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A New Paradigm About HERV-K102 Particle Production and Blocked Release to Explain Cortisol Mediated Immunosenescence and Age-associated Risk of Chronic Disease

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Abstract: The majority of chronic diseases in the aging adult are thought to relate to immune aging characterized by dominant immunosuppression and paradoxically, concomitant inflammation. This is known collectively as immunosenescence. The main change thought to be controlling immune aging is the age-related decline in dehydroepiandrosterone (DHEA) and corresponding increase in cortisol; the net effect which decreases the DHEA/cortisol ratio. Exactly how this translates to immunosuppression and concomitant inflammation remains unclear. Recently a new component of the human innate immune system has been discovered. Human endogenous retrovirus K102 (HERV-K102) is a replication-competent foamy retrovirus unique to humans which has been implicated in chronic diseases. Accumulating evidence suggests that HERV-K102 may defend the host against viral infections, as well as against breast and other cancers. Particles are produced in activated monocytes and released into vacuoles but do not bud through the cell surface. This renders macrophages foamy, while the release of particles is only through cell lysis. New evidence presented here suggests DHEA but not DHEA-S may specifically bind and inactivate alpha-fetoprotein (AFP). AFP is a well-established immunosuppressive factor which importantly, also blocks cell lysis induction in macrophages through the 67 kilodalton (kD) AFP receptor (AFPr). Here, it is proposed that a decreased DHEA/cortisol ratio may favor the accumulation of foamy macrophages reflecting the cortisol induction of HERV-K102 particle production concomitant with the blocked release of particles by secreted AFP. This is a new paradigm to explain how cortisol-mediated immunosenescence can result in the persistence of foamy macrophages, and how this relates to risk of chronic disease. [*Discovery Medicine* 20(112):379-391, December 2015]

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Introduction

Immunosenescence is a term used to describe the overall diminished functioning or dysregulation of the immune system associated with aging (Baylis *et al.*, 2013), the details of which have been described elsewhere (Aw *et al.*, 2007; Lindstrom and Robinson, 2010; Hsu *et al.*, 2005). Paradoxically, immunosenescence also incorporates a propensity for upregulation of the inflammatory response, which has been called 'inflammaging' as coined by Franceschi *et al.* in 2000. While the precise etiology of inflammaging remains largely unknown (Franceschi and Campisi, 2014), it is characterized by increased levels of IL-1, IL-6, TNF-alpha and CRP and reduced IL-10 in the blood (Baylis *et al.*, 2013; Bueno *et al.*, 2014; Lindstrom and Robinson, 2010) implying activated macrophages/dendritic cells in the process. Diseases linked with inflammaging include cardiovascular disease, cancer, dementia, Alzheimer's disease, autoimmune diseases, type-2 diabetes, HIV-1, and increased vulnerability to infectious disease (Baylis *et al.*, 2013; Heffner, 2011; Fulop *et al.*, 2013; Kuller *et al.*, 2008; Guaraldi *et al.*, 2011; Lo and Plutzky, 2012; Dillon *et al.*, 2013; Gruver *et al.*, 2007).

Immunosenescence is most closely related to adrenal aging (Valenti, 2004; Heffner, 2011; Lois *et al.*, 2014; Giefing-Kroll *et al.*, 2015). As humans age, the levels of dehydroepiandrosterone (DHEA) decrease and cortisol increase (Buford and Willoughby, 2008; Baylis *et al.*, 2013), and together they decrease the DHEA/cortisol ratio. DHEA is also known to blunt the cortisol stress response and to reduce cortisol levels (Miyake *et al.*, 2014). Accordingly, as we age we do not handle stress

as well as before when younger. In addition to increased serum cortisol, macrophages express increased glucocorticoid receptor mRNA associated with aging or stress (Kizaki *et al.*, 2002). Decreased DHEA/cortisol ratios in addition to reduced DHEA are detected in a number of chronic diseases (Baylis *et al.*, 2013) including those which generally affect younger adults, such as HIV-1 (Christeff *et al.*, 2000; Dillon *et al.*, 2013). Premature immunosenescence is known to occur with chronic psychological stress, chronic inflammation or exposure to certain persistent viral infections, with the latter appearing to elevate cortisol (Bauer *et al.*, 2015).

