Latest Clinical Discoveries on Retroviruses and Chronic Disease

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Agenda

- **1. What are retroviruses (RVs)?**
  - Endogenous and exogenous RVs
  - Conditions they are associated with
- 2. Towards detecting the activation of RVs
- 3. The treatment of retroviral activation/infection
  - Proven biological remedies
  - Pharmaceuticals

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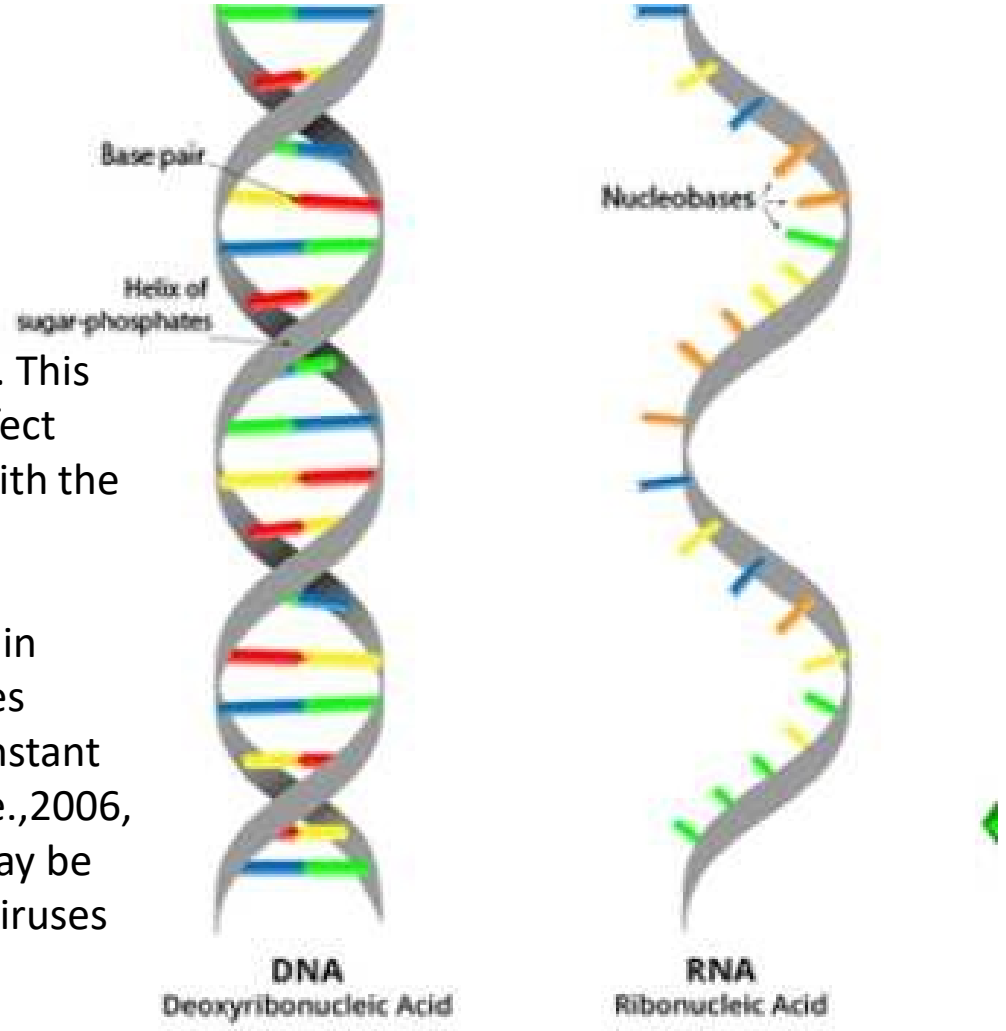
What are retroviruses?

Retroviruses are viruses composed not of DNA (a double-stranded helix: deoxyribonucleic acid), but of RNA (single-stranded: ribonucleic acid).

They use an enzyme called reverse transcriptase that gives them the unique property of transcribing their RNA into DNA after entering a cell. Once inside the cell, it uses that enzyme to force the cell to create viral DNA. This viral DNA becomes integrated into the host-cell DNA.[1] Retroviruses are in effect retrograde, because the flow of genetic information is reversed compared with the normal pathway of molecular biosynthesis—DNA → RNA → protein.

"Subsequent retrotransposition events amplified these sequences, resulting in approximately 8% of the human genome being composed of HERV sequences today."[2] – passed down to us from our ancestors as battle-scars from our constant encounter with an often hostile microbial and virus-rich environment (Stoyle.,2006, Mayer et al.,2011; Li et al.,2001). A retrovirus integrated into our genome may be passed from mother to child during pregnancy (Sakuma et al.,2012). These viruses are referred to as **Human Endogenous Retroviruses or HERVs**.



Source: *1. www.medicinenet.com; 2. Li W, Lee MH, Henderson L, Tyagi R, Bachani M, Steiner J, Campanac E, Hoffman DA, von Geldern G, Johnson K, Maric D, Morris HD, Lentz M, Pak K, Mammen A, Ostrow L, Rothstein J, Nath A. Human endogenous retrovirus-K contributes to motor neuron disease. Sci Transl Med. 2015 Sep 30;7(307) being composed of HERV sequences today.*

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Endogenous retroviruses can be beneficial …

Endogenous retroviruses are subdivided into different categories, e.g., Beta Retroviruses: HERV-K; Gamma Retroviruses: HERV-H and HERV-W.
The length of HERV-DNA in one person: 150,000 times round the earth.
DNA methylation and acetylation suppress the expression of endogenous retroviral genes.
"If our DNA were an airplane carry-on bag (and essentially it is), it would be bursting at the seams. We lug around 100,000 retrovirus sequences inside us; all told, genetic parasites related to viruses account for more than 40 percent of all human DNA. Our body works hard to silence its viral stowaways by tying up those stretches of DNA in tight stacks of proteins, but sometimes they slip out."[2]

Many HERVs have been present in our genome for considerable time and some are of benefit to the human host. E.g. pregnancy would be impossible without retroviral activity.

PRIMER

The placenta goes viral: Retroviruses control gene expression in pregnancy

Edward B. Chuong*

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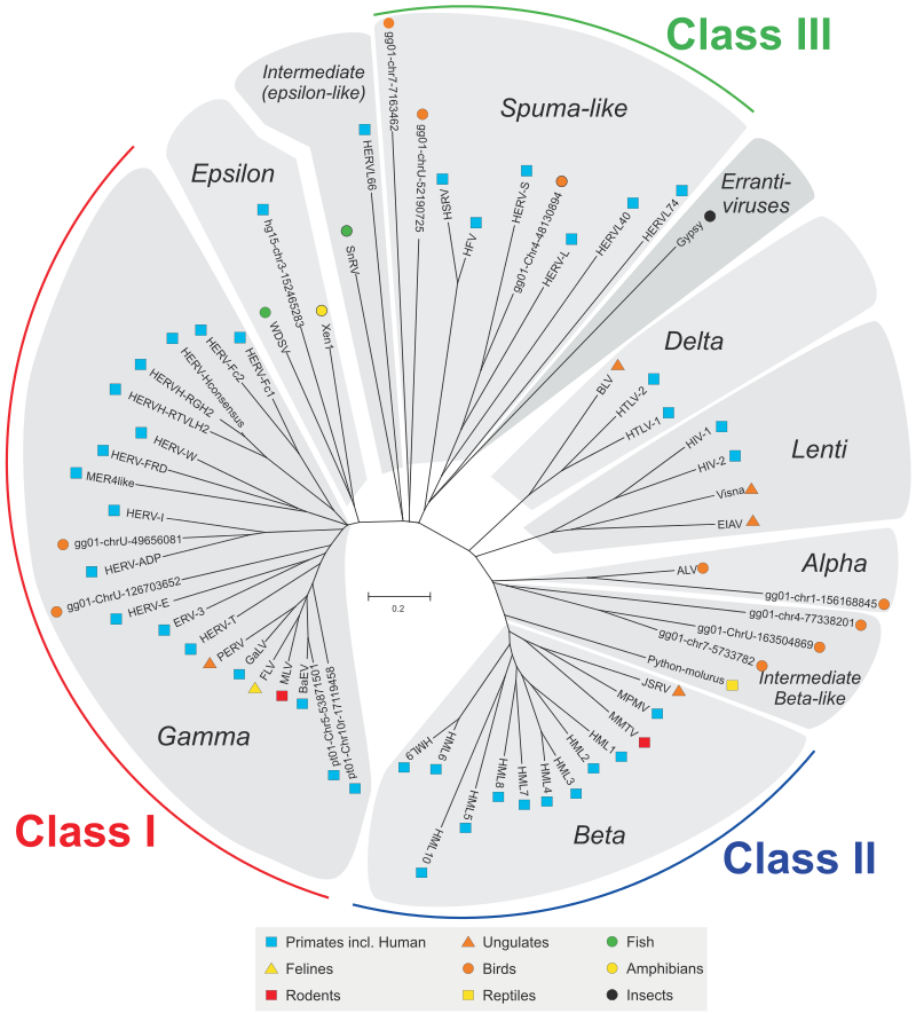
Abstract

The co-option of endogenous retroviruses (ERVs) is increasingly recognized as a recurrent theme in placental biology, which has far-reaching implications for our understanding of mammalian evolution and reproductive health. Most research in this area has focused on ERV-



Dendrogram of various classes of endogenous retroviruses[1]

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Source: 1. *Classes_of_ERVs.jpg: Jern P, Sperber GO, Blomberg J derivative work: Fgrammen (talk), Wikipedia*

… but HERVs are also implicated in disease

HERVs have however been implicated in the etiology and pathology of disease, too. They can be triggered under certain circumstances, e.g. by inflammation, oxidative stress and microbial infection. Put the name of a disease and "retrovirus" into the web, and you will see the peer-reviewed scientific literature that often emerges.

Immunity. 2001 Oct;15(4):579-89.

Epstein-Barr virus transactivates the human endogenous retrovirus HERV-K18 that encodes a superantigen.

Sutkowski N[1], Conrad B, Thorley-Lawson DA, Huber BT.

Author information

1 Department of Pathology, Tufts University School of Medicine, Boston, MA 02111, USA.

Abstract

Superantigens (SAgs) are proteins produced by pathogenic microbes to elicit potent, antigen-independent T cell responses that are believed to enhance the microbes' pathogenicity. Here we show that the human lymphotropic herpesvirus Epstein-Barr virus (EBV) transcriptionally activates the env gene of an endogenous retrovirus, HERV-K18, that possesses SAg activity. SAg activity was demonstrated by MHC class II

Retrovirology. 2013 Nov 12;10:132. doi: 10.1186/1742-4690-10-132.

Human cytomegalovirus (HCMV) induces human endogenous retrovirus (HERV) transcription.

Assinger A, Yaiw KC, Göttesdorfer I, Leib-Mösch C, Söderberg-Nauclér C[1].

Author information

1 Center for Molecular Medicine, Department of Medicine, Karolinska Institutet, SE-171 76 Stockholm, Sweden. cecilia.naucler@ki.se.

Abstract

BACKGROUND: Emerging evidence suggests that human cytomegalovirus (HCMV) is highly prevalent in tumours of different origin. This virus is implied to have oncogenic and oncomodulatory functions, through its ability to control host gene expression. Human endogenous retroviruses (HERV) are also frequently active in tumours of different origin, and are supposed to contribute as cofactors to cancer development. Due to the high prevalence of HCMV in several different tumours, and its ability to control host cell gene expression, we sought to define whether HCMV may affect HERV transcription.

"Numerous studies have established links between HERV activation and infections with viruses such as the Epstein Barr virus (EBV)." Gruchot et al. Front. Genet., 11 July 2019

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HHV6 and RVs an important role in the pathogenesis of inflammatory diseases

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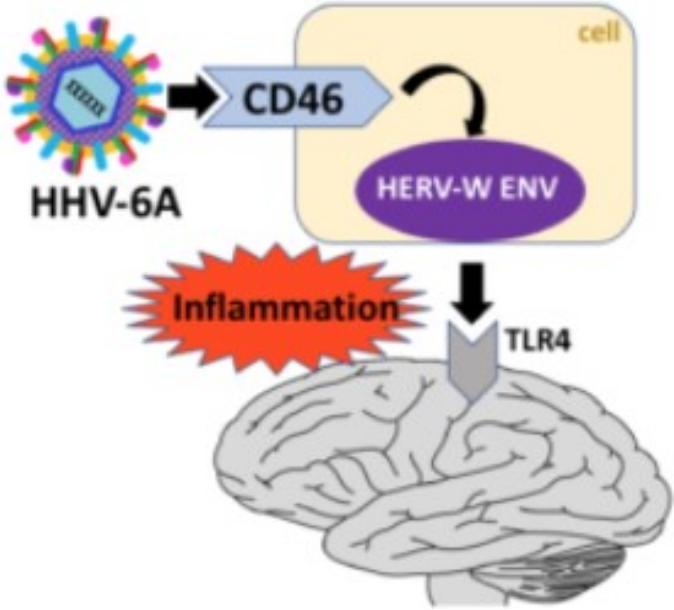
HHV-6A receptor CD46 identified as a critical factor in 'awakening' endogenous retrovirus

Both infectious and UV-inactivated HHV-6A activate endogenous retrovirus envelope protein – but so does selective stimulation of HHV-6A's CD46 receptor. This "cross-talk" between HHV-6A and endogenous retrovirus appears to play an important role in the pathogenesis of inflammatory diseases.

