Situation of XMRV and Blood Transfusion

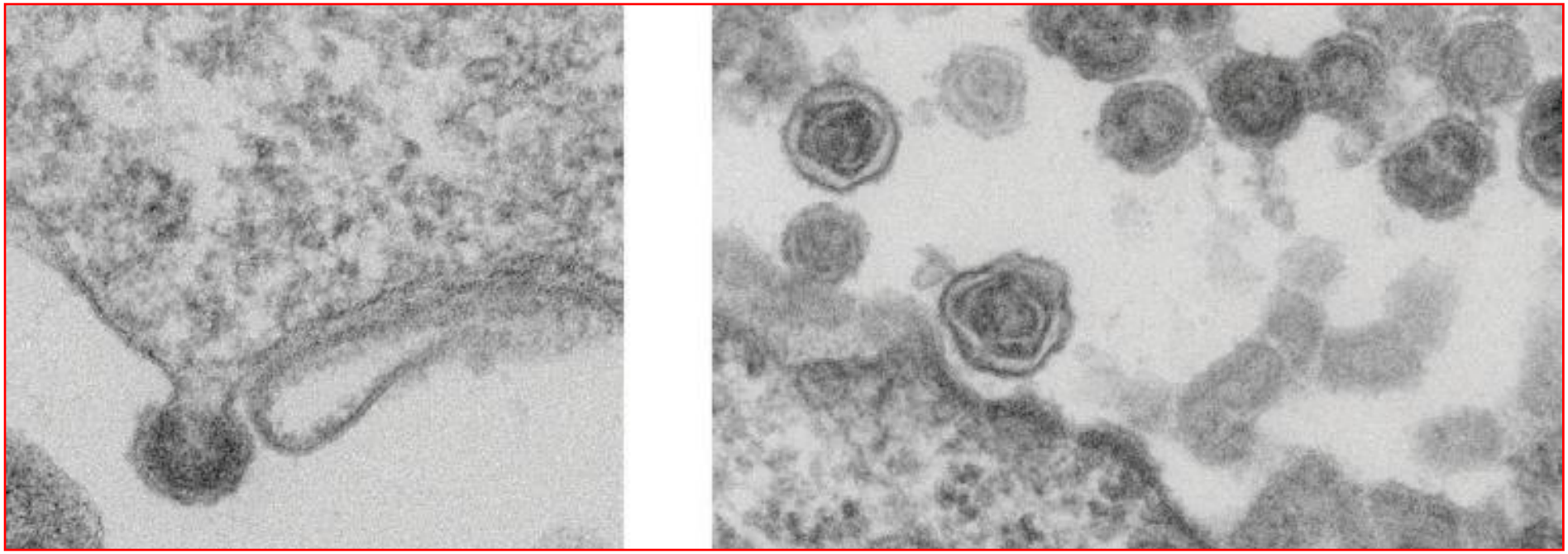
Celso Bianco, MD
ISBT Working Party on TTID
Lisbon, June 19, 2011

America's Blood® Centers
It's About *Life.*

Sciencexpress Report

Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

Vincent C. Lombardi,[1]* Francis W. Ruscetti,[2]* Jaydip Das Gupta,[3] Max A. Pfost,[1] Kathryn S. Hagen,[1] Daniel L. Peterson,[1] Sandra K. Ruscetti,[4] Rachel K. Bagni,[5] Cari Petrow-Sadowski,[6] Bert Gold,[2] Michael Dean,[2] Robert H. Silverman,[3] Judy A. Mikovits[1]†



Lombardi et al. *Science 326, 585 (2009)*

Conclusions: CFS and XMRV

- XMRV found in 67% of CFS patients
- An immune response to the virus was detected in some CFS patients
- Data suggest that the human population is at risk from infection with XMRV (3.7% of controls are DNA positive)
- *Given that infectious virus is present in plasma and in blood cells, blood-borne transmission is a possibility."*
- Coffin and Stoye. *Science*. 2009.

Common Names

- Chronic Fatigue Syndrome (CFS, U.S. Health and Human Services Committee)
- Myalgic encephalomyelitis (ME, EU)
- Chronic fatigue and immune dysfunction syndrome (CFIDS, US Association)
- Post-viral fatigue syndrome
- Post-infectious fatigue syndrome
- X-associated neuroimmune disease (XAND)
- Worldwide prevalence 0.4-1%; US 1.2-4 million individual

CFIDS Association (U.S.)

