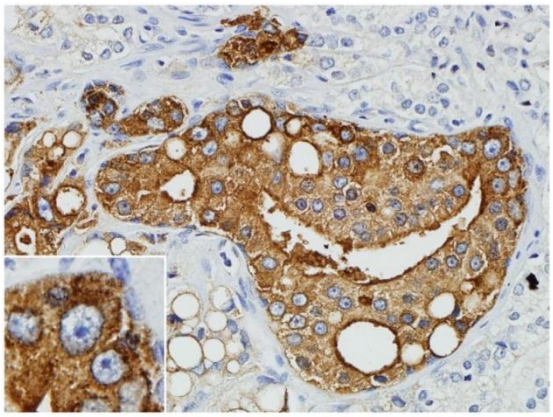
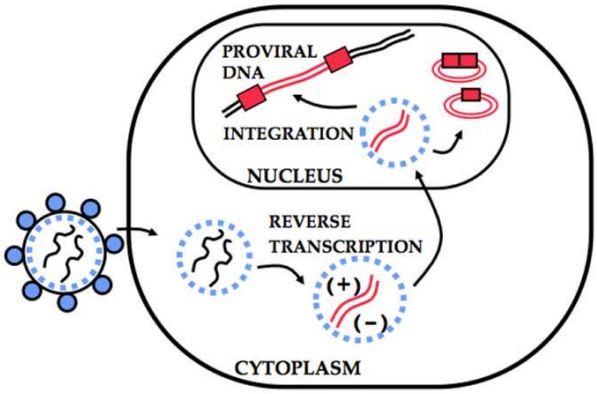
Vrije Universiteit Brussel

XMRV, a recently discovered human retrovirus: What do we know about it?

June 9th, 2011, 16:00 -19:00
Auditorium Brouwer
Faculty of Medicine and Pharmacy
Vrije Universiteit Brussel (Free University of Brussels)
Chair: Kenny De Meirleir M.D., PhD (Vrije Universiteit Brussel)





Francis W. Ruscetti PhD (National Cancer Institute, USA)
XMRV: What do we know?

Judy A. Mikovits PhD (WPI, University of Nevada, USA)
XMRV and other gammaretroviruses (HGRVs) in neuro-immune diseases.

Maureen Hanson PhD (Cornell University, USA)
Assays for MLV-like virus sequences in a New York CFS cohort.

Cecilia Cabrera PhD (IrsiCaixa Foundation, Badalona, Spain)
Infection of Xenotropic murine leukemia virus-related virus in human lymphoid tissue.

Disclaimer:

Although this summary has been made with great care, misinterpretations cannot be excluded. This document focuses on remarkable issues and new insights, an is not intended to summarize the four presentations integrally.

