Emerging Concepts

• Recombination events in animal and human cells can generate families of infectious related gamma retroviruses

• Greatest concern is that they have acquired the ability to infect humans as our data shows consistently 6% in control populations

• Are XMRV-like sequences and proteins important in human disease pathogenesis?
Clinical features of patients infected with human retroviruses:

- Reduced Natural Killer (NK) cell numbers and NK cell cytotoxicity
- Abnormal CD4$^+$ and CD8$^+$ T cell numbers and ratio
- Reduced T cell response to antigens
- Presence of auto antibodies
- Alteration in cytokine profile
- Hyperactivation of T cells
- Decreased Antibody dependent cellular cytotoxicity (ADCC)
Chapter VI

Innate Immune Changes in the Peripheral Blood of Chronic Fatigue Syndrome Patients: Risk Factors for Disease Progression and Management

Deborah L. S. Goetz\textsuperscript{1}, Judy A. Mikovits\textsuperscript{2}, Jamie Deckoff-Jones\textsuperscript{3} and Francis W. Ruscetti\textsuperscript{2}

\textsuperscript{1}LANDRES Management Consultant LLC, Nevada, US
\textsuperscript{2}MAR Consulting Inc., California, US
\textsuperscript{3}Private CFS Practice, Arizona, US
Experimental Design

- Investigate the nature of the immune cells in peripheral blood patients with Neuroimmune Disease
  - Phenotypic analysis using Multi-parameter Flow Cytometry.
  - Multiplex proteomic analysis on the Luminex platform

GOAL

- Determine phenotypic and cytokine signatures that could be used as biomarkers of infection and disease subgroups.
Summary of Phenotypic Results

<table>
<thead>
<tr>
<th></th>
<th>ME/CFS, CLD vs. healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cellularity</td>
<td>No Difference</td>
</tr>
<tr>
<td>CD45+ Leukocytes</td>
<td>No Difference</td>
</tr>
<tr>
<td>CD45+ Lymphocytes</td>
<td>Reduced in ME/CFS</td>
</tr>
<tr>
<td>CD45+ CD3- Lymphocytes</td>
<td>Reduced in ME/CFS</td>
</tr>
<tr>
<td>CD3+ CD56+ NKT cells</td>
<td>Increased in ME/CFS, CLD</td>
</tr>
<tr>
<td>CD3- CD56+ NK cells</td>
<td>Reduced in ME/CFS, CLD</td>
</tr>
<tr>
<td>CD56&lt;sup&gt;DIM&lt;/sup&gt; NK subpopulation</td>
<td>Reduced in ME/CFS</td>
</tr>
<tr>
<td>CD56&lt;sup&gt;DIM&lt;/sup&gt; CD16+ NK subpopulation</td>
<td>Reduced in ME/CFS</td>
</tr>
<tr>
<td>CD19+ B cells</td>
<td>Reduced in ME/CFS</td>
</tr>
<tr>
<td>CD19+ CD20+ CD23+ B subpopulation</td>
<td>Increased in ME/CFS</td>
</tr>
<tr>
<td>CD33+ CD14+ CD123- myeloid Population</td>
<td>Reduced in ME/CFS</td>
</tr>
<tr>
<td>Activated APCs</td>
<td>Increased in ME/CFS, CLD</td>
</tr>
</tbody>
</table>
Chronic innate immune activation leads to chronic immunosuppression

• Presence of CD20+ CD23+ B cells, not normally seen in healthy subjects, and activated APCs in some ME/CFS, CLD patients are similar to the myeloid and B cell defects described in HIV.
• The significant changes in the myeloid compartment including phenotypes are suggestive of activation of Antigen Presenting Cells (APCs).
• Increased γδT Cells clonality in ME/CFS, CLD, CLL, MCL
  □ Increased NKT compartment together with increased NKT to NK ratio.