The relative balance of Th1 versus Th2 responses also shifts with aging towards the latter and this imbalance is also seen in psychological stress (Heffner, 2011). A Th2 response is associated with autoimmune IgG antibody production, which increases with age (Nagele *et al.*, 2013). Immunosenescence leads to dysregulation of the immune system and thus, autoimmune disease with autoimmune antibodies frequently increases with aging consistent with its Th2 dominance (Mills, 2015). Sharon and Mason (2015) assert that autoimmune disease generally is associated with immunodeficiency and the inability to clear pathogens and highlights the notion of dysregulated immunity in immunosenescence.

There are three main types of cells which mediate non-specific immunosuppression (Baert *et al.*, 2015; Steinberg *et al.*, 2014): the non-antigen specific suppressor T cells now referred to as regulatory T cells (Tregs), myeloid derived suppressor cells (MDSC) which are precursors to macrophages/dendritic cells (Draghiciu *et al.*, 2015), and immunosuppressive tumor associated macrophages (TAMs). Interestingly, these immunosuppressive cell types can also display inflammatory properties (Bowdish, 2013; Schmitt *et al.*, 2012; Baert *et al.*, 2015) and as alluded to, may be associated with autoimmunity (Crook and Liu, 2014; Stevenson *et al.*, 2014; Schmitt *et al.*, 2012).

In this review article a new paradigm is being proposed in an attempt to explain how both immunosuppression and inflammatory activation in macrophages may be achieved by excess, unopposed cortisol associated with aging. Cortisol is proposed to directly induce particle production of human endogenous retrovirus K102 (HERV-K102) in monocytes/macrophages rendering them foamy and at the same time to transactivate expression of alpha-fetoprotein (AFP) mRNA. Secreted AFP then is postulated to bind back to the 67 kD AFP receptor (AFPr) expressed on partially activated macrophages. This blocks cell death induction thereby directly preventing the release of HERV-K102 particles (see Figure 1) and may also prevent the further differentiation of macrophages. The blocked release of HERV-K102 particles in foamy macrophages by AFP is hypothesized to result both in diminished protection by HERV-K102 against infectious agents and tumors, and the lingering of foamy macrophages. It is the persistence of these foamy macrophages which contributes to inflammation and it is the release of AFP which contributes to immunosuppression. Together these are postulated to cause "immunosenescence" where the immune system is dysfunctional. In this state, the host is at higher risk of inflammatory diseases such as autoimmune conditions, cancer, neurological disorders, and most directly an increased risk of atherosclerosis. As will also be developed below, in part the reduction in dehydroepiandrosterone (DHEA) which is also associated with stress and/or aging and which is commonly found in chronic diseases, provides less ability of the host to control or inhibit the active levels of AFP in the system further contributing to immunosenescence.

Alpha-fetoprotein Is Immunosuppressive, Promotes Apoptosis Resistance and Contributes to Immunosenescence

Historically, alpha-fetoprotein (AFP), an oncofetal antigen, was the first tumor marker discovered and the first immunosuppressive factor described (Abelev *et al.*, 1963; Murgita and Tomasi, 1975; respectively). However a role of AFP in cancer progression, in immune aging, and in immunosuppression, while well established (reviewed in Toyoda *et al.*, 2015; Laderoute, 1991; 1994; 1996; Deutsch, 1991; Terentiev and Moldogazieva, 2013), is no longer well appreciated. This change followed two reports which erroneously claimed the immunosuppressive effects of the AFP and TGF-b complex from amniotic fluids was merely due to TGF-b and that AFP did not contribute to immunosuppression (Lang and Searle, 1994; Altman *et al.*, 1990). In contrast, it had been concluded previously that AFP and a second immunosuppressive factor may co-exist in amniotic fluids (Suzuki and Tomasi, 1979). To resolve this dilemma, Semeniuk *et al*. (1995) expressed human and mouse AFP in bacterial, baculovirus and yeast expression systems. The highly purified recombinant AFPs were demonstrated to mediate immunosuppression in *in vitro* assays confirming immunosuppression is in fact, an intrinsic property of the AFP molecule. In clinical substantiation that AFP is immunosuppressive and may play a role in immunosenescence, ataxia telangiectasia involves premature aging with multisystemic progeric changes (Boder, 1985) and involves immunosuppression associated with abnormal elevated AFP expression in the vast majority

of the cases (Woods and Taylor, 1992). As well, that AFP levels may directly correlate with HIV-1 viral load (Gross *et al.*, 2003) would be consistent with the notion that premature aging may also be associated with elevated AFP as described in HIV-1 infected patients (Jenny, 2012).

