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# New Insights on Mechanisms of Foamy Macrophage (FM) Induction and Persistence

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Immune System Management

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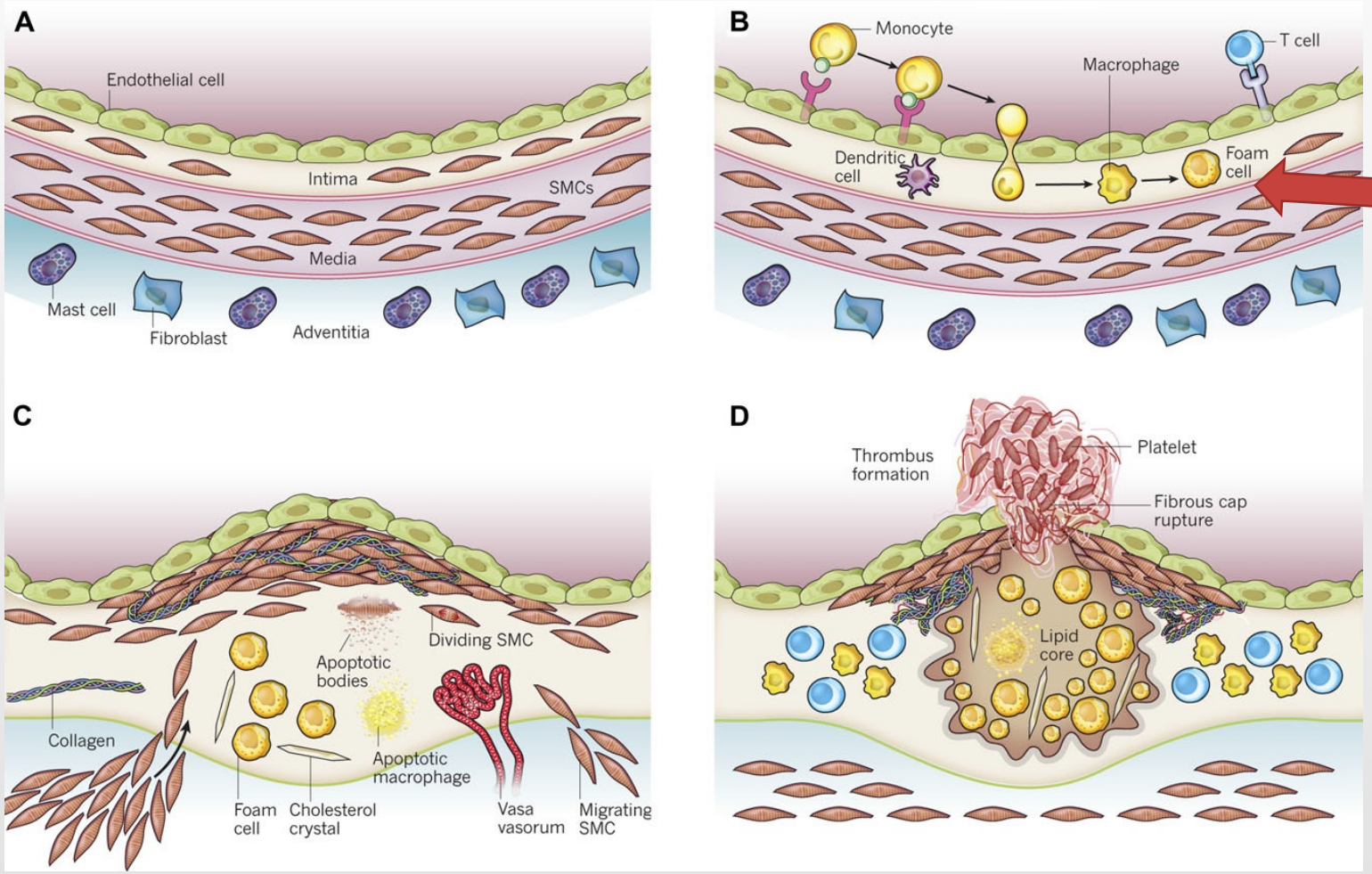
# Atherosclerosis (hardening of arteries) initiates with foamy macrophages




Foamy Macrophage (FM)

From: Lo J & Plutzky J. J Inf Diseases 2012; 205:S368-74.