A team in France led by Dr. Branka Horvat at the International Center for Infectiology Research (CIRI) in Lyon, in collaboration with Dr. Patrice Marche (INSERM, Grenoble) and Dr. Herve Perron (GeNeuro Inovation, Lyon) demonstrated that HHV-6A as well as stimulation of transmembrane glycoprotein CD46 induced the expression of an envelope protein of MSRV or multiple sclerosis associated retrovirus, a member of the human endogenous retrovirus (HERV-W) family. Specifically, they found that engagement of extracellular domains SCR3 and SCR4 of the CD46-Cyt1 isoform is required for the transactivation. In addition, they found that specific monoclonal antibodies and the C3b component of complement also caused transactivation.



Source: *Charvet, B., Reynaud, J. M., Gourru-Lesimple, G., Perron, H., Marche, P. N., & Horvat, B. (2018). Induction of Proinflammatory Multiple Sclerosis-Associated Retrovirus Envelope Protein by Human Herpesvirus-6A and CD46 Receptor Engagement. Frontiers in immunology, 9, 2803.*

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Identified in Sjogren's, RA, SLE, MS , inflammatory vascular disease …

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Review

Demystified . . . Human endogenous retroviruses FREE

P N Nelson[1], P R Carnegie[2], J Martin[1], H Davari Ejtehadi[1], P Hooley[1], D Roden[1], S Rowland-Jones[3], P Warren[1], J Astley[1], P G Murray[4]

HERVS AND AUTOIMMUNITY

In 1990, an article appeared in the Times newspaper (24 November) with the title "AIDS-like virus may cause arthritis". The report focused on Robert Garry's research that identified retroviral particles in lip biopsies taken from patients with primary Sjogren's syndrome (SS).[41] Similarly, in other autoimmune rheumatic diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), a plethora of articles added to this intriguing observation by providing evidence of retroviral antigens at the site of disease, or the presence of antiretroviral antibodies in the sera of patients.[6,42–44] A novel report in 1994 used both PCR (using consensus primers) and serological tests to investigate the presence of retroviruses in a cross section of patients with rheumatoid diseases, including RA, SS, and SLE.[45] Interestingly, PCR failed to amplify products relating to HTLV-I or HIV-1, although antibodies to retroviral antigens were detected in the sera of patients. Consequently, there appeared to be a conundrum: antibodies to retroviral products were present but no evidence to implicate exogenous retroviruses could be found. Between 1996 and 1999, some research groups used so called "degenerate" retroviral primers in their PCR reactions.[7,46,47] These primers cater for modest variations within two segments common to all retroviruses within the reverse transcriptase encoding pol region and provide an intervening "fingerprint region", which permits DNA sequencing. In brief, these studies[7,46,47] revealed nucleotide homologies to endogenous retroviral families, including viruses with similarity to known exogenous retroviruses. Thus, it was plausible that the presence of HERVs could provide an explanation for the presence of antiretroviral antibodies in certain rheumatoid diseases.[6,7,48] HERVs have also been implicated in other autoimmune diseases, such as multiple sclerosis (HERV-W, HERV-H) and insulin dependent diabetes mellitus (IDDM) (HERV-K, IDDM22), in addition to inflammatory vascular diseases.[49–54] However, in the case of

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Involved in neurological diseases …

Neural Cell Responses Upon Exposure to Human Endogenous Retroviruses

*Joel Gruchot, David Kremer and Patrick Küry**

Department of Neurology, Neuroregeneration, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany,

Human endogenous retroviruses (HERVs) are ancient retroviral elements, which invaded the human germ line several million years ago. Subsequent retrotransposition events amplified these sequences, resulting in approximately 8% of the human genome being composed of HERV sequences today. These genetic elements, normally dormant within human genomes, can be (re)-activated by environmental factors such as infections with other viruses, leading to the expression of viral proteins and, in some instances, even to viral particle production. Several studies have shown that the expression of these retroviral elements correlates with the onset and progression of neurological diseases such as multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Further studies provided evidence on additional roles for HERVs in schizophrenia (SCZ). Since these diseases are still not well understood, HERVs might constitute a new category of pathogenic components that could significantly change our understanding of these pathologies. Moreover, knowledge about their mode of action might also help to develop novel and more powerful approaches for the treatment of these complex diseases. Therefore, the main scope of this review is a description of the current knowledge on the involvement of HERV-W and HERV-K in neurological disease specifically focusing on the effects they

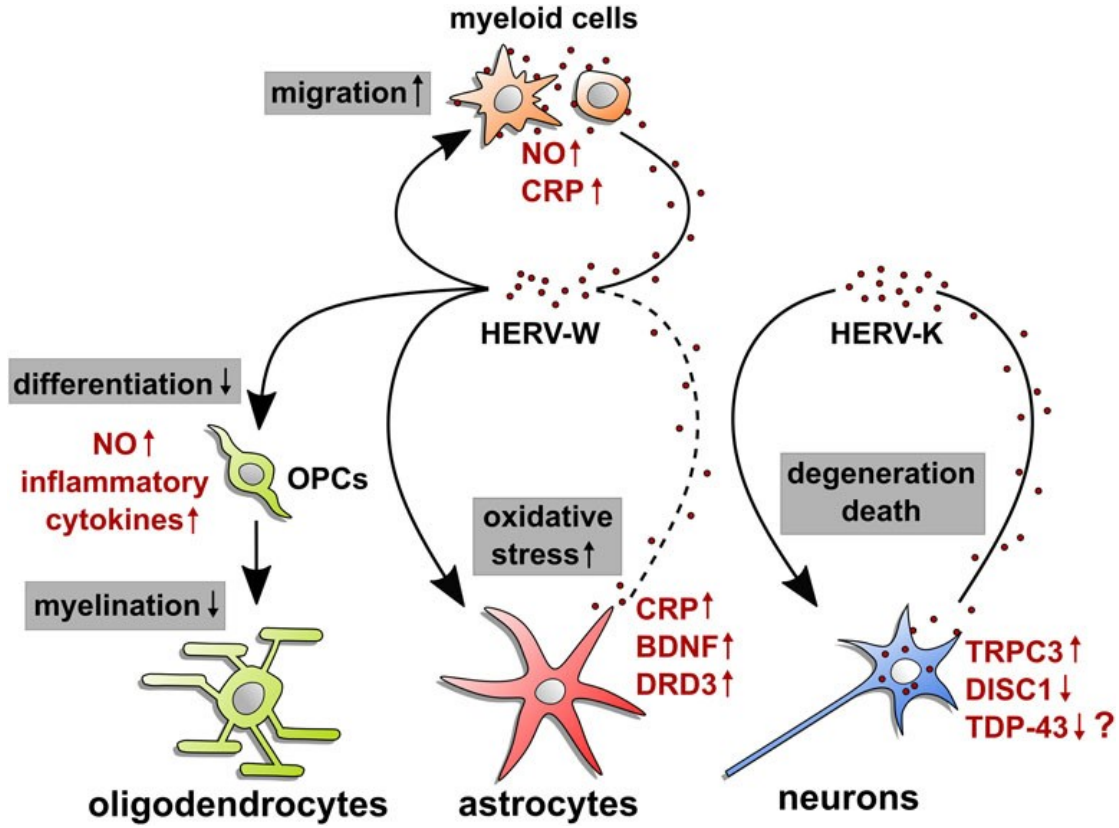

Figure 1 HERV-mediated effects on neural cells. This illustration summarizes origin and observed molecular effects of HERW-W and HERV-K on cells of the central nervous system.

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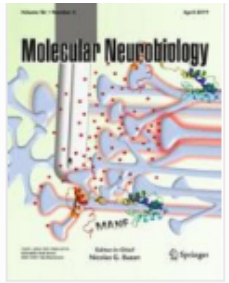
Source: *Gruchot, J., Kremer, D., & Küry, P. (2019). Neural Cell Responses Upon Exposure to Human Endogenous Retroviruses. Frontiers in genetics, 10, 655. doi:10.3389/fgene.2019.00655: https://www.frontiersin.org/files/Articles/466148/fgene-10-00655-HTML-r2/image_m/fgene-10-00655-g001.jpg*

Found in the brain and cerebrospinal fluid …



Molecular Neurobiology
April 2019, Volume 56, Issue 4, pp 2590–2605 | Cite as

Do Human Endogenous Retroviruses Contribute to Multiple Sclerosis, and if So, How?

Authors | Authors and affiliations

Gerwyn Morris, Michael Maes, Marianna Murdjeva, Basant K. Puri

mechanisms. HERV virions, RNA, cDNA, Gag and Env, and antibodies to HERV transcriptional products, have variously been found in the blood and/or brain and/or cerebrospinal fluid of MS patients, with the HERV expression level being associated with disease status. Furthermore, some HERV-associated single nucleotide polymorphisms (SNPs), such as rs662139 T/C in a 3-kb region of Xq22.3 containing a HERV-W *env* locus, and rs391745, upstream of the HERV-Fc1 locus on the X chromosome, are associated with MS susceptibility, while a negative association has been reported with SNPs in the tripartite motif-containing (TRIM) protein-encoding genes *TRIM5* and *TRIM22*. Factors affecting HERV transcription include immune activation and inflammation, since HERV promoter regions possess binding sites for related transcription factors; oxidative stress, with oxidation of guanine to 8-oxoguanine and conversion of cytosine to 5-hydroxymethylcytosine preventing binding of methyl groups transferred by DNA methyltransferases; oxidative stress also inhibits the activity of deacetylases, thereby favouring the acetylation of histone lysine residues favouring gene expression; interferon beta; natalizumab treatment; impaired epigenetic regulation; and the sex of patients.

Both **human and animal retroviruses can infect the CNS**. These are associated with many diseases of the CNS causing neurological disease directly through infection of immune cells which traffic to the brain and indirectly through increases in proinflammatory cytokines and chemokines

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… even identified in ALS (Amyotropic lateral sclerosis, Lou Gherig's disease)

Ann Neurol. 2011 Jan;69(1):141-51. doi: 10.1002/ana.22149.

Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis.

Douville R[1], Liu J, Rothstein J, Nath A.

Author information

1 Department of Neurology, Johns Hopkins University, Baltimore, MD, USA.

"We have identified a specific pattern of HERV-K expression in ALS, which may potentially define the pathophysiology of ALS. Targeting of activated genome-encoded retroviral elements may open new prospects for the treatment of ALS."[1]

Abstract

OBJECTIVE: Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of motor neurons, of unknown etiology. Previous studies showed reverse transcriptase in serum of ALS patients at levels comparable to human immunodeficiency virus-infected patients; however, the source and significance of the retroviral elements is uncertain.

INTERPRETATION: We have identified a specific pattern of HERV-K expression in ALS, which may potentially define the pathophysiology of ALS. Targeting of activated genome-encoded retroviral elements may open new prospects for the treatment of ALS.

