How politics corrupts scientific research

Particularly research involving human subjects

“they find what they want to find”

No funding = no associations

Difference between associations found in research associations found in MEDIA
Key Contributors to Chronic Diseases

Sub Clinical (damage from a distance) → Disease
- immune deficiency
- inflammation
- mutation
- disease development

Microbial Infection (the switch)

Oxidative Stress (ROS/RNS)

Inflammation (NFkB & COX2)

cytokine storm

chronic inflammation

immune deficiency

hit and run

trigger

activation
Systems Biology Approach to Chronic Disease

Clinicians

Consultation
Consultation and Diagnosis

Clinical labs/databases

Clinical Data and Samples

Immunological Profiling

Immunophenotyping

Immune Protein arrays/ Cytokines and chemokines

ME/CFS Database and Sample Repository @ UNR
Cells, cell lines, DNA, RNA

Results and Discussion

BASIC Research

Epidemiology Patient family registries

Gene Expression mRNA, MicroRNA

Molecular Data Analysis/Bioinformatics

Viral/Pathogen Arrays and deep sequencing technologies

Epigenetic Profiling

Research Gateway
Web-based, open-access Bio-Informatics
The Environment ME/CFS, ASD
All Chronic Disease?

• More than 200 genes associated with Autism
• 2008 Kerr associated 88 genes with ME
• Many subtypes
• Pesticides
• Toxins
• EMF
• Lessons learned from Other human retroviral Infections
• Zoonotic transmission exposures
• Heavy metals in water-Example from the Silver state
• GMO
• Vaccinations-The Anti-hygiene Theory
• Microbiome.

ALL ON TOP OF THE GENETICS
As much as 15% of human genome is made up of endogenous Retroviruses that have been crippled by the immune system. That is, they are not replication competent. Retroviruses Integrate into genome...forever part of DNA of host cell.

As much as 15% of human genome is made up of endogenous Retroviruses that have been crippled by the immune system. That is, they are not replication competent.
Infection with Human Immunodeficiency Virus Type 1 Upregulates DNA Methyltransferase, Resulting in De Novo Methylation of the Gamma Interferon (IFN-γ) Promoter and Subsequent Downregulation of IFN-γ Production

Judy A. Mikovits, Howard A. Young, Paula Vertino, Jean-Pierre J. Issa, Paula M. Pitha, Susan Turcoski-Corales, Dennis D. Taub, Cari L. Petrow, Stephen B. Baylin and Francis W. Ruscetti

Mechanisms of Pathogenesis:

◆ Lessons learned from 30 years of Human retrovirus study

◆ Lessons learned from 40 years of MLV study
**HTLV-I: Pathogenesis**

**Genus:** Deltaretrovirus (complex)

**Genome:** Multiple spliced RNAs for regulatory and accessory proteins

**Pathogenesis:**
- Asymptomatic in majority of individuals
- 5-8% lifetime risk of developing types of disease:
  - Adult T cell leukemia
    - Clonal malignancy of CD4⁺ T cells.
    - Long latency; Immune deficiency
    - Tax and HBZ needed for transformation
  - Inflammatory syndromes
    - HTLV-I associated myelopathy/Tropical spastic paraparesis
    - Uveitis
    - Arthropyathy
Infectious vs. Mitotic transmission of HTLV-1
Increased Cytokine/Chemokine Production in plasma from ATL patients

<table>
<thead>
<tr>
<th>Concentration in culture supernatant (pg/ml)</th>
<th>ATL Patient</th>
<th>Uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12p40</td>
<td>130</td>
<td>36</td>
</tr>
<tr>
<td>IL-6</td>
<td>2800</td>
<td>17</td>
</tr>
<tr>
<td>IL-1β</td>
<td>162</td>
<td>---</td>
</tr>
<tr>
<td>TNF-α</td>
<td>600</td>
<td>---</td>
</tr>
<tr>
<td>IP10</td>
<td>130</td>
<td>---</td>
</tr>
<tr>
<td>MCP-1</td>
<td>770</td>
<td>150</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>450</td>
<td>90</td>
</tr>
<tr>
<td>IL-8</td>
<td>8500</td>
<td>420</td>
</tr>
</tbody>
</table>

