Curr Allergy Asthma Rep
DOI 10.1007/s11882-010-0106-2

Xenotropic Murine Leukemia Virus-Related Virus in Chronic Fatigue Syndrome and Prostate Cancer

James N. Baraniuk

Abstract Xenotropic murine leukemia virus-related virus (XMRV) is a γ retrovirus that has been associated with chronic fatigue syndrome (CFS) and prostate cancer. The search for viral causes of these syndromes was reignited by the finding that RNase L activity was low in hereditary prostate cancer and some CFS patients. The six strains of XMRV that have been sequenced have greater than 99% identity, indicating a new human infection rather than laboratory contamination. DNA, RNA, and proteins from XMRV have been detected in 50% to 67% of CFS patients and in about 3.7% of healthy controls. XMRV infections could be transmitted to permissive cell lines from CFS plasma, suggesting the potential for communicable and blood-borne spread of the virus and potentially CFS. This troubling concept is currently under intense evaluation. The most important steps now are to independently confirm the initial findings; develop reliable assays of biomarkers; and to move on to investigations of XMRV pathophysiology and treatment in CFS, prostate cancer, and potentially other virus-related syndromes, if they exist.

Keywords XMRV · Xenotropic murine leukemia virus-related virus · Chronic fatigue syndrome · CFS · Prostate cancer · Gamma-retrovirus · Pseudoallergy · Nonallergic rhinopathy

Introduction

The relatively recently described xenotropic murine leukemia virus-related virus (XMRV) [1] is a γ retrovirus that has been associated with chronic fatigue syndrome (CFS) [2••, 3] and prostate cancer [4]. This is of importance because CFS patients are commonly seen by allergists, immunologists, rhinologists, and many other physicians for their neurological nonallergic rhinitis, fatigue, pain, generalized hyperalgesia and allodynia, dyspnea without airway obstruction, drug intolerances, irritable bowel, irritable bladder, postural orthostatic tachycardia and hypotension, and other autonomic dysfunction syndromes. These are in addition to the unexplained fatigue plus at least four of the following eight conditions required for CFS case designation: 1) poor concentration or memory, 2) sleep disturbances, 3) exertional exhaustion (a small increase in activity level leads to a relapse of fatigue), 4) arthralgia, 5) myalgia, 6) sore throat, 7) sore lymph nodes, and 8) headaches [5]. Because reliable biomarkers have not been identified, this illness is often dismissed by physicians as a figment of the somatopathic patient's mind.

The new finding of XMRV in CFS must make us pause and take a fresh look at these frustrated and disillusioned victims of medical neglect. At the same time, we must maintain skepticism about this viral etiology given the history of other inferred causative agents, such as Epstein-Barr virus; enterovirus; cytomegalovirus; neurotropic herpes types 6 and 8; mycoplasma; Q fever; Murray Valley encephalitis; other microbes; specific genotypes; and an array of immune deficits ranging from low CD4, CD8, and natural killer cell activities to various antinuclear antibodies to cytokine imbalances [6–8].

RNase L and Rationale for XMRV Infection

The rationale for looking for retroviruses in prostate cancer came from reports of viral DNA detected by microarray (Virochip; Biotherapix, Madrid, Spain) in families who were

J. N. Baraniuk (✉)
Georgetown University,
Room 3004-F, 3rd Floor, PHC Building,
3800 Reservoir Road NW,
Washington, DC 20007-2197, USA
e-mail: baraniuj@georgetown.edu

Published online: 07 April 2010

homozygous for the loss of function R462Q (nucleotide G1385A) allele of RNase L (hereditary prostate cancer-1 gene) [4]. RNase L cleaves a single strand of RNA at UpUp and UpAp nucleotide sites in ribosomal RNA and various viruses. The low-activity, homozygous QQ RNase L genotype has a prevalence of 13% and does not act as a risk factor for prostate cancer in the general US population. Instead, the link to prostate cancer may be an androgen response element in the 5 untranslated region of XMRV [9]. A link to androgens does not fit with the 4:1 female-to-male ratio for CFS in the general population unless estrogen–or progesterone-related steroids can also activate this site.