Source: *1. Douville, R., Liu, J., Rothstein, J., and Nath, A. (2011). Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Ann. Neurol. 69, 141–151; Li W, Lee MH, Henderson L, Tyagi R, Bachani M, Steiner J, Campanac E, Hoffman DA, von Geldern G, Johnson K, Maric D, Morris HD, Lentz M, Pak K, Mammen A, Ostrow L, Rothstein J, Nath A. Human endogenous retrovirus-K contributes to motor neuron disease. Sci Transl Med. 2015 Sep 30;7(307)*

and ME/CFS …

Clinical Therapeutics/Volume 41, Number 4, 2019

Review

Epigenetic Components of Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome Uncover Potential Transposable Element Activation

Eloy Almenar-Pérez, MSc[1]; Tamara Ovejero, PhD[2]; Teresa Sánchez-Fito, MSc[1]; José A. Espejo, BSc[3]; Lubov Nathanson, PhD[4,5]; and Elisa Oltra, PhD[2,6]

[1]Escuela de Doctorado, Universidad Católica de Valencia San Vicente Mártir, Valencia, Spain; [2]School of Medicine, Universidad Católica de Valencia San Vicente Mártir, Valencia, Spain; [3]School of Experimental Sciences, Universidad Católica de Valencia San Vicente Mártir, Valencia, Spain; [4]Kiran C. Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, Florida, USA; [5]Institute for Neuro Immune Medicine, Nova Southeastern University, Fort Lauderdale, Florida, USA; and [6]Unidad Mixta CIPF-UCV, Centro de Investigación Príncipe Felipe, Valencia, Spain

ABSTRACT

Purpose: Studies to determine epigenetic changes associated with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) remain scarce; however, current evidence clearly shows that methylation patterns of genomic DNA and noncoding RNA profiles of immune cells differ between patients and healthy subjects, suggesting an active role of these epigenetic mechanisms in the disease. The present study compares and contrasts the available ME/CFS epigenetic data in an effort to evidence overlapping pathways capable of explaining at least some of the dormant transposons and structured cellular RNA interactions, triggering the activation of the innate immune system without a concomitant active infection.

Implications: Repetitive sequence filters (ie, RepeatMasker) should be avoided when analyzing transcriptomic data to assess the potential participation of repetitive sequences ("junk repetitive DNA"), representing >45% of the human genome, in the onset and evolution of ME/CFS. In addition, transposable element screenings aimed at designing cost-effective, focused empirical assays that can confirm or disprove the suspected involvement of

"We centered our attention on examining the existing evidence regarding: (1) differential DNA methylation patterns and histone modifications or histone modifier activities in ME/CFS ……
Unexpected associations between altered microRNA (miRNA or miR) profiles, differentially expressed (DE) very long ncRNAs, and changed DNA methylation prints in ME/CFS with potential transcriptional activation of transposable elements (TEs) emerged, **leading to the hypothesis for an active role of such retroviral and other repetitive elements with this disease."**

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Sou

- **ME/CFIDS**: The connection of ME/CFIDS with retroviruses has been firmly established by the work of F. Ruscetti and J. Mikovits *(Mikovits. Plague: One Scientist's Intrepid Search for the Truth about Human Retroviruses and Chronic Fatigue Syndrome (ME/CFS), Autism, and Other Diseases. Skyhorse Publishing Inc., 2017)*
- **Non-Hodgkin's Lymphoma**: The likelihood of a CFS sufferer contracting Non-Hodgkin's Lymphoma has been deemed to be 250 times higher than that of a typical healthy person.
- **Cancer**: The relationship of retroviral activity and cancer is well established, but inconvenient. The association of the XMRVs (xenotropic murine retrovirus) with prostate cancer was found in 23% of patients with the cancer, but those who tested positive for XMRV were the ones with the aggressive form of it (Schlaberg et al.,2009). The research team on this discovery, led by Dr. Ila Singh of the University of Utah, reported, "The presence of virus in malignant cells invokes classical pathways for retroviral pathogenesis, i.e. inactivation of a tumor suppressor or activation of an oncogene by retroviral integration, as possible mechanisms of tumorigenesis."[1]
- **Prostate cancer**: Schlaberg, Robert et al. "XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors." *Proceedings of the National Academy of Sciences of the United States of America* vol. 106,38 (2009): 16351-6. doi:10.1073/pnas.0906922106

Multiple Sclerosis caused by retroviruses**:**

- HERV-W - also referred to as MSRV - is involved in MS (also HERV-H). The activity and presence of MSRV in MS is well-published: in active plaques in every MS brain examined to date on both macrophages and microglia, also in astrocytes in MS lesions of the brain, as well as in the inner lining of the venous system. The highly neurotoxic MSRV envelope protein was found in the serum of 73% of MS patients and not in controls [Perron et al.,2012].
- "Expression of sequence variants of endogenous retrovirus RGH in particle form in multiple sclerosis". Lancet 1998; 352: 1033. T Christensen et al
- "Expression of endogenous retrovirus in blood mononuclear cells in brain tissue from multiple sclerosis patients". Acta Neural Scand 1995; 169: 38-44. HB Rasmussen et al.

Breast cancer caused by retroviruses:

- "Human endogenous retrovirus expression and reverse transcriptase activity in the T47D mammary carcinoma cell line". J Virol 1996; 70:2654-7. C Patience et al
- G C Buehring, H M Shen, H M Jensen, D L Jin, M Hudes, G Block. Exposure to Bovine Leukemia Virus Is Associated with Breast Cancer: A Case-Control Study. PLoS One. 2015 Sep 2;10(9):e0134304.
- G C Buehring, H M Shen, H M Jensen, K Y Choi, D Sun, G Nuovo. Bovine leukemia virus DNA in human breast tissue. Emerg Infect Dis. 2014 May;20(5):772-82.
- J F Ferrer, S J Kenyon, P Gupta. Milk of dairy cows frequently contains a leukemogenic virus. Science. 1981 Aug 28;213(4511):1014-6.
- G C Buehring, P M Kramme, R D Schultz. Evidence for bovine leukemia virus in mammary epithelial cells of infected cows. Lab Invest. 1994 Sep;71(3):359-65.

As many as 37 percent of breast cancer cases may be attributed to exposure to the RV bovine leukemia virus

RESEARCH ARTICLE Open Access

Bovine leukemia virus discovered in human blood

Gertrude C. Buehring[1*], Anne DeLaney[2], HuaMin Shen[1], David L. Chu[3], Niema Razavian[4], Daniel A. Schwartz[5,6], Zach R. Demkovich[7] and Michael N. Bates[1]

Abstract

Background: Bovine leukemia virus (BLV) infection is widespread in cattle globally and is present in marketed beef and dairy products. Human infection with BLV has been reported in breast and lung cancer tissues and was significantly associated with breast cancer in 3 case-control studies. The purpose of this current research was to determine if BLV is present in human blood cells and if antibodies to BLV are related to blood cell infection.

Methods: Standard liquid PCR and Sanger DNA sequencing were used to test for BLV in buffy coat cells (leukocytes and platelets) of blood specimens from 95 self-selected female subjects.
Enzyme-linked immunosorbent assay (ELISA) for IgG, IgM, and IgA was used to detect antibodies to BLV in the plasma of the corresponding blood samples.

Results: BLV DNA was detected in the buffy coat cells of blood in 33/95 (38%) of the subjects by PCR and DNA sequencing. IgG antibodies were detected in 30/95(32%), IgM in 55/95(58%), and IgA in 30/95(32%) of the subjects. There was no significant correlation between presence of the antibodies and presence of BLV DNA.

Conclusions: This first report of BLV in human blood raises the question of whether infection of leukocytes could conceivably lead to leukemia as it does in infected cattle. Also, system wide circulation of infected blood cells could facilitate BLV transit to various internal tissues/organs with potential for their infection and subsequent development of cancer. The most likely route of BLV transmission to humans would be zoonotic, as a foodborne infection. Although eradicated from cattle in some countries, BLV still has a high rate of infection in the Americas, the Middle East, and parts of Europe and Asia. This report of BLV in the blood layer containing human leukocytes/platelets adds important information which could be useful to elucidate possible routes of transmission of BLV to humans and to prevent further human infection.

Keywords: Bovine leukemia virus, Human blood, Zoonotic infection

Exposure to Bovine Leukemia Virus Is Associated with Breast Cancer: A Case-Control Study

Gertrude Case Buehring[1]*, Hua Min Shen[1], Hanne M. Jensen[2], Diana L. Jin[1], Mark Hudes[3], Gladys Block[4]

1 Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, California, United States of America, **2** Department of Pathology and Laboratory Medicine, University of California, Davis Medical Center, Sacramento, California, United States of America, **3** Atkins Center for Weight and Health, University of California, Berkeley, California, United States of America, **4** Division of Community Health and Human Development, School of Public Health, University of California, Berkeley, California, United States of America

* buehring@berkeley.edu

Abstract

Background

Age, reproductive history, hormo
cancer, but the agents that initiat
stood. We previously detected b
cattle, in the breast epithelium of
whether the presence of BLV DN
cancer.

The frequency of BLV DNA in mammary epithelium from women with breast cancer (59%) was significantly higher than in normal controls (29%) (multiply- adjusted odds ratio = 3.07, confidence interval = 1.66–5.69, p = .0004, attributable risk = **37%**)

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Source: *Buehring, G.C., DeLaney, A., Shen, H. et al. Bovine leukemia virus discovered in human blood. BMC Infect Dis* ***19,*** *297 (2019)*

Autoimmunity caused by retroviruses

- “Immunity and retroviral superantigens in humans” Trends Immunol 2002: 23; 57-8. DL Woodland
- “The potential role of endogenous retroviruses in autoimmunity”. Immunol Rev 1996; 152:194-236

Diabetes Type 1: is Ritchie Shoemaker’s “dreaded mould type” really a patient with activated retroviruses?

- “Retroviral superantigens and type 1 diabetes mellitus”. Cell 1998; 95:9-11
- “A human endogenous retroviral superantigen as candidate autoimmune gene in type 1 diabetes”. Cell 1997; 90: 303-13
- “Endogenous retroviral long terminal repeats of the HLA-DQ region are associated with susceptibility to insulin-dependent diabetes mellitus. Hum Immunol 1996; 50: 103-10. K Badenhoop et al

CCSVI and other vascular problems caused by retroviruses

- “Cytokine regulation of env gene expression of human endogenous retrovirus-R in human vascular endothelial cells”. Clin Immunol 1999; 93: 75-80

Rheumatoid Arthritis caused by retroviruses

- A positive correlation between HERV K levels and objective markers of disease activity in rheumatoid arthritis (Raynier et al.,2009) and Sjögren's Syndrome (Ohyama et al.,1998)
- "Retroviruses in rheumatic diseases". Ann Rheum Dis 1995; 55:441-2
- "Retrovirus-associated rheumatic syndromes". Current Opin Rheumatol 1998; 10:347-54
- "An endogenous retroviral long terminal repeat at the HLA-DQB1 gene locus confers susceptibility to rheumatoid arthritis". Hum Immunol 1999; 60: 63-8. C Seidl et al

Sjögren's Syndrome caused by retroviruses

- "Detection of serum antibodies to retroviral proteins in patients with primary Sjögren's Syndrome (autoimmune exocrinopathy). Arthritis Rheum 1990; 33:774-81

Lupus and retroviruses:

- There is a robust body of evidence implicating HERV-caused DNA hypomethylation in the etiology of SLE (Zhou et al.,2008; Talaber et al.,2014).

Other chronic illnesses caused or perpetuated by retroviruses

ADHD and retroviruses

Balestrieri, Emanuela, Mariabernarda Pitzianti, Claudia Matteucci, Elisa D'Agati, Roberta Sorrentino, Antonia Baratta, Rosa Caterina et al. "Human endogenous retroviruses and ADHD." *The World Journal of Biological Psychiatry* 15, no. 6 (2014): 499-504. *Conclusions.* Since the ADHD aetiology is due to a complex interaction of environmental, biological and genetic factors, **HERVs may represent a link among these factors and the clinical phenotype of ADHD.**

Schizophrenia and Retroviruses

O'Reilly, Richard L., and Shiva M. Singh. "Retroviruses and schizophrenia revisited." *American Journal of Medical Genetics Part A* 67, no. 1 (1996): 19-24. From the abstract: "**the involvement of an endogenous retrovirus would be compatible with some of the puzzling epidemiological findings in schizophrenia.**"

"A number of studies have found increased levels of reverse transcriptase activity in the cerebrospinal fluid of patients with the diagnosis" (Yolken et al.,2004).

Inflammatory brain diseases

Johnston, James B., et al. "Monocyte activation and differentiation augment **human endogenous retrovirus expression: implications for inflammatory brain diseases**." *Annals of neurology* 50.4 (2001): 434-442.: **three- to ninefold increases in HERV-W, HERV-K, and HERV-H RNA levels**. In addition, **elevated reverse transcriptase activity**

Autism and Retroviruses

Torres, Anthony R., Alma Maciulis, and Dennis Odell. "The association of **MHC genes** with **autism**." Front Biosci 6 (**2001**): D936-43.

Giles, T., Washington SJ Boyer-Lehnert, and Ohio P. Bassin. "Is **A Retrovirus The Root Cause Of The Autism** Epidemic?." (**2011**).