# Human Macrophages Undergo Spontaneous Foam Cell Formation Without the Need for Lipid or TLR Signalling

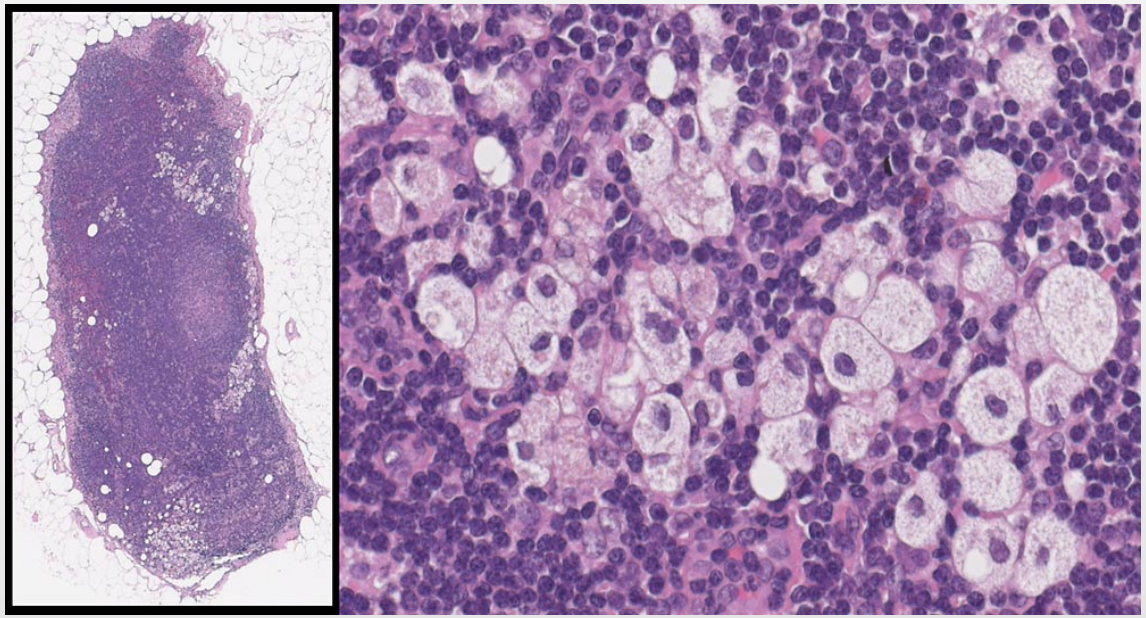
M1 human monocyte-derived macrophages (GM-CSF) undergo spontaneous foam cell formation (when cells cultured in DMEM).

Spontaneous foam cell formation is not found in murine systems nor in human monocytic leukemic cell lines, which instead requires oxLDL and/or Toll Like Receptor (TLR) signaling.

*Keyel PA et al,* Coordinate stimulation of macrophages by microparticles and TLR ligands induces foam cell formation. *J. Immunol, 2012 189:4621.*

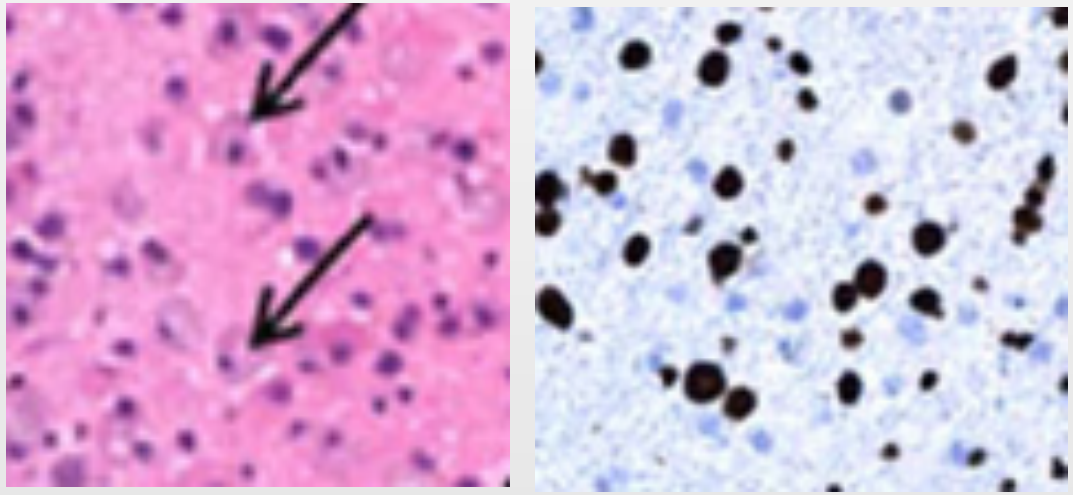
# Foamy Macrophages are Also Associated with Tumors and Viral Infections