- Many cytokines such as IL-4, IL-5, IL-7 and type 1 interferons are not expressed in blood of infected patients
Dysregulated Cytokine/Chemokine Production plasma from ME/CFS patients

<table>
<thead>
<tr>
<th>CYTOKINES/ CHEMOKINES</th>
<th>Patient N = 156</th>
<th>Control N=140</th>
<th>P value</th>
<th>FUNCTION IN INFLAMMATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>1067</td>
<td>11.1</td>
<td>&lt;0.0001</td>
<td>RNase L and CMV activated</td>
</tr>
<tr>
<td>IL-13</td>
<td>28</td>
<td>86</td>
<td>&lt;0.0001</td>
<td>Inhibits inflammatory cytokine production</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>1840</td>
<td>157</td>
<td>&lt;0.0001</td>
<td>Elevated in Neurodegenerative disease</td>
</tr>
<tr>
<td>TNF-α</td>
<td>109</td>
<td>12.8</td>
<td>&lt;0.0001</td>
<td>Stimulates chronic inflammation</td>
</tr>
<tr>
<td>MCP-1</td>
<td>468</td>
<td>421</td>
<td>0.003</td>
<td>Elevated in chronic inflammatory diseases</td>
</tr>
<tr>
<td>IL-7</td>
<td>21.1</td>
<td>82</td>
<td>&lt;0.0001</td>
<td>Stimulates proliferation of B and T lymphocytes and NK cells</td>
</tr>
<tr>
<td>IFN-α</td>
<td>35</td>
<td>60</td>
<td>&lt;0.0001</td>
<td>Stimulates macrophages and NK cells to elicit an anti-viral response</td>
</tr>
<tr>
<td>IL-6</td>
<td>271</td>
<td>29</td>
<td>&lt;0.0001</td>
<td>Stimulates chronic inflammation</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>673</td>
<td>91</td>
<td>0.0062</td>
<td>Elevated in Neurodegenerative disease</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>108</td>
<td>166</td>
<td>&lt;0.0001</td>
<td>Stimulates proliferation of B and T lymphocytes and NK cells</td>
</tr>
</tbody>
</table>
3 B-Cell Lines Derived Directly From CFS Patients’ PBMCs

- CFS patient PBMCs were cultured; 3 samples developed into immortalized cell lines.
- All three showed high CD20+ expression and two showed high CD23+ expression.
- All three showed strong similarity to B cells seen in patients.

<table>
<thead>
<tr>
<th>Marker</th>
<th>MCL</th>
<th>WPI 1125</th>
<th>WPI 1186</th>
<th>WPI 1143</th>
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<tbody>
<tr>
<td>CD5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD19</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FMC7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD7</td>
<td>+</td>
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<td>-</td>
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</tr>
<tr>
<td>CD8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD38</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD45</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD122</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lambda</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kappa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

These Cell lines were developed from CFS patients. One, (1125) developed MCL; one (1186) was developed from a bone marrow biopsy, 3rd a CLL.
Extended PBMC cell culture without manipulation shows XMRV \textit{gag} infection (DNA) in samples negative for XMRV \textit{gag} RNA.
Taken together, these data demonstrate the first direct isolation of infectious XMRV from humans and implicate a role for XMRV infection in the pathogenesis of CFS.”

The original abstract of the Science article which was published on October, 8, 2009
Microglia Activation in Neurodegeneration

**Stimuli**
Loss of cell:cell communication, matrix breakdown, infections, vascular damage, others

**Homeostasis**
Phagocytosis of debris, neurotrophins

**Damage**
Neurotoxins, phagocytosis of normal neurons, apoptosis

**Neurodegenerative disorders**
- Parkinson’s disease
- Alzheimer’s disease
- Multiple sclerosis

Fetler, L and S Amigorena, Science 2005, 309:392
Model for the Induction of Neurodegeneration by one strain of MLV in an animal model

These immune pathways seen in ASD and Other Chronic neurological diseases
Two important lessons learned from studying MuLVs

- While insertional mutagenesis by MuLVs can result in transformation of cells and the development of leukemias and lymphomas, the envelope proteins encoded by these viruses can also have profound biological effects. So it’s important to study the biological effects of the XMRV envelope protein.

