DEMYSTIFYING MEDICINE

DE-DUCTION

MYSTIFYING MEDICINE

FROM CONFUSION TO
UTTER INCOMPREHENSION

DILEMMA

- The viruses, XMRV and pMLV , have not been accepted as human invaders/pathogens by most of the scientific community
- Disease associations between these agents and prostate cancer and chronic fatigue syndrome (CFS) have not as yet progressed to establish causality
- The disease, chronic fatigue syndrome, does not have an established etiology and is not universally accepted as being a medical, rather than a psychological condition

THE ANSWER IS……

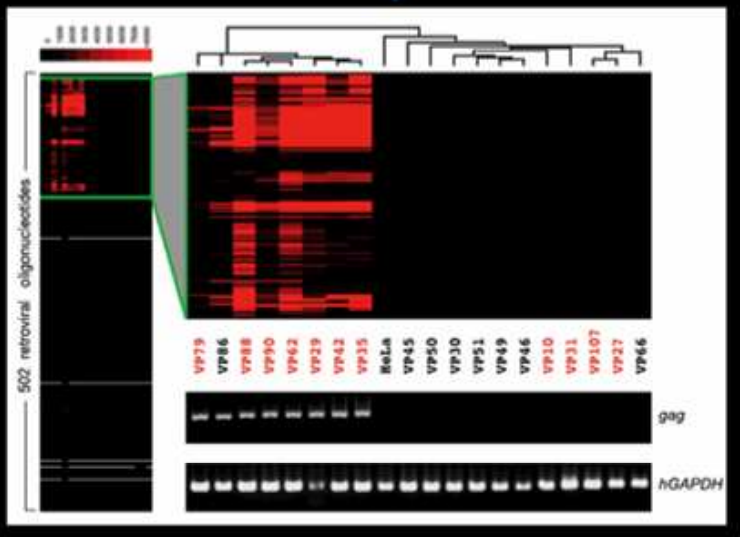
NOT KNOWN

Retrovirus Discovery in Prostate Cancer

Prostate Cancer Tissue

RNA/DNA

Virochip



Hybridization Pattern & RT- PCR

Red VP= homozygous for QQ RNase L Variant

Urisman, et al. PLoS Pathogens 2006

Association of XMRV with RNase L genotype in 86 prostate cancer cases

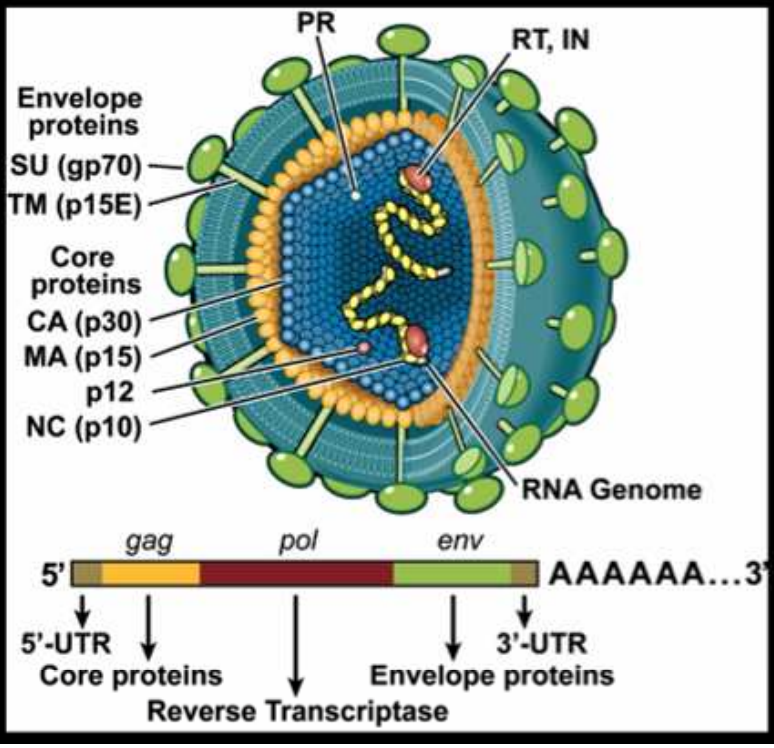
RNase L genotype	XMRV positive	
Homozygous (RR)	1/52	1.9%
Heterozygous (RQ)	0/14	0%
Homozygous (QQ)	9/20	45%
Total	10/86	11.6%