## Foamy Macrophages in Lymph Nodes Adjacent to Tumor



PathoPic
www.unibas.ch/patho

## Foamy Macrophages in Brain with Re-activated John Cunningham Virus (JCV)

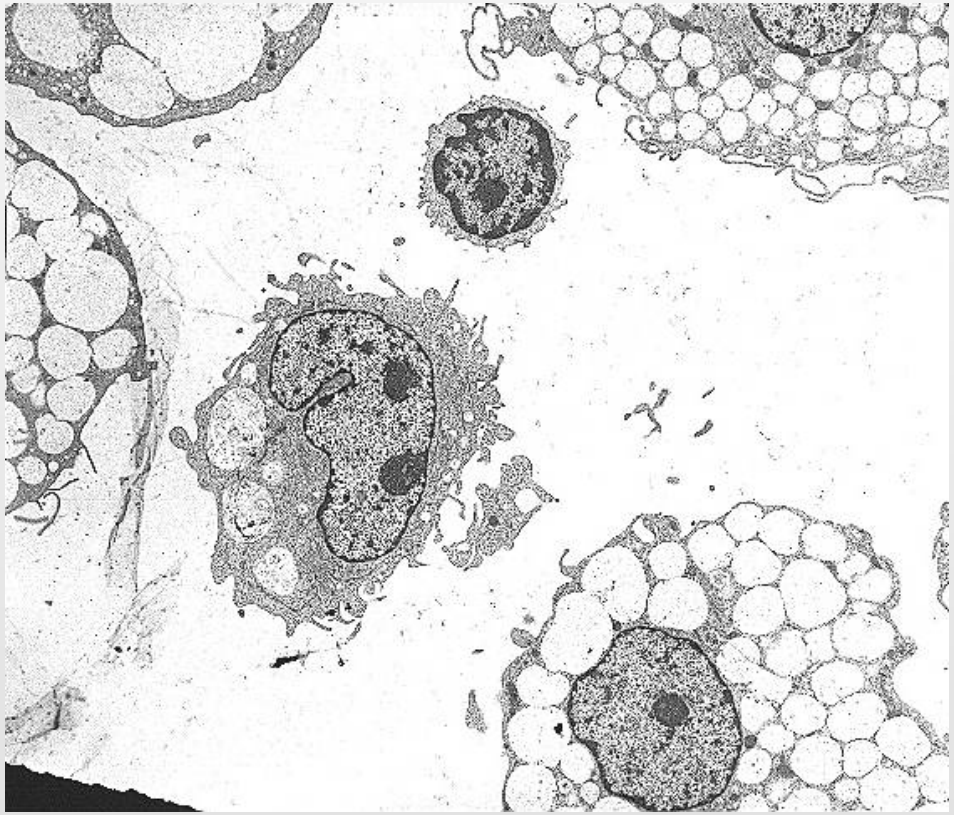


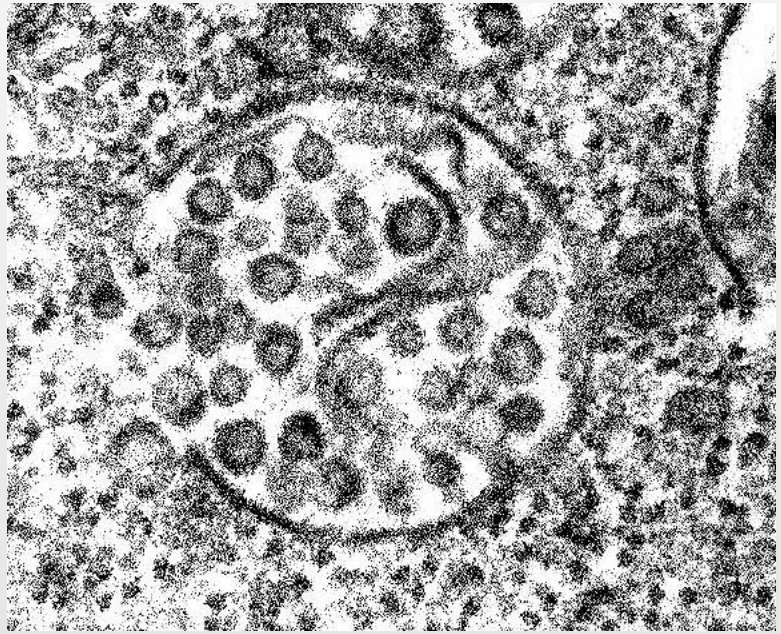
From: Vaklavas C *et al*, Virol J 2010, 7:256.

# So what causes foamy macrophages in humans?

**One cause of a certain type of foamy macrophage appears to be the induction of endogenous (foamy) retrovirus particles.**

Culture of CB in IMDM Media **instead of RPMI**





Electron Microscopy of Cord Blood mononuclear cells (CB)

**No viral budding from cell surface, therefore RELEASE of Particles is ONLY through cell lysis.**

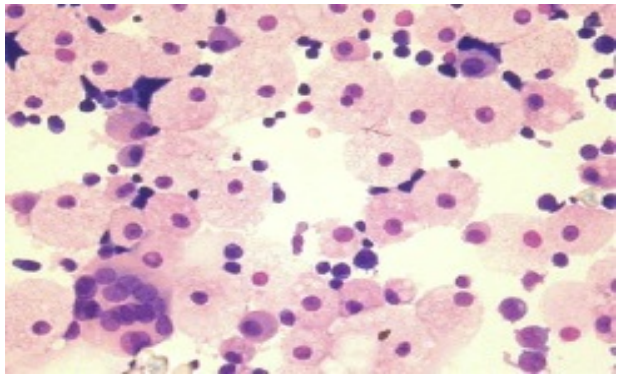
# Human Endogenous Retroviruses (HERVs)

- 8% of human genome involves HERVs
- named according to the amino acid transfer RNA used for reverse priming for integration into host genome
- HERV-K HML-2 proviruses are the most recent and **biologically active**
- Antibodies to HERV-K antigens found in many diseases
- The foamy retrovirus of humans has not been discovered, but most mammals have their own