XMRV protein was found in 23%, but its DNA was found in only 6% of 334 prostate cancer specimens from a New York population [10]. High-grade tumors were more likely to show evidence of XMRV. Significantly, XMRV was detected in only 1 of 105 prostate cancer specimens, and 1 of 70 benign prostate specimens in one German study [11]. A second German group did not detect XMRV by polymerase chain reaction, Western blotting, or serological tests in 589 prostate cancer specimens. This raises the possibility that XMRV-infected prostate cancer may be geographically restricted to the United States [12].

Low RNase L activity has been found in patients with CFS. Proteolytic cleavage of high molecular weight RNase L by elastase was associated with decreased exercise performance [13]. Low RNase L activity in CFS and hereditary prostate cancer provided the insight that XMRV or a related virus may be found in CFS [2••, 4].

XMRV Biology

XMRV is xenotropic because highly similar viruses exist in the germline of mice, but their envelope (env) proteins no longer have a receptor on murine cells [14••]. Although it cannot reproduce in mice, the virus can bind to receptors in other foreign (Greek *xenos*) species, including humans [15]. Similar remnants of past retrovirus infections now form transposable elements that account for up to 8% of the human genome. The precursor to the murine leukemia virus and its related virions probably invaded mice about 1 million years ago. It is not known when XMRV began to infect humans, but it may have been within the last few hundreds of generations given the 99% identity between the six genomes that have been sequenced to date from activated CFS leukocytes and advanced prostate cancers [2••, 3, 14••]. These differ by only about 30 of 8000 nucleotides, with many of the replacements occurring in nontranslated regions of the virus, or as silent polymorphisms in the third position of codons that do not change protein sequences. Xenotropic infection may have occurred during a period in which mice were a morsel in the human diet. It is not known if XMRV has entered the human germline or if it is communicably transmitted in the population. Humans are susceptible because XMRV can bind to human xenotropic and polytropic retrovirus receptor-1 (XPR-1) [16]. This protein may be a phosphate transporter or sensor and may act in coordination with prostate-specific antigen [17].

Binding to XRP-1 and potentially to its co-receptors leads to endocytosis. The invaginating phagosome peels off the virus' plasma membrane envelope to reveal the capsid. This is composed of matrix proteins and contains the RNA genome strands, integrase, and reverse transcriptase enzymes. These move to the nucleus, where the RNA is reversely transcribed into insertion sites in the host DNA. Infectious XMRV tends to insert into the human genome at transcription start sites, CpG islands, DNase-hypersensitive sites, and gene-dense regions [18]. In prostate cancer, it integrates at cancer break points, common fragile sites, microRNA, and some cancer-related genes. These regions share unfolded chromatin with structurally open transcription regulatory sequences. Conversely, nucleosome-rich condensed chromatin would not offer suitable integration sites. Incorporated XMRV DNA is then transcribed to serve as mRNA for the formation of new proteins and genomic RNA for infectious virions. Two mRNAs are spliced. One codes for the 3 upstream regions, contiguous gag-pro-pol coding region and is spliced to the 5 untranslated region, whereas the other has the 3 region spliced to the envelope code regions and the contiguous 5 untranslated sequence. Both proteins are cleaved by the viral pepsin to form the matrix, integrase, reverse transcriptase, and transmembrane spanning stem and disulfide-linked XPR-1 binding envelope glycoprotein. The virus is assembled at the membrane; the RNA-containing capsid buds out and off the cell surface.

XMRV Detection and Transmissibility in CFS

XMRV proteins, RNA, and DNA were detected in plasma, phytohemagglutin (PHA), plus interleukin-2–activated leukocytes from CFS patients [2••, 3]. DNA for the Gag (capsid matrix protein) sequence was detected in the leukocytes of 68 of 101 (67%) CFS specimens that had been banked as long ago as 1984. DNA sequencing identified 736 nucleotides from XMRV Gag and 352 from Env genes. In contrast, Gag sequences were identified in activated leukocytes from 8 of 218 (3.7%) healthy control specimens. Only 1 of 11 controls had positive polymerase chain reaction for Gag, and there were none for Env. Five XMRV genomes were identified that had greater than 99% sequence identity. Their sequences were phylogenetically distinct from any other known xenotropic γ retrovirus, indicating that a human-specific virus was detected.

