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XMRV as a Human Pathogen?

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Xenotropic murine leukemia virus-related virus (XMRV) has been proposed to be associated with prostate cancer and chronic fatigue syndrome (CFS). This proposition has been controversial because many investigators have failed to replicate the reported associations. Here, we explore whether XMRV is an authentic human pathogen in the light of recent findings that indicate otherwise.

Background

Xenotropic murine leukemia virus-related virus (XMRV) is a recently described gammaretrovirus that was originally detected in and molecularly cloned from a human prostate cancer (Urisman et al., 2006). A later paper reported that XMRV was present in the blood of 67% of patients with chronic fatigue syndrome (CFS) and in 4% of healthy controls (Lombardi et al., 2009). Since these publications, some investigators have detected XMRV in human prostate cancer samples while others have not (Silverman et al., 2010; Aloia et al., 2010). Aside from a single study that reported finding polytropic murine leukemia virus sequences (a virus related to but different from XMRV) in 87% of CFS samples (Lo et al., 2010), all other reports (at least seven) have failed to detect XMRV in CFS patients from Europe, China, and the U.S. (van der Kuyl et al., 2011).

The current state of XMRV as a human pathogen is controversial and remains confounding even to the most knowledgeable retrovirology aficionados. In this context, it is important to frame two separate issues for consideration. First, is XMRV a physiologically prevalent pathogen in humans? Second, based on where the virus has been detected in humans, is XMRV a passenger microbe or is it causal for prostate cancer and/or CFS?

XMRV Can Infect Human Cells

The receptor for XMRV is XPR1, a cell-surface protein ubiquitously expressed in many human and animal cells. Although XMRV was originally described as infecting human prostate stromal cells (Urisman et al., 2006), in vitro assays show that the virus can infect and replicate in various human, feral mouse, mink, monkey, and bovine cell lines (Stieler et al., 2010). A recent intravenous infection study of eight adult Indian rhesus macaques (five infected and three mock-infected controls) used immunohistochemical staining and in situ hybridization-based assays to show that the virus was widely disseminated in vivo. Infected tissues included lymphoid organs (CD4-positive cells) as well as the prostatic epithelium and reproductive tract (Onlamoon et al., 2011). These results show that XMRV has the capability to infect humans. Nevertheless, some of the controversy surrounding XMRV centers on the possibility that detection of this virus in human samples is due to contamination from mouse cells (Smith, 2010). A significant argument against this stance was the identification of 14 XMRV integrated proviral sequences in 9 human prostate cancers (Kim et al., 2008). However, a recent report showed that 2 of these 14 integrated proviral sequences were contaminants from an experimentally infected cell line that was propagated in the laboratory, raising the question of whether the other 12 reported XMRV prostate-cancer integration sequences may not also be erroneously tainted results (Garson et al., 2011).

Unlike other human pathogenic/oncogenic viruses such as human T cell leukemia virus (HTLV-1) or human papilloma virus (HPV) (Table 1), there is currently no conclusively reproducible epidemiological link for an XMRV-related human disease (prostate cancer or CFS) or a natural XMRV reservoir in humans, mice or other animals (Figure 1). In contrast to other "newly discovered" viral infections such as the severe acute respiratory syndrome (SARS) coronavirus or avian influenza virus, the infection source of XMRV for humans has remained elusive. Indeed, some investigators have argued that the high conservation of XMRV genome sequences reported from different geographic locales by multiple laboratories are inconsistent with significant infection or extensive replication of this virus in humans (Hué et al., 2010).

Table 1. Comparative Virus-Cancer Associations

Viruses	HTLV-1	HPV	XMRV
Disease	Adult T cell leukemia	Cervical cancer	Prostate pancer
Transmission	Human intimate contact (a)	Human intimate contact (b)	?
Epidemiology	Consistent with disease	Consistent with disease	?
Viral copies	$\geq$ 1/cell	$\geq$ 1/cell	$<<$ 1/cell
Mechanism	Yes (oncogene, Tax)	Yes (oncogene, E6, E7)	?
Natural reservoir	Human	Human	?

(a) Breast feeding, sexual contact; (b) sexual contact.