Balestrieri, E., Arpino, C., Matteucci, C., Sorrentino, R., Pica, F., Alessandrelli, R., Coniglio, A., Curatolo, P., Rezza, G., Macciardi, F. and Garaci, E., **2012**. HERVs expression in autism spectrum disorders. PloS one, 7(11), p.e48831. From the discussion: "***To the best of our knowledge, this is the first evidence linking retrotransposon activity and ASD***"

Balestrieri, E., Cipriani, C., Matteucci, C., Capodicasa, N., Pilika, A., Korca, I., ... & Coniglio, A. (**2016**). Transcriptional activity of human **endogenous retrovirus** in Albanian children with **autism spectrum disorders**. The new microbiologica, 39(3), 228.

Cipriani, Chiara, Laura Ricceri, Claudia Matteucci, Alessia De Felice, Anna Maria Tartaglione, Ayele Argaw-Denboba, Francesca Pica et al. "***High expression of Endogenous Retroviruses from intrauterine life to adulthood in two mouse models of Autism Spectrum Disorders***." Scientific reports 8, no. 1 (**2018**): 629.

How do we become infected? Why do HERVs become activated and pathogenic?

Exogenous Retroviruses can be introduced into a system as an aerosol (inhaled), inadvertently injected via vaccines (review Miyazawa et al.,2010) or via blood-based products (IVIG, etc.), acquired via intercourse (HIV) and many other avenues. HERVs have been in our system since conception.

This author has good evidence from his clinical experience that the current permanent **2.4 Gigahertz microwave irradiation** of our system (cellphone radiation) is the main driver of chronic inflammation, a precondition for the explosion of the viruses within and is probably silencing our methylation and acetylation enzymes. Several toxins present everywhere in our world are suspected as cofactors**: glyphosate** (from food), **nanonized aluminium** and lead (from polluted air), and **mercury** (from fish and dental amalgam fillings).

HERVS can also become activated via a number of other influences such as a **viral infection** and **chronic inflammation** involving elevated cytokine production with upregulation of NF-kappaB and STAT-3 (Manghera and Douville,2013). The ubiquitous **Epstein Barr Virus** induces expression of the HERV-K envelope gene and transactivation of the Multiple Sclerosis retrovirus (Mameli et al.,2007; Sutkowski et al.,2001). *Liu, Yang, et al. "The induction of Epstein-Barr Virus early antigen expression in Raji cells by GSM mobile phone radiation." Biomed Environ Sci 26.1 (2013): 76-8.*

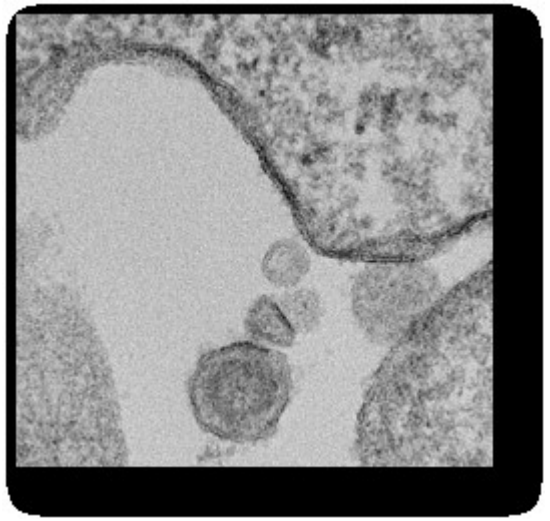
Herpes Simplex type 2 activates members of the HERV-W family. These and other mechanisms are likely responsible for the transactivation of HERVs seen in RA, SLE , Sjögren's disease, schizophrenia, autism, MS and cancer.

We have observed that the confusion of the immune system in **Lyme** sufferers consistently triggers the lasting activation of HERVs.

Retroviruses can also be acquired: exogenous RVs

Retroviruses can also be exogenous – acquired – and possess a similar genomic organisation to endogenous retroviruses/HERVs. Today they are present in the saliva of most biting insects and can be transferred to the host in the company of bacteria (Borrelia, Bartonella) or viruses: flaviviruses (tick-borne encephalitis, zika, some flus, dengue, etc.), EBV, HSV-1/2. Vaccines have also tested positive for retroviruses. Babies and infants do not have a competent blood brain barrier or immune system to contain retroviruses.

"In animals, exogenous retroviruses are responsible for some of the deadliest diseases known. Yet it wasn't until 1980 that Poiesz and Ruscetti isolated the first human disease-causing retrovirus, then called Human T-cell Leukemia Virus as it was shown to cause an aggressive cancer called Adult T-cell leukemia (ATL)."[1]



Electron micrograph (EM) of the gamma retrovirus isolated by J. Mikovits from human blood in 2009

Source: *1. Dr. Judy Mikovits, https://vaccineimpact.com/2017/retroviruses-in-vaccines-are-we-altering-the-genes-of-future-generations-in-unknown-ways/;*
"The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences"; Proc Natl Acad Sci USA 1996; 93: 5177-84
"Demystified: Human endogenous retroviruses"; Mol Pathol 2003 Feb; 56(1): 11-

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The overlooked co-infection in Lyme: the saliva of biting insects contains **retroviruses**

Transmission of retroviruses by arthropods

LD Foil, CJ Issel - Annual Review of Entomology, 1991 - annualreviews.org

Kim, A., C. Terzian, P. Santamaria, A. Pelisson, N. Purd'homme, and A. Bucheton. 1994. **Retroviruses in invertebrates**: the gypsy retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 91:1285-1289.

Song, S. U., M. Kurkulos, J. D. Boeke, and V. G. Corces. 1997**. Infection of the germ line by retroviral** particles produced in the follicle cells: a possible mechanism for the mobilization of the gypsy retroelement of *Drosophila*. Development 124:2789-2798.

Terzian, C., A. Pélisson, and A. Bucheton. 10 August 2001, posting date. Evolution and phylogeny of **insect endogenous retroviruses**. BMC Evol. Biol. 1:3. [Online.] http://www.biomedcentral.com.

Teysset, L., J. C. Burns, H. Shike, B. L. Sullivan, A. Bucheton, and C. Terzian. 1998. A Moloney murine **leukemia virus-based retroviral vector** pseudotyped by the insect retroviral *gypsy* envelope can infect *Drosophila* cells. J. Virol. 72:853-856.

Dual Infection: most Lyme patients are co-infected with retroviruses

"Viral Association with the Elusive Rickettsia of Viper Plague from Ghana, West Africa" From the abstract: *A type D **retrovirus** was observed in vacuoles in all infected cells. **The virus and rickettsia infection was associated*** with transfer of cytopathic effect

Annals of the New York Academy of Sciences. 15 December 2008

https://doi.org/10.1196/annals.1428.092

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Most biting insects harbour **insect retroviruses**. This includes ticks, stinging flies, fleas and spiders

- Pearson, Margot N., and George F. Rohrmann. "Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae **(insect retrovirus**) families." *Journal of virology* 76.11 (2002): 5301-5304.
- Recent research suggests that members of the *Baculoviridae* family can be divided into two groups on the basis of their envelope fusion proteins. One group utilizes proteins related to GP64. Homologs of GP64 are also used by the thogotoviruses, a genus of the *Orthomyxoviridae* family. Members of the other group of baculoviruses utilize envelope fusion proteins related to a protein called LD130. LD130 has been shown to be related to the envelope protein of insect **retroviruses** in the genus *Errantivirus* (family *Metaviridae*).
- In this review, the evidence for these data is outlined and possible pathways of transfer, incorporation, and substitution are discussed.

Cat fleas not only give you Bartonella but also a multitude of **retroviruses,** with life-long consequences

Vobis, M., J. D'haese, H. Mehlhorn, and N. Mencke. "The feline leukemia virus (FeLV) and the cat flea (Ctenocephalides felis)." *Parasitology research* 90, no. 3 (2003): S132-S134.

Abstract

The feline leukemia virus (FeLV) is a naturally occurring and widespread retrovirus among domestic cats. The virus is mainly transmitted horizontally through saliva, blood and other body fluids by close contact between cats. Other vectors than cats, e.g. blood sucking parasites, have not been reported. This study tested the vectors potential of the cat flea (*C.felis*) for FeLV. In a first feeding, fleas were fed for 24 hours with blood from a FeLV-infected cats with persistent viremia. FeLV could be detected in the fleas, as well as in their faeces. Fleas were then divided in two populations and fed in a second feeding for 5 and 24 hours, respectively, with uninfected non-viremic blood. **The FeLV was again detected in the fleas and their faeces.** In addition, the two resulting blood samples of the second feeding were subsequently tested for FeLV, and both samples were positive for FeLV-RNA. The cat flea transmitted the feline leukemia virus from one blood sample to another. In a third feeding, the same populations of fleas were fed again with uninfected blood for 5 and 24 hours, respectively. This time, samples FeLV was detectable neither in the fleas, nor in the faeces or blood. Results show that cat fleas are potential vectors for the feline leukemia virus RNA *in-vitro* and probably also *in-vivo*.

Vaccines today contain retroviral sequences with predictable adverse consequences

Issues Law Med. 2016 Fall;31(2):221-234.

Insertional mutagenesis and autoimmunity induced disease caused by human fetal and retroviral residual toxins in vaccines.

Jarzyna P[1], Doan NV[1], Deisher TA[1].

Article excerpt

Introduction

Major concern of vaccination regarding childhood diseases in terms of Insertional Mutagenesis and Autoimmunity

The potential consequences of injecting our children with human fetal DNA contaminants include two well-established pathologies:

1) Insertional mutagenesis in which fetal DNA incorporates into the child's DNA **causing mutations**.

2) **Autoimmune disease triggered by the human fetal DNA in vaccines** leading a child's immune system to attack his or her own body.

Vaccines: The United States manufacturing process and vaccine history of using human fetal cell lines: In January 1979, the Rubella manufacturing switch from animal-based to the human fetal cell line WI-38 was approved by the FDA

Agenda

- 1. What are retroviruses (RVs)?
  - Endogenous and exogenous RVs
  - Conditions they are associated with
- **2. Towards detecting the activation of RVs**
- 3. The treatment of retroviral activation/infection
  - Proven biological remedies
  - Pharmaceuticals

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Currently for us physicians no direct testing for retroviruses is available.
We rely on 6 indirect parameters

- 1. Low white blood count (below 5000) – should elicit a search!
- 2. Symptoms and high level of suspicion (clinical experience)
- 3. Elevated levels of IgG for EBV, HHV-6, CMV and HSV-II
- 4. Elevated levels of NAGALASE
- 5. Lowered levels of CD 26
- 6. Elevated levels of RANTES (IMD lab in Berlin only)

Maina, Ayub K., et al. "The Potential for **DPP-4/CD26 usage as a surrogate marker for Antiretroviral Therapy Efficacy** in HIV Infected populations." *African Journal of Pharmacology and Therapeutics* 5.4 (2017).

Background: Human Immunodeficiency Virus (HIV) viral load and $CD4^+$ cell counts are the most commonly used markers for monitoring efficacy of anti-retroviral therapy (ART) in HIV infected individuals. The high cost of viral load monitoring limits its usage in resource limited countries, often leaving the use of CD4+ T cell counts as the only alternative. Though cheaper and more readily available, CD4+ cell counts as a measure of detecting treatment failure, is an unreliable predictor of disease progression. Hence, there is a need for more sensitive alternative, but less costly techniques for detecting treatment failure which can be used in resource limited settings.

Objective: To evaluate the feasibility of using plasma CD26/Dipeptidyl peptidase IV (DPPIV) as a novel marker for clinical evaluation of treatment efficacy in HIV infected children.

Method: Blood samples collected from HIV^+ children (n=76) before and after initiation on ART, were assessed for HIV RNA (viral load), CD4+ T-cell count and DPPIV/CD26 levels. Viral load levels were analyzed using Roche Amplicor HIV-1 Monitor Test kit; CD4+ T-Cell Counts were analyzed using BD FACS Calibur flow cytometer while DPPIV/CD 26 levels were analyzed using Human DPPIV/CD26 Quantikine ELISA kit (R&D Systems, Minneapolis MN).

Results: The plasma DPPIV/CD26 levels increased significantly in children after ART initiation ($p = 0.017$), while the viral load levels declined after ART initiation with subsequent CD4+ cell counts increase. **The DPPIV/CD 26 increase positively correlated with viral load decrease** while negatively correlating to the CD4+ cell count increase.

Conclusion: These findings demonstrate **an inverse relationship between DPPIV/CD26 levels and HIV viral load** and the direct proportionality of CD4+ Cell counts and DPPIV/CD26 levels, suggesting potential for use of DPPIV/CD26 as a surrogate marker for evaluating HIV disease progression in children receiving anti-retroviral therapy.

