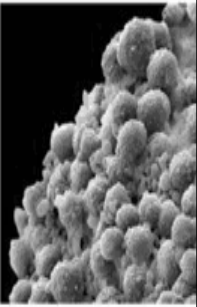


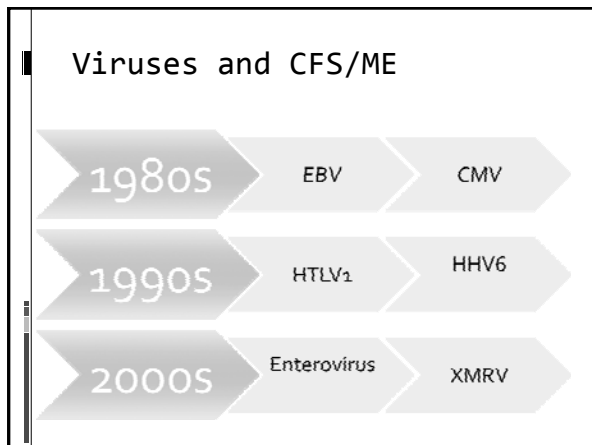
**Gulf War Illness
And XMRV :**

GWI Implications



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Gulf War Illness	Chronic Fatigue Syndrome***
Fatigue	Disabling fatigue
Depression	Exercise induced relapse
Arthralgia	Arthralgia
Myalgia	Myalgia
Sleep disturbance	Non restorative sleep
Cognitive dysfunction	Cognitive dysfunction
Headache	Headache
Diarrhea, intermittent	Sore throat
Wheezing, Cough, Chest pain, Shortness of breath*	Tender lymph nodes
Weight loss, low grade fever**	



A new virus: XMRV

- XMRV – previously described in up to 40% of prostate CA samples in two US surveys, but not in a European study
- 3-4% of normals in US are seropositive in 3 published studies
- Recent Science article (Lombardi et al Oct 8 09) found 67% of CFS subjects both seropositive and PCR positive.

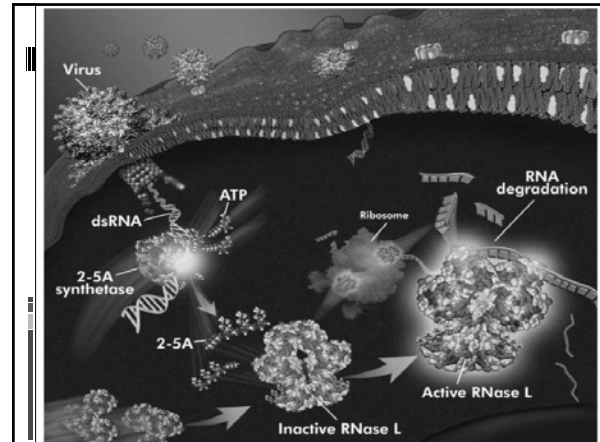
- Human strain very recently discovered (2005)
- Initial work by oncologists working in prostate cancer
- Result have varied, but several studies have shown up to 40% of prostate cancers have a strain of XMRV in samples. European (Germany, Ireland) groups have seen none.
- Reasons may include assay variability, population variability – lessons for CFS/ME

XMRV – a newly discovered retrovirus

- the pathogenicity of the virus and its distribution in the human population are not yet know
- XMRV was discovered by investigators at the Cleveland Clinic (Silverman et al) and UCSF (Derisi et al) in men with a deficiency in the IFN pathway gene encoding RNase L
- Further studies suggest a subset of men with prostate cancer have XMRV infection, though this has only been seen in US studies, and has not been confirmed in studies from Germany and the UK

XMRV – a newly discovered retrovirus

- Dr Robert Silverman's group found that RNase L is required for a complete IFN antiviral response.
- They also have shown the presence of a functional androgen response element (ARE) in the U₃ region of the XMRV LTR.
- XMRV integration into prostate cells could cause androgen-stimulation of pro-inflammatory genes and proto-oncogenes leading to cancer.



Tropism

Qui et al CROI 2010

XMRV inoculation resulted in low transient plasma viremia, although proviral DNA persisted in circulating peripheral blood mononuclear cells for several weeks.

Of interest, the earliest leukocyte targets were CD₄⁺ T cells and NK cells followed by CD₈⁺ enriched T and CD₂₀⁺ enriched B cells (50% positive); CD₁₄⁺ monocytes were negative.