Immunofluorescent cell sorting of activated peripheral blood mononuclear cells was performed with monoclonal and polyclonal antibodies to Gag and Env proteins. These antibodies recognized XMRV-immunoreactive proteins on cells infected with the VP62 prostate cancer–related XMRV strain. A total of 19 of 30 sets of activated CFS peripheral blood mononuclear cells expressed immunoreactive Gag and other XMRV proteins. Sixteen healthy control cells were negative for all antigens. Western blots were positive for Gag and Env proteins in CFS but in none of the five controls. The odds ratio for a positive association between XMRV proteins and CFS was 54.1 (95% CI, 23.8–122) compared with results for controls.

One CFS patient had his CD4 T and B cells purified. After PHA plus interleukin-2, the cells were positive for Gag by cell sorting. This status was maintained for 42 days in culture.

Infectious XMRV were demonstrated by activating peripheral blood mononuclear cells from CFS patients and plating them with the LNCaP prostate cancer cells. This cell line has defective JAK-STAT and RNase L pathways and permits XMRV infections. After mixing the peripheral blood mononuclear cells and LNCaP cells, cultured LNCaP cells showed positive Western blots for Gag and Env proteins. Transmission electron microscopy showed budding of typical 90–to 100-nm diameter type C virions.

Ultracentrifuged plasma from 10 of 12 CFS patients was also able to infect LNCaP cells. None of the 12 control plasma samples were infectious. Thus, both infected and activated CFS leukocytes and their plasma could transmit XMRV infection to a permissive cell line. This begs the question of whether XMRV can be transmitted between humans. The relationship to human prostate cancer and murine leukemia suggests a neoplastic potential. However, epidemiologic evidence of increased incidences of leukemias and lymphomas in CFS patients has not been documented by agencies such as the Centers for Disease Control and Prevention despite multiple investigations of large populations.

Serum antibody responses to XMRV were detected in 9 of 18 CFS specimens but none of 7 healthy controls. The assay was novel, as it relied on showing human antibodies bound to a mouse B-cell line engineered to express spleen focus–forming virus envelope protein, but not the parent cell line. Human serum antibodies were not shown to bind to individual XMRV proteins.

Pandora's Box?

The proposed pathobiology of XMRV-related viruses in mice and other species opens a Pandora's box of medical issues. Does XMRV induce a primary immunodeficiency? Are lymphocytes the primary target cells in the periphery, with microglial or other support cells playing that role in the central nervous system? Is XMRV neurotropic and capable of inducing the cognitive, autonomic, and nociceptive changes typical of CFS? Are there readily detectable plasma or cerebrospinal fluid biomarkers of XMRV infection, activation, and transmissibility?

How is XMRV spread? Do the current data implicate transmission by plasma, leukocytes, gammaglobulin, or other blood products? Blood products prepared with detergent and organic solvents should not contain viable virions because their plasma membrane envelopes will be dissolved and ribonucleocapsid dissociated. Naked retrovirus RNA is not thought to be infective. Screening of intravenous drug users who share needles may reveal XMRV infection. Are there indications of fatiguing illnesses in clusters of previously healthy individuals who have received blood transfusions? Is XMRV transmitted sexually or via saliva?

Evidence of infection in 3.7% of the healthy population was presented. Are these individuals at risk for CFS, fibromyalgia, related syndromes, or even leukemia or prostate cancer? Does XMRV play a role in the CFS that follows immunocompromise by chemotherapy for cancer or lupus?

Is XMRV Integrated Into Human Germline DNA?