# An Inducible Endogenous Human Foamy Virus from Normal Cord Blood (CB) Identified as HERV-K102

**Methods: Laderoute MP *et al*, AIDS 2007**

A



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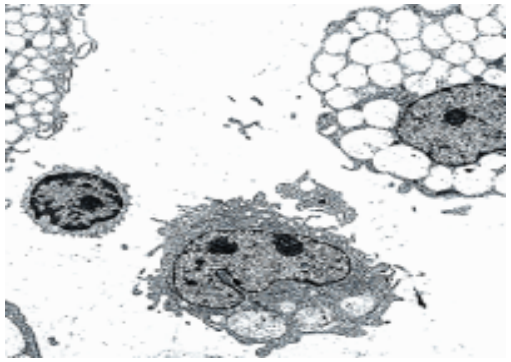
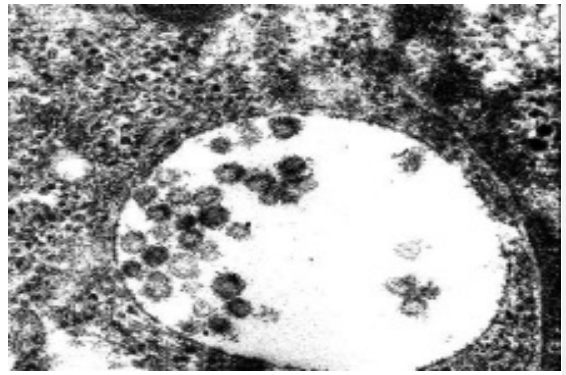
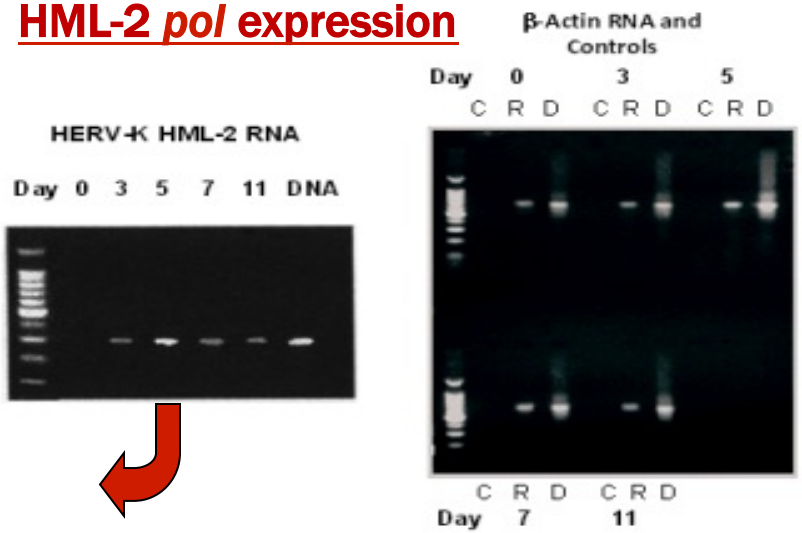