Chronic Fatigue and Immune Dysfunction Syndrome

- CFIDS
  - http://www.cfids.org/default.asp
- CFIDS Research 1st
  - http://www.research1st.com/2011/06/01/xmrv-trials-and-tribulations/
- Position on blood donation
  - There are numerous medical reasons why people with CFS should not donate blood…. The CFIDS Association of America has long advised against CFS patients donating blood [or organs].”
  - http://www.cfids.org/cfidslink/2009/120204.asp

AABB Task Force Report

COMMITTEE REPORT

Xenotropic murine leukemia virus–related virus (XMRV) and blood transfusion: report of the AABB interorganizational XMRV task force

Harvey G. Klein, Roger Y. Dodd, F. Blaine Hollinger, Louis M. Katz, Steven Kleinman, K. Kimberly McCleary, Robert H. Silverman, and Susan L. Stramer for the AABB Interorganizational Task Force on XMRV

TRANSFUSION 2011;51:654-661

Association Bulletin #10-03 - Chronic Fatigue Syndrome & Blood Donation (06-18-10)

"…AABB recommends that blood collecting organizations make educational information available regarding the reasons why an individual diagnosed with CFS should not donate blood…."

Resources from AABB

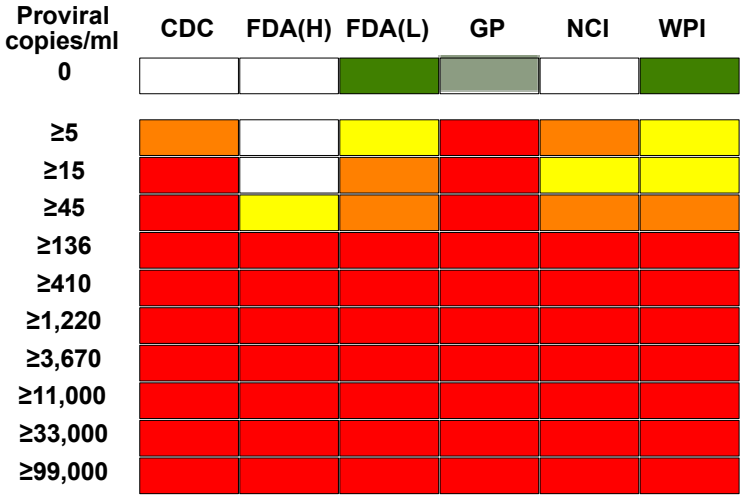
- AABB Bulletin, June 18, 2010 recommends educational materials discouraging individuals with CFS from donating blood
- Fact Sheet (March 2011)
  - http://www.aabb.org/resources/bct/eid/Documents/xmrvfactsheet.pdf
- Table of Published Studies (April 27, 2011)
  - http://www.aabb.org/resources/bct/eid/Documents/xmrvtable.pdf

U.S. National Heart Lung and Blood Institute (NHLBI) Scientific Working Group

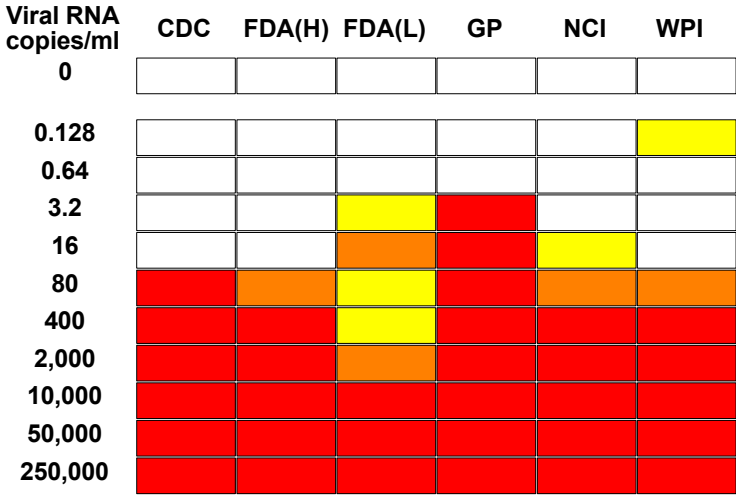
- Analytical NAT panel development:
  - analysis of labs with assays (CDC, FDA, NCI, WPI, BSRI) abilities to find/quantify XMRV from productive cell line in blinded positive and control panel in spiked *whole blood and plasma* samples
- Clinical NAT panel development:
  - Plasma and whole blood panels for XMRV prevalence in 400 blood donors from BSRI (Reno), 25 XMRV pos. CFS patients from WPI, 25 controls (+ and -) each
- Serology validation after nucleic acid
- Epidemiology study design