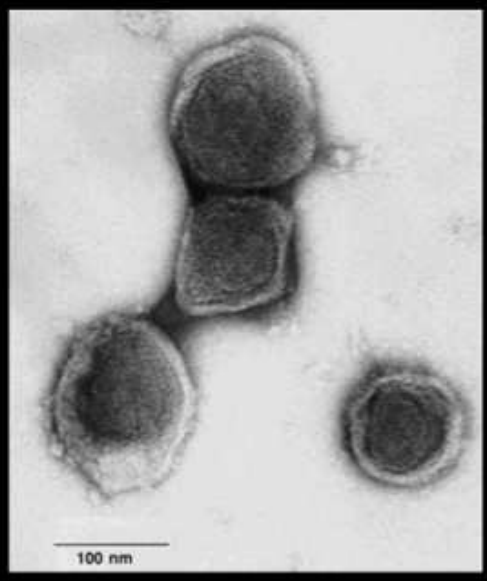
P<.00002 for association of QQ and XMRV

Urisman et al. *PLoS Pathogens*. 2006

XMRV

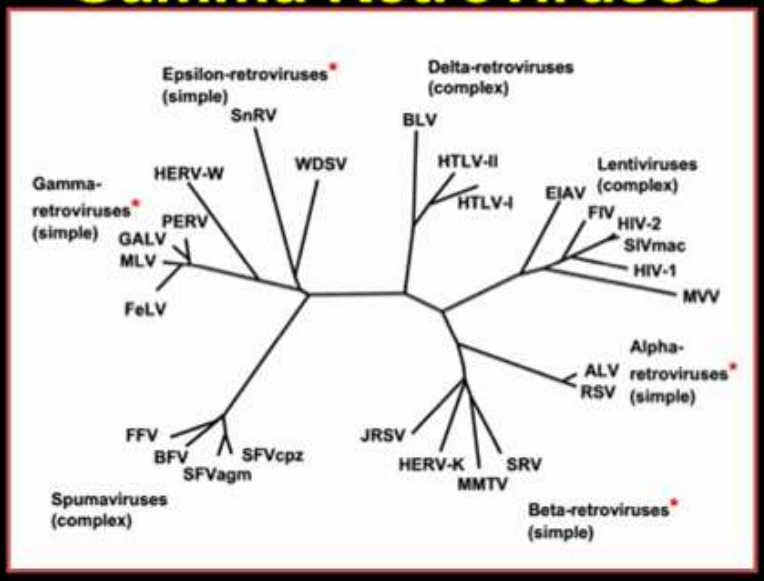
Xenotropic **M**urine Leukemia Virus-**R**elated **V**irus