Key words: CD26/Dipeptidyl peptidase IV (DPPIV), ELISA, Surrogate marker, Viral Load, CD4 Count, antiretroviral.

Bradstreet, James Jeffrey, Emar Vogelaar, and Lynda Thyer. "Initial observations of elevated alpha-N-acetylgalactosaminidase activity associated with autism and observed reductions from Gc protein--macrophage activating factor injections." *Autism Insights* 4 (2012): 31.

Abstract Background: Autism spectrum disorders (ASD) are developmental disorders affecting 1:88 children, and which appear to be associated with a variety of complex immune dysregulations including autoimmunity. The enzyme, alpha-N-acetylgalactosaminidase (Nagalase) deglycosylates serum Gc protein (vitamin D3 – binding protein) rendering it incapable of activating macrophage defenses. **Increased Nagalase activity has been associated with a variety of malignancies, immune disorders and viral infections**. Macrophage activating factor (GcMAF) has been repeatedly published as an intervention to lower serum Nagalase activity for a variety of cancer and **HIV patients**. GcMAF is a naturally occurring protein with well-established safety and therapeutic benefit(s) supported by numerous human studies. Methods: Initially, parents of 40 individuals with ASD sought testing for Nagalase serum activity as part of an evaluation of immune dysregulation. Nagalase enzyme activity measurement was performed by the European Laboratory of Nutrients (ELN), Bunnik, the Netherlands, using an end-point enzymatic assay of a chromogenic substrate. Some parents of patients with elevated Nagalase activity opted for weekly GcMAF injections provided by Immuno Biotech Ltd., Guernsey UK (www.gcmaf.eu). GcMAF is purified from human serum obtained from the American Red Cross using 25-hydroxyvitamin D3-Sepharose high affinity chromatography. The protein is then further diluted to obtain therapeutically appropriate levels for patients based on their clinical presentations. Results: Individuals with ASD (32 males and 8 females, n = 40, ages: 1 year 4 months - 21 years 2 months) had initial and post treatment assessment of Nagalase activity. Dosing of GcMAF was recommended based on previously reported response curves adjusted by the treating clinician for age, weight, and Nagalase levels. The average pre-treatment Nagalase activity of the autism group was 1.93 nmol/min/mg of substrate. This was well above the laboratory reported normal range of ,0.95 nmol/min/mg. For the ASD group the average level at the time of second testing was 1.03 nmol/min/mg, reflecting an average reduction of 0.90 nmol/min/mg (P , 0.0001). Apart from the likely immunological benefits of lowering the Nagalase activity of these individuals, uncontrolled observations of GcMAF therapy indicated substantial improvements in language, socialization and cognition. No significant side-effects were reported during the course of injections.

Conclusions: In this first report of **Nagalase activity in patients with autism**, it appears that most individuals have **substantially higher levels** than the expected healthy ranges. Although Nagalase is a nonspecific marker of immune dysregulation, its observed levels in autism may have both etiological and therapeutic significance. Importantly, this is also the first report of reduction of Nagalase activity in an autism population with GcMAF injections.

RANTES is a marker for retroviral virulence

Gordon, Cynthia J., et al. "Enhancement of human immunodeficiency virus type 1 infection by the CC-chemokine RANTES is independent of the mechanism of virus-cell fusion." *Journal of virology* 73.1 (1999): 684-694.

ABSTRACT

We have studied the effects of CC-chemokines on human immunodeficiency virus type 1 (HIV-1) infection, focusing on the **infectivity enhancement caused by RANTES**. High RANTES concentrations increase the infectivity of HIV-1 isolates that use CXC-chemokine receptor 4 for entry. However, RANTES can have a similar enhancing effect on macrophagetropic viruses that enter via CC-chemokine receptor 5 (CCR5), despite binding to the same receptor as the virus. Furthermore, **RANTES enhances the infectivity of HIV-1** pseudotyped with **the envelope glycoprotein of murine leukemia virus** or vesicular stomatitis virus, showing that the mechanism of enhancement is independent of the route of virus-cell fusion. The enhancing effects of RANTES are not mediated via CCR5 or other known chemokine receptors and are not mimicked by MIP-1α or MIP-1β. The N-terminally modified derivative aminooxypentane RANTES (AOP-RANTES) efficiently inhibits HIV-1 infection via CCR5 but otherwise mimics RANTES by enhancing viral infectivity. There are two mechanisms of enhancement: one apparent when target cells are pretreated with RANTES (or AOP-RANTES) for several hours, and the other apparent when RANTES (or AOP-RANTES) is added during virus-cell absorption. We believe that the first mechanism is related to cellular activation by RANTES, whereas the second is an increase in virion attachment to target cells.

Regulated upon activation, normal T-cell expressed, and secreted (RANTES)

The relevance of treating retroviral activity in chronic illness was recognized by this author with the use of the muscle-tension biofeedback method A.R.T. (developed by D. Klinghardt) – with the delicate guidance of Judy Mikovits

This study shows that ART is both reliable and valid in allergy testing

Altern Ther Health Med. 2018 Jan 15. pii: AT5703. [Epub ahead of print]

Autonomic Response Testing Compared With Immunoglobulin E Allergy Panel Test Results: Preliminary Report.

Frandsen A, McClure M, Chung MK, LaRiccia PJ.

Abstract

Chronically ill patients who have failed standard medical assessment and therapies are often assessed by integrative medical providers for atypical manifestations of allergies as the possible source or contributing factor(s) to their condition. Skin testing and immunoglobulin E (IgE) allergy panels increase the cost of care in these patients. Objective • The objective of this study was to determine the accuracy of autonomic response testing (ART) as compared with IgE allergy panel blood tests.

Design • This study was a retrospective chart review of patients who had ART and blood drawn for an IgE allergy panel at the same office visit. Outcome Measures • Sensitivity, specificity, positive predictive value, negative predictive value, overall accuracy, phi coefficient, and Cohen's kappa were calculated.

*Results • A total of 14 charts were reviewed. All measures of accuracy were of either useful or excellent strength. The **strength of association measures** of the phi coefficient and Cohen's kappa **were strong**. Conclusion • This first and preliminary evaluation of the allergy assessment utility of ART is very promising and reveals the need for more vigorous follow-up studies.*

Frandsen, A., McClure, M., Chung, M. K., & LaRiccia, P. J. (2018). Autonomic Response Testing Compared With Immunoglobulin E Allergy Panel Test Results: Preliminary Report. *Alternative therapies in health and medicine*. 2018 - ncbi.nlm.nih.gov

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In this clinical study we were able to show that when A.R.T. finds dysfunction in an organ or tissue, ultrasound can confirm the dysfunction in all cases. A.R.T. is a reliable tool for detecting tissue dysfunction, establishing an accurate diagnosis and effective treatment protocols

The Ruggiero-Klinghardt (RK) Protocol for the Diagnosis and Treatment of Chronic Conditions with Particular Focus on Lyme Disease

American Journal of Immunology, March 2017

Dietrich Klinghardt and Marco Ruggiero

Abstract: Here we describe the Ruggiero-Klinghardt (RK) Protocol that is based on **integration of Autonomic Response Testing (ART) with diagnostic ultrasonography** and on application of therapeutic ultrasounds; the latter are used as a provocation tool and as an instrument to optimize drug uptake and utilization in specific areas of the body. This protocol consists of a precise sequence of diagnostic and therapeutic procedures with the ultimate goal of improving sensitivity and specificity of diagnosis at the same time evaluating and optimizing efficacy of treatments in chronic conditions including, but not limited to, persistent Lyme disease. The RK Protocol represents **a paradigm shift in diagnostics and therapeutics: Thus, compartmentalized microbes, transformed cells, toxins and metabolites could be detected using a safe and non-invasive method**. In addition, the RK Protocol allows optimization of efficacy of drugs and other therapeutic interventions. Although the RK Protocol was initially developed for persistent Lyme disease, it shows significant potential in conditions ranging from cancer to neurodegenerative diseases and autism. In oncology, the RK Protocol may serve to facilitate early diagnosis and to increase sensitivity of cancer cells to the killing effects of a variety of remedies ranging from conventional radio- and chemotherapy to more recent forms of immunotherapy. Thus, the 1st goal of the RK Protocol is diagnostic: That is, to make pathogens, toxins, transformed cells and cells infected by viruses that are inaccessible to conventional diagnostic and therapeutic tools, “visible” to the therapist who can detect them with laboratory methods and deal with them with appropriate interventions; and also to make them “visible” to the immune system that can fight them in a physiological manner. The 2nd goal is to optimize drug uptake and utilization in the organs and tissues studied and targeted with these procedures.

Keywords: Lyme, Ultrasound, Autonomic Response Testing, Immune System, Imaging, Brain

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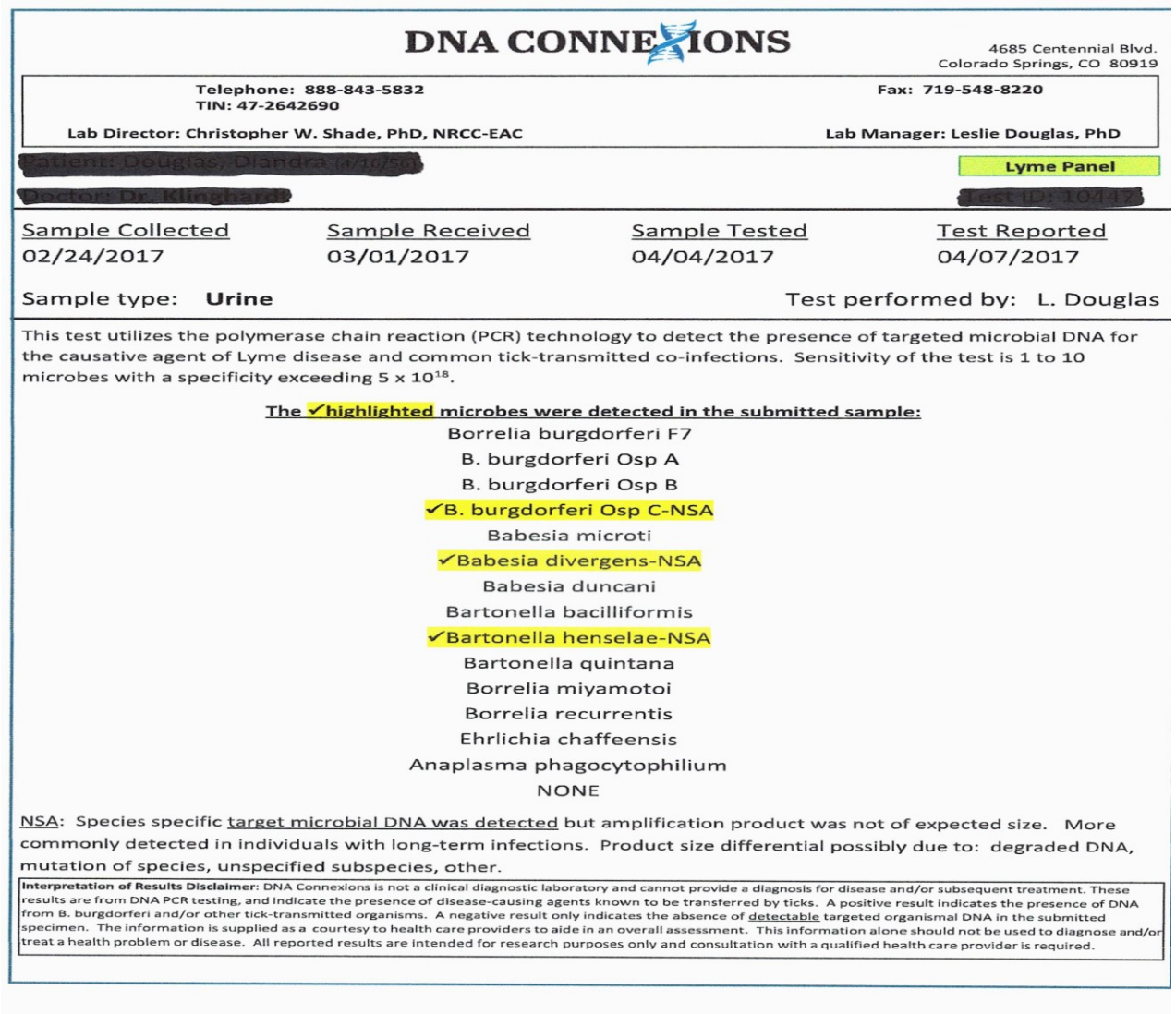
25-year-old woman with POTS and severe fatigue and brain fog. Tested negative with the Western Blot and appropriate IgG/IgM tests, Immunfluorescence - but positive for all 3 pathogens with A.R.T.



