

Questions and Answer Session with:

Dr. Judy Mikovits: Principal Investigator, Whittemore-Peterson Institute.

Q: From what I read, XMRV is infecting immune cells. If patients take nutraceuticals that have been shown to increase natural killer cell count, could some of this increase in NK cells come from dividing infected cells, thus risking a heavier load of XMRV?

Andrew Bokelman

A: Because XMRV research is in its infancy, little is known about the tissue reservoirs of the virus, while many cell types can be infected (because the XPR1 entry molecule/receptor is on every cell in the body), few cells support high levels of replication of XMRV. You could potentially increase the viral load by increasing the NK cell count. However, we have done studies with compounds that increase NK cell killing without increasing NK replication or XMRV viral loads so NK cells kill better and can therefore be efficacious in killing XMRV infected cell.

An extremely minor species of HIV becomes a dominant species when a large mixture of the viruses (quasi-species) from the blood of AIDS patients is cultured in the laboratory. As much as 18% of our human genome (3 billion genetic codes) are made of retrovirus sequences. The sequence of XMRV is 95% homologous to the human endogenous retrovirus (HERV) sequence in our human genome. Is the 5% genetic sequence variation enough to call XMRV a new virus? Is XMRV a minor quasispecies that is selected out and predominantly amplified from the tissue cultures?

The 6 full length XMRV clones: 3 from prostate cancer tissue biopsies from men in Cleveland and 3 from leukocytes from female CFS patients in the Western United States drawn in 2006-2008..were 99% similar. We did not despite repeated attempts isolate ANY other variants from those individuals suggesting that the virus is very low replicating and unlike HIV does not have quasi species. As much as 18% of the human genome is retroviral elements or endogenous retroviruses. THESE ARE QUITE DISTINCT FROM EXOGENOUS VIRUSES! PRIOR TO THE SCIENCE PAPER THERE were ONLY 2 EXOGENOUS human retrovirus families, HTLV (1,2) and HIV (1,2). HERVs are not infectious exogenous retroviruses...The main point of the science paper was the demonstration that XMRV was the third human exogenous retrovirus and that it was blood borne and transmissible (can isolate it and do primary and secondary infections of primary human cells) Moreover, the full sequence of XMRV was blasted against the entire human and mouse genome and NEITHER the mouse genome or the HUMAN genome contained XMRV..proving it was NOT a mouse virus. XMRV was on the basis of our data proven to be a NEW HUMAN Blood borne infectious and transmissible retrovirus. Finally the proof of an immune response in CFS patients from whom the virus had been isolated and sequenced showed that the XMRV in CFS patient was not a mouse contaminant or a human lab contaminant.

Q: It has been said in the new paper article that XMRV is transmitted by blood, sex and body fluid. The author may have meant that this is what happens in mice but is not yet defined for human. Sexual transmission (men who have sex with men) and transmission through blood (hemophiliacs) were long suspected based on epidemiologic data before HIV was found to be cause of AIDS in 1984. ME/CFS does not have typical pattern of sexual transmission although there are rare mentions of transmission between couples. Most of my patients have developed ME/CFS after flu-like illness with respiratory and/or gastrointestinal symptoms, which would speak against sexual and blood transmission. Furthermore, why are children developing this illness and why clusters of cases occurred in Incline village and other areas, if the transmission is as stated. Please clarify this issue.

Thank you,

John K. Chia M.D.

A: Newspapers are notorious for extrapolating data and drawing conclusions. We and Dr. Stuart LeGrice attempted several times in writing to correct the mis-statements in the Wall Street journal and the NY Times. The Science paper showed that XMRV was blood borne, infectious and transmissible SUGGESTING blood and body fluid transmission although NO studies have proven transmission of any kind. The authors meant this is the mode of transmission for the other two human exogenous retroviruses HTLV-1 and HIV. XMRV is NOT a Mouse virus and NO ONE knows how it got into the human population). This author has never worked with mice.