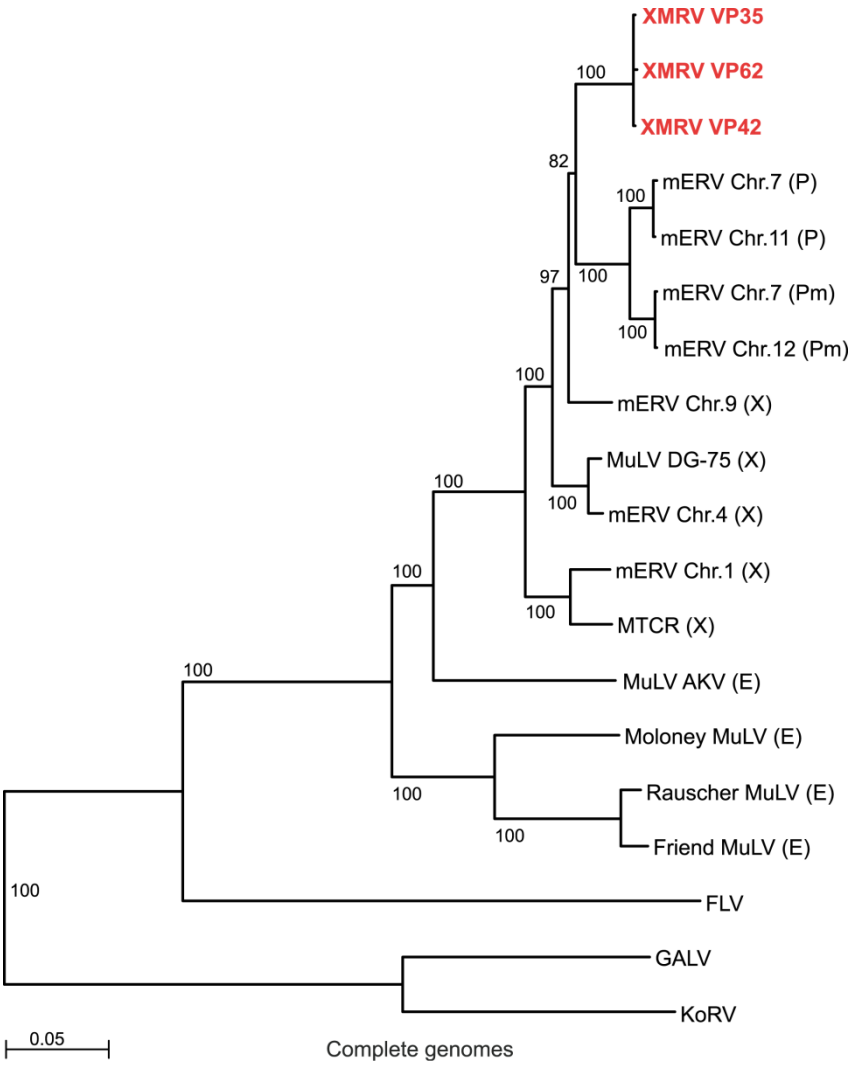
Summaries of the lectures can also be found at another site: click here.

Francis W. Ruscetti PhD (National Cancer Institute, USA)
XMRV: What do we know?

Frank Ruscetti, introduced as the father of retroviruses, is married with Sandra Ruscetti (Head of the Retroviral Pathogenesis Section, Laboratory of Cancer Prevention), one of co-authors of the Lombardi/Mikovits Science study with and one of the two men who first isolated HTLV in Robert Gallo's lab.

He outlined the course of events with relation to XMRV, from the discovery of XMRV in prostate cancer in men with a specific RNase-L allele (R462Q variant) by Silverman et al. to the recent controversy and contamination issues.

Ruscetti talked about the three strains of "XMRV" isolated by Silverman et al. (vp35, vp62 en vp42), the discovery of other MLV variants later on and the relation between ecotropic MLV (MLV capable of infecting mouse cells in culture), xenotropic MLV/"XMRV" (infecting non-mouse species) and (modified) polytropic MLV (infecting a range of hosts including mice). How xenotropic/polytropic MLV jumped species (from mice to men) is unknown. MLV can cause immune deficiency, neurodegenerative diseases and cancer in mice.



Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog 2(3): e25. doi:10.1371/journal.ppat.0020025. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, Malathi K, Magi-Galluzzi C, Tubbs RR, Ganem D, Silverman RH, DeRisi JL.

There are 4 possible mechanisms for viral oncogenesis (cancer induced by viruses): insertional mutagenesis, a pro-inflammatory environment induced by the viral infection, oncogenic (viral) proteins and immunosuppression.