Phase I Analytical Panel - Results

Whole Blood Panel



Plasma Panel



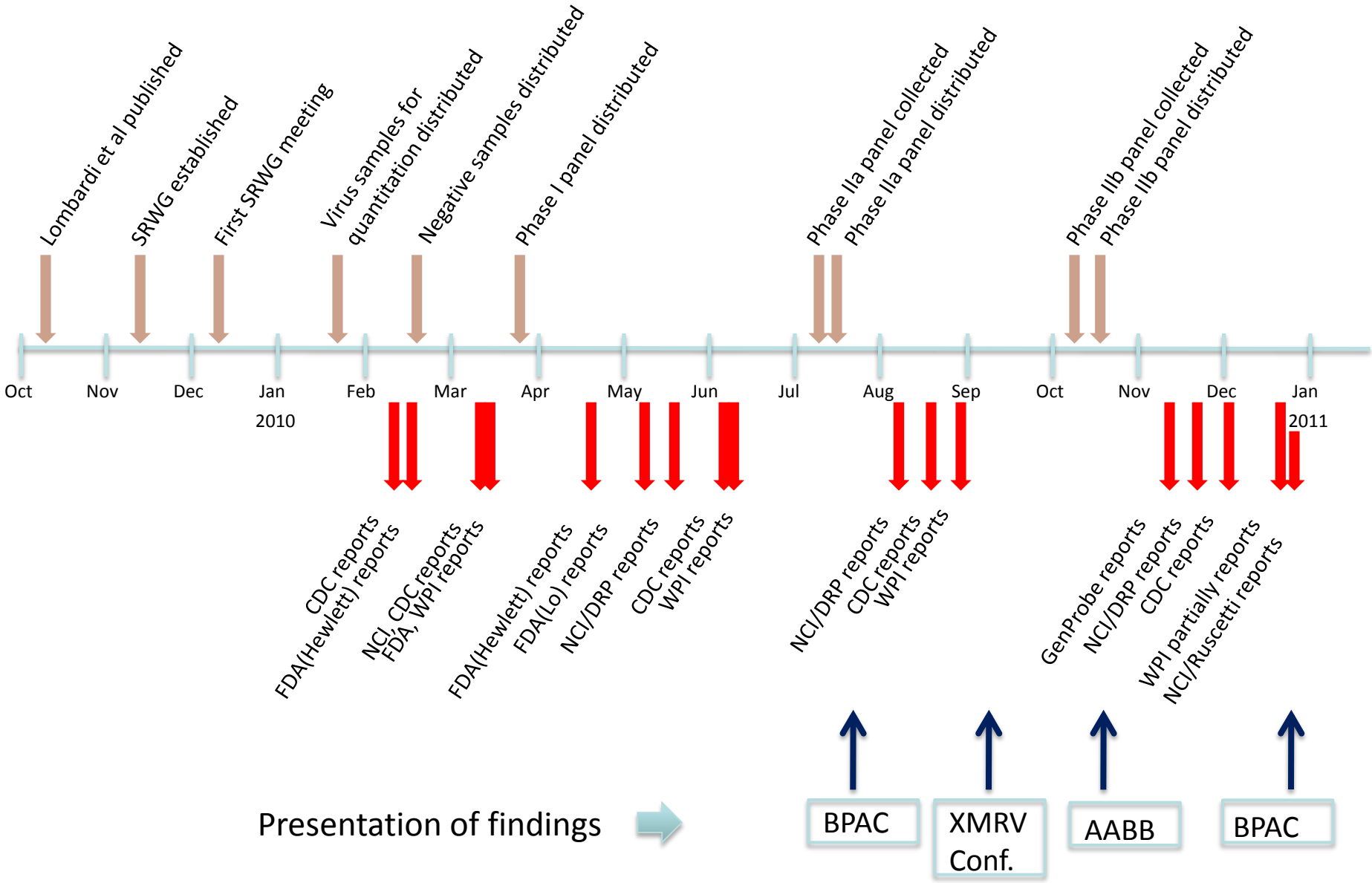
- 0/3 (for dilution series) or 0/6 (for negatives) replicates called positive
- 1/12 negative controls called positive (panels run twice - once each by two different operators)
- 1/6 negative controls called positive (identified as non-specific band of human genomic origin by sequencing subsequent to decoding of results)
- 1/3 replicates called positive
- 2/3 replicates called positive
- 3/3 replicates called positive