It is important NOT to think only of HIV when considering modes of transmission. Body fluid means ANY body fluid, blood was proven in the Science paper, but one can also consider saliva, vomit, urine and feces. XMRV is a gammaretrovirus (simple) and these tend to be much more stable than HIV and HTLV1 which are complex retroviruses. XMRV has been demonstrated by us in the science paper to be very stable and easily transmissible CELL Free unlike its cousins HTLV (which is tightly cell associated) and HIV, which is very labile and not stable in vomit feces or urine and while it can be shed and theoretically transmitted in saliva this is rare as it is labile cell free as well.. the flu like illness and gastrointestinal issues are consistent with stability in other body fluids and particularly in acidic environments such as the gut and urine...We have isolated infectious XMRV from saliva and prostatic secretions to date and tested its stability. These are unpublished data and we await the development of a quantitative viral load assay, but when we can quantify the stability, my hypothesis is that XMRV is the most stable human retrovirus to date and while no human retrovirus (or animal retrovirus is transmitted airborne) our hypothesis is that we will find the transmission is in other body fluids and outbreaks occur because of the stability of XMRV in other body fluids

Q: Do you think that any of the new biotech drugs for MS could be useful for CFIDS?
Thank you. Pat Turello

A: My (limited knowledge of the new MS drug is that they target the hyperactive immune response and trafficking of white blood cells from lymph nodes so if a reservoir of XMRV is the lymph nodes and the hyperactive immune response (as we suspect) plays a role in disease progression through increased replication of XMRV or trafficking of white blood cells harboring XMRV to sites of inflammation, then these drugs could be useful for XMRV associated CFIDS.

Are there any official (or unofficial) records of forested areas near and around Incline Village being sprayed for 'insect control' prior to the outbreak of CFS there ?

There is no data or records that I am aware of and no evidence that XMRV is spread through insects (in fact there is no evidence of how XMRV got into the human population)

Q: Is it possible that XMRV is (or related to) an 'escaped,' genetically- engineered laboratory research retrovirus?

A: NO laboratory researchers are not smart enough to engineer new human retroviruses. XMRV has never been detected in ANY other animal in nature except humans.

Q: Please explain the significance of "xenotropic" in the transmission or infectivity of XMRV ?
Nancy Allen

A: Xenotropic viruses are not able to infect cells derived from laboratory mice (or the mice themselves but they can infect cell lines from a number of other species including humans and wild field mice...more proof that this is not a lab engineered or escape variant.

Q: Hey guys! miss you! I am keeping things hopping here in Miami, but it is weird being off the board. I noticed VIP Dx has dropped the PCR part of the XMRV testing, in favor of culture. Why?
Nancy Klimas

A: PCR of genomic DNA was found by the WPI and its NCI collaborators to be the least sensitive method of detecting XMRV because of the low copy number of XMRV and the realization by the negative studies that only 1 in 1million resting white blood cells harbors a copy of the XMRV provirus. Thus VIPDx was getting too many false negatives..ie the failure to detect XMRV. The same reason that other companies using sensitive PCR failed to detect XMRV from a spot of blood or 1 ml of blood. Retroviruses multiply only in dividing cells so VIPDx dropped the first PCR in favor of biological amplification of XMRV before PCR detection. Not only are the results more reliable but the test is considerably cheaper. The most important thing is to get the

Q: Do you feel that XMRV is necessary and sufficient or necessary but not sufficient or not necessary or sufficient to cause chronic illnesses like CFS?

A: *Our hypothesis is that chronic XMRV infection creates results in an immune deficiency that is necessary but not necessarily sufficient to result in chronic illnesses like CFS. As with HIV/AIDS copathogens define AIDS, CMV retinitis, kaposi's sarcoma are AIDS defining co-pathogens. That said XMRV is a type C retrovirus and Type C retroviruses are associated with neurovirulence in a range of host species, including humans, cats, rodents and birds with or without concurrent immunological disease suggesting XMRV can be Necessary and sufficient to cause CFS.*

Q: What are your current % positives in CFS? In controls?