Ruscetti discussed several XMRV/MLV landmark studies about

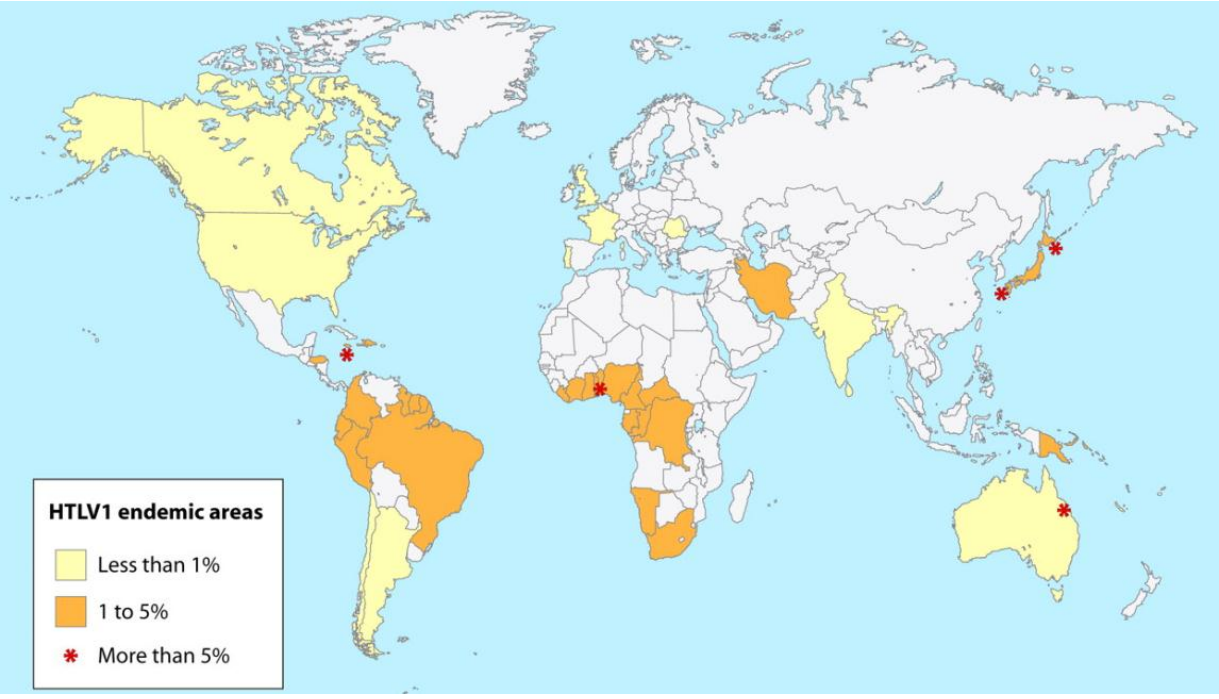
- the XPR-1 receptor enabling XMRV to infect cells: click here,
- the proviral integration site preferences: click here,
- the observation that XMRV is present in malignant prostatic epithelium and is associated with prostate cancer: click here,
- the presence of XMRV RNA in urine of prostate cancer patients: click here,
- fluorescence in situ hybridization (FISH), an alternative diagnostic method for detecting and/or confirmating XMRV infection: click here, and
- the direct isolation of XMRV/MLV in the plasma of ME/CFS patients by immunoprecipitation with anti-X-MLV-antibodies by Mikovits et al.

The macaques studies by Villinger et al. have shown that plasma XMRV/MLV RNA can have many genetic variations (in comparison with the vp62 strain), in contrast with DNA of "XMRV/MLV" in peripheral mononuclear blood cells.

Ruscetti proposed three possible reasons for the negative studies:

- XMRV is not associated with disease!
- XMRV detection is very sensitive to the processing methods and laboratory techniques (PCR: variations in sequences/strains, serology)
- There is a specific geographical distribution of XMRV/MLV infections as also seen in the case of HTLV (next page).

The pathogenesis of HTLV could give clues to the consequences of XMRV infection: HTLV infection is asymptomatic in most people and only 5-8% have a life time risk of adult T cell leukemia or an inflammatory syndrome.



In chronic disease viruses, e.g. HIV infection, seldom come alone (War and peace between microbes: HIV-1 interactions with coinfecting viruses. Lisco A, Vanpouille C, Margolis L. Cell Host Microbe. 2009 Nov 19;6(5):403-8.

Whether immune deficiency results into XMRV and other infections (EBV, HHV-6, enteroviruses etc.) or XMRV infection induces immune deficiency is yet unknown. Future studies should focus on the role of the host response to infection in the pathogenesis (like in the case of HPV and HTLV, see above).

The things we don't know, according to Ruscetti:

- The role of "XMRV": cause? coincidence? passenger virus? pathogenicity?
- The incidence and prevalence of XMRV/MLV infections.
- The worldwide distribution of infections.
- The mode of transmission.
- The origin of XMRV/MLV.