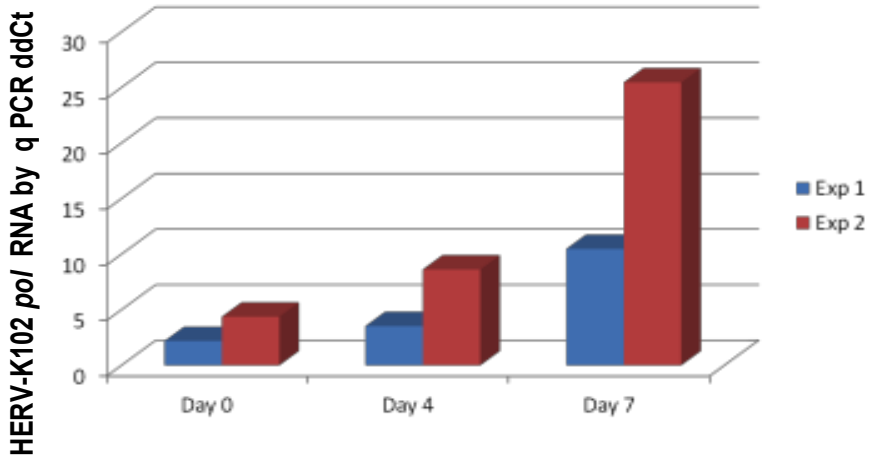
C



D **HML-2 *pol* expression**



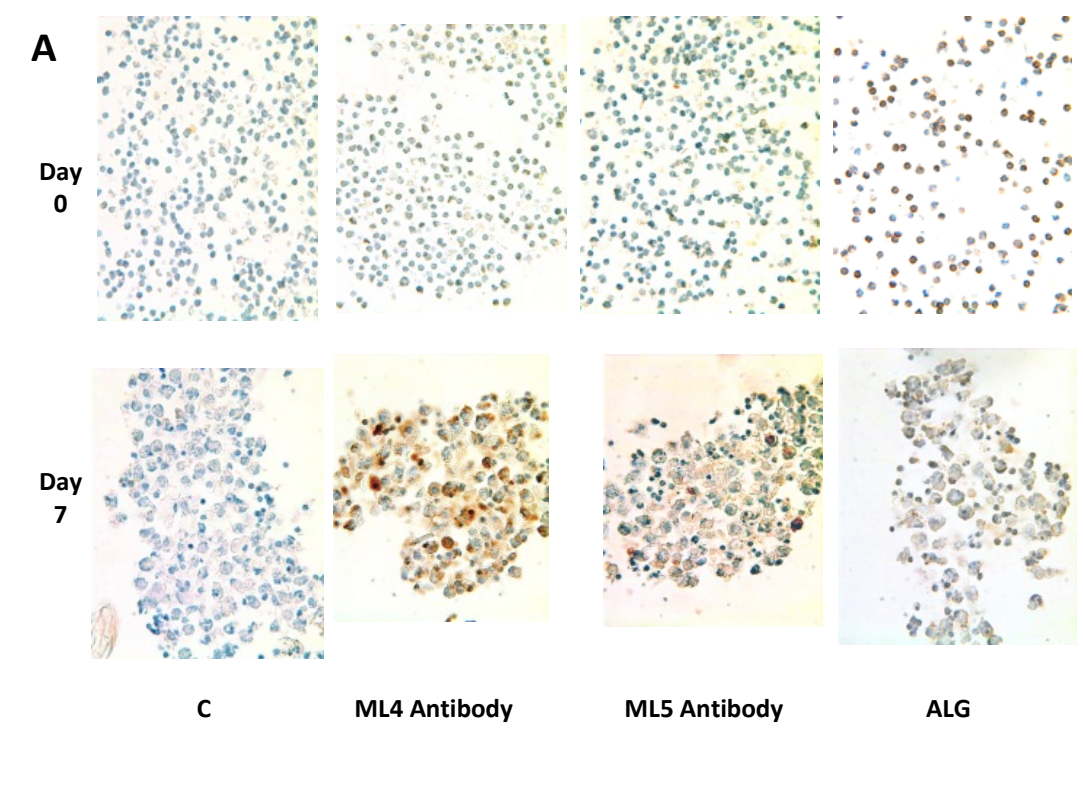

E **HERV-K102 *pol* RNA expression confirmed by qPCR ddCt**

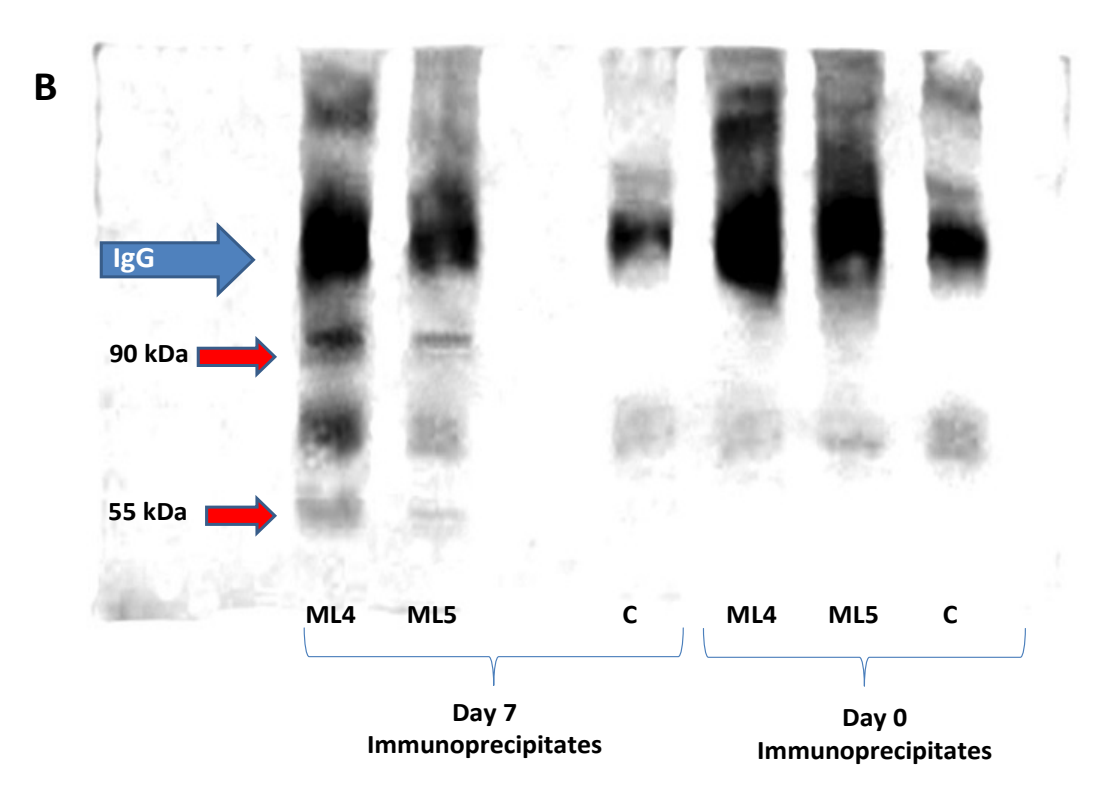



Sequencing of excised ***pol*** bands revealed only HERV-K102 *pol* (6/6 CB samples)

# HERV-K102 Env Expression and Env Processing were Detected (key for particle production and infectivity, respectively, of foamy viruses)