The Case of XMRV and Prostate Cancer

In developed economies, prostate cancer is the most common noncutaneous malignancy in men. The prevalence of prostate cancer is associated with age. Thus, approximately 50% of men over the age of 70 have asymptomatic in situ prostate cancer that does not significantly impact longevity. Given the large at-risk population for prostate cancer, a potential association with XMRV reported in two studies from the U.S. (Urisman et al., 2006; Schlaberg et al., 2009) is significant, since it suggests the possible use of antiretroviral drugs (ARVs) as a therapeutic or preventive measure. On the other hand, because ARVs are not devoid of serious side effects, one must not use such drugs if XMRV is not the cause of disease. Therefore, it is vital to unravel mechanistic explanations regarding how XMRV could be oncogenic in order to carefully consider the plausibility of a virus-prostate cancer link.

Although data from two laboratories (R. Silverman and I. Singh) were instrumental in raising a connection between XMRV and prostate cancer, there are critical details on which the two studies differ (Urisman et al., 2006; Schlaberg et al., 2009). For example, Silverman and colleagues (Urisman et al., 2006) reported that XMRV infection was restricted to prostatic stromal fibroblasts that surround malignant prostatic epithelial tissue, while Singh and coworkers (Schlaberg et al., 2009) identified XMRV-positive cells primarily in the cancerous prostatic epithelium. A nonparsimonious interpretation of the two studies is that XMRV causes prostate cancer through more than one mechanism. Another conundrum is that one of the two studies reported an association between XMRV-cancer and a function-attenuating R462Q polymorphism in the interferon-induced *RNASEL* gene in patients (Urisman et al., 2006), while the other study found no such association (Schlaberg et al., 2009).

A further puzzle is the extremely low copy number of XMRV genomes present in putatively virus-positive prostate cancers. It is well accepted that retroviral transformation of cells occurs in one of two ways: transduction by the virus of an oncogene into the cell (whether a cellular oncogene or a virus-encoded oncogene such as HTLV-1 Tax, Table 1) or insertional mutagenesis by the retrovirus that results in the activation of an endogenous cellular oncogene. Both mechanisms are known to result in virus-induced clonal tumors that contain one or more copies of the proviral DNA sequence per transformed cell. The finding that XMRV sequences in virus-positive prostate cancers exist at fewer than one copy per cancer cell (Danielson et al., 2010) posits that either XMRV employs a transformation mechanism unprecedented for other retroviruses or that virus infection of the cell per se is not the direct cause of cancer. In this context, it is relevant (1) that recent findings have suggested that XMRV may itself have resulted from a recombination event involving murine leukemia virus ancestors (van der Kuyl et al., 2011) (abstracts from CROI 2011 meeting) that produced a virus that infected human prostate tumor cells being experimentally passaged in mice, and (2) that multiple new studies have failed to detect XMRV sequences in prostate cancer biopsies.

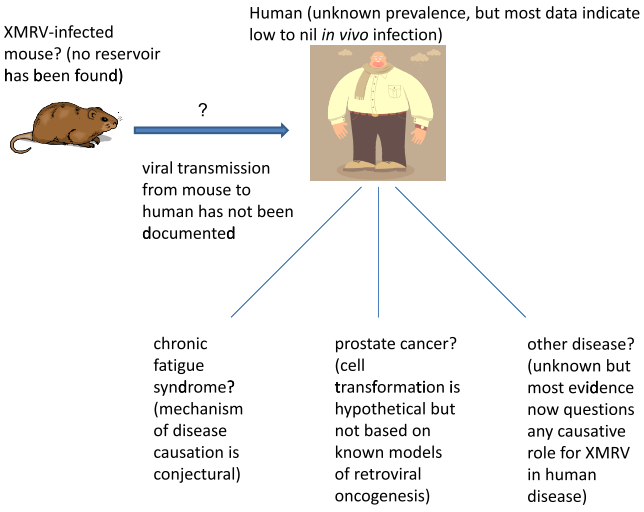

Figure 1. Unproven Transmission of XMRV from Mice to Humans
The viral reservoir and mode of transmission of XMRV are currently unknown. The ability of XMRV to replicate in humans in vivo and the link between XMRV and potential diseases have been questioned by many investigators.