Nevertheless, the elucidation of the biological activity of AFP protein has been hampered by various technical difficulties as reviewed elsewhere (Laderoute, 1996). AFP exists in active and inactive states potentially related to its role in the transport of various molecules across the placenta (Deutsch, 1991) and active AFP may circulate in plasma complexed with a binding protein (Biddle and Sarcione, 1987) which masks its presence. This AFP binding protein appears to be the soluble form of the 67 kD cell surface AFP receptor (AFPr) (Laderoute, 1991; Moro *et al.*, 1993; 2012). ELISA based technologies do not generally demonstrate excess AFP in cancer patient sera; for example, as recently reported for an AFP producing colorectal cancer (Anzai *et al.*, 2015). This helps to explain the inconsistencies reported in the literature for the detection of AFP in sera from cancer patients. It would be useful if new screening methods would be developed which were capable of detecting active (immunosuppressive) AFP species (Lester *et al.*, 1976) to help resolve this issue of the involvement of AFP in immunosenescence.

Fortunately, the immunosuppressive effects of AFP binding to the 67 kD AFPr on macrophages validated through the use of monoclonal antibodies (MAbs) to the AFPr (Laderoute, 1991; Moro *et al.*, 1993) which behaved as AFP agonists. These MAbs blocked the mixed lymphocyte reaction, blocked the Con-A T cell response, and inhibited macrophage anti-tumor activity

