EDITORIAL COMMENTARY

Current Status of Xenotropic Murine Leukemia Virus–Related Retrovirus in Chronic Fatigue Syndrome and Prostate Cancer: Reach for a Scorecard, Not a Prescription Pad

Mary Kearney and Frank Maldarelli
HIV Drug Resistance Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

(See the article by Danielson et al, on pages 1470–1477, the brief report by Henrich et al, on pages 1478–1481, and the brief report by Barnes et al, on pages 1482–1485.)

Xenotropic murine leukemia virus–related retrovirus (XMRV) is a newly discovered member of the gammaretrovirus genus of retroviruses, which has been recently associated with 2 human disorders, prostate cancer and chronic fatigue syndrome [1]. Since it was first reported in 2006, XMRV has been intensely investigated, but no clear picture of prevalence, geographic distribution, or disease association has emerged. In this issue of the Journal, 3 studies shed new light on the presence of XMRV in human populations.

XMRV was first identified by Silverman et al [2] during efforts to investigate a role for viruses in prostate cancer. At that time it was known that a subset of individuals with prostate cancer carry a susceptibility variant of the RNAse L gene that impairs innate immune responses to viral infection, but no viral candidates had been identified. Viral gene chip surveys of prostate cancers detected sequences similar to murine leukemia virus, a gammaretrovirus [2]. Mice have been hosts to retroviruses for millions of years and have accumulated hundreds, perhaps thousands, of retroviruses in their genome; a number of phylogenetic groups with distinct genealogies and virologic characteristics have been identified [3, 4]. The viral sequences identified by Silverman et al in prostate cancer were distinct, but closely related to the xenotropic murine retroviruses, and, thus, was designated xenotropic murine leukemia virus–related retrovirus, or XMRV. Singh et al [5] subsequently identified XMRV in patients with prostate cancer but found no association with RNase L variants. Because individuals with chronic fatigue syndrome also have impaired RNase L-dependent responses, new studies were initiated to investigate the presence of XMRV in patients with chronic fatigue syndrome. In 2009, Lombardi et al [6] analyzed 101 samples from a chronic fatigue syndrome sample repository and detected XMRV in 67% of individuals with severe chronic fatigue syndrome according to Centers for Disease Control and Prevention criteria. XMRV was also detected in 3.7% of controls, prompting concerns about the possibility of iatrogenic or transfusion-associated transmission.

Molecular and biological characterization of XMRV has provided some support for its role in human disease. Human cells, especially prostate cells, are highly susceptible to an XMRV infectious molecular clone [7–10]. Like other replication-competent murine leukemia viruses, XMRV does not appear to encode an oncogene and is detected in some, but not all, prostate cancer cells in tumor tissue, suggesting oncogenic mechanisms more complex than those used by other gammaretroviruses (ie, oncogene activation or promoter insertion). Despite close similarities with xenotropic viruses, XMRV is genetically distinct from all known xenotropic murine leukemia viruses, having a 24-nucleotide deletion in gag and a 2-nucleotide deletion in the long terminal repeat. Analyses of XMRV sequences from different sources have revealed limited genetic diversity among patients. Because retrovirus diversity is dependent on cycles of error prone viral replication, the absence of XMRV genetic variability suggests that multiple cycles of infection are not taking place in humans. This lack of genetic diversity is in stark contrast to human im-
mumodeficiency virus (HIV) infection, in which viral diversity rapidly accumulates. Instead, XMRV appears more similar to human T-lymphotropic virus (HTLV), a virus that infects millions but has low genetic diversity because it undergoes limited cycles of virus replication after infection and is maintained largely by division of provirus-containing cells. XMRV is readily susceptible to hypermutation by the APOBEC system [11], suggesting that XMRV infection would likely take place in cells with low or absent APOBEC activity. Taken together, these data suggest that XMRV had zoonotic origins in mice but has replicated to only a limited degree in humans.