A: *In CFS disease satisfying the Canadian consensus criteria, we have isolated virus from more than 300 patients of ~400 tested but we have serological evidence in another ~10% of CFS patients fulfilling CCC criteria, from whom we have not isolated virus, clear evidence of infection but the significance of which is not known.*

Q: Is there an indication that the virus is active and being transcribed in CFS? What percentage of positives are being transcribed?

A: *The Science paper clearly showed active transcription of XMRV in >75% of the patients tested and similar suggestion of latent infection or evidence of infection without isolation of virus in 10-15%.*

Q: With the known % of CFS patients positive for Mycoplasma species (~60% in multiple studies), Chlamydia pneumoniae (~10% in multiple studies), HHV-6 (~30% in some studies) and other infections, is there any concordance with XMRV positivity?

A: *We have only done those analyses on the 101 in the original study, HHV6A was 10%, EBV ~14% and nothing else more than 10%. We are working with several groups at Lyme and those numbers may approach 30%-40% of those tested.*

Q: Do you feel that XMRV could act to cause dysfunction of the immune system, allowing opportunistic infections (such as in 4, above), similar to HIV-1 in AIDS?

A: *Absolutely that is our working hypothesis*

Q: Do you think that treatment of XMRV might be useful or possibly too late in CFS to restore patients to pre-infection health status?

A: *I am extremely optimistic that treatment of XMRV or its immune target(s) will restore CFS patients to at least 85% of the original health status even in the sickest of the sick. I base this optimism solely on the level of health that was achieved in HIV AIDS patients with the advent of highly active antiretroviral therapy (HAART) and XMRV is much less cytopathic than HIV, its effects on the immune system are more subtle. I think the evidence is that some recover with out ever treating the XMRV or maintain relative health for years between "crashes".*

Q: When you assess signs/symptoms of patients with positive XMRV, are there any that track or don't track well with XMRV positivity compared to CFS patients as a group?

A: *I am not a clinician and right now we don't have sensitive quantitative assays to monitor viral load so we have not actually tracked symptom severity and XMRV positivity. For Science paper we did correlation analyses on every clinical marker of immune status, that we had available to us including RNAase L, NK (number and function, co-infections, cytokines and chemokines and NOTHING significantly correlated with a CFS diagnosis (the referees and editors felt these data were not important for the paper as they were all negative data. The only one they published was the R462Q RNaseL variant as this was the original hypothesis under which XMRV was discovered. The ONLY immune marker which correlated with XMRV infection 100% was decreased Interferon alpha.*

Q: In families with more than one CFS (or other chronic illness), is there any indication that XMRV was transmitted to immediate family members?

Prof. Garth Nicolson
The Institute for Molecular Medicine

A: *YES...lots of them and I have not looked at everyone but my knowledge of the 101 in the Science paper is that more than 2/3 of them have family member who are infected and MOST are ill perhaps with another overlapping chronic illness..for instance FM and CFS or CFS mom and autistic kids and the cancer in some of the families is frightening.*

Q: I read where 75 top researches met on 16 Dec. 2009 to discuss the current research findings about XMRV. I have been looking, but unable to find any information about what they had found. Hope you will include this.

William Hays

A: *I am not aware of that meeting nor am I aware that on December 16th of 2009 there were 75 top researchers working on XMRV! On November 10 the Cleveland Clinic sponsored a meeting where ~15 scientists presented their data including the WPI) on XMRV in cancer and CFS. There were 75 people in attendance and it was an interesting meeting but honestly I would be surprised is there are yet 75 top researchers in the world working on XMRV.*

Q: How do you see HHV-6 and XMRV interacting?