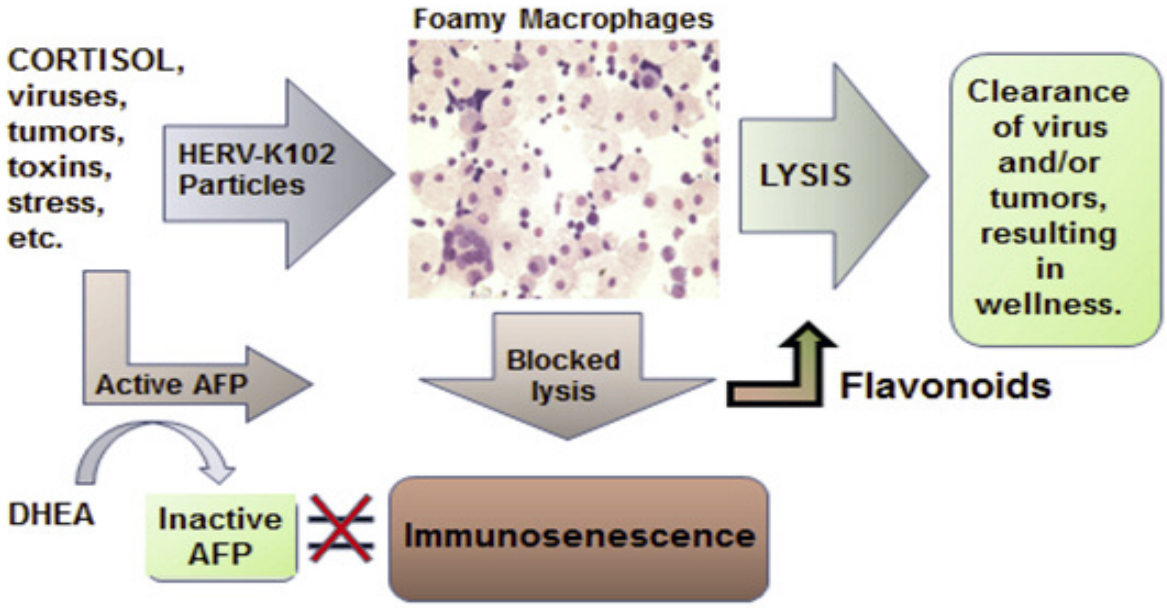

Figure 1. Model for how cortisol may activate HERV-K102 particle production producing foamy macrophages and how blocked release may lead to immunosenescence and chronic diseases. Image is an H&E stain of day 7 cord blood mononuclear cells (CB) cultured in IMDM media showing foamy macrophages (image reproduced with permission from Laderoute *et al*., 2015) which resemble the CD14++ CD16+ macrophages as described under similar conditions for cultured CB by Stec *et al*. (2007). Cortisol, stress, toxins, tumors, viruses and other intracellular pathogens are thought to induce HERV-K102 particle production in activated macrophages rendering them foamy. Cortisol may directly induce HERV-K102 particle production and AFP expression in activated macrophages. Secreted AFP binds back to the 67 kD AFP receptor on the foamy macrophages, blocking lysis (Laderoute and Pilarkski 1994), and thus, the lytic release of HERV-K102 particles is inhibited. The failed release of HERV-K102 particles not only abrogates protection against viruses and tumors which is known to be associated with aging (Gruver *et al*., 2007), but results in persistence of foamy macrophages. DHEA may bind and inactivate AFP (Table 1), and thus, may mitigate immunosenescence and allow for release of HERV-K102 particles enabling its proposed protection against tumors and intracellular pathogens. The health benefits of flavonoids have been long appreciated (Yao *et al*., 2004) and are known to reduce cardiovascular deaths (Yochum *et al*., 1999). Flavonoids may protect against cardiovascular disease through various mechanisms (Bhardwaj and Khanna, 2013) including overcoming apoptosis resistance (Dastjerdi *et al*., 2015; Bouic and Lamprecht, 1999). Here, it is suggested that flavonoids and/or DHEA may abrogate apoptosis resistance not only allowing for the release of HERV-K102 particles but hypothetically, for augmenting the oncolytic and virolytic capabilities of HERV-K102 particles.

(Laderoute, 1991) all which rely on the central role of monocytes/macrophage activation. In substantiation of our seminal work with the AFP-agonist MAbs, others have shown similar immunosuppressive effects of AFP in human systems as mediated also through a 67 kD AFPr on monocytes/macrophages (Wang and Alpert, 1995; Suzuki *et al.*, 1992; Pardee *et al.*, 2014). Thus, while the literature reports several types of AFP receptors (reviewed in Mizejewski, 2011), the 67 kD AFPr appears to mediate immunosuppression on monocytes/macrophages, and as detailed below, also conveys apoptosis resistance.

A role of AFP through binding to 67 kD AFP receptors in conferring apoptosis resistance was first demonstrated in a monocyte cell line HL-60 cells (Laderoute and Pilarski, 1994). With increased passage number, HL-60 cells lost expression of the 67 kD AFPr and demonstrated earlier adherence- mediated apoptosis. This strongly suggested a role of AFP and AFP receptors in anti-cellular senescence (Laderoute, 1994). Using a different strategy of AFP knockdown, in hepatoma cell lines, it was demonstrated intracellular AFP may block TNF-related apoptosis inducing ligand (TRAIL) by binding and blocking caspase-3 activation in the apoptotic cascade (Li *et al.*, 2009). Other similar gene silencing research has shown AFP promotes apoptosis resistance through antagonizing the p53/Bax/cytochrome c/capase-3 signaling pathway in hepatoma cell lines (Yang *et al.*, 2008; Zhang and Xu, 2000).

Anti-aging at the cellular level such as evidenced by the inhibition of apoptosis, contributes to physiological aging found at the whole host level (reviewed in Laderoute, 1994; Monti *et al.*, 2000; Pasparakis and Vandenabeele, 2015). At first this may seem paradoxical, but the following may help to illustrate its meaning. With age, our skin becomes more dull or flaky such as on the face. This is because the apoptosis of skin cells is impaired, and dead and dying cells linger. The signaling of AFP by binding to its 67 kD AFPr appears to be unusual since it imparts a negative signal through the 67 kD AFPr. By blocking other incoming signal transductions (Laderoute, 1991; 1994) means these cells are not behaving normally and may be dysfunctional. A common complaint with advanced aging is that bodily functions just do not work the way they used to. However, it remains to be determined if AFP and/or AFP receptors play a more general role in aging such as skin aging or reduced bodily functions.

Historically, AFP was also the first factor shown to induce antigen non-specific suppressor cells in T cell and non-T cell compartments (Murgita *et al.*, 1977; Gershwin *et al.*, 1978; Alpert *et al.*, 1978; Sibbitt *et al.*, 1978; Toder *et al.*, 1983; Hoskin and Murgita, 1989; Sharma *et al.*, 2015) and in monocyte-enriched populations (Peck *et al.*, 1982; Harimoto *et al.*, 2013). Thus, AFP likely plays a central but underappreciated role in immunosuppression associated with immunosenescence.

