor viral lysis are likely to be negatively selected during the course of evolution.

At first, nucleotide changes such as the ones observed here will be neutral, in that the single polymorphisms do not code for amino acid changes (synonymous mutations) on account of the redundancy in our triplet codons. However, as viral pressure on our genomes leads to new mutations (and their discovery), amino acid coding changes will occur, and such mutations are unlikely to become fixed unless they confer some degree of fitness on their hosts. Studies in the retroviral arena have provided striking insights here. Humans and chimpanzees have similar genomic structures in their antigen-presentation molecules and display 98.7% similarity at the nonrepetitive DNA level, having probably shared an ancestor ∼5–6 million years ago [23, 24]. Many millennia ago, a cataclysmic event occurred in chimpanzees that led to a severe repertoire reduction at the orthologues of the HLA-A, -B, and -C loci. This loss of variability, which predated the chimpanzee subspeciation, has had no marked effects on other gene systems. Because chimpanzees have a natural resistance to the development of AIDS, the authors concluded that a retrovirus caused this ancient selective sweep [25]. Hence, the contemporary populations represent the offspring of AIDS-resistant chimpanzees—the survivors of an HIV-like pandemic that took place in the distant past. Although there are few direct data that the HLA molecules that were preserved were those that produced particularly strong antiviral responses, an equally catastrophic series of events is now

occurring in the human race, with the strong likelihood that profound genetic changes in a considerably reduced human population could be present in the future.

Although it is unlikely that ancient herpesviruses can exert such profound selective pressure on humans, the finding of a susceptible host has a number of implications. First, it enables an understanding of the viral and cellular Th1 and Th2 cytokine genes and gene products that are important for HHV-8 antibody production and the control of lytic infection. All chronic infections result in a shift in the Th1 and Th2 cytokine balance, and clinical KS likely occurs in the context of a Th1 response. Second, it highlights that the neoplastic consequences of HHV-8 infection may be significantly more likely to be apparent in those individuals with the susceptible haplotype. This, in turn, emphasizes the connection among cytokines, growth factors, and survival—a situation that large randomized clinical studies are espousing [26]. Thus, the discovery of important host immunogenetic control mechanisms helps to elucidate new therapeutic targets. This clearly has implications for tumors caused by the closest relative of HHV-8, Epstein-Barr virus. Third, it raises the specter of viral selective pressure on our genome, analogous to the situation with retroviruses and chimpanzees. Individuals with the susceptible HHV-8 haplotype are probably more likely to develop tumors, and HHV-8 will therefore exert negative pressure in them, despite a more conducive replicative milieu. The discovery of host immunogenetic factors also has implications for human evolution in the context of other viral infections.

Several decades ago, after successes with polio and smallpox vaccines, William Stewart, the Surgeon General of the United States, said that infectious disease had been largely conquered and that more attention should, in the future, be devoted to chronic diseases such as cancer. How wrong he was. The AIDS epidemic, which has so far killed at least 28 million people, and the severe acute respiratory syndrome coronavirus and avian flu epidemics, which have killed considerably fewer, have produced widespread panic across the world [27, 28]. Although attention has been concentrated until now on attacking viral proteins, the study of host immunogenetics represents a new, complex, and revealing side to the host-pathogen equilibrium.

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