DNA CONNEXIONS

4685 Centennial Blvd.
Colorado Springs, CO 80919

Telephone: 888-843-5832
TIN: 47-2642690
Fax: 719-548-8220

Lab Director: Christopher W. Shade, PhD, NRCC-EAC
Lab Manager: Leslie Douglas, PhD

[redacted]
Lyme Panel
[redacted]

Sample Collected	Sample Received	Sample Tested	Test Reported
02/24/2017	03/01/2017	04/04/2017	04/07/2017

Sample type: **Urine**
Test performed by: L. Douglas

This test utilizes the polymerase chain reaction (PCR) technology to detect the presence of targeted microbial DNA for the causative agent of Lyme disease and common tick-transmitted co-infections. Sensitivity of the test is 1 to 10 microbes with a specificity exceeding 5×10^{18}.

The ✓highlighted microbes were detected in the submitted sample:

Borrelia burgdorferi F7
B. burgdorferi Osp A
B. burgdorferi Osp B
✓B. burgdorferi Osp C-NSA
Babesia microti
✓Babesia divergens-NSA
Babesia duncani
Bartonella bacilliformis
✓Bartonella henselae-NSA
Bartonella quintana
Borrelia miyamotoi
Borrelia recurrentis
Ehrlichia chaffeensis
Anaplasma phagocytophilium
NONE

NSA: Species specific target microbial DNA was detected but amplification product was not of expected size. More commonly detected in individuals with long-term infections. Product size differential possibly due to: degraded DNA, mutation of species, unspecified subspecies, other.

Interpretation of Results Disclaimer: DNA Connexions is not a clinical diagnostic laboratory and cannot provide a diagnosis for disease and/or subsequent treatment. These results are from DNA PCR testing, and indicate the presence of disease-causing agents known to be transferred by ticks. A positive result indicates the presence of DNA from B. burgdorferi and/or other tick-transmitted organisms. A negative result only indicates the absence of detectable targeted organismal DNA in the submitted specimen. The information is supplied as a courtesy to health care providers to aide in an overall assessment. This information alone should not be used to diagnose and/or treat a health problem or disease. All reported results are intended for research purposes only and consultation with a qualified health care provider is required.

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Agenda

- 1. What are retroviruses (RVs)?
  - Endogenous and exogenous RVs
  - Conditions they are associated with
- 2. How A.R.T. helped to identify that RVs can be treated
- **3. The treatment of retroviral activation/infection**
  - **Proven biological remedies**
  - Pharmaceuticals

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Herbal anti-retroviral preparations

RetroV Powder (www.KiScience.com)

Baikalin extract from Scullcap root/Scuttalaria

- Li, B. Q., T. Fu, Y. D. Yan, N. W. Baylor, F. W. Ruscetti, and H. F. Kung. "Inhibition of HIV infection by baicalin--a flavonoid compound purified from Chinese herbal medicine." *Cellular & molecular biology research* 39, no. 2 (1993): 119-124.
- Zhao, Qing, Yang Zhang, Gang Wang, Lionel Hill, Jing-Ke Weng, Xiao-Ya Chen, Hongwei Xue, and Cathie Martin. "A specialized flavone biosynthetic pathway has evolved in the medicinal plant, Scutellaria baicalensis." *Science advances* 2, no. 4 (2016): e1501780.

Bitter Melon

- Trivedi, R. V., Wadher, K. J., Taksande, J. B., Mahore, J. G., & Umekar, M. J. (2011). Momordica charantia: A Natural and Safe Approach for the Treatment of HIV Infection. *Int J of Pharm Tech Research*, *3*, 1660-1666.
- 4 Ng T B, Wong C M, Li W W, Yeung H W. Isolation and characterization of a galactose binding lectin with insulinomimetic activites from the seeds of the bitter gourd *Momordica charantia* (Cucurbitaceae). International Journal of Peptide and Protein Research. 1986; 28 163-72

Green Tea

- Nakane, H., Hara, Y., & Ono, K. (1994). Tea polyphenols as a novel class of immunodeficiency virus reverse transcriptase.

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Reishi Mushroom

- Min, B. S., Nakamura, N., Miyashiro, H., BAE, K. W., & Hattori, M. (1998). Triterpenes from the spores of Ganoderma lucidum and their inhibitory activity against HIV-1 protease. *Chemical and Pharmaceutical Bulletin*, *46*(10), 1607-1612.

Stinging Nettle

- Balzarini, J., Neyts, J., Schols, D., Hosoya, M., Van Damme, E., Peumans, W., & De Clercq, E. (1992).The mannose-specific plant lectins from Cymbidium hybrid and Epipactis helleborine and the (N-acetylglucosamine) n-specific plant lectin from Urtica dioica are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication in vitro. *Antiviral research*, *18*(2), 191-207**.**

Olive Leaf

- Lee-Huang, S., Zhang, L., Huang, P. L., Chang, Y. T., & Huang, P. L. (2003). Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochemical and Biophysical Research Communications*, *307*(4), 1029-1037.

Saffron

Sepehr Soleymani, Rezvan Zabihollahi, Sepideh Shahbazi and Azam Bolhassani. Crocin, a carotenoid pigment of saffron inhibits the replication of HSV and HIV in vitro. J Prot Bioinform 2017; 10:12

Plus **Lomatium Root**, **Stevia**

Dosage: 1 teaspoon twice daily (has shown potent anti retroviral effects)

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Whole Leaf Licorice or intravenous glycyrrhizic acid against retroviral replication (www.kiScience.com/compounding pharmacies)

Amsar Private Limited. Phytochemicals--Monoammonium glycyrrhizinate. http://www.amsar.com/PhytoChemicals/monoammonium.htm

Cantelli-Forti G, Maffei F, Hrelia R, Bugamelli F, Bernardi M D'Intino R, Maranesi M, and Raggi MA. Interaction of Licorice on Glycyrrhizin Pharmacokinetics. *Environmental Health Perspectives.* 1994 Nov;102 Suppl 9:65-8. Presented at the IV European ISSX Meeting on Toxicological Evaluation of Chemical Interactions: Relevance of Social, Environmental and Occupational Factors held 3-6 July 1992 in Bologna. http://www.ncbi.nlm.nih.gov/pubmed/7698088

Fiore C, Eisenhut M, Krausse R, Ragazzi E, Pellati D, Armanini D, Bielenberg J. Antiviral effects of Glycyrrhiza species. *Phytother Res.* 2008 Feb;22(2):141-8. http://www.ncbi.nlm.nih.gov/pubmed/17886224

Ikegami N., S. Kinoshita, T. Kanesaki, K. Uno, K. Akatani, T. Kishida. Glycyrrhiza glabra. Evaluation of long-term treatment with glycyrrhizin and of combination therapy with glycyrrhizin and AZT or DDI on HIV-1 carriers. *Antiviral Research.* Volume 30, Issue 1, pp. A33-A33, April, 1996. http://www.ingentaconnect.com/content/els/01663542/1996/00000030/00000001/art80277

Ito M, Sato A, Hirabayashi K, Tanabe F, Shigeta S, Baba M, De Clercq E, Nakashima H, Yamamoto N. Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). *Antiviral Res.* 1988 Dec 11;10(6):289-98.http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=18974890

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- **Saidi** H, Melki M-T, and Gougeon M-L. HMGB1-Dependent Triggering of **HIV-1** Replication and Persistence in Dendritic Cells as a Consequence of NK-DC Cross-Talk *PLoS ONE.* 2008; 3(10): e3601. Published online 2008 October 31. doi: 10.1371/journal.pone.0003601.

  http://www.ncbi.nlm.nih.gov/pubmed/18974890

- **Melki** MT, Saidi H, Dufour A, Olivo-Marin JC, Gougeon ML.. Escape of **HIV-1**-infected dendritic cells from TRAIL-mediated NK cell cytotoxicity during NK-DC cross-talk--a pivotal role of HMGB1. *PLoS Pathog.* 2010 Apr 15;6(4):e1000862.

  http://www.ncbi.nlm.nih.gov/pubmed/20419158

- **Yao** WH, Zhao W, Wu YW, Zhao H, Wei HX, Cheng C, Zhu P, Chi Y. Effect of compound glycyrrhizin on peripheral T-lymphocyte subset in **AIDS** patients. *Zhonghua Nan Ke Xue.* 2006 Jul;12(7):598-601.

  http://www.ncbi.nlm.nih.gov/pubmed/16894934

- **Yuan** H, Ji WS, Wu KX, Jiao JX, Sun LH, Feng YT. Anti-inflammatory effect of Diammonium Glycyrrhizinate in a rat model of **ulcerative colitis**. *World J Gastroenterol.* 2006 Jul 28;12(28):4578-81.

  http://www.ncbi.nlm.nih.gov/pubmed/16874877

At SHI we frequently and successfully use compounded glycyrrhizin intravenously

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Sardinian Cistus Incanus (kiScience.com)

Stephanie Rebensburg, Markus Helfer, Martha Schneider, Herwig Koppensteiner, Josef Eberle, Michael Schindler, Lutz Gürtler, Ruth Brack-Werner. **Potent in vitro antiviral activity of Cistus incanus extract against HIV and Filoviruses targets viral envelope proteins**. *Scientific Reports*, 2016; 6: 20394 DOI: 10.1038/srep20394

Scientists at the Helmholtz Zentrum München discover that extracts of the medicinal plant *Cistus incanus* (Ci) prevent human immunodeficiency viruses from infecting cells. Active antiviral ingredients in the extracts inhibit docking of viral proteins to cells. Antiviral activity of *Cistus* extracts also targets Ebola- and Marburg viruses.

HIV: broad activity, no resistance

The Brack-Werner team found potent activity of Ci extracts acted against a broad spectrum of clinical HIV-1 and HIV-2 isolates. This also included a virus isolate resistant against most available drugs. "Antiviral ingredients of Ci extracts target viral envelope proteins on infectious particles and prevent them from contacting host cells," Brack-Werner explains. No resistant viruses were detected during long-term treatment
(24 weeks) with Ci extract, indicating that Ci extract attacks viruses without causing resistance. Since the antiviral activity of Ci extracts differs from all clinically approved drugs, Ci-derived products could be an important complementation to current established drug regimens.

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Cistus

Rauwald, H. W., et al. "On the **antispirochaetal activity** of manoyloxides and carvacrol from the oleoresin labdanum of Cistus creticus L." Planta Medica 79.13 (2013): PN53.

Bouamama, H., et al. "Antibacterial and **antifungal activities** of **Cistus incanus** and C. monspeliensis leaf extracts." Therapie 54.6 (1999): 731-733..

Cistus is also a very effective **biofilm breaker**, unmasking persistent infections:

Hannig, Christian, et al. "Effects of **Cistus-tea** on bacterial colonization and enzyme activities of the in situ pellicle." Journal of dentistry 36.7 (2008): 540-545.