Animals sacrificed at the acute stage showed evidence of viral replication in spleen, lung, lymph nodes and liver.

In contrast, sacrifice of 2 animals at 19 weeks post XMRV re-inoculation showed greater dissemination of XMRV DNA and RNA in various organs including the GI and urinary tract as well as in vaginal tissue of the one female.

Tropism

Qui et al (paper 151) CROI 2010

By Western blot analysis, all 3 chronically infected macaques developed antibody responses to env and gag proteins. Preliminary results showed evidence of detectable reactivity to all 3 antigens in a low proportion (~0.1%) of US blood donors.

Conclusions: These data suggest that lymphocytes are a primary target for replication persistence (low grade replication) of XMRV in the absence of detectable plasma viremia.

This study identified specific serological markers useful for detection of antibodies induced by XMRV infection. The prototype antibody assays will facilitate large-scale epidemiological studies.

Tropism

- Sharma et al (paper 150 LR; CROI 2010) in a study of Macaques, showed a wide dissemination of replicating virus even when the plasma viral load was undetectable.
- Of interest was the finding that isolated lymphoid cells and primarily CD₄⁺ T cells were found positive in most lymphoid organs including spleen, lymph nodes, and gastrointestinal tract, while in lung, XMRV⁺ cells exhibited a macrophage morphology.
- The frequency of infected cells appeared to decrease in spleen while increasing in the gastrointestinal tract from acute to chronic infection.

Tropism

- XMRV infection was not restricted to bone marrow derived cells, but showed distinct target specificities in various organs.
- Foci of infected epithelial cells were detected in prostate, seminal vesicles and epididymis while XMRV⁺ cells in the testes were interstitial.
- In the lone female animal, XMRV⁺ epithelial and fibroblast like cells were detected in the vagina and cervix suggesting that the virus may be transmitted sexually.
- While XMRV dissemination was complete at day 6 post infection, the prostate was positive only during the acute infection in these healthy animals, while other reproductive organs were similarly positive during the chronic phase.

Detection of Infectious Xenotropic MuLV-Related Virus (XMRV) in Blood Cells From Patients With Chronic Fatigue Syndrome

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CFS Study Cohort Reported in Science:

- Study cohort from the WPI national repository.
- Repository samples include samples from NV, CA, OR, FL, NC and NY as well as international CFS patients.
- Repository inclusion criteria:
 - CFS diagnosis, regardless of severity
 - 19-75 years of age
- Study characteristic:
 - 67% women, reflecting gender incidence of CFS
 - Mean age: 55
 - 320 control samples from same geographic locations

XMRV in CFS/ME

It is a different strain than that seen in mice
Most viruses vary considerably within a strain, reflecting accumulation of mutations that happen over time – this virus does not vary much, suggesting from an evolution viewpoint it is relatively new to humans, possibly in the last century.

From an individual viewpoint, the virus may not be replicating very fast, which might suggest quite different treatment strategies

Presence of XMRV Sequences in Human DNA

A CFS Patients

Leader
WPI-1104
WPI-1107
WPI-1115
WPI-1118
WPI-1124
WPI-1125
WPI-1134
WPI-1175
WPI-1178
WPI-1179
No DNA

B Normal Controls

Leader
Control 1
Control 2
Control 3
Control 4
Control 5
Control 6
Control 7
Control 8
Control 9
Control 10
Control 11
No DNA

gag
env
gapdh

68/101 (67%) 12/320 (3.75%)

These results are representative of the 101 patients tested. They reflect the presence of virus (DNA PCR).

Transmission Electron Micrograph of C-type Retrovirus Particles Transmitted from CFS patient T-cells to LNCaP

Infectious whole virus budding from the cell membrane

Phylogenetic analysis revealed that XMRV isolates from prostate cancer and CFS form a distinct branch within non ecotropic MLVs

WPI-1104
VP62
WPI-1106
WPI-1178
VP35
VP42

- Not a mouse contaminant
- XMRV is a new human retrovirus
- Not prostate cancer XMRV

- **Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome.** Lombardi VC, Ruscetti FW, Gupta JD, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Silverman RH, Mikovits JA. *Science* 8 October 2009. 1179052.
- **Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome.** Erlwein O, Kaye S, McClure MO, Weber J, Willis G, Collier D, Wessley S, Cleare A. (2010) *PLoS ONE* 5(1):e8519. doi:10.1371/journal.pone.0008519
- **Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome.** Groom HCT, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, Gow JW, Mattes FM, Breuer J, Kerr JR, Stoye JP, Bishop KN. *Retrovirology*. 15 February 2010. 10.1186/1742-4690-7-10

Adapting to the host

Evade detection by the immune system in order to survive. (e.g. low VL, intracellular infection, protected compartments)

Sabotage the immune response.:

- French investigators showed that retroviruses, including XMRV, contain an immunosuppressive domain (ISD) in envelope protein.
- This ISD allows the virus to infect the cell but prevents the body from mounting an immune response and developing neutralizing antibodies to the virus.