A: *There are no data to suggest that HHV6 and XMRV directly interact in any way. XMRV is a simple retrovirus so it encodes only structural proteins and not transacting factors like HIV and HTLV. We are testing the hypothesis that like HIV, the transmission of XMRV and the progression of XMRV disease are accompanied by de novo infection by other microbes or by activation of microbes that were present in the host in homeostatic equilibrium before XMRV infection. In recent years, data have accumulated on the interactions of these coinfecting microbes—viruses in particular—with HIV. Coinfecting viruses generate negative and positive signals that suppress or upregulate HIV-1, suggesting that the signals generated by these viruses may largely affect XMRV transmission and pathogenesis as they do with HIV transmission and pathogenesis.*

Q: The Brits have commented that both M.E., and the U.S. cluster outbreaks of the 1980s, seem to spread like an enterovirus (a polio-like virus), not like a herpes virus.

A: *XMRV is a retrovirus not a herpesvirus so I don't understand the comment. HIV causes leaky gut and disruption of the gut microbiome and an open blood brain barrier allowing pathogens access to areas of the body that these pathogens normally would not have access...the same could happen with XMRV...I guess I cannot answer this because more than 75 of the 101 patients in our study were not involved in cluster outbreaks. Whether that criticism holds, it certainly seems the outbreaks did not follow the pattern set by HIV. While our knowledge of HIV disease is useful in developing hypotheses and studying potential mechanisms of HIV pathogenesis, XMRV is totally unlike HIV and HTLV-1 in that it is a simple retrovirus the first ever identified human exogenous simple retrovirus and the family of viruses most closely related to XMRV causes neurological diseases and cancer very by mechanisms distinct in many aspects from HIV and HTLV. We discussed earlier XMRV may be much more stable in body fluids other than blood and cell free and would completely explain clusters and sporadic transmission as seen in CFS both in the US and UK*

Q: How would teachers in the same school, for example (who were not misbehaving), contract the disease from each other?

A: *See above but what if XMRV is more stable in body fluids such as saliva, urine, vomit such that exchange of body fluids less directly than sexual contact and blood borne direct infection were the mode of transmission. This hypotheses would satisfy the familial and close contact such as a school particularly if a co-pathogen enhanced transmission and progression as was the case of HIV and HSV in Africa.*

Q: What does this mean for young women who wish to become pregnant, but who might have XMRV?

A: *Gammaretroviruses can be transmitted vertically and are transmitted in breast milk. The hormone responsiveness of the virus suggests that lactating moms express more XMRV in breast milk. HTLV 1 was endemic in Japan so simply preventing breast feeding reduced HTLV-1 associated neuroimmune disease and*

the reservoirs of virus low.. Stop the XMRV from being expressed and spread to healthy cells keep the XMRV provirus from integrating into long lived cellsthe variation was so small and the lack of quasi species and high levels of replication suggest that vaccine development will be easier with this retrovirus that with the complex retroviruses, HIV and HTLV. The most important thing to do is identify everyone infected and stop the spread and replication of the virus in every individual prevent disease in those who are well and stop the progression and restore the immune system in those who are ill.

Q: Given the association of XMRV with patients whose blood was stored at WPI, how many other docs have sent samples that are positive?

A: Contrary to the assumptions and misinformation about the samples in the WPI repository. These samples were NOT solely from the incline village outbreak. All samples were drawn from patients coming to Sierra Internal medicine between 2006 AND 2008. Fewer than 20 of the 101 were from the original outbreak and more than 75 were sporadic cases of CFS from patients who came to Dr. Peterson from 12 states and Canada. The 101 were representative of patients satisfying CCC and Fukuda criteria throughout the US. At least 5 doctors from across the US including Dr Klimas and Dr Cheney had patients in the repository and in the study. The 101 patients were selected at random from the hundreds in the repository. The main consideration was that we had samples that could be cultured (retroviruses cannot multiply unless a cell divides and several samples from a given patient).

Q: Are your "hit rates" different in the samples sent to you since the Science paper?