Key to the discussion of immunosenescence is the evidence provided in Table 1 that DHEA, but not its sulfated inactive derivative (DHEA-S), may specifically bind and inactivate AFP. This observation is important as the loss of DHEA with aging would be consistent with the notion that AFP activity might also increase with aging. Moreover, while glucocorticoid steroids may diminish AFP expression in murine models, in direct contrast, cortisol increases AFP expression in humans (Nakabayashi *et al.*, 1989; 1991). Thus, the increased bioactivity of AFP associated with diminished DHEA levels and with increasing cortisol levels may contribute to chronic diseases in aging adults. This is through its immunosuppressive properties and also by conferring apoptosis resistance on cells bearing the 67 kD AFPr. Cells known to express the 67 kD AFPr include cells of the monocyte/macrophage lineage, possibly gamma-delta T cells, neurons, as well as tumors such as the common adenocarcinomas where the AFPr is strongly upregulated (Laderoute, 1991; Laderoute *et al.*, 1994). Accordingly, AFP increased activity and/or expression may be related to loss of DHEA and, increased cortisol levels, respectively, which are associated with the age-related changes to the DHEA/cortisol ratio.

It is very relevant to this paradigm that increased circulating levels of AFP have been found to be prognostic for progression of a number of viral infections and/or tumors (Zhu *et al.*, 2015; Cheng *et al.*, 2014; Mizejewski, 2001; Terentiev and Moldogazieva, 2013). As well p53 and TGF-beta repress AFP expression (Zhang and Xu, 2000; Sakata *et al.*, 2014) consistent with its role in oncogenesis and in dysregulated immunity.

The Discovery of HERV-K102 as a Functional Foamy Retrovirus of Humans and Its Activity in Human Diseases

Our research team serendipitously discovered an endogenous foamy retrovirus (FV) of humans producing particles in foamy macrophages and T cells from cord blood when mononuclear cells were cultured in Iscove's Modified Dulbecco's media (Laderoute *et al.*, 2006; 2007; 2015). We characterized this putative FV as human endogenous retrovirus-K102 (HERV-K102) and showed it was replication competent in HIV-1

infected patients. HERV-K102 is a HERV-K HML-2 type 1 provirus specific to humans (Subramanian *et al.*, 2011). It is noteworthy that HML-2 members are the most biologically active and the most recent acquisitions in the human genome, but their role in health or disease remains to be established. However, as outlined below, accumulating evidence appears to suggest HERV-K102 activation and/or particle production may be part of the innate immune system and as a non-pathogenic foamy retrovirus, may be protective.

HERV-K102 has hallmark sequence motifs and features of FV analogous to the well-studied prototypic foamy virus (PFV) (Laderoute *et al.*, 2015). FV are unconventional retroviruses in that they have a reversed life-cycle to HIV where particles contain predominately DNA genomes and thus, are more genetically stable (Linial, 1999; Yu *et al.*, 1999; Meiering and Linial, 2001; Rethwilm, 2003). They do not use a Rec-like domain to export genomes from the nucleus (Bodem *et al.*, 2011). While they are not pathogenic, they are naturally oncolytic (Heinkelein *et al.*, 2005) and virolytic (Mikovits *et al.*, 1996). HERV-K102 may be the first functional foamy retrovirus of humans identified and may be the only HERV shown to be naturally replication competent *in vivo* (Laderoute *et al.*, 2007) and *in vitro* (Laderoute *et al.*, 2015). Importantly particles isolated from plasma contained predominately DNA genomes and in the plasma samples from HIV-1 patients, excess DNA signals over genomic were confirmed to be cDNA sequences. Dube *et al.* (2014) recently validated that some HERV-K HML-2 particles have DNA genomes and retain infectivity in substantiation of our work. For foamy viruses, assembly of the immature capsids uniquely occurs in the cytoplasm, and the budding site can be an intracellular compartment such as the endoplasmic reticulum or alternatively, the cell surface membrane such as found for PFV (Hutter *et al.*, 2013). This mechanism characterizes B/D-type retroviruses including mouse mammary tumor virus and HERV-K HML group members. HERV-K HML members are distantly related to mouse mammary tumor viruses which is how they were discovered (Ono, 1986). For HERV-K102, assembly of the immature capsids seemed to occur in the cytoplasm

Table 1. DHEA But Not DHEA-S May Abrogate the Ability of AFP to Block Programmed Cell Death in In Vitro Developing Multi-Negative Human Thymocytes.
(% Non-viable Cells by Flow Cytometry on Day 7)