- MuLVs can be expressed in the CNS, triggering an inflammatory response that can cause severe neurological damage. Since similar inflammatory responses are associated with ME/CFS, XMRV could be playing a role.
XMRV Controversy

- What happened?
- What did we learn?
- Where do we go from here?
Lots of risk factors, but there is a genetic predisposition to prostate cancer. For maybe about 10 percent of prostate cancer, there are folks predisposed to it.

And the first gene that was mapped in association studies for this, early-onset prostate cancer, was this gene called RNASEL. What is that? It’s an antiviral defense enzyme. So, we’re sitting around and thinking, “Why would men who have this mutation—a defect in an antiviral defense system—get prostate cancer? It doesn’t make sense,--unless, maybe, there’s a virus?” So, we put tumors—on our array . . . And what I’m telling you from the signal is that men who have a mutation in this antiviral defense enzyme, and have a tumor, often have—40 percent of the time—a signature which reveals a new retrovirus. OK, that’s pretty wild. What is it?
So, we clone the whole virus

Schematic of Plasmid containing XMRV/VP62
It’s a classic gamma retrovirus, but it’s totally new. Nobody’s ever seen it before. Its closest relative is, in fact, from mice, and so we would call this a xentotropic retrovirus, because it’s infecting a species other than mice.

Dr. Joseph De Risi: Hunting the Next Killer Virus – February 2006 – Monterey, CA – TED Talks

*Plague* Chapter 4 p41
Six WPI DNA Samples shown in Fig. 1 of the original study analyzed by the Silverman Lab in 2009 contained VP-62 plasmid.
Original DNA Samples were negative for XMRV plasmid

◆ Pitfall: Choose your collaborators wisely!!
Cell-Free Transmission of XMRV from PCR-negative CFS Patients’ Plasma to LNCaP cells
Independent Reanalysis of archival samples used in Original Study Detected XMRV gag without plasmid or mouse contamination

PCR performed with USB HotStart-IT FideliTaq Master Mix

94°C 2 min
45 cycles:
94°C 30 sec, 54.8°C 30 sec, 72°C, 30 sec
72°C 3 min.

All three are negative for IAP and negative for CMV385F/XMRV528R primers for VP62 junction fragment

Sequencing of bands:

↑ Non-specific (Human DNA)
↑ XMRV Gag
Direct Isolation of XMRV Protein From Plasma of CFS Patients By Immunoprecipitation with Anti-X-MLV Antibodies

IP: Goat anti-X-MLV (BALB-V2)

Blot: Goat anti-R-MuLV Gag

p30 gag

p12 gag
Clones of XMRV Env SU Similar to Polytropic XMRVs

- The main XMRV/ in this patient is unlikely to be VP-62
Phylogenetic Tree of XMRV(P) and Other Gammaretroviruses detected in original cohort
N-Terminus of SFFV ENV allows recognition of most potential XMRVs using monoclonal antibody 7C10
Plasma from CFS patients block binding of SFFV Env rat mAb to the B cell line expressing SFFV Env, demonstrating specificity.

An ANTIBODY POSITIVE RESULT DOES NOT NECESSARILY SHOW THE PRESENCE OF A REPLICATION COMPELNT RETROVIRUS.
Sequence and phylogenetic analysis of a novel xenotropic XMRV-like MLV B4rv.