Ruscetti discussed three potential sources of contamination in PCR studies:

- PCR reagents (polymerase etc.).
- Genomic DNA in humans of murine origin.
- cwr22v1 humor tumor cell lines.

According to Ruscetti there are two possibilities:

1. The virus has spread through several labs due to contamination
2. People are infected with a human gamma retrovirus

Whether XMRV originates from cancer research depends on the answer whether XMRV can be traced back to a date back before 1992-1994 or not?

Things that cannot be explained by contamination: proviral integration sites, detection of XMRV sequences by in situ hybridization and histochemistry and antibody reactivity of infected patients.

The macaques studies give hints about tissue reservoirs of XMRV and latency.

In answer to a question about studies on XMRV in tissue of ME/CFS patients, Ruscetti emphasized a greater risk of contamination in tissue studies.

Judy A. Mikovits PhD (WPI, University of Nevada, USA)
Do XMRV and other gammaretroviruses (HGRVs) play a role in ME/CFS?

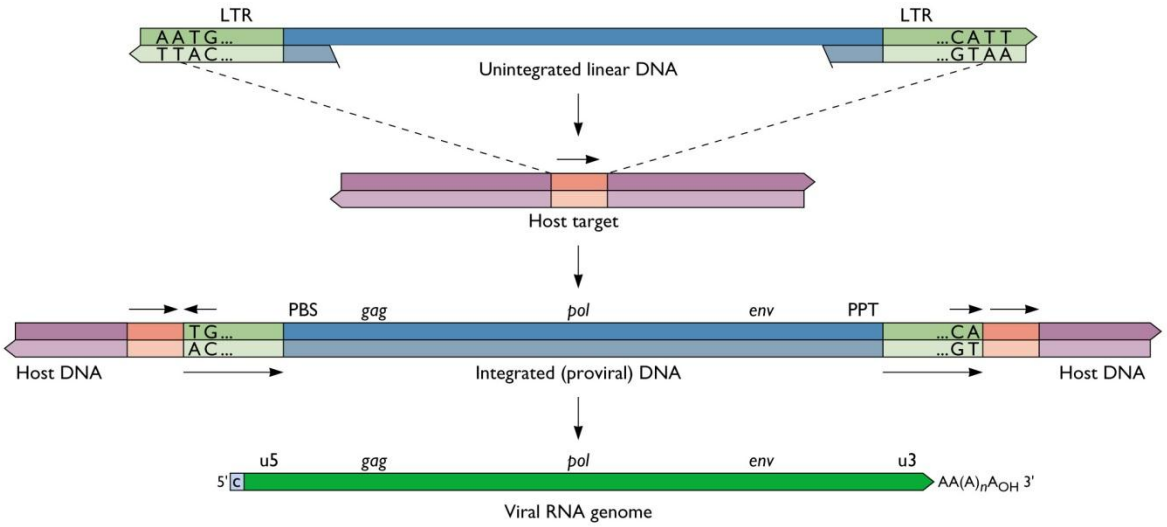
Judy Mikovits started her presentation with the rationale for the quest "to XMRV in ME/CFS (a multisystem disorder with a diagnosis of exclusion):

- RNase-L dysfunction in ME/CFS, as also was established in prostate cancer
- NK cell dysfunction (numbers and functionality: cytotoxicity)
- Innate immune activation (T cells and pro-inflammatory cytokines)
- Multiple active infections, many pathogens (HHV-6, EBV etc.)

What do we know about "XMRV"?

- XMRV sequences were detected in men with prostate cancer with a specific immunological allele (RNase L R462Q)
- XMRV has shown to have preferential integration sites in men
- XMRV is genetically closely related to xenotropic MLVs, polytropic MLVs, and other MLVs, but XMRV is not an endogenous retrovirus.
- How XMLV jumped species (from mice to men) still remains unknown.
- XMRV expression is stimulated by androgens and by inflammation (NF-kB).