Conclusion
Results suggest a similar Disease cycle of innate immune activation leading to an immune dysregulation and chronic immunosuppression and may guide future research towards the development of biomarkers and treatment targets.
### Chronic Diseases Potentially Associated with Human Retroviral Infection

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Auto-Immune Diseases</th>
<th>CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate*</td>
<td>Lupus</td>
<td>ME/CFS*</td>
</tr>
<tr>
<td>Breast*</td>
<td>Crohn’s*</td>
<td>Gulf War Syndrome*</td>
</tr>
<tr>
<td>Non Hodgkin’s Lymphoma*</td>
<td>Hashimoto’s Thyroiditis*</td>
<td>Autism*</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukemia*</td>
<td>Polymyositis</td>
<td>MS*</td>
</tr>
<tr>
<td>Mantle Cell Lymphoma*</td>
<td>Sjogren’s syndrome</td>
<td>Parkinson’s*</td>
</tr>
<tr>
<td>Hairy Cell Leukemia</td>
<td>Bechet’s Disease*</td>
<td>ALS*</td>
</tr>
<tr>
<td>Bladder*</td>
<td>Primary Biliary Cirrhosis*</td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* RT Activity, RV sequences or proteins, antibodies to RV proteins
Detection of serum reverse transcriptase activity in patients with ALS and unaffected blood relatives

A.J. Steele, PhD; A. Al-Chalabi, PhD, FRCP; K. Ferrante, BA; M.E. Cudkowicz, MD, MSc; R.H. Brown, Jr., MD, DPhil; and J.A. Garson, MD, PhD

NEUROLOGY 2008;64:454–458

Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate

Neurology® 2008;70:278–283

That Novel Candidate was XMRV
Pathways shared in Autoimmune and Neuroimmune Disease

Autoimmunity

- p53
- NF-KappaB
- Cerebral hypoperfusion
- Activated microglia & astrocytes
- HPA axis

Brain Mitochondria Damage & low ATP

- TNF-α
- IL-1 Beta
- IL-6
- TGF-β1
- IL-8

Neuroprogression

- Disabling fatigue
- Flu like malaise
- Brain Fog

Neurocognitive symptoms

- Loss of autonomic control & homeostasis, e.g. Glucose metabolism

Vagus nerve

Sleep architecture Circadian rythm

Low Glutathione

- Inos NO peroxynitrite
- Glutamate
- Quinolinic acid

Gut periphery Mitochondria Damage & low ATP

Methylation cycle block (also NO)

Th17

Autoimmunity

- NK
- Foxp3
- IL-2
- Treg

Pathogen

- DC
- TLR
- IL-6
- STAT3
- TGF-β1
Review

Autoimmune disease: A role for new anti-viral therapies?

David H. Dreyfus *

Pediatrics, Yale SOM, United States
ARVs provide therapeutic benefit in some patients with autoimmune, Neuroimmune Disease and Cancer

Beneficial Effects could be against:

◆ An exogenous Replication Competent Retrovirus

◆ An expressed endogenous virus in an immune compromised individual

◆ A defective virus expressing only viral proteins

◆ Aberrantly expressed cellular RNA including microRNA (regulatory)
miR-155 a new target in ME/CFS?

- miR-155 has distinct expression profiles
- Plays crucial role in various physiological and pathological processes such as hematopoietic lineage differentiation, immunity, inflammation, cancer, and cardiovascular diseases; miR-155 has been implicated in chronic DNA viral infections
- Been implicated in viral infections, particularly in those caused by DNA viruses.

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**ORIGINAL ARTICLE**

Annals of Neurology 2013

**miR-155 as a Multiple Sclerosis–Relevant Regulator of Myeloid Cell Polarization**

Craig S. Moore, PhD,¹ Vijayaraghava T.S. Rao, PhD,¹ Bryce A. Durafourt, MSc,¹ Barry J. Bedell, PhD, MD,² Samuel K. Ludwin, MD,³ Amit Bar-Or, MD,¹ and Jack P. Antel, MD¹
Endogenous retroviruses (sleeping giants) are reactivated in immune deficient individuals (ME/CFS, CLD, CLL, ASD, HIV/AIDS), likely because of dysregulated DNA methylation.