Even though dysfunctional RNase L does not seem to be a risk factor for XMRV-related CFS or prostate cancer, are there other human genes, haplotypes, or alleles that place some individuals at increased risk of developing XMRV infections? Candidate restriction factors for HIV-1 infection include apolipoprotein B mRNA editing enzyme 3G, bone marrow stromal cell antigen-2, cyclophilin A, tripartite motif protein 5-α (TRIM5A), and cellular microRNAs [19]. TRIM5A and cyclophilin A bind the capsid matrix proteins. This complex of host proteins bound to the viral capsid becomes ubiquinated, which targets these proteins and the capsid virion complex for proteolytic degradation in the proteosome. Nonnucleoside reverse transcriptase inhibitors may be useful given the high similarity of the xenotropic retrovirus sequences. Drugs directed at preventing docking of XMRV to XPR-1 are also conceivable. However, before thoughtfully planned antiviral drug therapy clinical trials are started in patients with CFS and, potentially, prostate cancer, we must consider the potential risk of developing drug-resistant XMRV if single antiviral agents are used.

Conclusions

Identification of a new retrovirus in CFS and prostate cancer patients and in 3.7% of the healthy American population is an important new finding. Its urgency is apparent from the Department of Health and Human

Services [20]; National Cancer Institute [21]; National Heart, Lung, and Blood Institute's Retrovirus Epidemiology Donor Study; Centers for Disease Control and Prevention Division of HIV/AIDS Prevention Laboratory, US Food and Drug Administration; and Red Cross taskforces that have been formed to rapidly investigate XMRV. It is critical to develop rapid, reliable tests for biomarkers of XMRV to determine if there is widespread infection of CFS and prostate cancer groups and to assess the safety of the nation's blood supply. CFS patients have been advised not to give blood for some time because a potential infectious cause for the syndrome has not been ruled out. Profiteering by offering nonvalidated tests should not be tolerated. It is critical to independently confirm the presence of XMRV as a causative agent in at least a subset of CFS patients, and potentially also in overlapping syndromes such as fibromyalgia and irritable bowel syndrome. This is vital before serious consideration is given to large-scale treatment studies. This is especially true given three polymerase chain reaction and serology studies from Britain and the Netherlands that have failed to verify the original American observations [22, 23, 24••]. Reconciliation of these discrepancies will almost surely lead to additional insights into the potential pathophysiological role(s) and difficulties in shown causality for XMRV in CFS, prostate cancer, and other syndromes [25••].

Acknowledgments This work was supported by US Public Health Service Award RO1 ES015382 from the National Institute of Environmental and Health Sciences; Department of Defense Congressionally Directed Medical Research Program W81XWH-07-1-0618; and M01-RR13297 from the General Clinical Research Center Program, National Center for Research Resources, National Institutes of Health.

Disclosure No potential conflict of interest relevant to this article was reported.