Summary of Phase IIb NAT and Antibody Results

Subject ID (description)	NCI	G-P	CDC	WPI	NCI* Ab	CDC Ab
Subject 1 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 1 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Negative	Negative
Subject 1 - PBMC - day 0	Negative	Non reactive	Negative	Positive		
Subject 1 - PBMC - day 2	Negative	Non reactive	Negative	Negative		
Subject 2 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Negative	Negative
Subject 2 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Negative	Negative
Subject 2 - PBMC - day 0	Negative	Non reactive	Negative	Negative		
Subject 2 - PBMC - day 2	Negative	Non reactive	Negative	Positive		
Subject 3 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 3 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 3 - PBMC - day 0	Negative	Non reactive	Negative	Negative		
Subject 3 - PBMC - day 2	Negative	Non reactive	Negative	Positive		
Subject 4 - Plasma - day 0	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 4 - Plasma - day 2	Negative	Non reactive	Negative	Negative	Positive	Negative
Subject 4 - PBMC - day 0	Negative	Non reactive	Negative	Negative		
Subject 4 - PBMC - day 2	Negative	Non reactive	Negative	Negative		
Pedigreed Negative - Plasma - day 0	Negative	Non reactive	Negative	Negative	Negative	Negative
Pedigreed Negative - Plasma - day 2	Negative	Non reactive	Negative	Negative	Positive	Negative
Pedigreed Negative - PBMC - day 0	Negative	Non reactive	Negative	Positive		
Pedigreed Negative - PBMC - day 2	Negative	Non reactive	Negative	Negative		

* Ruscetti

Blood XMRV Scientific Research Working Group Activities



Blood XMRV Scientific Research Working Group

COMMENTARY

The Blood Xenotropic Murine Leukemia Virus–Related Virus Scientific Research Working Group: mission, progress, and plans

Graham Simmons, Simone A. Glynn, Jerry A. Holmberg, John M. Coffin, Indira K. Hewlett, Shyh-Ching Lo, Judy A. Mikovits, William M. Switzer, Jeffrey M. Linnen, and Michael P. Busch for the Blood XMRV Scientific Research Working Group

TRANSFUSION 2011;51:643-653.

Question for the Blood Products Advisory Committee of FDA (December 14, 2010):

Do the scientific data support asking donors about a medical history &/or diagnosis of CFS as a basis for indefinite deferral?

9 yes, 4 no, no abstentions

Hué et al. Retrovirology 2010, 7:111
http://www.retrovirology.com/content/7/1/111

RETROVIROLOGY

RESEARCH Open Access

Disease-associated XMRV sequences are consistent with laboratory contamination

Stéphane Hué[1†], Eleanor R Gray[1†], Astrid Gall[2†], Aris Katzourakis[3], Choon Ping Tan[1], Charlotte J Houldcroft[2], Stuart McLaren[2], Deenan Pillay[1], Andrew Futreal[2], Jeremy A Garson[1], Oliver G Pybus[3], Paul Kellam[1,2*], Greg J Towers[1*]

Abstract

Background: Xenotropic murine leukaemia viruses (MLV-X) are endogenous gammaretroviruses that infect cells from many species, including humans. Xenotropic murine leukaemia virus-related virus (XMRV) is a retrovirus that has been the subject of intense debate since its detection in samples from humans with prostate cancer (PC) and chronic fatigue syndrome (CFS). Controversy has arisen from the failure of some studies to detect XMRV in PC or CFS patients and from inconsistent detection of XMRV in healthy controls.