**Methods: Laderoute MP *et al*, AIDS 2007**



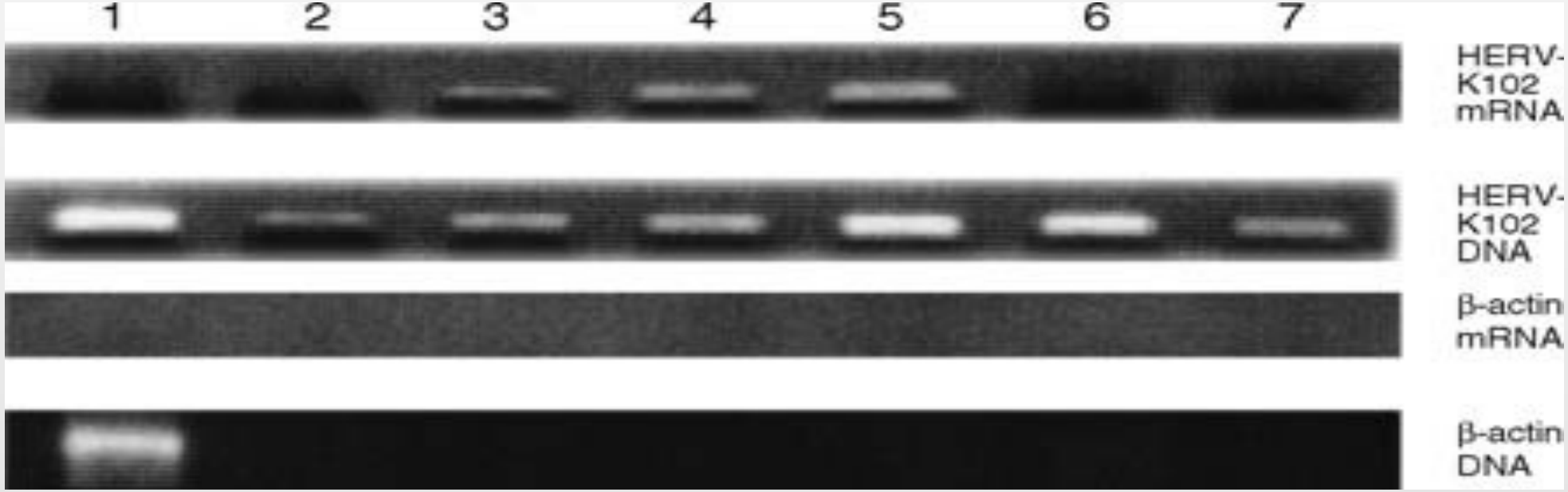




**Altogether these *in vitro* results suggested HERV-K102 might form particles *in vivo* and be replication competent.**

- HERV-K102 particles can be isolated from plasma during acute disease which disappear upon remission: not isolated from 30 normal adult plasma samples.
- The genomes are predominately DNA (cDNA) confirming they are foamy retroviruses (FV) with a reversed life cycle to most other retroviruses.

NB: 2 of 4 normal CB had HERV-K102 particles consistent with known non-pathogenicity of FV

Spiked Control* CFS Acute EBV MS initial MS prog CB#1 CB#2




* Normal plasma spiked with 500,000 PBMCs (uninduced) then processed with the plasma virus isolation kit.

# HERV-K102 particles are also produced in response to viral infections

## (HERV-K102 *pol* ddCt ratios on plasma DNA ).

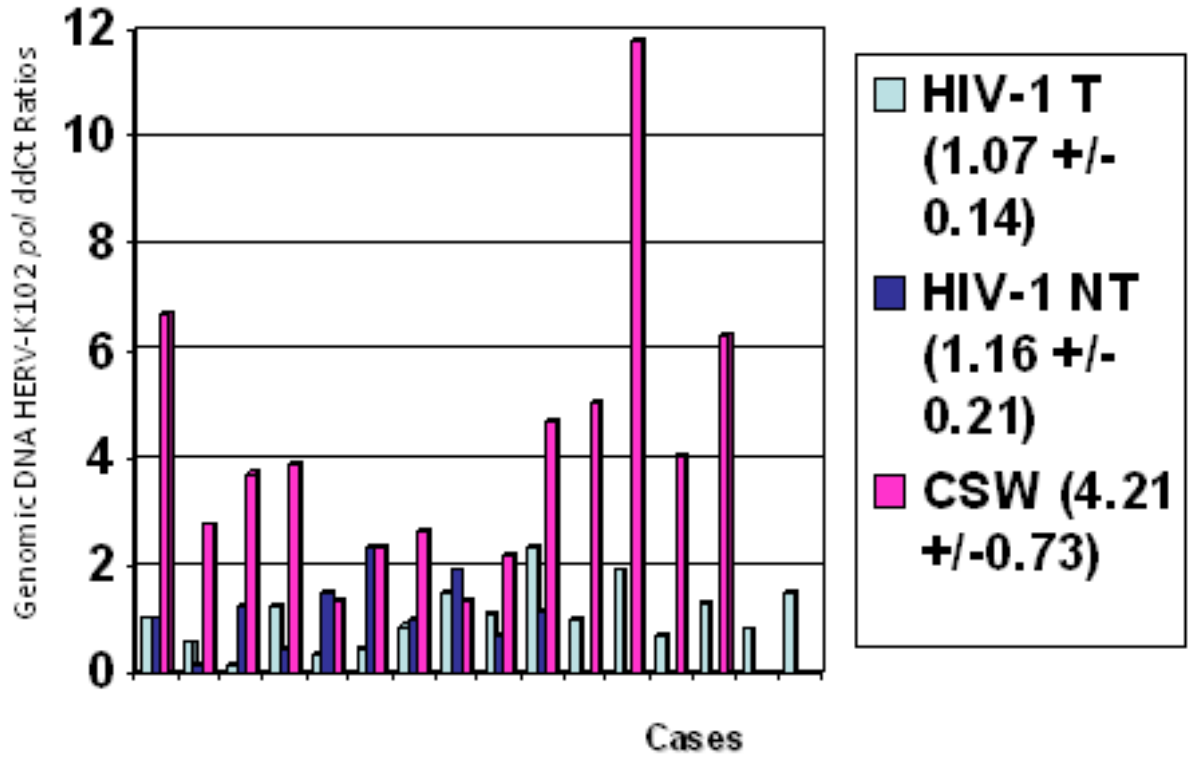
| Cohort | % Positive Ratios (positive/total cutoff 1.60) | HERV-K102 *pol* ddCt ratio - RANGE - |
|---|---|---|
| Normal | 3.3% (1/30) | 0.41 to **1.74** [a] |
| Hepatitis B and C | 78.6% (22/28) * | 0.81 to **$4.32 \times 10^9$** |
| Herpes | 61.9% (13/21) * | 0.24 to **$2.02 \times 10^9$** Antagonism |
| HIV-1 | 75.7 % (28/37)* | 0.49 to **$1.22 \times 10^2$** |