References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Raisch KP, Pizzato M, Sun HY, et al.: Molecular cloning, complete sequence, and biological characterization of a xenotropic murine leukemia virus constitutively released from the human B-lymphoblastoid cell line DG-75. Virology 2003, 308:83–91.
2. •• Lombardi VC, Ruscetti FW, Das Gupta J, et al.: Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science 2009, 326:585–589. *This is the key article describing a link between XMRV and CFS.*
3. Lombardi VC, Ruscetti FW, Gupta JD, et al.: Supporting online material for detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science 2009 Oct 8 (Epub ahead of print).
4. Urisman A, Molinaro RJ, Fischer N, et al.: Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog 2006, 2:e25.
5. Fukuda K, Straus SE, Hickei I, et al.: The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Intern Med 1994, 121:953–959.
6. Kerr JR, Burke B, Petty R, et al.: Seven genomic subtypes of chronic fatigue syndrome/myalgic encephalomyelitis: a detailed analysis of gene networks and clinical phenotypes. J Clin Pathol 2008, 61:730–739.
7. Lorusso L, Mikhaylova SV, Capelli E, et al.: Immunological aspects of chronic fatigue syndrome. Autoimmun Rev 2009, 8:287–291.
8. Fletcher MA, Zeng XR, Barnes Z, et al.: Plasma cytokines in women with chronic fatigue syndrome. J Transl Med 2009, 7:96.
9. Dong B, Silverman RH: Androgen stimulates transcription and replication of XMRV (xenotropic murine leukemia virus-related virus). J Virol 2009 Nov 11 (Epub ahead of print).
10. Schlaberg R, Choe DJ, Brown KR, et al.: XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. Proc Natl Acad Sci U S A 2009, 106:16351–16356.
11. Fischer N, Hellwinkel O, Schulz C, et al.: Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer. J Clin Virol 2008, 43:277–283.
12. Hohn O, Krause H, Barbarotto P, et al.: Lack of evidence for xenotropic murine leukemia virus-related virus (XMRV) in German prostate cancer patients. Retrovirology 2009, 6:92.
13. Nijs J, Meeus M, McGregor NR, et al.: Chronic fatigue syndrome: exercise performance related to immune dysfunction. Med Sci Sports Exerc 2005, 37:1647–1654.
14. •• Coffin JM, Stoye JP: Virology. A new virus for old diseases? Science 2009, 326:530–531. *Coffin brings his 40 years of experience with retroviruses to this informative editorial that introduces the findings of Lombardi et al. [2••].*
15. Levy JA: Xenotropism: the elusive viral receptor finally uncovered. Proc Natl Acad Sci U S A 1999, 96:802–804.
16. Battini JL, Rasko JE, Miller AD: A human cell-surface receptor for xenotropic and polytropic murine leukemia viruses: possible role in G protein-coupled signal transduction. Proc Natl Acad Sci U S A 1999, 96:1385–1390.
17. Hong S, Klein EA, Das Gupta J, et al.: Fibrils of prostatic acid phosphatase fragments boost infections with XMRV (xenotropic murine leukemia virus-related virus), a human retrovirus associated with prostate cancer. J Virol 2009, 83:6995–7003.
18. Kim S, Kim N, Dong B, et al.: Integration site preference of xenotropic murine leukemia virus-related virus, a new human retrovirus associated with prostate cancer. J Virol 2008, 82:9964–9977.
19. Strebel K, Luban J, Jeang KT: Human cellular restriction factors that target HIV-1 replication. BMC Med 2009, 7:48.
20. CFIDS Association of America: HHS coordinating xenotropic murine leukemia virus-related virus (XMRV) scientific activities. Available at http://www.cfids.org/cfidslink/2009/120203.asp. Accessed December 15, 2009.
21. FightingFatigue.org: The National Cancer Institute's interim guidelines for XMRV. Available at http://www.fightingfatigue.org/?p=7871. Accessed October 23, 2009.
22. Erlwein O, Kaye S, McClure MO, Weber J, Wills G, Collier D, Wessely S, Cleare A. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. *PLoS One*. 2010 Jan 6;5(1)
23. Groom HC, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, Gow JW, Mattes FM, Breuer J, Kerr JR, Stoye JP, Bishop KN. Absence of xenotropic murine leukaemia virus-

related virus in UK patients with chronic fatigue syndrome. *Retrovirology*. 2010 Feb 15;7(1):10.

24. •• van Kuppeveld FJ, de Jong AS, Lanke KH, Verhaegh GW, Melchers WJG, Swanink CMA, Bleijenberg G, Netea MG, Galama JMD, van der Meer JWM. Prevalence of xenotropic murine leukaemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort. *British Medical Journal* 2010 (Accessed 25 February 2010) •• No evidence of XMRV was found. The absence of XMRV infections in Europe or differences in sample handling may contribute to the negative finding.
25. •• Voisset C, Weiss RA, Griffiths DJ: Human RNA "rumor" viruses: the search for novel human retroviruses in chronic disease. Microbiol Mol Biol Rev 2008, 72:157–196. *This is a cautionary tale of the inference of retroviruses in human disease and the difficulties of proving cause and effect. Will XMRV become another rumor, or be conformed as causing human disease?*