Whole leaf Stevia has been shown in a study by Northwest University in the US to be as effective or more effective in the treatment of Lyme disease than triple antibiotic therapy, including the use of Daptomycin:

Effectiveness of **Stevia Rebaudiana Whole Leaf Extract** Against the Various Morphological Forms of **Borrelia Burgdorferi** in Vitro. Eur J Microbiol Immunol (Bp). 2015 Dec ;5(4):268-80. Epub 2015 Nov 12; P A S Theophilus, M J Victoria, K M Socarras, K R Filush, K Gupta, D F Luecke, E Sapi

Broccoli sprout extract

Broccoli Synergy Powder, kiscience.com: 1tsp twice daily

- Singh K, Zimmerman AW. Sulforaphane Treatment of Young Men with Autism Spectrum Disorder. CNS Neurol Disord Drug Targets. 2016;15(5):597-601.
- Singh, K., Connors, S. L., Macklin, E. A., Smith, K. D., Fahey, J. W., Talalay, P., & Zimmerman, A. W. (2014). Sulforaphane treatment of autism spectrum disorder (ASD). *Proceedings of the National Academy of Sciences*, October 2014; *111*(43), 15550-15555. from the text: . “Sulforaphane, which showed negligible toxicity, was selected because it **upregulates genes that protect aerobic cells against oxidative stress, inflammation, and DNA-damage**, all of which are prominent and possibly mechanistic characteristics of ASD”
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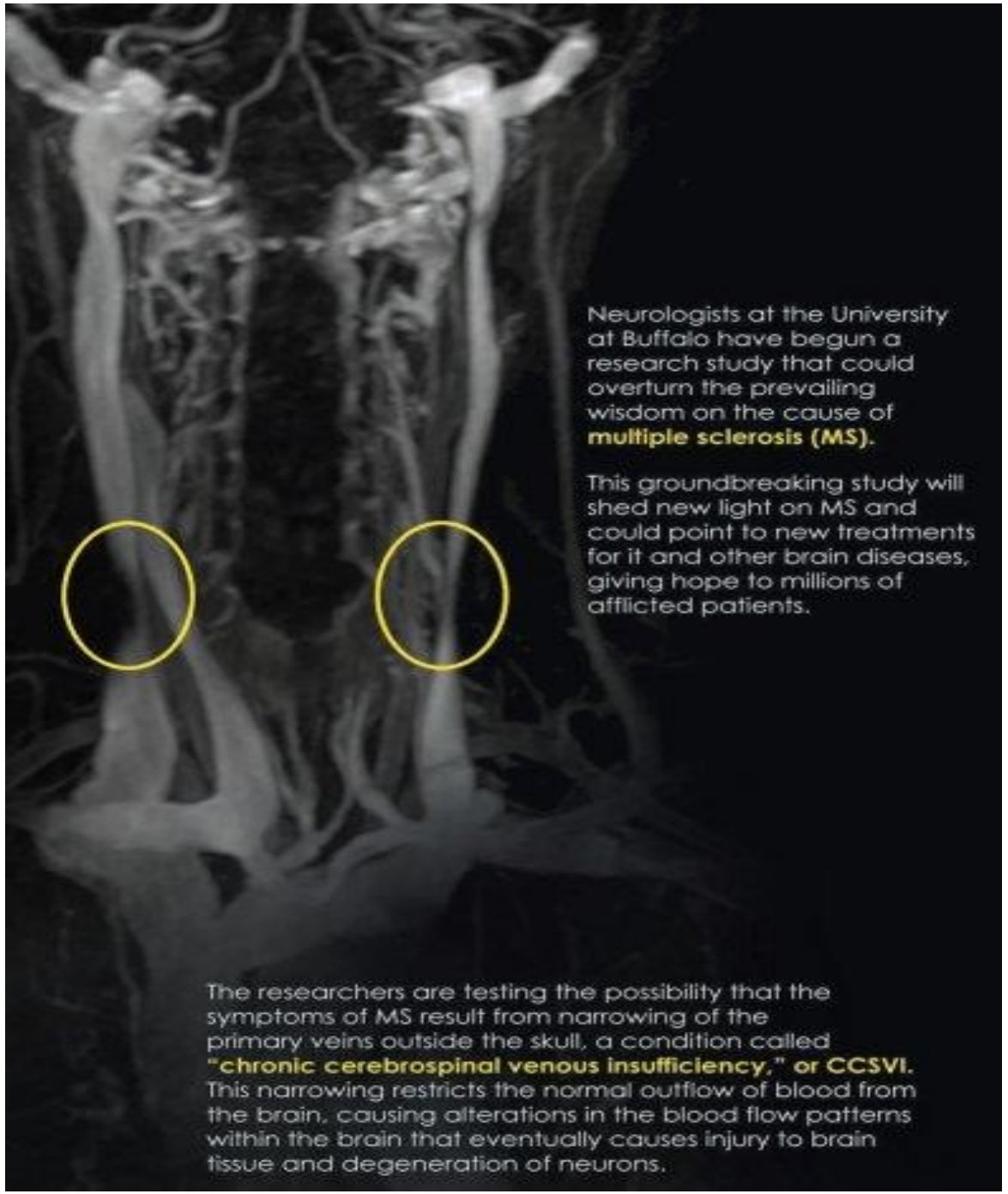
CCSVI/TVAM treatment

- CCSVI - chronic cerebrospinal venous insufficiency
- Outcome of chronic infections affecting the endothelium
- **TVAM (Transvascular Autonomic Modulation)** is an endovascular procedure in which a catheter is inserted via a small incision and threaded up into the jugular vein.
  - Treatment involves stretching the vein with small catheters, activating autonomic nerve fibers located within the outer tissues of the vein.
  - By stimulating the nerve fibers, we activate the venous distension reflex leading to increased sympathetic tone

Treatment: **Transdermal creams**: ***"Ki Circulation Cream"*** *(kiscience.com):* Apply 2 times daily to front of neck (bioactive molecules/often rapid decrease of retroviral markers) with the Klinghardt technique



Dietary supplements to treat HERVs

Melatonin – a must in treating retroviral infections without drugs

Boga, Jose Antonio, et al. "Beneficial actions of melatonin in the management of viral infections: a new use for this “molecular handyman”?." *Reviews in medical virology* 22.5 (2012): 323-338.

Melatonin (N-acetyl-5-methoxytryptamine) is a multifunctional signaling molecule that has a variety of important functions. Numerous clinical trials have examined the therapeutic usefulness of melatonin in different fields of medicine. Clinical trials have shown that melatonin is efficient in preventing cell damage under acute (sepsis, asphyxia in newborns) and chronic states (metabolic and neurodegenerative diseases, cancer, inflammation, aging). The beneficial effects of melatonin can be explained by its properties as a potent antioxidant and antioxidant enzyme inducer, a regulator of apoptosis and a stimulator of immune functions. **These effects support the use of melatonin in viral infections, which are often associated with inflammatory injury and increases in oxidative stress. In fact, melatonin has been used recently to treat several viral infections, which are summarized in this review.** The role of melatonin in infections is also discussed herein.

At SHI we may use transdermal melatonin in very high doses as a treatment for all infections, including retroviral activity

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Zhang, Z., et al. "Prevention of immune dysfunction and vitamin E loss by **dehydroepiandrosterone and melatonin supplementation during murine retrovirus infection**."

Immunology 96.2 (1999): 291.

ABSTRACT

Female C57BL/6 mice infected with the LP-BM5 leukaemia retrovirus developed murine acquired immune-deficiency syndrome (AIDS). Dehydroepiandrosterone (DHEA) and melatonin (MLT) modify immune dysfunction and prevent lipid peroxidation. We investigated whether DHEA and MLT could prevent immune dysfunction, excessive lipid peroxidation, and tissue vitamin E loss induced by retrovirus infection. Retrovirus infection inhibited the release of T helper 1 (Th1) cytokines, stimulated secretion of Th2 cytokines, increased hepatic lipid peroxidation, and induced vitamin E deficiency. Treatment with DHEA or MLT alone, as well as together, largely prevented the reduction of B- and T-cell proliferation as well as of Th1 cytokine secretion caused by retrovirus infection. Supplementation also suppressed the elevated production of Th2 cytokines stimulated by retrovirus infection. DHEA and MLT simultaneously reduced hepatic lipid peroxidation and prevented vitamin E loss. The use of DHEA plus MLT was more effective in preventing retrovirus-induced immune dysfunction than either DHEA or MLT alone. **These results suggest that supplementation with DHEA and MLT may prevent cytokine dysregulation, lipid oxidation and tissue vitamin E loss induced by retrovirus infection.** Similarly, hormone supplementation also modified immune function and increased tissue vitamin E levels in uninfected mice.

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Pantethine (only the fat soluble form of B5 works!) activates gene and histone acetylation and slows replication of retroviral DNA

Kristensson, Krister. "Microbes' roadmap to neurons." *Nature Reviews Neuroscience* 12.6 (2011): 345

Abstract: The nervous system is protected by barriers that restrict the invasion of pathogens. Nevertheless, mechanisms have evolved by which microbes can pass these barriers, enter and exit neurons and target various regions of the nervous system. In the brain, immune responses to pathogens are generally not robust, so microbes can hide and survive or, conversely, cause severe uncontrolled infections. Depending on their sites of entry and the regions that they target, microbes can cause diverse nervous system dysfunctions and even influence host behaviour to their own advantage. This review discusses routes by which microbes can reach the nervous system and cause persistent or life-threatening infections.

From the text: "… dysfunctions in the neurovascular units. Administration of a low-molecular-weight thiol, **pantethine**, interrupts overproduction of microparticles and **prevents signs of cerebral malaria** in a mouse model."

The cautious use of glutathione – but do not use it until parasites, protozoa, fungi and bacteria are under control!

Sprietsma, J.E., 1999. Cysteine, glutathione (GSH) and zinc and copper ions together are effective, natural, intracellular inhibitors of (AIDS) viruses. *Medical hypotheses*, *52*(6), pp.529-538. Abstract

Sufficient essential nutrients such as methionine, cysteine, copper, selenium, zinc and vitamins C and E are indispensable for the maintenance of optimal (immune) cell functions. Parasitic organisms such as protozoa, fungi, bacteria and viruses also depend on these essential nutrients for their multiplication and functioning. An evolutionarily developed optimal distribution of available nutrients between host (cells) and parasitic organisms normally prevents diseases, the nature of which will depend on genetic and environmental factors. The way in which the right amount of cysteine, glutathione (GSH), and copper and zinc ions made available in the right place at the right time and in the right form can prevent an unchecked multiplication of (AIDS) viruses in a more passive or active way forms the basis for the AIDS zinc-deficiency hypothesis (A–Z hypothesis) presented in this article.

Zinc and copper ions stimulate/inhibit/block in a concentration-dependent way the (intracellular) activation of essential protein-splitting enzymes such as HIV proteases. Zinc and copper ions as 'passive' virus inhibitors. Apart from this, zinc ions directly or indirectly regulate, via zinc finger protein molecular structures, the activities of virus-combating Th-1 cells such as cytotoxic T-cells (CTLs). Zinc ions act as regulators of the active, virus-combating Th-1 cells. **Zinc and copper ions that remain available in sufficient amounts via cysteine/GSH are effective natural inhibitors/combaters of (AIDS) viruses** and thereby prevent the development of chronic virus diseases that can lead to AIDS, autoimmune diseases, (food) allergies and/or cancer.

A safe, relatively inexpensive and extensively tested medicine such as **N-acetylcysteine (NAC) can help in supplying extra cysteine**. The anti-HIV peptide T22, synthesized on the basis of two natural peptides from the Tachypleus tridentatus and Limnus polyphemus crabs, appears to be able to serve as supplier/carrier molecule of cysteine and zinc and/or to hinder the entry of HIVs into cells by way of the CD4 receptor.

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Brundu, S., Palma, L., Picceri, G. G., Ligi, D., Orlandi, C., Galluzzi, L., ... & Mannello, F. (2016). Glutathione depletion is linked with Th2 polarization in mice with a retrovirus-induced immunodeficiency syndrome, MAIDS: role of pro-glutathione molecules as immunotherapeutics. *Journal of virology*, JVI-00603. Glutathione GSH, NAC and OSR against retroviruses. But beware of compounded "GSH". The FDA has made sure that commercially available glutathione is not GSH – this institution ruled a few years ago that during the compounding of GSH the vial cannot be topped up with nitrogen – causing the GSH to be oxidized, turning it into the GSH antidote rather than the active principle.

Masutani, H., S. Ueda, and J. Yodoi. "The thioredoxin system in retroviral infection and apoptosis." *Cell death and differentiation* 12.S1 (2005): 991. Glutathione and related thioredoxins (OSR, NAC) are low in those infected with retroviruses

Breitkreutz, R., Pittack, N., Nebe, C., Schuster, D., Brust, J., Beichert, M., ... & Dröge, W. (2000). Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. *Journal of Molecular Medicine*, *78*(1), 55-62. NAC is low in infected individuals

Herzenberg, Leonore A., et al. "Glutathione deficiency is associated with impaired survival in HIV disease." *Proceedings of the National Academy of Sciences* 94.5 (1997): 1967-1972. NAC supplementation is helpful in HIV, probably also in other retroviral infections

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Roederer, Mario, et al. "Cytokine-stimulated **human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine.**" *Proceedings of the National Academy of Sciences* 87.12 (1990): 4884-4888.

Fraternale, A., et al. "Erythrocytes as carriers of reduced glutathione (GSH) in the treatment of retroviral infections." *Journal of Antimicrobial Chemotherapy* 52.4 (2003): 551-554.

Dröge, W., & Breitkreutz, R. (2000). Glutathione and immune function. *Proceedings of the Nutrition Society*, *59*(4), 595-600. Give cysteine where cysteine is needed!

Palamara, Anna T., et al. "Inhibition of murine AIDS by reduced glutathione." *AIDS research and human retroviruses* 12.14 (1996): 1373-1381.

Palamara, Anna Teresa, et al. "Glutathione inhibits HIV replication by acting at late stages of the virus life cycle." *AIDS research and human retroviruses* 12.16 (1996): 1537-1541.