Results: Here we demonstrate that Taqman PCR primers previously described as XMRV-specific can amplify common murine endogenous viral sequences from mouse suggesting that mouse DNA can contaminate patient samples and confound specific XMRV detection. To consider the provenance of XMRV we sequenced XMRV from the cell line 22Rv1, which is infected with an MLV-X that is indistinguishable from patient derived XMRV. Bayesian phylogenies clearly show that XMRV sequences reportedly derived from unlinked patients form a monophyletic clade with interspersed 22Rv1 clones (posterior probability >0.99). The cell line-derived sequences are ancestral to the patient-derived sequences (posterior probability >0.99). Furthermore, *pol* sequences apparently amplified from PC patient material (VP29 and VP184) are recombinants of XMRV and Moloney MLV (MoMLV) a virus with an envelope that lacks tropism for human cells. Considering the diversity of XMRV we show that the mean pairwise genetic distance among *env* and *pol* 22Rv1-derived sequences exceeds that of patient-associated sequences (Wilcoxon rank sum test: $p = 0.005$ and $p < 0.001$ for *pol* and *env*, respectively). Thus XMRV sequences acquire diversity in a cell line but not in patient samples. These observations are difficult to reconcile with the hypothesis that published XMRV sequences are related by a process of infectious transmission.

Conclusions: We provide several independent lines of evidence that XMRV detected by sensitive PCR methods in patient samples is the likely result of PCR contamination with mouse DNA and that the described clones of XMRV arose from the tumour cell line 22Rv1, which was probably infected with XMRV during xenografting in mice. We propose that XMRV might not be a genuine human pathogen.

Oakes et al. Retrovirology 2010, 7:109
http://www.retrovirology.com/content/7/1/109

RETROVIROLOGY

RESEARCH Open Access

Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences

Brendan Oakes[1,2], Albert K Tai[1], Oya Cingöz[3,4], Madeleine H Henefield[1], Susan Levine[5], John M Coffin[3,4], Brigitte T Huber[1*]

Abstract

Background: In 2006, a novel gammaretrovirus, XMRV (xenotropic murine leukemia virus-related virus), was discovered in some prostate tumors. A more recent study indicated that this infectious retrovirus can be detected in 67% of patients suffering from chronic fatigue syndrome (CFS), but only very few healthy controls (4%). However, several groups have published to date that they could not identify XMRV RNA or DNA sequences in other cohorts of CFS patients, while another group detected murine leukemia virus (MLV)-like sequences in 87% of such patients, but only 7% of healthy controls. Since there is a high degree of similarity between XMRV and abundant endogenous MLV proviruses, it is important to distinguish contaminating mouse sequences from true infections.

Results: DNA from the peripheral blood of 112 CFS patients and 36 healthy controls was tested for XMRV with two different PCR assays. A TaqMan qPCR assay specific for XMRV *pol* sequences was able to detect viral DNA from 2 XMRV-infected cells (~ 10-12 pg DNA) in up to 5 μg of human genomic DNA, but yielded negative results in the test of 600 ng genomic DNA from 100,000 peripheral blood cells of all samples tested. However, positive results were obtained with some of these samples, using a less specific nested PCR assay for a different XMRV sequence. DNA sequencing of the PCR products revealed a wide variety of virus-related sequences, some identical to those found in prostate cancer and CFS patients, others more closely related to known endogenous MLVs. However, all samples that tested positive for XMRV and/or MLV DNA were also positive for the highly abundant intracisternal A-type particle (IAP) long terminal repeat and most were positive for murine mitochondrial cytochrome oxidase sequences. No contamination was observed in any of the negative control samples, containing those with no DNA template, which were included in each assay.

Conclusions: Mouse cells contain upwards of 100 copies each of endogenous MLV DNA. Even much less than one cell's worth of DNA can yield a detectable product using highly sensitive PCR technology. It is, therefore, vital that contamination by mouse DNA be monitored with adequately sensitive assays in all samples tested.