Urisman et al., *PLoS Pathogen*, 2006; Dong et al., PNAS, 2007

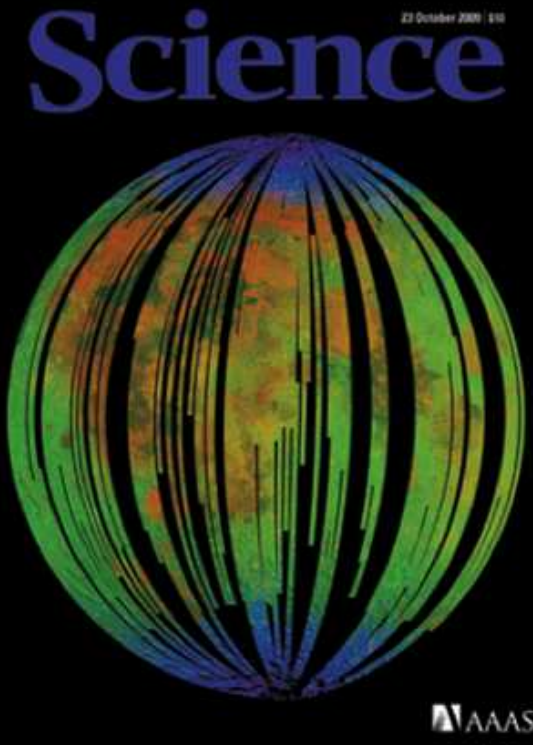
Gamma Retroviruses



- Includes Murine Leukemia Virus, Feline Leukemia Virus
- Simple retroviruses with no accessory genes
- Can be exogenous or endogenous
- Infection leads to sustained viremia
- Induce solid cancers, immune dysfunction and neurological disorders
- MLV gene therapy vectors induced leukemia in SCID-X1 patients

Why look for XMRV in CFS?

- CFS onset often temporally associated with apparent acute infection
- Apparent outbreaks of CFS
- Cohort studies of acute infections suggest that a proportion will develop CFS
- Alterations of RNAse-L pathway seen in both CaP and CFS
- Patients with CFS often have active herpesvirus infections suggesting an underlying immune deficiency



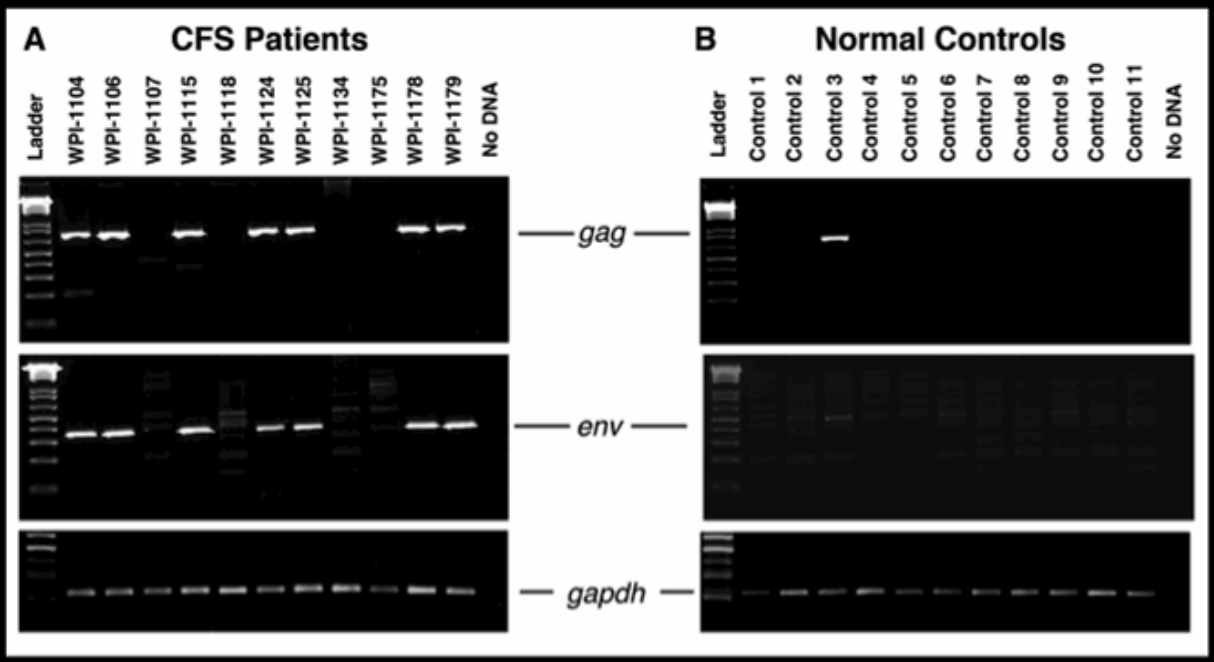
Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

Vincent C. Lombardi,[1]* Francis W. Ruscetti,[2]* Jaydip Das Gupta,[3] Max A. Pfost,[1] Kathryn S. Hagen,[3] Daniel L. Peterson,[3] Sandra K. Ruscetti,[4] Rachel K. Bagni,[5] Cari Petrow-Sadowski,[6] Bert Gold,[7] Michael Dean,[7] Robert H. Silverman,[3] Judy A. Mikovits[1]†