Co-infections, reactivated viruses, GMOs, genetic susceptibilities can create perfect storm of aberrant methylation immune activation (including microglia and inflammation seen in ASD, ME/CFS, other neuroimmune disease and cancer.
Hypothesis

Aberrant evolution of the human genome by:

- Increased zoonosis of novel retroviruses in human and animal populations
Human endogenous retroviruses and the nervous system

RENÉE N. DOUVILLE\textsuperscript{1} AND AVINDRA NATH\textsuperscript{2,*}

\textsuperscript{1}Department of Microbiology, University of Winnipeg, Winnipeg, Manitoba, Canada

\textsuperscript{2}Section of Infections of the Nervous System, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD, USA

Human endogenous retrovirus (HERV) families associated with neurologic disease

<table>
<thead>
<tr>
<th>HERV</th>
<th>Family</th>
<th>Neurologic diseases</th>
<th>Known triggers</th>
<th>Pathogenic contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERV-W</td>
<td>Gammaretrovirus</td>
<td>Multiple sclerosis</td>
<td>Herpesviruses</td>
<td>MSRV virions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Schizophrenia</td>
<td>\textit{Toxoplasma}</td>
<td>Env superantigen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proinflammatory cytokines</td>
<td>Env alters glial function</td>
</tr>
<tr>
<td>HERV-H/F</td>
<td>Gammaretrovirus</td>
<td>Multiple sclerosis</td>
<td>Unknown</td>
<td>Virions</td>
</tr>
<tr>
<td>HERV-K</td>
<td>Betaretrovirus</td>
<td>Schizophrenia</td>
<td>Herpesviruses</td>
<td>Env superantigen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALS</td>
<td>HIV</td>
<td>Env superantigen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV infection</td>
<td>HTLV-1</td>
<td>Neoeptopes for CTLs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toxoplasma</td>
<td></td>
</tr>
</tbody>
</table>
The replicative activity of human endogenous retrovirus K102 (HERV-K102) with HIV viremia

Marian P. Laderoute\textsuperscript{a, b}, Antonio Giulivi\textsuperscript{a, b}, Louise Larocque\textsuperscript{a}, Deana Bellfoy\textsuperscript{a}, Yangxun Hou\textsuperscript{a}, Hong-Xing Wu\textsuperscript{a}, Keith Fowke\textsuperscript{c}, Jun Wu\textsuperscript{a} and Francisco Diaz-Mitoma\textsuperscript{d}

Results: Both the peptide serology and ddCt qPCR excess ratio methods suggested the activation of HERV-K102 in about 70—80\% of HIV viremic cases whereas only 2—3\% of normal healthy adults had marginally activated HERV-K102 ($P < 0.0001$). Moreover, by the end of the pilot study, the number of patients with HERV-K102 cDNA production in their serum increased to 100\%.

Conclusions: Our work uniquely suggests the common activation of HERV-K102 with HIV viremia and may be first to directly demonstrate HERV-K102 cDNA production \textit{in vivo}. The potential implications of the induction of HERV-K102 activation and replication for the prevention and control of HIV are discussed.
Effects of environmental change on zoonotic disease risk: an ecological primer

Agustín Estrada-Peña¹, Richard S. Ostfeld², A. Townsend Peterson³, Robert Poulin⁴, and José de la Fuente⁵,⁶

¹ Department of Animal Pathology, Faculty of Veterinary Medicine, Miguel Servet, 177, 50013-Zaragoza, Spain
² Cary Institute of Ecosystem Studies, Millbrook, NY 12545-0129, USA
³ The University of Kansas Biodiversity Institute, Lawrence, KS 66045-7593, USA
⁴ Department of Zoology, University of Otago, Dunedin 9016, New Zealand
⁵ SaBio, IREC, Ronda de Toledo s/n, 13071 Ciudad Real, Spain
⁶ Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA
The New Genetics and Natural versus Artificial Genetic Modification

Mae-Wan Ho

Institute of Science in Society, 29 Tytherton Road, London N19 4PZ, UK;
E-Mail: m.w.ho@i-sis.org.uk; Tel./Fax: +44-0-20-7272-5636

Received: 2 August 2013; in revised form: 9 October 2013 / Accepted: 10 October 2013 / Published: 4 November 2013
### Hazards of GMOs