Agent Added at Culture Initiation	Controls	DHEA Plus AFP	DHEA-S Plus AFP	DHEA Plus 167H.1 MAb to AFP Receptor
None[a]	74%			
AFP	19%			
HSA	54%			
167H.1 MAb	35%			
167H.4 MAb	74%			
Steroid 10^{-7}		32%	25%	28%
Steroid 10^{-8}		50%	24%	30%
Steroid 10^{-9}		74%	25%	32%
Steroid 10^{-10}		64%	28%	30%
Steroid 10^{-11}		20%	21%	33%
Steroid 10^{-12}		13%	18%	27%

Note: a, media control. Multi-negative ($CD3^-4^-8^-$; $CD19^-$) human thymocytes (MN) were isolated by depletion as previously described (Pilarski *et al*., 1995) and cultured in 5 % FCS-RPMI (no phenol red) at 1 x 10^6/ml in 100 µl of media in 96 well flat bottom plates (Linbro) for 7 days. Freshly prepared and purified alpha-fetoprotein (AFP) or a homologous control, human serum albumin (HSA, Sigma), were used at 30 µg/ml. In the afternoon of the 7th day of culture, a majority of the thymocytes undergo cell death. AFP or the 167H.1 MAb was pre-incubated with the indicated concentration of steroid at 37ºC for one hour prior to adding to the cultures. Variance was less than 3%. Purified MAbs were used at 50 µg/ml as previously described (Laderoute and Pilarski, 1994). Steroids were purchased from Sigma-Aldrich. It is known the immunoenhancing effects of DHEA shows an optimal concentration at 10^{-9} M and that DHEA-S has no biological activity in vitro (Daynes *et al*., 1990; Straub *et al*., 1998). The results demonstrate AFP and the 167H.1 MAb, but not HSA, nor the 167H.4 MAb, can block programmed cell death (PCD) as reported previously for HL-60 cells, a human monocytic cell line (Laderoute and Pilarski, 1994). Most probably cells expressing the 167H.4 AFPr isoform are not committed to cell death and thus, this AFP-agonist MAb cannot reverse apoptosis. DHEA appears to specifically bind AFP and inactivate it, since the level of PCD detected at 10^{-9} M was the same as the media control (74 %) and DHEA-S, the inactive form, was found to have no appreciable effect. As well the finding DHEA did not affect the ability of the 167H.1 MAb to rescue the thymocytes from PCD, implies the interference by DHEA is most likely through specifically binding and inactivating AFP. Results are representative of 3 similar experiments.

outside vacuoles, and budding was exclusively into vacuoles and not the cell surface as shown by electron microscopy in foamy macrophages. Therefore, HERV-K102 is clearly a foamy retrovirus by this definition.

HERV-K102 HML-2 particles were isolated from 50 % of cord blood plasma samples, but not from 30 normal adult plasma samples. This showed HERV-K102 particle production was not constitutive in adults. Since the adaptive immune system is not matured at birth, and particles are produced in activated macrophages (Figures 1 and 2), strongly implies HERV-K102 particle production is part of innate immunity. By flow cytometry, highly granular cells found on day 6 but not on day 0 of cultured cord blood mononuclear cells, were found to express CD14, CD3 but not CD19 (unpublished data), implying T cells may also produce HERV-K102 particles. However, depletion of CD14 cells by magnetic beads, abrogated the induction of foam cell formation and the relative increase in HERV-K102 DNA over the 7 days of culture suggesting particle production initiates in monocytes/macrophages or that they are intimately required for production (unpublished data). The foamy macrophages morphologically resembled the CD14++ CD16+ macrophages described by Stec *et al.* (2007).

The finding of HERV-K102 particles in patients with active disease, such as chronic fatigue syndrome, acute EBV infection and in multiple sclerosis, but which disappeared during remission (Laderoute *et al.*, 2007), implies that HERV-K102 particles are induced, released, and may possibly mediate recovery. The HERV-K102 genome contains two glucocorticoid response elements (GRE) (Ono, 1986). Therefore HERV-K102 full length transcripts are likely induced by cortisol and/or progesterone which both use the same GRE (Chan *et al.*, 1989). It is noteworthy that the stimulation of HERV-K HML-2 genome expression by progesterone has been observed after estradiol treatment in a human breast cancer cell line (Ono *et al.*, 1987). Thus, HERV-K102 particle induction generating foamy macrophages and/or subsequently T cells (Figure 1) may be a novel innate immune mechanism and likely plays a key role in cortisol-mediated immunosenescence. In particular, foamy macrophages are known to initiate atherosclerosis (Crowe *et al.*, 2010; Lo and Plutzky, 2012; Falk *et al.*, 2013). Since cardiovascular disease accounts for significant levels of morbidity and mortality worldwide (GBD 2013 Mortality and Causes of Death Collaborators, 2015) this attests to the potential importance of HERV-K102 blocked particle release in human health. The discovery that HERV-K102 particle production may induce foamy macrophages rather than high cholesterol per se, may substantiate the notion that abnormalities of lipoproteins may be only surrogate indicators of the underlying risks (Fuchs *et al.*, 2012). Indeed, elevated cortisol may increase cholesterol production (Anagnostis *et al.*, 2010). This suggests in part, it may be elevations in cortisol moreso than cholesterol that may initiate foamy macrophages and/or may cause them to persist.