a) Mean ddCt ratio was 0.88 +/- 0.37 in 30 serologically negative normals, and no particles could be isolated

* p<0.0001 Fisher exact test when compared to normal by nonparametric proportions.

# Evidence of HERV-K102 Particle Production in the Antagonism of HIV-1 Replication *In Vivo*

**Study of HERV-K102 *pol* gene copy numbers in HESN commercial sex trade workers (CSW) versus HIV-1 infected individuals by real time PCR on plasma DNA**




**P =0.0005 (Normal control ddCt ratios =0.88 +/- 0.37)**

1. Confirms HERV-K102 likely replication competent and/or infectious *in vivo*

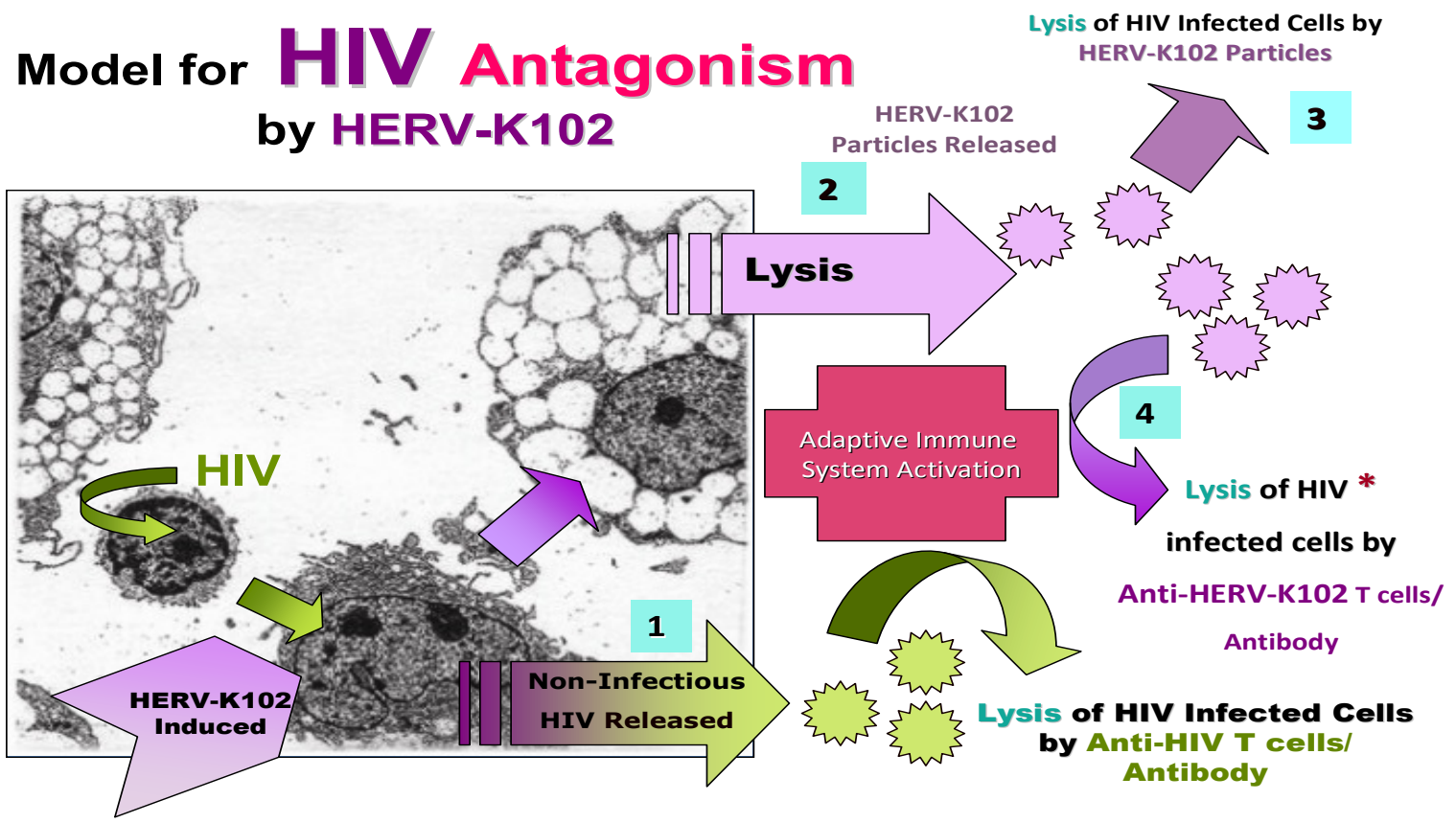
2. HERV-K102 particle production/activity might antagonize HIV-1 replication/transmission