93% overall homology
Frequent detection of infectious xenotropic murine leukemia virus (XMLV) in human cultures established from mouse xenografts

Yu-An Zhang,† Anirban Maitra,‡ Jer-Tsong Hsieh,§ Charles M. Rudin,¶ Craig D. Peacock,* Collins Karikari,‡ Rolf A. Brekken,† Victor Staatsn,† Boning Gao,† Luc Girard,† Ignacio Wistuba,§ Eugene Frenkel,§ John D. Minna† and Adi F. Gazdar†,*

†Hamon Center for Therapeutic Oncology Research; ‡Department of Urology; §Division of Hematology-Oncology; ¶The University of Texas Southwestern Medical Center at Dallas; Dallas, TX USA; ‡Departments of Pathology and Oncology; The Sol Goldman Pancreatic Cancer Research Center; *Department of Oncology; Johns Hopkins University School of Medicine; Baltimore, MD USA; §Department of Pathology; MD Anderson Cancer Center; Houston, TX USA

The Name Game and the Immaculate Recombination

How many have we created, John? How many retroviruses are out there Judy Mikovits asking a question to Dr. John Coffin at the Ottawa IACFS ME/CFS meeting 23 September 2011

Plague Chap 17 p 284
Antibodies to XMRV ENV Reproducibly Detected in Human Population

<table>
<thead>
<tr>
<th>Lab site</th>
<th>Analysis</th>
<th>Sample</th>
<th>CFS/ME cases (n = 147)</th>
<th>Controls (n = 146)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total studied</td>
<td>No. positive (%)</td>
</tr>
<tr>
<td>CDC</td>
<td>RT-PCR</td>
<td>Plasma</td>
<td>147</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>FDA</td>
<td>RT-PCR</td>
<td>Plasma</td>
<td>121</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>PBMC</td>
<td>121</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mikovits, Ruscetti, and Hanson</td>
<td>PCR of cultured PBMC</td>
<td>PBMC</td>
<td>117</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mikovits and Ruscetti</td>
<td>Serology</td>
<td>Plasma</td>
<td>147</td>
<td>9 (6.1)</td>
</tr>
</tbody>
</table>

|                  |                |            | Total studied         | No. positive (%)   |
|                  |                |            |                       |                   |
| CDC               |                |            | 146                   | 0 (0.0)           |
| FDA               |                |            | 110                   | 0 (0.0)           |
|                   |                |            | 111                   | 0 (0.0)           |
| Mikovits, Ruscetti|                |            | 126                   | 0 (0.0)           |
| Mikovits and Ruscetti|           |            | 146                   | 9 (6.2)           |

* Numbers represent all samples available for analysis at that site.

* Fifty samples (30 cases; 20 controls) were unable to be assayed because at least one of two aliquots from each set of subject PBMC did not grow in tissue culture.
Partial molecular cloning of the JHK retrovirus using gammaretrovirus consensus PCR primers

Brian D Halligan¹, Hai-Yuan Sun², Vladimir M Kushnaryov² & Sidney E Grossberg*²

¹Biotechnology & Bioengineering Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA
²Department of Microbiology & Molecular Genetics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA
*Author for correspondence: Tel.: +1 414 276 8194 = segrossb@gmail.com

The JHK virus (JHKV) was previously described as a type C retrovirus that has some distinctive ultrastructural features and replicates constitutively in a human B-lymphoblastoid cell line, JHK-3. In order to facilitate the cloning of sequences
Although it is highly unlikely that either XMRV VP62 or B4Rv themselves infect humans and are pathogenic, the results suggest that xenograft approaches commonly used in these studies of human cancer promote the evolution of novel retroviruses with pathogenic properties. Similar retroviruses may have evolved to infect humans!
Are two RCRs made by passing human prostate tissue through mouse; XMRV, BRV4 (second recombinant infectious virus occurring in human cells)

Additional XMRV-like viruses may exist

They do not have to be the exact sequence of XMRV (VP62)

Whether we fail to see the clever virus which does not kill its host, but has learned to live with it

When a disease takes so much from a patient but stops just short of death, how does the medical community respond?

Will the scientific community have the courage to answer the question of whether these diseases Might have been of their own creation” ????”
CONCLUSION

Taken together these data suggest there are additional human gamma retroviruses which may be involved in the Pathogenesis of neuroimmune disease and cancer!

“The question, which urgently needs to be answered is whether the plague feared by Coffin and Stoye has already arrived, but we do not Recognize it...

THEY SEE WHAT THEY WANT TO SEE AND THAT’S THE REAL PLAGUE”

Plague  Chapter 21 p382
Thank You

Judy A. Mikovits, PhD
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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