XMRV is a simple virus (the genome is primarily made up of LTR: the promoter or transcriptional enhancer of the retrovirus, gag, pol and env genes). It needs the cellular machinery of the host to survive and replicate.



http://www.virology.ws/wp-content/uploads/2011/01/retroviral_int.jpg

Mikovits discussed the Lombardi/Mikovits Science study (2009), in which 67% of the ME/CFS patients and 4% of the healthy controls were found to be XMRV positive, the Alter/Lo study (2010) in which less specific, but more sensitive gag sequences, various (modified) polytropic MLV strains, were detected and 86.5% of the ME/CFS patients and 6.8% of the healthy controls were positive, and the Mikovits et al. study (2010), in which the original Science 2009 cohort was studies with a combination of detection methods: (1) nested PCR for gag sequences from LNCaP cells co-cultured with subject's plasma or activated PBMCs, (2) antibodies to XMRV Env in subject's plasma, (3) gag products by nested PCR on stimulated PBMCs or viral proteins expressed by activated PBMCs with appropriate antisera, (4) nested RT-PCR of plasma nucleic acid or PCR from cDNA from unactivated PBMCs and (5) PCR of DNA from unactivated PBMC prepared from the subject's blood.

After the Science study Mikovits et al. have established that a patient can carry various strains of HGV (XMRV, ecotropic and (modified) polytropic MLVs).

The genetic variations and low number of virus copies in the blood were proposed as two reasons for failure to detect "XMRV" by several researchers.

For that reason WPI and collaborators are not only trying to improve the XMRV detection methods but are also looking for footprints of the virus.

- Anti-XMRV antibodies (using the SFFV env)
- XMRV envelope induced gene expression
- XMRV proteins (in collaboration with Ruscetti/NCI): the plasma of XMRV positive ME/CFS patients seems reactive to multiple XMRV proteins)
- 2D gels and mass spectrometry (to detect reactive proteins in sera).

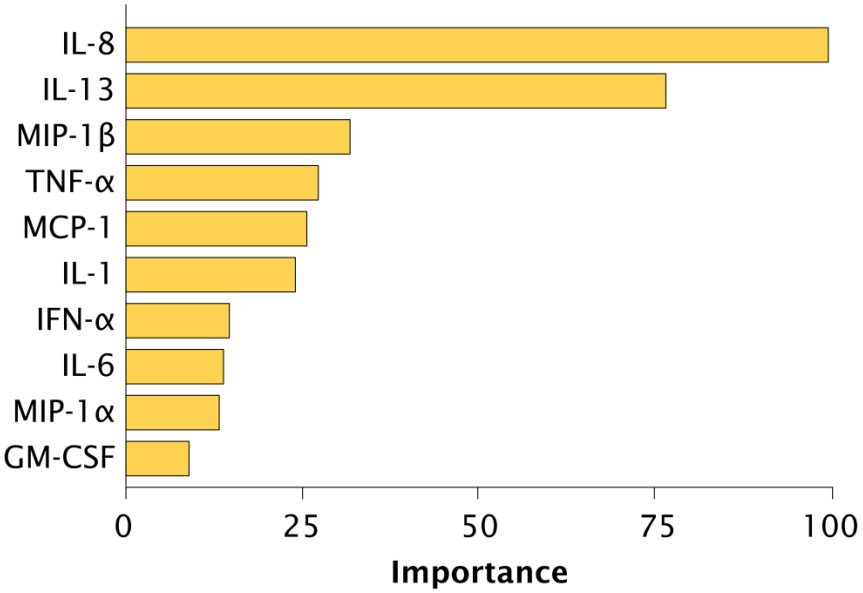
Mikovits discussed HIV, AIDS and the host/immune response-model as a possible explanation why not all XMRV-positive people get sick. If you have AIDS you are HIV-positive, but not all HIV-positive people have AIDS. Can HGV and ME/CFS compared with HIV and AIDS (including secondary infections)?

Some things about the XMRV pathogenesis already known:
replication of "XMRV" is enhanced

- by androgens (cortisol/stress hormone, estrogens, testosterone etc.) and
- by inflammation (NF-kB).