HERV-K102 envelope RNA (*env*) expression was first reported in breast cancers (Wang-Johanning *et al.*, 2001) and full length genomic sequences and *env* splicing were also subsequently demonstrated in breast cancers (Wang-Johanning *et al.*, 2003). Both T and B cell responses to HERV-K102 antigens were described in breast cancer patients (Wang-Johanning *et al.*, 2008). More remarkably, antibodies to HERV-K102 envelope protein (Env) epitopes were found to directly induce apoptosis in human breast cancer cells *in vitro* and *in vivo* in xenotransplanted human tumors cancers (Wang-Johanning *et al.*, 2012). Thus, while controversial (Hohn *et al.*, 2013; Downey *et al.*, 2015), HERV-K102 activation in tumors may be protective.

HERV-K HML-2 group members have also been implicated in other diseases such as autoimmune conditions (Brodziak *et al.*, 2012; Volkman and Stetson, 2014). HERV-K HML-2 type 1 plasma RNA expression but not type 2, correlated with disease activity in rheumatoid arthritis patients (Reynier *et al.*, 2009). In addition, TNF-α and IL-6 increased the transcription rate of HML-2 (Freimanis *et al.*, 2010) and are known to be elevated in autoimmune diseases. HERV-K activation has also been associated with neuropathogenesis (Christensen, 2010), and in viral infections including HIV-1 and CMV (Laderoute *et al.*, 2007; Bergallo *et al.*, 2015). Both CMV (Stevenson *et al.*, 2014) and HIV-1 infection (Crowe *et al.*, 2010; Guaraldi *et al.*, 2011; Lo and Plutzky, 2012) as well as other intracellular pathogens (Chatzidimitriou *et al.*, 2012) are associated with increased atherosclerosis risks. HERV-K HML-2 activation has been best studied in HIV-1 infection where it appears to be protective (Ormsby *et al.*, 2012).

A model outlining the main mechanisms by which HERV-K102 may antagonize HIV-1 replication is presented in Figure 2. There is now compelling evidence that HIV-1 infection or alternatively, the Tat protein (Gonzalez-Hernandez *et al.*, 2012) induces the transcription of HERV-K HML-2 proviruses (Contreras-Galindo *et al.*, 2006; 2008; 2012) including HERV-K102 proteins (Brinzevich *et al.*, 2014; Vincendeau *et al.*, 2015) or particles (Laderoute *et al.*, 2007; Contreras-Galindo *et al.*, 2012). Molecular interference by HERV-K proteins resulting in the production of non-infective HIV-1 particles has been demonstrated by sev-