Luteolin as a potent anti-retroviral remedy (GliaLia from KiScience or Mirica/OptiPEA, Netherlands)

Paterniti, Irene, et al. **"Neuroprotection by association of palmitoylethanolamide with luteolin** in experimental **Alzheimer's disease** models: the control of neuroinflammation." *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* 13.9 (2014): 1530-1541.

Alzheimer's disease (AD) is the most common neurodegenerative disorder. Its neuropathological hallmarks include deposition of beta amyloid (Aβ) fibrils in senile plaques. Numerous biochemical events, leading to Aβ neurotoxicity in AD, have been proposed and it seems that neuroinflammation plays a prominent role among these. Thus, since inflammatory processes and oxidative stress are considered to play an important role in neuroinflammatory disorders and in AD pathology, in the present work we decided to test a new composite, which is a formulation constituted of an anti-inflammatory compound such as palmitoylethanolamide (PEA) and the well recognized antioxidant flavonoid luteolin (Lut), subjected to an ultra-micronization process, here designated co-ultraPEALut. We investigated the effect of co-ultraPEALut in both an in vitro and ex vivo organotypic model of AD. For the in vitro model, we used human neuronal cells, obtained by differentiating SH-SY5Y neuroblastoma cells into sustainable neuronal morphology. …. Differentiated SH-SY5Y cells were pre-treated with co-ultraPEALut (reference concentrations: 27, 2.7 and 0.27 µM PEA) for 2 h. AD features were induced by Aβ1-42 stimulation (1 µM). Twenty-four hours later cell vitality was evaluated by the colorimetric MTT assay, whereas the neuroinflammation underling AD was observed by Western blot analysis for IKBα degradation and nuclear factor-KB traslocation, as well as glial fibrillary acidic protein expression. For the organotypic model of AD, hippocampal slice cultures were prepared from mice at postnatal day 6 and after 21 days of culturing the slices were pre-treated with co-ultraPEALut (reference concentrations: 27, 2.7 and 0.27 µM PEA) for 2 h and then incubated with Aβ1-42 (1 µg/ml) for 24 h. Pretreatment with co-ultraPEALut significantly reduced inducible nitric oxide synthase and glial fibrillary acidic protein expression, restored neuronal nitric oxide synthase and brainderived neurotrophic factor and reduced the apoptosis. **Taken together our results clearly showed that co-ultra PEALut is able to blunt Aβ-induced astrocyte activation and to exert a markedly protective effect on glial cells. These findings suggest that the association of co-ultraPEALut may provide an effective strategy for AD.**

Ko, Yeon-Ju, et al. **"Flavonoids as potential inhibitors of retroviral enzymes**." *Journal of the Korean Society for Applied Biological Chemistry* 52.4 (2009): 321-326.

Mehla, Rajeev, Shalmali Bivalkar-Mehla, and Ashok Chauhan. "A flavonoid, **luteolin, cripples HIV-1** by abrogation of tat function." *PLoS One* 6.11 (2011): e27915.

Palmitoyl-Ethanolamide to help regulate cannabinoid receptors and re-regulate microglia
Zeidán-Chuliá, Fares, et al. "The **glial perspective of autism spectrum disorders**." *Neuroscience & Biobehavioral Reviews* 38 (2014): 160-172.

Bertolino, Bartolomeo, et al. **"Beneficial Effects of Co-ultramicronized Palmitoylethanolamide/luteolin in a Mouse Model of Autism** and in a Case Report of Autism." *CNS neuroscience & therapeutics* 23.1 (2017): 87-98.

PEA antidotes glia activation
Sarnelli, Giovanni, et al. "HIV-1 Tat-induced diarrhea is improved by the PPAR-alpha agonist, **palmitoylethanolamide, by suppressing the activation of enteric glia."** *Journal of neuroinflammation* 15.1 (2018): 94.

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Ecklonia Cava (brown ocean algae; www.KiScience.com)

- Ahn, Mi-Jeong, et al. "Inhibition of HIV-1 reverse transcriptase and HIV-1 integrase and antiviral activity of Korean seaweed extracts." *Journal of Applied Phycology* 14.5 (2002): 325-329.

Abstract: Forty-seven species of marine macroalgae from the coast of Korea havebeen screened for the presence of inhibitory compounds against humanimmunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and HIV-1integrase (IN). One of 4 Chlorophyta, 8 of 17 Phaeophyta and 6 of 26 Rhodophytashowed inhibitory **activity against HIV-1 reverse transcriptase**. Five species(***Ecklonia cava,*** *Ishige okamurae*,*Sargassum confusum*, *Sargassumhemiphyllum*, *Sargassum ringgoldianum*) belongingto Phaeophyta showed to inhibit the 3′-processing activity of HIV-1integrase. In cell-based assays, the methanol extracts of *Bossiella* sp. and *Chondriacrassicaulis* inhibited cytopathogenecity of HIV-1 at a concentrationbelow that cytotoxic for MT4 cells.

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- Ahn, Mi-Jeong, et al. "Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga Ecklonia cava." *Biological and Pharmaceutical Bulletin* 27.4 (2004): 544-547.

Selenium to silence retroviruses

- Bologna, Rosa, et al. "Selenium and immunity in HIV-1 infected pediatric patients." *Journal of Nutritional immunology* 3.1 (1994): 41-49.
- Baum, Marianna K., et al. "High risk of HIV-related mortality is associated with selenium deficiency." *JAIDS Journal of Acquired Immune Deficiency Syndromes* 15.5 (1997): 370-374.
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Vagal nerve stimulation as an anti-inflammatory strategy

Trends in Molecular Medicine, September 2016, Vol. 22, No. 9

MicroRNA Regulators of Anxiety and Metabolic Disorders

Chanan Meydan; Shani Shenhar-Tsarfaty and Hermona Soreq

Biomedical research views the ANS as fulfilling a more extended role than classically imagined, and ANS modulation of inflammation via cholinergic activities has become implicated in MetS [54]. The inflammatory reflex associated with cholinergic activity plays a central part in keeping inflammation under control [54]. The administration of nicotine, for example, has been known to ameliorate ulcerative colitis, with clinical and histologic improvement relative to placebo [55]. This outcome reflects a nicotinic anti-inflammatory effect, which may theoretically be mediated by miR-132 and modified AChE activity [28,51,56]. **Autonomic influence also ameliorates sepsis, as demonstrated in rats exposed to endotoxin and then to external stimulation of vagus nerves** [57]. The anti-inflammatory activities of the ANS and endocrine system presumably limit the inflammatory response to the local level, avoiding a widespread systemic reaction. In parallel, the efferent reaction to inflammation is led by the parasympathetic arm of the ANS, and ACh acts to inhibit target cells in both the innate immune system (e.g., in macrophages) and in non-immune cells which participate in inflammation (such as vascular smooth muscle cells [58]). **At the cellular level, the parasympathetic system negates inflammation by inhibiting the recruitment, migration, and activation of innate immune cells** [56]. This effect operates by reducing proinflammatory cytokines, such as tumor necrosis factor (TNF), IL-1, IL-6, and IL-18, and signaling molecules such as MCP-1 and HMGB-1, while also elevating anti-inflammatory cytokines [46,56,57,59]. This bidirectional process may involve miRNA-mediated control over the cholinergic surveillance of inflammation, contributing to the cholinergic-mediated bridge between anxiety and metabolic…..

Cannabis as an anti-retroviral medicine

Milloy, M-J., et al. "High-intensity cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load among recently infected people who use injection drugs." *Drug and alcohol review* 34.2 (2015): 135-140.

Introduction and Aims: Cannabis use is common among people who are living with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS). While there is growing pre-clinical evidence of the immunomodulatory and anti-viral effects of cannabinoids, their possible effects on HIV disease parameters in humans are largely unknown. Thus, we sought to investigate the possible effects of cannabis use on plasma HIV-1 RNA viral loads (pVLs) among recently seroconverted illicit drug users.

Results: Between May 1996 and March 2012, 88 individuals seroconverted after recruitment and were included in these analyses. Median pVL in the first 365 days among all seroconverters was 4.66 log10 c mL^{-1}. In a multivariable model, at least daily cannabis use was associated with 0.51 log10 c mL^{-1} lower pVL ($\beta = -0.51$, standard error = 0.170, *P* value = 0.003).

Discussion and Conclusions: Consistent with the findings from recent *in vitro* and *in vivo* studies, including one conducted among lentiviral-infected primates, we observed a strong association between cannabis use and lower pVL following seroconversion among illicit drug-using participants. Our findings support the further investigation of the immunomodulatory or antiviral effects of cannabinoids among individuals living with HIV/AIDS. *[Milloy M-J, Marshall B, Kerr T, Richardson L, Hogg R, Guillemi S, Montaner JSG, Wood E. High-intensity* ***cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load*** *among recently infected people who use injection drugs*. Drug Alcohol Rev *2015;34:135–40]*

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Agenda

- 1. What are retroviruses (RVs)?
  - Endogenous and exogenous RVs
  - Conditions they are associated with
- 2. How A.R.T. helped to identify that RVs can be treated
- **3. The treatment of retroviral activation/infection**
  - Proven biological remedies
  - **Pharmaceuticals**

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Pharmaceuticals

Dreyfus, David H. "Autoimmune disease: a role for new anti-viral therapies?." *Autoimmunity reviews* 11.2 (2011): 88-97.

Abstract: Many chronic human diseases may have an underlying autoimmune mechanism. In this review, the author presents **a case of autoimmune** CIU (chronic idiopathic urticaria) **in stable remission after therapy with a retroviral integrase inhibitor**, raltegravir (Isentress). Previous reports located using the search terms “autoimmunity” and “anti-viral” and related topics in the pubmed data-base are reviewed suggesting that novel anti-viral agents such as retroviral integrase inhibitors, gene silencing therapies … may provide new options for anti-viral therapy of autoimmune diseases. Cited epidemiologic and experimental evidence suggests that increased replication of epigenomic viral pathogens such as Epstein–Barr Virus (EBV) in chronic human autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus Erythematosus (SLE), and multiple sclerosis (MS) may activate endogenous human retroviruses (HERV) as a pathologic mechanism. Memory B cells are the reservoir of infection of EBV and also express endogenous retroviruses, thus depletion of memory b-lymphocytes by monoclonal antibodies (Rituximab) may have therapeutic anti-viral effects in addition to effects on B-lymphocyte presentation of both EBV and HERV superantigens. Other novel anti-viral therapies of chronic autoimmune diseases, such as retroviral integrase inhibitors, could be effective, although not without risk.

At SHI we good experience working with Truvada, starting with ¼ tablet (50/75 mg). Other anti-retroviral approaches for chronic illness are now in the pipeline.

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The use of **Quinacrines** (a group known for their anti-Malaria effects)

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Curaxin CBL0100 Blocks HIV-1 Replication and Reactivation through Inhibition of Viral Transcriptional Elongation.

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Despite combination antiretroviral therapy (cART), acquired immunodeficiency syndrome (AIDS), predominantly caused by the human immunodeficiency virus type 1 (HIV-1), remains incurable. The barrier to a cure lies in the virus' ability to establish a latent infection in HIV/AIDS patients. Unsurprisingly, efforts for a sterilizing cure have focused on the "shock and kill" strategy using latency-reversing agents (LRAs) to complement cART in order to eliminate these latent reservoirs. However, this method faces numerous challenges. Recently, the "block and lock" strategy has been proposed. It aims to reinforce a deep state of latency and prevent sporadic reactivation ("blip") of HIV-1 using latency-promoting agents (LPAs) for a functional cure. Our studies of curaxin 100 (CBL0100), a small-molecule targeting the facilitates chromatin transcription (FACT) complex, show that it blocks both HIV-1 replication and reactivation in *in vitro* and *ex vivo* models of HIV-1. Mechanistic investigation elucidated that CBL0100 preferentially targets HIV-1 transcriptional elongation and decreases the occupancy of RNA Polymerase II (Pol II) and FACT at the HIV-1 promoter region. In conclusion, CBL0100 is a newly identified inhibitor of HIV-1 transcription that can be used as an LPA in the "block and lock" cure strategy

https://www.ncbi.nlm.nih.gov/pubmed/29089933

We have used compounded Quinacrine in small doses successfully in adults with chronic illness. Loading dose: 200 mg every 15 minutes to a total of 800 mg. Followed by 100 mg t.i.d for 10 days. Premedicate with B vitamins and R-Lipoic acid to avoid side effects. Amazing results!

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Suramin (available as a frequency-imprinted remedy: Homeo-K APT from KiScience)

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In my Swiss practice I have successfully used Suramin at the published dose of 20 mg/kg every 6 weeks. Surprising results, especially in children with autism. In the rest of the world we use Homeo-K APT

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