Mikovits then discussed the chemokine/cytokine study in XMRV-positive ME/CFS patients and healthy controls, which identified a "signature" of 10 cytokines and chemokines which correctly identifies XMRV/CFS patients with 93% specificity and 96% sensitivity (increased expression of IL-8, MIP-1β, TNF-α, MCP-1, IL-6, MIP-1α, decreased expression of IL-13, IL-7, IFN-α en GM-CSF).

The 10 most relevant cytokines/chemokines (adapted from Xenotropic murine leukemia virus-related virus-associated chronic fatigue syndrome reveals a distinct inflammatory signature. Lombardi VC, Hagen KS, Hunter KW, Diamond JW, Smith-Gagen J, et al. In Vivo. 2011 May-Jun;25(3):307-14, figure 2):



Mikovits conclude her presentation with white blood cell abnormalities in XMRV-positive ME/CFS patients:

- increased numbers of CD3-CD56+ NK cells
- decreased number of CD56+ NKT cells *1, *2, and
- increased numbers of CD20+ CD23+ B cells,
  which could explain the positive effect of B cell depletion therapy in ME/CFS using Rituximab as was established by Fluge and Mella in 2009).

*1 Is the ratio of number of NK+ cells/number of NKT+ cell a good marker?
*2 2 of the three persons on retrovirals showed an improvement in this ratio.

APOBEC3G, present in many if not all cells (including B and T cells), is a key player in the retroviral defense. It inactivates viral RNA by modifying its sequences *, transforming an infectious virus into a non-infectious virus). The role of APOBEC3G in the viral defense against XMRV was explored by Groom et al. (2010) and Prapotka et al. (2010). Prapotka et al .established extensive G-A hypermutation (*) in B cell lines from XMRV-positive patients.

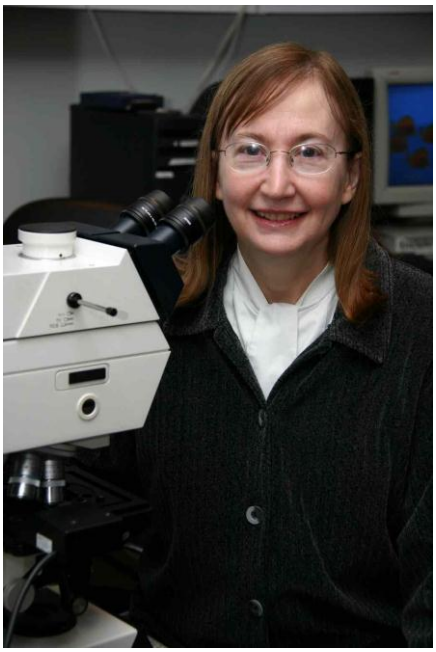
In answer to a question afterwards Mikovits stated that WPI disposes of a large (blood) samples repository and that the interest of pharmaceutical companies to conduct studies on XMRV are hindered by the controversy/

A study into the chemokine/cytokine profile of XMRV negative ME/CFS patients is being conducted, which could establish which cytokines/chemokines are increased/decreased in all ME/CFS patients and which cytokines/chemokines are differentially expressed only in XMRV positive (ME/CFS) patients.

Mikovits also stated that some XMRV “positive” studies are near publication and that, when looking at the contamination-and-recombination controversy , is more efficient that non-WPI researchers publish their results, since XMRV “positive” studies from the WPI will be received with sepsis on short notice.

Only if WPI (and others) are not able to find XMRV/MLV in the Lipkin study (she is convinced they will), Mikovits is prepared to reconsider her position.

Maureen Hanson PhD (Cornell University, USA)
Assays for MLV-like virus sequences in a New York CFS cohort.



Cornell University

Photo:
http://mbg.cornell.edu/cals/mbg/faculty-staff/faculty/images/MH-Head-at-Microscope-2009-Low-Res_1.JPG

Maureen Hanson, summarized the preliminary result of the 20/20 study, in which she and her colleagues have tested 20 ME/CFS patients of dr. Bell, 10 severe cases and 10 "recovered" patients, and 20 healthy controls on XMRV. The patients fulfill the Fukuda criteria, but Hanson stated they would most likely also meet the Canadian criteria since dr. Bell had diagnosed the patients.