# Summary of Literature on Human Endogenous Retrovirus K102 (HERV-K102)

- HERV-K102 has hallmark features and genetic motifs of **non-pathogenic foamy** retroviruses (FV)
- is replication competent reaching $10^{12}$ particles per ml of plasma in just a few days (dns)
- HERV-K102 is unique to humans, not found in other species
- Accumulating evidence suggests HERV-K102 is protective and may be an inflammatory (innate immunity) response to viruses, tumors, toxins and/or stress and may also induce autoimmune reactivity (T and B cell responses) against abnormal cells (tumor transformed or infected) (Wang-Johanning, Nixon, Markovitz, Laderoute)
- HERV-K102 has two GREs (Oh, 1986) and thus, likely is directly induced by cortisol

# HERV-K HML-2 activation has been best studied in HIV-1 infection.

**Hypothetical Model (2005)**

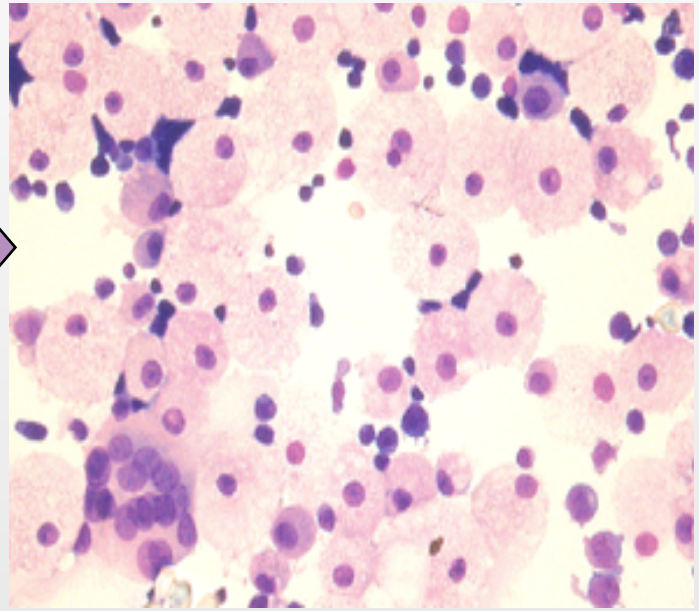



1. Molecular Antagonism
2. Lysis of Transformed Cell Producing HERV-K102 Particles
3. Lytic Infection of Abnormal Cells (oncolytic and virolytic) and Increased Proviral Copy Number in Normal Cells (arming)
4. Expansion of Autoimmune T and B Cells to HERV-K Antigens (TLR mediated?), the latter which Behave as Surrogate Antigens for Targeting Transformed Cells

# Working Model for
# Foamy Macrophage (FM) Persistence in Atherosclerosis

Viruses, tumors, toxins, stress, etc

HERV-K102 Particle Production



Lysis

HERV-K102 particle release

Pathogen/ tumor Clearance/ control

Wellness

Immunosuppressed Host

BLOCKED LYSIS

Flavonoids

ATHEROSCLEROSIS

Good evidence for pathogen mediated FM persistence, eg. HCMV, *Stevenson EV et al, Viruses, 2014 and heart attacks with flu season in aged humans (>65 yrs), Foster ED et al, Epi & Infection 2013*

# Flavonoids Are Known to Reduce Cardiovascular Deaths,

Yochum L et al, Am J Epi 1999, 149:943-949.

## may protect against cardiovascular disease as has been shown for the following: (see review by Bhardwaj P et al, 2013),

Atherosclerosis, Hypertension, Endothelial dysfunction, Ischemic heart disease, Cardiomyopathy, Congestive heart failure, Inflammatory responses, Oxidative Stress, Platelet aggregation, Proliferation of vascular smooth muscle cells

## And, may lead to normalization of the DHEA:cortisol ratio as well as rebalance immunoreactivity favoring Th1 over Th2 associated with a decline in IL-6.

Bouic P & Lamprecht J, Alternative Medicine Review 1999, 4: 170-177.

# Summary

Induction of foamy macrophages can be a normal host inflammatory response involving particle production of an endogenous FOAMY virus, identified as HERV-K102 in response to intracellular pathogens and/or tumors.

However, foamy macrophage persistence and resulting atherosclerosis might signify active immunosuppression, stress, and/or persistent pathogens which should be eliminated or treated, and not necessarily high cholesterol per se.

# FOAMY MACROPHAGE INDUCTION & PERSISTENCE

For more details on "Endogenous Foamy Virus Theory of Foamy Macrophage Accumulation in Atherosclerosis: 2014", *see*

## Why is February Heart Month?

http://www.aminomics.com/professionals/HERVK.htm

# THANK YOU!