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No difference in antibody titers against Xenotropic MLV Related Virus in prostate cancer cases and cancer-free controls

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Dear Editor

The association of prostate cancer with polymorphisms in RNASE L, a gene involved in innate immunity, has given credibility to the hypothesis that the etiology of this disease is infectious¹. A scan (Is it possible to be more specific without adding too many words? for viruses in prostate cancer tissue uncovered the Xenotropic MLV Related Virus (XMRV)². This previously unknown virus has more than 90% homology to the Murine Leukemia Virus and is capable of replicating in human cell lines. The possible involvement of this virus in the development of prostate cancer has generated much attention since XMRV could be targeted by antiretrovirals and vaccines. PCR-based studies have found increased prevalence of XMRV infection in familial prostate cancer cases having the RNASEL (R462Q) allele^{2, 3}. A combined immunohistochemistry and PCR study also purported to show a link between XMRV and sporadic cases of prostate cancer, although the two tests were frequently discordant on a given tumor⁴. A recent study which measured neutralizing antibodies reported 27.5% of prostate cancer patients homozygous for the R462Q allele to be infected (exposed is a better term) with XMRV⁵. Contrary to these findings, a study from Germany found no evidence of XMRV in prostate cancer patients⁶. In summary, a conclusive case for XMRV being a pathologic agent in prostate cancer can not be made and it remains unclear if this agent is worth pursuing as a potential diagnostic or therapeutic target.

Due to various technical issues, PCR tests for infectious agents can give rise to inconsistent results, especially when standardized assays are lacking. Serological assays do not suffer from these limitations and may provide a more accurate measure for viral exposure. Hence, we developed two serological assays to test for serum antibody titers against XMRV. The XMRV env and gag sequences (env 5749–7683 and gag 611–2215 of genbank sequence EF185282.1) were codon optimized for expression in *Trichoplusia ni* insect cells (I am not sure but we may have to submit the codon optimized sequence to GenBank). Each synthesized sequence was subcloned into a modified pAB-GST vector (AB Vector, San Diego, CA) containing an N terminal GST tag, a C terminal His tag and, a 3' 60 nucleotide sequence tag encoding the bovine polyomavirus large T antigen (BPVLT) and a recombinant baculovirus was created. For protein expression, Hi-five cells were inoculated with high titer stocks of recombinant baculovirus constructs and allowed to grow for 3 days. The cells were then lysed and the crude extracts were collected. In addition, a baculovirus

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construct containing the empty vector was made. The expression of the fusion proteins were verified by Western Blot analysis using an anti-BPVLT antibody.

The cell lysates were thawed, diluted and added to ELISA plates coated with glutathione. The diluted lysates were incubated for an hour in order to allow the GST tag of the fusion protein to bind to the glutathione. The amount of protein to add was determined empirically based on the amount of immunoreactivity observed using the anti-BPVLT. The immobilized protein was then washed, blocked and incubated with patient sera diluted to 1/100 with PBS tween. The XMRV-gag, envelope and the blank vector were loaded into adjacent wells and the serum from the same sample was added to these three wells. Each plate used the anti-BPVLT as the positive control and negative control wells were incubated with PBS/tween buffer instead of patient sera. The serum reactivity to the empty vector was used to determine background levels for the XMRV assays and these background values were subtracted from the XMRV-gag and -env measurements.

Using this assay format we measured antibody reactivity from 200 individuals with prostate cancer and 200 non-cancer controls from the NCI Immunodiagnosis Serum Bank, originally collected from patients treated at the Mayo Clinic⁷. We observed low antibody reactivity against both antigens. We were unable to determine a cutoff point for seropositivity as we did not have any reference positive and negative control samples. However, reproducibility was good based on blinded replicate measurements for 50 of the samples, with intraclass correlation coefficients of 0.65 for the XMRV-gag assay and 0.46 for the XMRV-env assay. Hence, we analyzed the data by picking arbitrary (maybe exploratory sounds better) cutoff points and we also compared the antibody reactivity between cases and controls as a continuous variable. Regardless of the analytical method used, we did not detect a statistically significant difference in immunoreactivity between cases and controls for either the XMRV-env or the XMRV-gag antigen (figure 1A and 1B). In fact, some of the highest antibody reactivities against both XMRV antigens were seen in controls. We did find a statistically significant increase in XMRV reactivity with age (figure 1C and 1D). Individuals over the age of 30 had slightly higher XMRV-env antibody reactivities than younger individuals ($p=0.003$). However, age related increases in immunoreactivity for various antigens have been described previously and this observation may be unrelated to disease.

Our results do not support the involvement of XMRV in prostate cancer. Admittedly, the presumed sensitivity of our antibody assays has not been empirically confirmed, since we have not tested sera from individuals with proven XMRV infection. Nevertheless, our findings are consistent with a study of German prostate cancer patients which also did not find evidence of the virus by either antibody assays or PCR⁶. Our ELISA assay was slightly different in that we used the entire protein instead of partial XMRV-env and gag sequences. In addition, we expressed our proteins in insect cells instead of bacteria, which results in improved protein folding. The German group hypothesized that the absence of XMRV may be related to a low prevalence of the virus in Germany. However, our subjects came from the central United States, similar to subjects in the original report. We did not have genotyping data on our cohort but given that up to 15% of individuals with prostate cancer may be homozygous for the R462Q allele, we would expect to find 10 or more individuals with anti XMRV antibodies if XMRV is present in 40% of prostate cancer patients carrying this polymorphism, as originally reported¹. In summary, our findings are in contrast with studies reporting positive associations and it is difficult to reconcile this difference. It is possible that the prevalence of XMRV in prostate cancer is much lower than previously reported and a larger study may be needed to examine this association.

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Figure 1. XMRV-env and XMRV-gag Immunoreactivity
Histogram of XMRV-env (A) and XMRV-gag(B) immunoreactivity in prostate cancer cases and controls. Panels (C) and (D) show XMRV-env and XMRV-gag immunoreactivity based on age.