Following the first reports of XMRV in the United States, new studies of patients with chronic fatigue syndrome or prostate cancer were initiated that searched for the presence of XMRV in geographically diverse areas. A flurry of publications in the past year has uncovered major complexities in XMRV research. Several large studies of individuals with prostate cancer or chronic fatigue syndrome in Germany, the Netherlands, Mexico, and England did not detect any [12–14] or detected rare [15–17] evidence of XMRV. XMRV has also not been detected in individuals with other immune deficiencies, such as HIV infection [18]. In the United States, Arnold et al [19] detected anti-XMRV antibodies in ~27% of individuals with prostate cancer, and Schlaberg et al [5] reported infection in 23% of patients with prostate cancer and 4% of controls. In contrast, Switzer et al [20] reported no detectable XMRV in United States patients with chronic fatigue syndrome (n = 51), normal volunteers (n = 56), or blood donors (n = 121). Lo et al [21] recently reported the presence of mouse retroviral sequences in 86.5% of patients with chronic fatigue syndrome. Adding to the mystery, the sequences amplified from these patients were distinct from XMRV and appeared to be more closely related to different murine endogenous retroviruses.

In this issue of the Journal, several new studies have assessed the presence of XMRV in human populations. Danielsen et al [22] extracted prostate tissue from individuals who had radical prostatectomies at Baylor University Medical Center (Houston, Texas); they detected XMRV gag and env by polymerase chain reaction (PCR) amplification in samples containing ~4 × 10³ cells from 22.8% of these patients, a proportion of XMRV positivity that is similar to that in several studies of prostate cancer in the United States that did detect XMRV [5, 19]. In 57 individuals, tumor and normal tissue was available for analysis. Intriguingly, XMRV was detected in both normal and tumor tissue, suggesting that XMRV did not target prostate tumor cells specifically. In addition, the finding that XMRV positivity was coincident in normal and tumor tissue from specific patients and not uniformly distributed among normal and tumor samples in the patient population suggested that the XMRV signal they detected was not a random amplification event. Two other reports in this month’s Journal find no evidence of XMRV in different patient populations. Barnes et al [23] investigated patients with HIV or hepatitis C virus infection from Switzerland or the United Kingdom. Analysis of 230 patient samples revealed no evidence of XMRV by DNA PCR in peripheral blood mononuclear cells from HIV-infected (n = 84) or hepatitis C virus–infected (n = 67) individuals and no XMRV RNA in plasma of HIV-infected persons (n = 79). In addition, enzyme-linked immunosorbent assays demonstrated no responses to overlapping XMRV Gag peptides. Henrich et al [24] surveyed XMRV in patients with chronic fatigue syndrome and severe symptoms (n = 32), recent transplant recipients (n = 26), HIV infection (n = 43), rheumatoid arthritis (n = 97), and matched controls with use of PCR technology capable of detecting 10 copies of XMRV in 1 × 10³ cells. No XMRV was detected using this sensitive assay in any patient. From these data, the authors calculated the prevalence of XMRV in these populations to be <1.2%.

Several factors may contribute to the varied detection of XMRV in different populations, including geographic distribution, patient selection, analyte choice (DNA, RNA, antigen), and detection methodology. Geography may play a pivotal role. Henrich et al [24] studied samples from immunodeficient patients who were well characterized at facilities in Boston and found no evidence of XMRV, whereas Lombardi et al [6] studied similarly affected patients from a distinctly different geographic region and found substantial rates of XMRV. Assay sensitivity and specificity may certainly affect detection. Reports of no XMRV detection, including the 2 in this issue of the Journal, have superb sensitivity, generally in the range of 1–10 copies per 1 × 10³ cells or 10–100 copies/mL plasma. However, viremia may be chronically low (as it is in HTLV), transient, or episodic, complicating detection. XMRV detection is likely to be especially sensitive to potential false positive results by contamination with mouse-derived material. Many of the hundreds of mouse endogenous retroviruses present in the mouse genome may amplify with XMRV primers; as such, a few copies of the mouse genome may represent a substantial source of contamination.