Sato et al. Retrovirology 2010, 7:110
http://www.retrovirology.com/content/7/1/110

RETROVIROLOGY

SHORT REPORT Open Access

An Endogenous Murine Leukemia Viral Genome Contaminant in a Commercial RT-PCR Kit is Amplified Using Standard Primers for XMRV

Eiji Sato[1], Rika A Furuta[2], Takayuki Miyazawa[1*]

Abstract

During pilot studies to investigate the presence of viral RNA of xenotropic murine leukemia virus (MLV)-related virus (XMRV) infection in sera from chronic fatigue syndrome (CFS) patients in Japan, a positive band was frequently detected at the expected product size in negative control samples when detecting a partial *gag* region of XMRV using a one-step RT-PCR kit. We suspected that the kit itself might have been contaminated with small traces of endogenous MLV genome or XMRV and attempted to evaluate the quality of the kit in two independent laboratories. We purchased four one-step RT-PCR kits from Invitrogen, TaKaRa, Promega and QIAGEN in Japan. To amplify the partial *gag* gene of XMRV or other MLV-related viruses, primer sets (419F and 1154R, and GAG-I-F and GAG-I-R) which have been widely used in XMRV studies were employed. The nucleotide sequences of the amplicons were determined and compared with deposited sequences of a polytropic endogenous MLV (PmERV), XMRV and endogenous MLV-related viruses derived from CFS patients. We found that the enzyme mixtures of the one-step RT-PCR kit from Invitrogen were contaminated with RNA derived from PmERV. The nucleotide sequence of a partial *gag* region of the contaminant amplified by RT-PCR was nearly identical (99.4% identity) to a PmERV on chromosome 7 and highly similar (96.9 to 97.6%) to recently identified MLV-like viruses derived from CFS patients. We also determined the nucleotide sequence of a partial *env* region of the contaminant and found that it was almost identical (99.6%) to the PmERV. In the investigation of XMRV infection in patients of CFS and prostate cancer, researchers should prudently evaluate the test kits for the presence of endogenous MLV as well as XMRV genomes prior to PCR and RT-PCR tests.

Robinson et al. Retrovirology 2010, 7:108
http://www.retrovirology.com/content/7/1/108

RETROVIROLOGY

RESEARCH Open Access

Mouse DNA contamination in human tissue tested for XMRV

Mark J Robinson[1†], Otto W Erlwein[1†], Steve Kaye[1], Jonathan Weber[1], Oya Cingoz[2], Anup Patel[3], Marjorie M Walker[4], Wun-Jae Kim[5], Mongkol Uiprasertkul[6], John M Coffin[2], Myra O McClure[1*]

Abstract

Background: We used a PCR-based approach to study the prevalence of genetic sequences related to a gammaretrovirus, xenotropic murine leukemia virus-related virus, XMRV, in human prostate cancer. This virus has been identified in the US in prostate cancer patients and in those with chronic fatigue syndrome. However, with the exception of two patients in Germany, XMRV has not been identified in prostate cancer tissue in Europe. Most putative associations of new or old human retroviruses with diseases have turned out to be due to contamination. We have looked for XMRV sequences in DNA extracted from formalin-fixed paraffin- embedded prostate tissues. To control for contamination, PCR assays to detect either mouse mitochondrial DNA (mtDNA) or intracisternal A particle (IAP) long terminal repeat DNA were run on all samples, owing to their very high copy number in mouse cells.

Results: In general agreement with the US prevalence, XMRV-like sequences were found in 4.8% of prostate cancers. However, these were also positive, as were 21.5% of XMRV-negative cases, for IAP sequences, and many, but not all were positive for mtDNA sequences.

Conclusions: These results show that contamination with mouse DNA is widespread and detectable by the highly sensitive IAP assay, but not always with less sensitive assays, such as murine mtDNA PCR. This study highlights the ubiquitous presence of mouse DNA in laboratory specimens and offers a means of rigorous validation for future studies of murine retroviruses in human disease.

Absence of XMRV and other MLV-related viruses in patients with Chronic Fatigue Syndrome

Running Title: Absence of XMRV and other MLV-related viruses in CFS

Clifford H. Shin[1]
Lucinda Bateman[2]
Robert Schlaberg[1]
Ashley M. Bunker[3]
Christopher J. Leonard[1]
Ronald W. Hughen[4]
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J. Virol. doi:10.1128/JVI.00693-11

- qPCR and PCR on buffy coat and whole blood
- ELISA and Western blot on plasma
- Virus culture
- 100 CFS and 200 matched controls
- 14 CFS samples from Reno study

