



## Perspectives

### XMRV: Not a Mousy Virus

Antoinette C. van der Kuyl, \* Ben Berkhout

The newly discovered mouse-derived human retrovirus, xenotropic murine leukemia virus-related virus (XMRV), is currently fuelling both the scientific and public debate, where skeptics suggest that results are based on laboratory contamination, while the “believers” have already dressed the virus with highly pathogenic potential. Could the truth lie somewhere in the middle?

XMRV was discovered in 2006 in tumor tissue from patients with prostate cancer,<sup>1</sup> with a viral genome sequence highly similar to that of mouse xenotropic retroviruses. Sequence analysis suggested that XMRV is a novel recombinant derived from two fragmented endogenous murine viruses integrated in the mouse genome. XMRV was subsequently detected in other prostate cancer tissues and in blood from patients with CFS (chronic fatigue syndrome). However, most other studies failed to replicate these findings, especially outside the USA, suggesting either that the virus has a limited geographical spread, or that positive results were due to contamination of biological reagents or human samples with mouse DNA. Four recent papers indeed show that murine DNA sequences can be detected virtually everywhere,<sup>2–5</sup> and that extreme care should be taken when amplifying XMRV sequences. These results

certainly put into serious doubt some of the high prevalence results and proposed disease associations that could not be confirmed by others. However, these recent studies do not imply, and in fact did not intend to prove, that all positive results reported thus far are due to contamination, and that XMRV in humans can be dismissed as an artefact. The most important remaining question is: does XMRV infect and replicate in humans, in other words is it a genuine human virus apart from being an easy and frequent contaminant? The relevant subsequent questions would be: “how did XMRV end up in humans” and “is XMRV infection associated with disease”? It is of utmost importance to resolve these questions in a timely manner, as some CFS patients have begun taking antiretroviral drugs that can have side effects. Based on the original XMRV studies, it has also been proposed that CFS patients be banned from donating blood.

The best evidence for XMRV replicating in human cells is the detection of proviral integrations flanked by human genome sequences in prostate tissue from eleven patients.<sup>6,7</sup> The flanks are human and not mouse sequences, and the insertion sites differ for each clinical sample, thus ruling out a mouse-contamination and

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Laboratory of Experimental Virology, Department of Medical Microbiology, Centre for Infection and Immunity Amsterdam (CINIMA), Academic Medical Centre of the University of Amsterdam.

\*Correspondence to: Dr Antoinette C. van der Kuyl, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

E-mail: [a.c.vanderkuyl@amc.uva.nl](mailto:a.c.vanderkuyl@amc.uva.nl)

demonstrating independent infection cases. However, a recent paper suggested that the XMRV integration sites in the patient material could have resulted from laboratory contamination with artificial XMRV cell lined-derived integration sites.<sup>8</sup> In situ fluorescent hybridization of prostate cancer tissue has detected XMRV proviral genomes that correlated with neutralizing antibody reactivity and/or PCR results in patients.<sup>9</sup> These findings indicate that even though it is likely that many positive cases may have to be dismissed as representing contamination with mouse DNA, genuine human infections with XMRV seem to exist at a relatively low prevalence.

In retrospect, the method used in most studies for detection of XMRV, almost exclusively nested PCR amplification, has been a rather unlucky choice. In clinical virology, a putative virus infection is usually probed with a serological assay, although real time PCR assays are currently in use for infections in which a high copy number of viral nucleic acid (RNA or DNA) is known to correlate with disease. A simple detection PCR is rarely the first method of choice for several reasons, including the danger of getting false positives due to contamination. Serological assays for XMRV are currently being developed, and will be much needed to distinguish genuine infected patients from contamination cases. Serology will be more informative than running a control PCR for mouse DNA as contamination by XMRV particles (e.g. from protein preparations or contaminated cell cultures) will not show mouse DNA, other than the viral genome.

The route of XMRV transmission from mouse to human currently remains unclear. Patients could have been infected by isolated zoonotic transmissions from feral mice, through human to human transmission or, as we recently proposed, through the use of mouse-derived biological products.<sup>10</sup> The use of a mouse-derived vaccine as a single source of XMRV infection could explain the low genetic variation of XMRV sequences isolated from different individuals in different geographic locations. The global distribution of XMRV is currently ill-defined. Most prevalence studies were

performed in Northern America or Europe, with only a single report from Asia, specifically China.<sup>11</sup>

In conclusion, although evidence from several patients, all with prostate cancer, strongly suggests that XMRV is truly infecting and replicating in humans, it is much too early to speculate about any disease association of XMRV because the most basic questions have not been answered. Serological assays should be used to identify genuinely XMRV-infected patients that can subsequently be studied to elucidate the replication and pathogenic properties of XMRV infection in humans.

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