The Case of XMRV and CFS

The original study that identified a link between XMRV and CFS (Lombardi et al., 2009) remains unconfirmed and has been questioned in regard to the prevalence of in vivo XMRV infection and its causal disease relationship to CFS. Like the connection between XMRV and prostate cancer, several studies now claim that the identification of XMRV sequences in CFS samples has resulted from laboratory contamination involving either faulty primers that were used in PCR analyses or laboratory tainting of human-tissue samples by mouse cells containing XMRV sequences (Smith, 2010; van der Kuyl et al., 2011). Others have failed to identify antibodies to XMRV in the sera of individuals suffering from CFS (Satterfield et al., 2011). Moreover, there has been no clarification on the causal mechanism(s) by which XMRV is responsible for CFS. The fact that XMRV can infect lymphoid tissues, which is not contested, does not establish causality of CFS. This may, in fact, represent a wide array of discrete conditions of unknown etiologies that have been grouped together for reasons of convenience, much as many variations of human hepatitis were termed non-A, non-B for many years, simply because no other definitive hepatitis viruses had yet been identified.

Unresolved Issues and Future Perspectives

There is significant uncertainty in postulating a link between XMRV and either prostate cancer or CFS. Unless and until more definitive reports of in vivo prevalence and disease causality are published, it is probably judicious to assume that some of the currently reported findings may be erroneous. In the bigger picture of XMRV as a public health concern, the following risk-benefit considerations warrant discussion.

1. There is already a suggestion that blood agencies should immediately develop screening tools to prevent the potential transfusion of XMRV in human blood or the acquisition of XMRV through organ transplantation. However, there is still no consensus on the

serological- and nucleic acid-based methodologies that are most suitable for executing this screening. In the absence of conclusive evidence of in vivo prevalence and causality (risk) for diseases, it should be recognized that the potential development and implementation of such screenings would be very expensive and have unquantifiable benefits. Thus, it could be that countless units of blood would not be donated or would be sacrificed needlessly without avail. Decision makers should be scrupulously cautious that some individuals and/or companies might stand to gain financially from the notion that XMRV is a human pathogen. A potential conflict of interest was recently alleged in reports on the controversy surrounding the hypothesis that vaccination with the measles, mumps, rubella virus vaccine might cause autism. Lessons learned from this and other past episodes could inform cool-headed deliberations on future XMRV policies.

2. In regard to CFS, the possibility that XMRV may be a causal pathogen has led many affected individuals to turn to ARVs as possible remedies for their conditions. Usually, this has involved the use of nucleoside reverse transcriptase inhibitors (NRTIs), since these compounds have been shown to be active against the reverse transcriptase (RT) enzyme of XMRV as well as against those of HIV-1, HIV-2, and other retroviruses. At present, there are no clinical reports of benefit following such anecdotal use of ARVs in CFS. Even if XMRV is linked to some CFS cases, it is almost certain that many and perhaps most CFS cases are not caused by XMRV since CFS is a heterogeneous entity. Scientific and regulatory communities might have a responsibility to endorse the appropriate drug therapy if some cases of XMRV-CFS could be established and to strongly discourage the use of ARVs in cases where it is shown beyond a reasonable doubt that an XMRV-CFS link does not exist.
3. Decisions regarding therapeutic or preventive approaches to the XMRV-prostate cancer question are equally complex. As stated above, it has been argued that approximately 50% of men over the age of 70 have asymptomatic in situ prostate cancers that do not significantly impact longevity. Because 50% of men over the age of 70 are unlikely to acquire new XMRV infection, it is imperative that one asks how many, if any, of these age-associated cases are caused by XMRV. If XMRV does cause some prostate cancers, how many longevity-critical cases versus longevity-neutral cases are associated with this virus? In the long run, resource-consuming interventions require a clear cut risk-benefit justification.
4. An important general issue raised by the current XMRV-disease debate is how to approach future medical questions of this nature. Undoubtedly, real and rumor viruses will continue to be discovered in the coming years and probably at accelerated speed with the popularity of next-generation ultrasensitive deep-sequencing technology. In future episodes, what should suffice as proof of a virus-disease link? How hard should we chase down every one, two, or three reports of a pathogen-disease axis? Even more pertinent, how much counterevidence do we need to accumulate before we stop and close the file on a virus-disease hypothesis? If the current XMRV controversy helps to refine our future decision thinking, then the considerable energy, resources, and emotions that have been expended in this debate could have benefits beyond CFS and prostate cancer for yet undiscovered infectious diseases.

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