Dr. Davis S. Bell. Photo: Regina Klos.

She first outlined the measures investigated (SF-36, Bell Disability Scale, number of hours of upright daily activity etc.), after which she focused on the laboratory techniques for XMRV detection. Hanson et al. have employed a PCR assay for gag sequences of XMRV and polytropic MLV viruses. She stressed the importance of the test conditions (e.g. the lab room temperature).

Although exact figures were not presented, she stated that gag sequences were detected in both severe and recovered ME/CFS patients, as well as in some healthy controls, and that the sequences are more similar to the MLV-like sequences found by Lo than to the XMRV sequences reported by Lombardi.

She emphasized that primers must have the XMRV-specific glycogag deletion and discussed a checklist of measures her team employs to ensure high-quality results, e.g. water controls (positive results: something is wrong) and PCR tests for mouse mitochondrial DNA (mtDNA), 10000 copies per cell, and intracisternal A particle (IAP), 1000 per haploid genome in every type of cell.

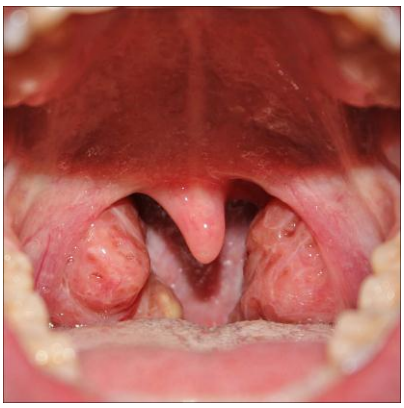
Hanson also pointed out 3 possible sources of contamination of samples:
- Contamination by samples used in the research lab.
- Contamination of taq polymerase / PCR reagents.
- Carryovers of PCR products employed in previous experiments.

Hanson concluded her presentation with the actions she and her team wants to perform in the near future to ensure high-quality outcomes of this study. Serological testing to MLV-related proteins has yet to be performed (antibodies tolerate more variation). Hansen et al. want to reexamine the blood and take other precautions and double check the results to avoid discussion. The study is expected to be finished at the end of the summer [In my view this means publication of this study cannot be expected for the end of 2011, FT].

Manson ended with four arguments used by HTLV naysayers as mentioned by Gallo at a conference in Leuven (history seems to repeats itself):
- There are numerous failed attempts to find the virus.
- The virus is highly replicating in animals (XMRV: mice!), so easy to find.
- There is little evidence of the virus in primates.
- The humans sera is lytic for many retroviruses.

Cecilia Cabrera PhD (IrsiCaixa Foundation, Badalona, Spain)
Infection of XMRV in human lymphoid tissue.

Dr. Cabrera summarized the results of a recent study on XMRV in human tonsil tissue presented at the 15th International Conference on Human Retrovirology: HTLV and Related Retroviruses (Leuven/Gembloux, 2001, June 5-8).



The two key questions of this study were:
- Does XMRV replicate in human lymphoid tissue?
- Are AZT and/or Raltegravir capable of suppressing the XMRV infection?

The most relevant findings of this study, in which human tonsils were obtained from healthy donors, dissected and infected with a 22Rv1 cell culture supernatant in the presence or the absence of AZT or Raltegravir:
- Cells migrating out the tissue and tissue cells were positive for XMRV gag.
- The number of gag copies increased exponentially in time.
- Both AZT and Raltegravir blocked the detection of viral DNA.
- XMRV infection did not modify the percentage of CD3 (T cells), CD4 (T helper cells), CD8 (cytotoxic T cells), or CD19 cells (B cells), neither the (number of naive cells/number of memory cells) ratio, nor the immune activation markers HLA-DR and CD38.
- CXCL8 and CLCX10 could be potential candidates as markers of infection.

XMRV is able to enter, to integrate and to replicate in human lymphoid cells. The presence of XMRV didn't result in changes of T or B cells nor in immune activation, suggesting lymphoid tissue could support latent XMRV infection.

The Irsicaixa XMRV research group has plans to study of XMRV infections in other tissues in the near future.

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