The Curious Incident of the Dog in the Nighttime: Does the Absence of Virus Replication in Epstein-Barr Virus–Transformed B Cells Point to an Important Feature of JC Virus Biology?

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(See the article by Chapagain and Nerurkar, on pages 184–191.)

The article by Chapagain and Nerurkar [1] in this issue of the Journal reports interesting findings of JC virus (JCV) expression in an Epstein-Barr virus (EBV)–transformed cell line. JCV is the etiologic agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease following the lytic infection of oligodendrocytes in patients who most often have cellular immune deficiencies, in particular, human immunodeficiency virus infection and/or AIDS [2]. PML follows reactivation of primary infection, which occurs in children and young adults. Progress in defining the sites of JCV latency/persistence and reactivation over the past 2 decades has led to a better understanding of the pathogenesis of PML.

The molecular biology of JCV requires that cells that support virus replication express host cell nuclear transcription factors that recognize the virus regulatory region. Rearrangement and alteration of the virus regulatory region are likely to be additional requirements [3]. The regulatory region controlling virus expression is the most variable portion of the JCV genome. Primary JCV infection occurs with the archetype strain, which has a regulatory region capable of supporting virus expression in uroepithelial cells but is inefficient in most other cell types. JCV isolated from brains of patients with PML and B lymphocytes have rearranged regulatory sequences, most often with 98–base pair tandem repeats, with and without insertions and deletions, which support virus replication in oligodendrocytes, leading to demyelination. The regulatory regions found in JCV isolated from the brains of patients with PML and B lymphocytes can be theoretically generated from the archetype sequence [4]. The site at which alterations in the archetype regulatory region required for efficient activity in glial and B cells has remained elusive. Once the altered regulatory regions are present, host cells supporting virus reactivation and replication must express nuclear transcription factors (NTFs) that recognize DNA sequences in the altered regulatory region and drive virus expression. The biology of B lymphocyte development and maturation suggests they may be an ideal site for these events to occur.

Replication of JCV in B lymphocytes was first described in 1988 by S.A.H. [5]. We reported 2 patients with PML, 1 with human immunodeficiency virus infection and AIDS, and the other with no identified underlying disease but who had polyclonal B cell activation, with evidence of JCV infection of B cells. JCV DNA was detected in B lymphocytes in the bone marrow and spleen by in situ hybridization. The viral genome was labeled with a biotinylated JCV DNA probe requiring 100–200 viral copies/cell for successful detection, supporting that virus replication was occurring in these cells. In these 2 patients and other patients with PML, JCV-infected B cells were found in the brain, associated with demyelinating lesions with use of the same techniques [6]. Taken together, these results support the hypothesis that B lymphocytes supporting JCV replication carry the virus into the brain, following which their physiologic apoptosis results in release of the virus with infection of oligodendrocytes and demyelination. In the
past, this “Trojan horse” hypothesis has been well established in the pathogenesis of other DNA and RNA viruses that infect the central nervous system.

Since our original findings, we and others, using polymerase chain reaction techniques, have reported JCV DNA in B lymphocytes in circulating peripheral blood lymphocytes, bone marrow, spleen, and tonsillar B cells, and in stromal cells in patients with and without PML [7]. When examined, the JCV regulatory region in brain, peripheral blood lymphocytes, bone marrow, and spleen from patients with PML have identical nucleotide sequences in the regulatory region, suggesting that JCV-infected B cells are the source for JCV infection in the brain [8]. Both the archetype and rearranged regulatory sequences have been detected in the bone marrow of patients with PML. Major et al [8] found JCV DNA in specimens of bone marrow and, in some instances, spleen collected up to 4 years prior to the development of PML. Taken together, these studies suggest JCV infection of B lymphocytes in cells of the bone marrow, spleen, and tonsil may provide a possible site for alterations of the virus regulatory region, as well as virus latency and reactivation that could lead to PML.