1. Uncontrollable, unpredictable impacts on safety due to the genetic modification process
   - Scrambling the host genome
   - Widespread mutations
   - Inactivating genes
   - Activating genes
   - Creating new transcripts (RNAs) including those with regulatory functions
   - Creating new proteins
   - Creating new metabolites or increasing metabolite to toxic levels
   - Activating dormant viruses
   - Creating new viruses by recombination of viral genes in GM insert with those in the host genome

2. Toxicity of transgene protein(s) introduced (intentionally or otherwise)
   - Transgene protein toxic
   - Transgene protein allergenic or immunogenic
   - Transgenic protein becoming allergenic or immunogenic due to processing
   - Unintended protein created by sequence inserted may be toxic or immunogenic

3. Effects due to the GM insert and its instability
   - Genetic rearrangement with further unpredictable effects
   - Horizontal gene transfer and recombination
     - Spreading antibiotic and drug resistance
     - Creating new viruses and bacteria that cause diseases
     - Creating mutations in genomes of cells to which the GM insert integrate including those associated with cancer

4. Toxicity of herbicides used with herbicide tolerant GM crops
What are the detection rates in 1000 blood donors using these serological assays?

Analyzed to CA, TM and SU

SU is the most reactive antigen (subjects vs donors)
- 4% reactive
- 13% 'indeterminate'
  - Values above cut-off, but also reactive to neg ctrl antigen

Test reactives and indeterminates by Western Blot: Found SU most unstable protein
by Intercept Blood System is consistent with results for other human Retroviruses

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Extent of Inactivation (log\textsubscript{10} reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelets\textsuperscript{a}</td>
</tr>
<tr>
<td>HIV-1 (cell associated)</td>
<td>&gt;6.1</td>
</tr>
<tr>
<td>HIV-1 (cell free)</td>
<td>&gt;6.2</td>
</tr>
<tr>
<td>HTLV-I</td>
<td>4.7</td>
</tr>
<tr>
<td>HTLV-II</td>
<td>5.1</td>
</tr>
<tr>
<td>XMRV &amp; MLV-Related virus</td>
<td>&gt;4.0\textsuperscript{e}</td>
</tr>
</tbody>
</table>
2014 December 1 FDA Approval

Platelets, Plasma and RBC

Platelets Plasma

Red Blood Cells

Amotosalen

Intercalation

Crosslinking

Targeting

Helical region of DNA and RNA

Linker

Effector

Anchor

Reaction

Degradation

Replication blocked

S-303 (Non-reactive)
Summary/Conclusions

- Data suggest there are different strains of Gamma Retroviruses that can infect humans
- Assays that capture the variation of these viruses in the blood supply are the best i.e. Serology and transmission
- Technologies can inactivate infectious strains of XMRV in Blood Components
- New Disease associations include leukemia, lymphoma and the platelet disorder, ITP
Ebola In The Air: What Science Says About How The Virus Spreads

DECEMBER 01, 2014 12:29 PM ET

MICHALEEN DOUCLEFF

Viruses can spread through the air in two ways: inside large droplets that fall quickly to the ground (red), or inside tiny droplets that float in the air (gray). In the first route, called droplet transmission, the virus can spread only about 3 to 6 feet from an infected person. In the second route, called airborne transmission, the virus can travel 30 feet or more.

Adam Cole/NPR

Here’s an Ebola puzzle for you: If the virus isn’t airborne, why do doctors and nurses need to wear full protective suits, with face masks, while treating patients?
BIOMARKERS

Distinct plasma immune signatures in ME/CFS are present early in the course of illness

Mady Hornig,1,2* José G. Montoya,3 Nancy G. Klimas,4 Susan Levine,5 Donna Felsenstein,6 Lucinda Bateman,7 Daniel L. Peterson,8 C. Gunnar Gottschalk,8 Andrew F. Schultz,1 Xiaoyu Che,1 Meredith L. Eddy,7 Anthony L. Komaroff,9 W. Ian Lipkin1,2,10

2015 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution Non-Commerical License 4.0 (CC BY-NC). 10.1126/sciadv.1400121
Thank You

Judy A. Mikovits, PhD
MAR Consulting Inc., Carlsbad CA
www.marconsultinginc.com
www.plaguethebook.com
jamikovits@me.com

Here we are not afraid to follow the truth wherever it may lead,
Nor tolerate error so long as freedom is left to combat it
Thomas Jefferson
mTOR a master regulator of Neuroimmune Disease?

http://www.discoverymedicine.com/David-Fernandez/files/2010...