The new studies in the Journal highlight the following measures needed to resolve conflicting reports on detection of XMRV:

1. Standardization of detection assays. This aspect is currently being addressed by a consortium of laboratories that will develop clear performance characteristics for testing.

2. Prospective epidemiologic surveys. In response to the initial identification of XMRV in patients with prostate cancer, the search for XMRV infection was driven by a few clues and the availability of local sample sets. Such “molecular geocaching” is an entirely appropriate first response, but now larger, prospectively designed and appropriately powered epidemi-
logic studies with longitudinal sampling and standardized sample processing are in order. The data from existing studies, especially power analyses from Henrich et al [24] presented in this issue of the Journal, represent useful starting benchmarks.

3. Sharing reagents and samples. Because XMRV has been reported in otherwise normal individuals, there are no a priori, gold standard XMRV-positive or -negative populations. A working set of standards will require sharing samples among groups to independently confirm results. This painstaking process will yield a reference panel that would be an invaluable resource to the research community. Researchers have already contributed a number of XMRV molecular biology reagents for general use through the National Institutes of Health AIDS Research and Reference Reagent Program. The establishment of a reference panel will propel research in this field.

4. Comprehensive and rigorous phylogenetic sequence analysis. Standard phylogenetic analyses established well described lineages for mouse viruses; adding all new sequences to a detailed phylogeny will yield useful observations [25].

5. Development of tractable animal models, such as macaques [26], will be necessary to dissect XMRV pathogenesis.

Defining any XMRV disease association is a distinct and more challenging prospect. Over the past 40 years, a number of retroviruses have been discovered to be bona fide infections of humans (HTLV-1–4, HIV-1, HIV-2, spumaviruses). Not all infections are associated with disease; no diseases have been unequivocally associated with infection by HTLV-II or spumaviruses. The XMRV disease association will require “shoe leather” epidemiology, exhaustive molecular biology, and gimlet-eyed statistical analysis to reach conclusions regarding etiology. As many virologists have long realized, disease association with retroviruses goes beyond traditional Koch’s postulates as benchmarks. As Rowe [27] summarized, Koch’s postulates assume an acute and persistent association of a readily detectable pathogen, criteria that poorly serve investigation of illnesses such as neoplasms and chronically debilitating diseases. The collective experience and utility of retroviruses for these studies [28] will provide invaluable support for XMRV investigations.

Reports of a viral agent in prostate cancer and chronic fatigue syndrome immediately established a new area of research. The XMRV field has strong magnetic properties, attracting basic researchers (particularly old time murine leukemia virus labs), clinicians, patients, and other interested parties to opposing poles regarding etiology, pathogenesis, and therapeutics. With accumulating publications, including those here, there are increasing discussions regarding possibilities for widespread testing and therapeutic intervention. XMRV may be detected using PCR or serologic techniques, and detection technologies are being made commercially available. None of the research or commercial assays has been exhaustively tested, and none is FDA approved. Further research is necessary before any of these tests can be used reliably; at this stage, discussions concerning blood donation deferral for patients with chronic fatigue syndrome are based on the general principle that there might be an infectious etiology, not on specific diagnostic testing. With respect to therapeutics, XMRV is sensitive to some antiretrovirals in vitro [11, 20, 29], and there are calls for use of antiretroviral agents for therapy, even though early observations suggest XMRV replication may be minimal in humans. At this time, such an approach is premature and medically indefensible outside the secure oversight of a well-controlled clinical trial. “Real world” coping with severe diseases like chronic fatigue syndrome and prostate cancer creates understandable desperation on the part of patients, caregivers, and health care professionals. Such pressures are not justification for testing of therapies in an uncontrolled manner. Indeed, because they are of no help whatsoever to other patients, physicians, pharmaceutical companies, or regulatory agencies, such uncontrolled therapy works directly against the goal of providing effective therapy to the million or more individuals experiencing these serious conditions.

Acknowledgments

We thank J. Coffin and J. Mellors for insightful comments and discussions.

References