Chronic fatigue syndrome (CFS) is a debilitating disease of unknown etiology that is estimated to affect 17 million people worldwide. Studying peripheral blood mononuclear cells (PBMCs) from CFS patients, we identified DNA from a human gammaretrovirus, xenotropic murine leukemia virus–related virus (XMRV), in 68 of 101 patients (67%) as compared to 8 of 218 (3.7%) healthy controls. Cell culture experiments revealed that patient-derived XMRV is infectious and that both cell-associated and cell-free transmission of the virus are possible. Secondary viral infections were established in uninfected primary lymphocytes and indicator cell lines after their exposure to activated PBMCs, B cells, T cells, or plasma derived from CFS patients. These findings raise the possibility that XMRV may be a contributing factor in the pathogenesis of CFS.

Presence of XMRV Sequences in Human DNA

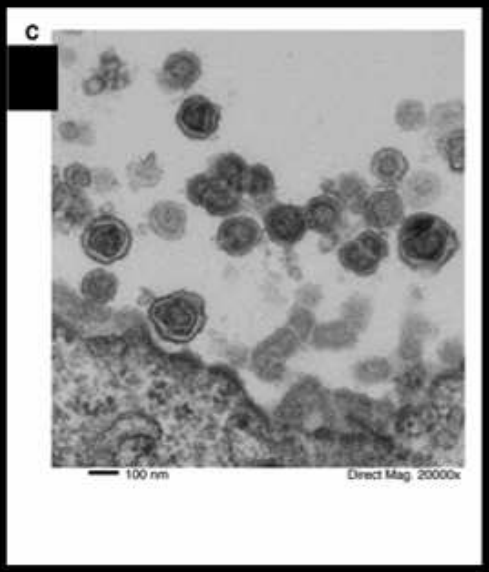
Single round PCR:

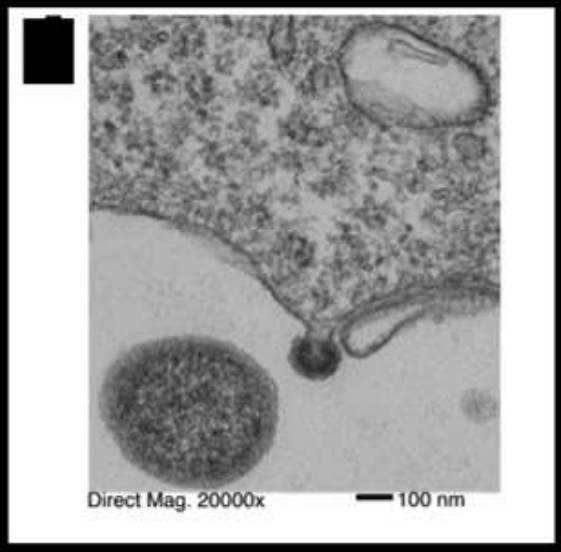


Nested PCR:

CFS: 68/101 (67%) **Controls: 12 /320 (3.75%)**

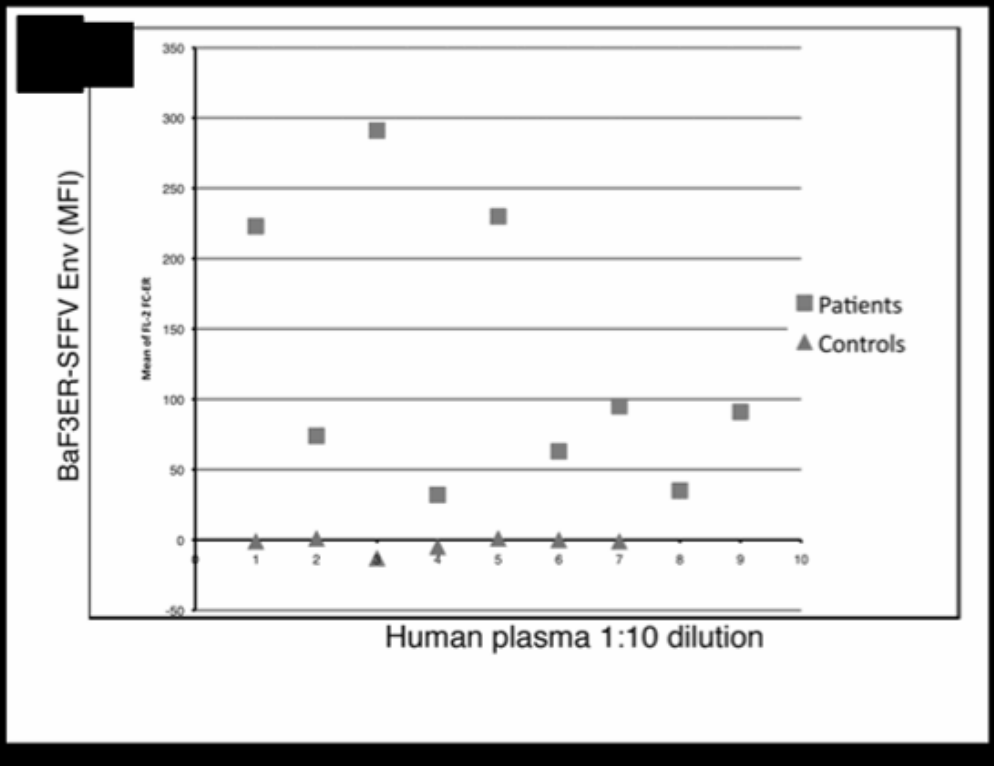
Transmission Electron Micrograph of C-type Retrovirus Particles Transmitted from CFS Plasma to LNCaP





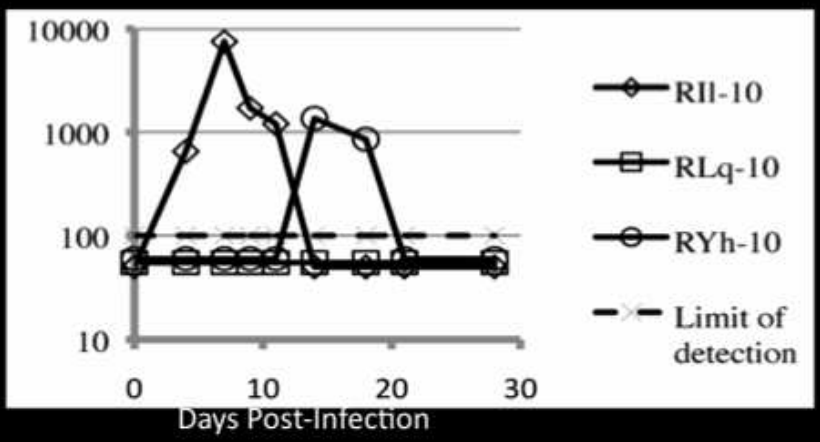
21/25 (84%) plasmas from XMRV+ CFS patients transmitted infection to LNCaP cells in culture

Detection of Antibodies to XMRV Env in CFS Patient Plasma

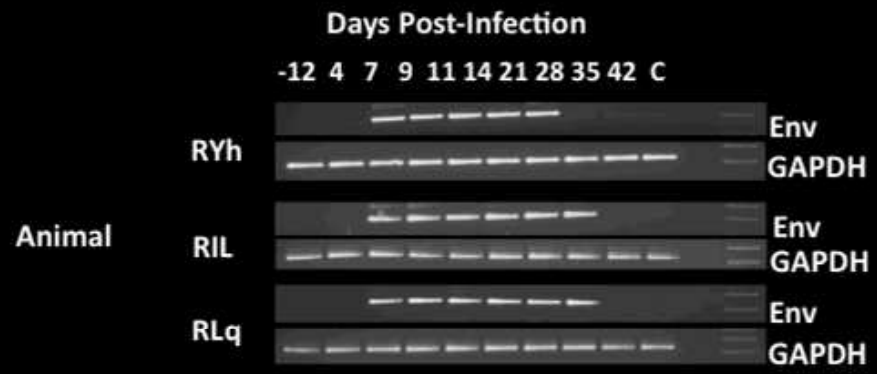


Productive XMRV Infection of Rhesus Macaque

Viremia:



PBMC Infection:



XMRV and CFS: DISCORDANT RESULTS

	Site	CFS	Controls
		# Pos/Tested	# Pos/Tested
Lombardi , Science 2009	US (WPI)	68/101 (67%)	8/218 (3.7%)
Erlwein PLosOne 2010	UK	0/186	
Groom et al., Retrovir. 2010	UK	0/142	0/157
Van Kuppefeld BMJ 2010	Neth.	0/32	0/43
Switzer et al Retrovir. 2010	US (CDC)	0/50	0/56
Kuntsman AIDS 2010	US (MACS)	0/996	

Lo S-C, Pripuzova N, Bingjie I, Hung G-C, Wang R, Alter HJ

Detection of MLV-related virus gag gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors

PNAS 2010;107:15874-79

Group	Sequence Confirmed Gag Gene Pos.
CFS Patients	32/37 (86%)
Blood Donors	3/44 (6.8%)

SUPPORTING DATA REPORTED AT NIH XMRV WORKSHOP

STUDY	SAMPLE SOURCE	NO. TESTED	N0. (%) POSITIVE XMRV/MLV	No. (%) ANTIBODY XMRV
Mikovits	England	50	24 (48%)*	31 (63%)*
Hanson	New York State	20	11 (55%)**	
Ruscetti	Various	101		82 (82%)

*39/50 (78%) pos. in DERSE culture assay ; antibody in 4% controls
** None had XMRV sequences; all had polytropic sequences

Contamination results in false positivity when detecting XMRV by PCR

- Evidence for contamination of human samples with mouse DNA
  - Robinson MJ, et al. Mouse DNA contamination in human tissue tested for XMRV. Retrovirology 2010; 7:108
  - Oakes B, et al. Contamination of human DNA samples with mouse DNA can lead to false detection of XMRV-like sequences. Retrovirology 2010; 7:109
  - Evidence for contamination of PCR reagents with MLV RNA
  - Sato E, et al. An endogenous murine leukemia viral genome contamination in a commercial RT-PCR kit is amplified using standard primers for XMRV. Retrovirology 2010; 7:110
  - Phylogenetic support for the contamination of the previous studies of XMRV
  - Hue S, et al. Disease-associated XMRV sequences are consistent with laboratory contamination. Retrovirology 2010; 7:111

FUTURE

DIRECTIONS

Residual Question #1

- Is XMRV/MLV actually replicating in patients with CFS or is the finding of gene sequences due to contamination of the sample either in the collection process or in the testing laboratory?
  - The diversity of sampling sites, the testing of vials never previously entered after initial separation, finding the agent in the same patients 15 years later and highly sensitive assays to exclude mouse contamination mitigate against this premise.

Residual Question #2

- If the virus is in the patient, as suggested by the simultaneous presence of antibody and the ability to culture XMRV from the WPI cohort, was it there prior to the onset of CFS or did it occur after the fact as an opportunistic infection or as a contaminant carried by a parenteral treatment modality unique to some CFS populations?
- In essence, is the infection primary (causative) or secondary ?

Proposed Resolution

- NHLBI and NIAID are conducting separate studies to assess the reproducibility and specificity of published findings.
- Classic cases of CFS are being identified in 6 US centers. With informed consent, large volume samples will be obtained and sent to a coordinating center .
- WB, plasma and sometimes PBMCs will be aliquoted and coded in duplicate or triplicate for distribution to testing labs
- Labs will use their published techniques including PCR, nested PCR, TMA and sometimes culture and antibody assays
- Codes will be broken at the coordinating centers and data will be published

Possible Outcomes of Panel Testing

- If XMRV/MLV cannot be found in these pedigreed patient samples by labs who previously reported such findings, then the original findings will be considered unconfirmed and contamination will be suspect
- If the panels show the consistent and specific finding of XMRV/MLV in this diverse and expanded CFS patient populations, then the published findings will have been confirmed.
- Confirmation will not establish causality, but will resolve the contamination issue
- Causality will be difficult to prove and may depend on controlled clinical trials with anti-retroviral agents