A: Not as long as the physicians sending the samples are diagnosing as Dr Peterson does on CCC criteria. In fact the hit rates from overlapping diseases more than we expected now ~35%.

Q: Given that a number of physicians who send you XMRV samples are also collecting HLA DR data, can we sort the positives and negatives by HLA DR haplotype, i.e., is there evidence of an increased relative risk for carriage of XMRV and presence of illness by HLA DR?

A: We established the original WPI research program to look at the underlying genetics from the exact same population from which we were looking for novel viruses and correlating immune profiles and gene expression patterns. Therefore our collaborators Mary Carrington and Michael Dean of the NCI's genomic diversity program and the geneticists who identified the HLA and KIR susceptibility and resistance loci in HIV/AIDS have all of these studies well underway...some of these data were presented at last year's IACFS meeting. At that time we did not know XMRV status of those patients. Those data are currently being analyzed.

Q: Do we know of any patients with acute acquisition of XMRV? How can you confirm a new case versus re-activation of transcription of intercalated viral material in DNA? What happens to their symptom with acquisition? What happens to their measures of innate immune responses?

A: No. None of these studies can be done yet. We are working on cases of suspected "transfusion acquired" CFS which could establish an acute infection. These studies will require quantitative assays. The requirement that a diagnosis of CFS require an individual to be ill for 6months has precluded anyone from having such data. Hopefully at least one archaic practice will soon be gone ..the worst predictor of HIV disease progression is the viral set point and reservoir viral load. Set the reservoirs as low as possible! That is identify the infection as soon as possible. This is the best chance to prevent disease development and progression.

Q: Do we know if genes carried by XMRV are being transcribed? If so would genomic data from samples collected by other labs be useful in a pooled analysis?

A: XMRV is a simple retrovirus and encodes and expresses only the gag pol and env genes. ALL are expressed and proven to make functional proteins. This was clearly demonstrated in our Science paper. Gamma retroviruses do not cart or pick up cellular genes? Hope I answered the question because I don't understand the second part.

Q: What regulatory mechanisms are felt to be present that would affect transcription of XMRV genetic material?

A: Expression of XMRV and all simple retroviruses are controlled only by cis acting elements in the upstream untranslated region. In XMRV 3 transcriptional control elements have been identified two glucocorticoid

response elements (a strong one responding to androgens and estrogens, a weaker one responding to the stress hormone cortisol and an NfKB site) All turn on XMRV.

Q: If XMRV is actively being transcribed, is there a difference in lab parameters and symptoms compared to those who carry XMRV but are without transcription?

A: We don't have these data yet but that is a testable hypothesis.

Q: If XMRV is present but inactive, are there any suggestions as to what could be a trigger for (re)-activation?

A: Estrogens, Androgens, Cortisol (stress) and inflammation.

Q: Given the problems with antigen presentation seen routinely in so many patients with chronic fatiguing illnesses, we would expect to see some patients with culture (+) XMRV who are antibody negative?

A: Yes! We see lots of those...these data are very interesting and suggest immune therapy including antibody therapy may be most useful in this disease.

Q: I heard that there may be another study in the U.K. that came to a different conclusion about a possible link between CFS and XMRV. Can you comment on the differing results, and whether the results of the two studies can be reconciled? Thank you.

A: The negative studies were technically flawed in that their methods were demonstrated NOT to be capable of detecting XMRV. Their patient populations likely did not satisfy CCC criteria and they looked only by PCR on genomic DNA the least sensitive way of detecting XMRV. To date none one has attempted to replicate our study. It is very clear that the prevalence of XMRV in UK is NOT ZERO and that XMRV has been detected in CFS patients in the UK.

Q: How might the finding of the XMRV virus relate to Lyme Disease?

A: We are seeing XMRV in Chronic Lyme patients sent to us from several physicians. The hypothesis that chronic XMRV infection creates an underlying immune deficiency is consistent with many co-pathogens including Lyme.