Ramesh et al
Dysfunction of the mTOR pathway is a risk factor for Alzheimer's disease

Sharon C Yates¹, Amen Zafar¹, Paul Hubbard¹, Sheila Nagy¹, Sarah Durant², Roy Bicknell², Gordon Wilcock³, Sharon Christie³, Margaret M Esiri⁴, A David Smith⁵ and Zsuzsanna Nagy¹*
# Immune Reconstitution Inflammatory Syndrome

<table>
<thead>
<tr>
<th>Form of IRIS</th>
<th>Progression of events leading to IRIS</th>
<th>Immune defect</th>
<th>High microbial load without normal inflammation</th>
<th>Clinical intervention</th>
<th>Full inflammation restored</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-associated</td>
<td>Infection or iatrogenic immunosuppression</td>
<td>HIV</td>
<td>↓ CD4$^+$ T cells</td>
<td>ART</td>
<td>↑ CD4$^+$ T cells</td>
<td>Many manifestations</td>
</tr>
<tr>
<td>Exacerbation of TB after withdrawal of TNF blockade</td>
<td>TNF blockade</td>
<td>↓ TNF</td>
<td>M. tuberculosis</td>
<td>Stop TNF blockade</td>
<td>↑ TNF</td>
<td>Lung pathology, lymphadenitis</td>
</tr>
<tr>
<td>PML-associated</td>
<td>α4 integrin blockade</td>
<td>↓ Immune cell migration into CNS</td>
<td>JC polyoma virus</td>
<td>Stop α4 integrin blockade</td>
<td>↑ Immune cell migration into CNS</td>
<td>Leukoencephalopathy</td>
</tr>
<tr>
<td>Organ transplant-associated</td>
<td>Anti-graft rejection treatment</td>
<td>Broad immunosuppression</td>
<td>C. neoformans</td>
<td>Decrease immunosuppressive treatment</td>
<td>Many factors?</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Mouse model</td>
<td>TCRαKO or scid mice</td>
<td>T cell deficiency</td>
<td>M. avium or P. carinii</td>
<td>Inject CD4$^+$ T cells</td>
<td>↑ CD4$^+$ T cells</td>
<td>Wasting, lung pathology</td>
</tr>
</tbody>
</table>
Detection of Murine Leukemia Virus in the Epstein-Barr Virus-Positive Human B-Cell Line JY, Using a Computational RNA-Seq-Based Exogenous Agent Detection Pipeline, PARSES

Zhen Lin, Adriane Puetter, Joseph Coco, Guorong Xu, Michael J. Strong, Xia Wang, Claire Fewell, Melody Baddoo, Christopher Taylor, and Erik K. Flemington

Tulane University Health Sciences Center and Tulane Cancer Center, New Orleans, Louisiana, USA, and University of New Orleans, New Orleans, Louisiana, USA

A. PARSES

RNA-seq reads

Novoalign/TopHat

Non-human reads

Human reads

Blast

MEGAN

ABYSS

Akata

Viruses

Human herpesvirus 4

Enterobacteria phage phiX174 sensu lato

C. JY

Enterobacteria phage phiX174 sensu lato

Human herpesvirus 4

Retroviridae

Mammalian virus group

Gamma retrovirus

Lymphocryptovirus

Human herpesvirus 4

Xenotropic MuLV-related virus

Murine leukemia virus

Friend spleen focus-forming virus

Mus musculus mobilized endogenous polytropic provirus

Murine xenotropic virus NZB

Enterobacteria phage phiX174 sensu lato
Adoptive T cell Transfer for Cancer Immunotherapy in the Era of Synthetic Biology

Michael Kalos and Carl H. June
Abramson Cancer Center and the Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-5156