Sciencexpress Report

No Evidence of Murine-Like Gammaretroviruses in CFS Patients Previously Identified as XMRV-Infected

Sciencexpress / www.sciencexpress.org / 31 May 2011 / Page 1/ 10.1126/science.1204963

Zhou,[3,4] John Hackett Jr.,[6] Xiaoxing Qiu,[6] Ka-Cheung Luk,[6] Gerald Schochetman,[6] Allyn Knox,[1] Andreas M. Kogelnik,[2] Jay A. Levy[5]*

Sciencexpress Report

Recombinant Origin of the Retrovirus XMRV

Tobias Paprotka,[1]* Krista A. Delviks-Frankenberry,[1]* Oya Cingöz,[3,4]* Anthony Martinez,[5] Hsing-Jien Kung,[5,6] Clifford G. Tepper,[5] Wei-Shau Hu,[2] Matthew J. Fivash Jr.,[7] John M. Coffin,[3,4] Vinay K. Pathak[1†]

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Sciencexpress / www.sciencexpress.org / 31 May 2011 / Page 1 / 10.1126/science.1205292

On the origin of XMRV

1992 Patient CWR22 CaP (No DNA/RNA)

CWR22 xenograft in nude mice

1992 CWR22 3rd pass. (gDNA)
CWR22 7th pass. (gDNA)
CWR22 unk. pass. (No DNA/RNA)

preXMRV 1 + 2
No XMRV

preXMRV 1 + 2
recombination

1996 CWR22 (total nucleic acid)
2152, 2524, 2272, 2274

1999 Cell line 22Rv1 (gDNA)

2001 Cell line CWR R1 (gDNA)

Late xenografts + cell lines XMRV positive

Pathak et al. CROI 2011.

Sciencexpress Letter

Editorial Expression of Concern

Bruce Alberts

Editor-in-Chief

Published online 31 May 2011;
10.1126/science.1208542

In the issue of 23 October 2009, *Science published the Report "Detection of an infectious retrovirus, XMRV, in blood cell of patients with chronic fatigue syndrome," a study by Lombardi et al. purporting to show that a retrovirus called XMRV (xenotropic murine leukemia virus-related virus) was present in the blood of 67% of patients with chronic fatigue syndrome (CFS) compared with 3.7% of healthy controls (1). Since then, at least 10 studies conducted by other investigators and published elsewhere have reported a failure to detect XMRV in independent populations of CFS patients. In this week's edition of Science Express, we are publishing two Reports that strongly support the growing view that the association between XMRV and CFS described by Lombardi et al. likely reflects contamination of laboratories and research reagents with the virus.*

The authors of the Lombardi study believe that it is premature to conclude that the negative studies are accurate or change the conclusions of the original studies and we fully agree," said Annette Whittemore, President of the Whittemore Peterson Institute. (WPI Press release, May 31, 2011)

Ongoing Studies

- NHLBI will continue to support the **XMRV blood study**. Results are expected in the Fall of 2011.,
- **The Lipkin** laboratory-based study is designed to rigorously evaluate whether the presence of XMRV/MLV nucleic acids in the blood is associated with CFS. Researchers, working with clinicians in six regions across the United States, will compare blood and plasma samples from patients diagnosed with CFS to samples from healthy people who have not been diagnosed with CFS and who are matched to the CFS patients by age, sex, and geography. Study results are anticipated later this year.

Proposed REDS-III XMRV Study

Supported by NHLBI

- **Blood donor prevalence over four decades using total of 10,000 samples from 4 NIH repositories:**
  - Transfusion Transmitted Viruses Study (TTVS)
  - Transfusion Safety Study (TSS)
  - Retrovirus Epidemiology in Donors Study (REDS) General Leukocyte and Plasma Repository (GLPR)
  - Viral Activation by Transfusions Study (VATS)
  - REDS Allogeneic Donor and Recipient Repository (RADAR)

- **Transfusion-Transmission using TTVS, VATS and RADAR repositories**
  - Rates of transfusion-transmission and correlations of transmission with viral and serologic findings in XMRV+ donations
  - Effect of routine blood filtration (leukoreduction) and blood component storage period on transmission
  - Limited data on mortality and morbidity

- **Utilize Abbott and Gen-Probe high-throughput screening assays for serology and NAT**

Thank you!

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