Retroviral infection in vivo requires an immune escape virulence factor encrypted in the envelope protein of oncoretroviruses. Schlecht-Louf G, Renard M, Mangeney M, Letzelter C, Richaud A, Ducos B, Bouallaga I, Heidmann T. *Proc Natl Acad Sci U S A*. 2010 Feb 8.

Testing for XMRV

- Tests to identify XMRV are new, imperfect and not standardized,
- PCR (polymerase chain reaction) identifies the viral DNA. (amplifies DNA/RNA, very sensitive and subject to contamination)
- Antibodies that are produced by the host to fight the virus can be measured. Can be nonspecific or confounded. Proves only prior infection, not specific for active or recurrent infection.
- The virus can be collected, put in a new medium, and cultured. Culturing viruses is time consuming and technically difficult, but is the "gold standard"
- The virus can also be seen by electron microscopy budding from cultured cells

Testing for XMRV

- U.S. Department of Health and Human Services [Blood XMRV Scientific Research Working Group](#) is developing analytical panels that will allow multiple laboratories to standardize methods to optimize sensitive detection of XMRV proviral DNA and viral RNA.
- Once methods are standardized, these same laboratories plan to test coded panels of blood samples obtained primarily from healthy blood donors and from CFS patients who have been reported to be positive for XMRV.

Questions:

- What cell types are infected?
- How is XMRV transmitted?
- Do infected individuals make an immune response?
- Does XMRV infection alter the risk of cancer development in infected individuals?
- Can we develop immune based therapies for CFS based on XMRV?
- Will existing antivirals work in this illness?

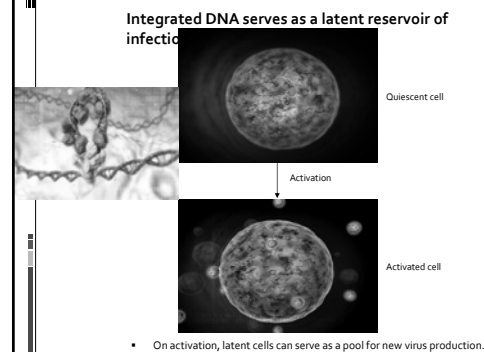
Question:

- Is XMRV participating in Gulf War Illness?

XMRV in GWI

- Study design:
- 35 GWI subjects 35 GW era veteran controls with extensive immune data
- Antibody (Western blot)
- PCR
- Culture
- Split samples coded and sent to 2 labs
- (Richard Sutton, New Haven VAMC
- Judy Mikowitz, Vince Lombardi WPI/Reno)

Active Virus vs. Latent Reservoir



The role of other viruses

- Often a virus is far more damaging when another virus is also at work "co-infection"
- Herpes family viruses have been shown to increase the severity of HIV infection and the immunologic damage
- A very real possibility in CFS/ME or GWI that if the XMRV work is validated, the relationship to HHV6 and EBV will need to be established

Conclusion

- The recent work linking XMRV to CFS/ME raises the question of a role in GWI
- The known tropism to NK, T, B, macrophage, lung, liver, prostate is intriguing given the abnormalities seen in GWI
- Controversy will surround this work until standard methods are developed and shared among investigators
- None the less, an early study of GWI is underway

Miami VAMC/UM GWI and CFS Research and Clinical Center- Research Protocols

- XMRV in GWI – pilot study (VA)
- GWI and CFS Gene Array studies (VA)
- System Biology Approach to Understanding GWI, submitted (DOD OCMR)
- GWI longitudinal study – biomarker discovery (VA)
- Dynamic Modeling in GWI (DOD)
- Telehealth SMART Energy Study CFS (CBT) (NIH)
- Pathogenesis of NK cell defect in CFS (NIH)
- Natural history of CFS (Foundation)
- CFS Biomarker discovery project (NIH)

Collaborators

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