The molecular features of B lymphocyte biology offer several intriguing features that may play important roles in the development of PML. B cell generation of antibody diversity involves rearrangements of the immunoglobulin gene sequences of the variable-diversity-joining sequences mediated by the recombinases RAG1 and RAG2 in pre-B cells [9]. Later in B cell development, encounters of B cells with their cognate antigens initiate secondary diversification processes of the immunoglobulin loci, including somatic hypermutation, immunoglobulin gene conversion, and class-switch recombination. Many of these processes are mediated by activation-induced cytidine deaminase [10]. Infection of pre-B cells with archetype or JCV strains during primary infection could allow modifications of the regulatory region during B cell development and maturation through expression of inducible RAG recombinases and activation-induced cytidine deaminase, as well as other possible molecular mechanisms leading to antibody diversity. Supporting this possibility are reports of both archetype and rearranged regulatory sequences of JCV at the site at which the RAG recombinases and activation-induced cytidine deaminase enzymes are expressed. Thus, B cell generation of antibody diversity may also alter JCV regulatory regions, generating viruses capable of expression in B lymphocytes and neuroglial cells.

The expression of inducible B lymphocyte nuclear transcription factors offers another possible level of control of JCV replication. It is well established that JCV expression is controlled, at least in part, by the binding of host cell NTFs to their appropriate nucleotide sequences. The nuclear factor–1X transcription factor has been shown to be involved in virus replication and is inducible in CD34 lymphocyte precursor cells driven to a lymphocyte but not a macrophage phenotype. Other host cell NTFs required for efficient function of the viral regulatory region include the c-jun/fos complex and SP1. On the other hand, expression of nuclear factor–1A down-regulates viral replication [11]. Some evidence suggests the lymphocyte NTFs, Oct1 and Oct2, may also bind to the JCV regulatory region and increase virus expression.

B cell development and maturation is associated with a variety of NTFs that are expressed at certain stages of B cell development. Expression of these inducible host cell NTFs during lymphocyte development may also recognize nucleotide sequences in the JCV regulatory region. If so, virus could remain latent until B cells reach a stage in which the needed NTFs are expressed. These findings illustrate that B lymphocytes offer mechanisms which could control JCV expression by providing a molecular mechanism for altering the regulatory region nucleotide sequences as well as controlling expression of host cell NTFs that up-regulate viral expression. It has not escaped our notice that immunosuppression can be associated with polyclonal B cell activation, which would likely increase the likelihood that these events could occur.

Conflicting results of JCV expression in transformed B cell lines have been reported. Unlike the report of Chapagain et al, others have reported JCV replication in B cell lines. Atwood et al [12] showed that JCV replication would occur in a small number of BJAB cells, a transformed B cell line, and Namalwa cells, an EBV-transformed cell line. In these experiments, JCV replication was determined by in situ hybridization using a biotinylated DNA probe. Infection of both cell lines could be initiated with whole virus in culture, but electroporation with JCV DNA was more efficient. Like the findings encountered in the bone marrow and spleen of patients with PML, only a few cells were found supporting virus replication in these experiments.

In the Sherlock Holmes story, Silver Blaze, the failure of the dog to bark at the time the horse disappeared led Holmes to a solution of the mystery. The absence of JCV replication in an EBV-transformed B cell line by Chapagain and Nerurkar may offer an analogous opportunity to solve a “mystery.” Why did they not see virus replication in their cultures? The absence of virus replication cannot be attributed to the Mad1 JCV strain, which has a regulatory region known to support virus expression in lymphocytes. What is missing? We would suggest several possible explanations. First, the EBV-transformed cell line used may not express NTFs necessary for expression of the JCV regulatory region. Secondly, their B cell line may express NTFs, such as nuclear factor–1A, that down-regulate JCV replication. Other possible molecular events that would prevent JCV expression are also possible. Characterization of the molecular features
of their EBV-transformed B cell line may offer an answer which could “solve the mystery” of why they did not see virus replication while making a significant contribution to our understanding of the molecular events surrounding JCV and B lymphocytes.

The complexity of B lymphocyte maturation and development provides molecular machinery that could generate JCV regulatory regions capable of supporting viral expression in neuroglial and B cells. The temporal expression of inducible NTFs at stages of B cell development offer possible insights into virus latency and reactivation. Stages of B cell development in which the needed NTFs are not expressed would support latency, regardless of the sequences of the regulatory region. Once B cells are activated and develop, inducible NTFs expressed by these cells could also activated JCV expression by binding to the viral regulatory region. Current research into the questions raised and areas discussed in this editorial should expand our understanding of the pathogenesis of this fatal viral infection of the nervous system.

References