eral groups (Monde *et al.*, 2012; Padow *et al.*, 2000) and could also serve to assist adaptive immune responses to HIV-1 antigens (Figure 2, Mechanism 1). The release of particles in HIV-1 infected cells by lysis such as occurs on day 7 in uninfected cultures is postulated to destroy the HIV-1 infected cells (Figure 2, Mechanism 2). As mentioned previously, HERV-K102 particles are proposed to enter and undergo lytic infections in virus infected cells in analogy to PFV (Mikovits *et al.*, 1996) (Figure 2, Mechanism 3). However, direct evidence of HERV-K102 lytic infections in HIV-1 infected cells or tumor cells is lacking and needs to be addressed. In contrast, in normal cells, HERV-K102 may simply integrate. That HERV-K102 integration occurs *in vitro* and *in vivo* has been demonstrated and is consistent with its high replicative activity. About 5 fold higher genomic copy numbers were reported to be associated with protection against HIV-1 acquisition (Laderoute *et al.*, 2015) indicating HERV-K102 particle production may be an early and potent innate response to viral infections. In HIV-1 patients, B cell and T cell responses to HERV-K HML-2 antigens (Figure 2, Mechanism 4) have been demonstrated (Boller *et al.*, 1997; Laderoute *et al.*, 2007; Garrison *et al.*, 2007; Michaud *et al.*, 2014a) and have been shown to be protective against HIV-1 replication and/or associated with elite controller status (SenGupta *et al.*, 2011; Jones *et al.*, 2012; Michaud *et al.*, 2014b). Remarkably these HML-2 antigens are not expressed on normal cells but present on the cell surface of virally infected or tumor transformed cells and appear to operate as beacons allowing for clearance of altered cells. However, since endogenous retroviruses such as HML-2 are known to activate the innate immune system through Toll-like receptors (TLR) (Hurst and Magiorkinis, 2015), this raises the notion that the generation of autoreactive T and B cell responses to HERV-K102 antigens may use alternative immune signalling pathways possibly involving TLRs as described by Pone *et al.* (2015) or by Zeng *et al.* (2014). This would help explain why HERV-K antibody production is only temporary and does not seem to involve an amnestic response, as was shown in patients with germ cell tumors (Boller *et al.*, 1997; Kleinman *et al.*, 2004).

Although Bhardwaj *et al.* (2014) recently provided evidence that "RNA" bearing HERV-K HML-2 particles were not detectable in plasma samples from HIV-1

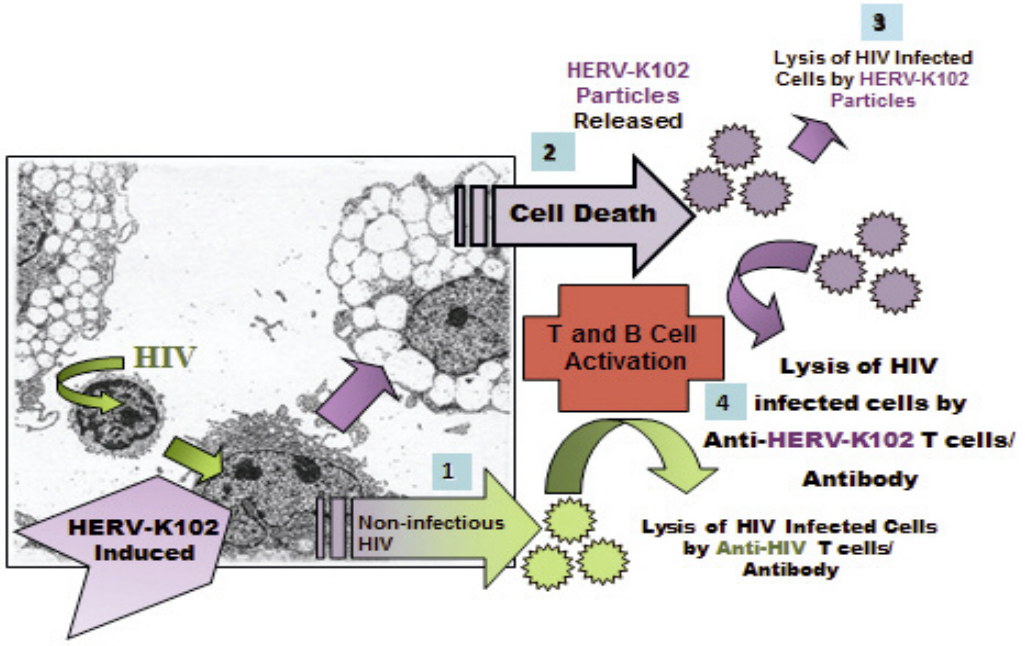

Figure 2. Hypothetical Model for How HERV-K102 Replication may Protect Against HIV-1. The image provided is an electron micrograph of HERV-K102 associated vacuolation (reproduced with permission from Laderoute *et al.*, 2015). According to the model, upon HIV infection HERV-K102 RNA is induced, proteins are made, particles are produced, and particles are released by lysis on day 7. In HIV-1 infected cells, HERV-K102 particles are postulated to undergo lytic infection which might be enhanced by flavonoids, the latter which have also been shown to block HIV-1 replication (Pasetto *et al.*, 2014). When HERV-K102 particles enter innate immunity cells, sensing of retroviral DNA may activate TLRs causing an up regulation of various antiviral mechanisms. It may also allow triggering of autoreactive T and B cells to HERV-K antigens possibly through TLRs (i.e., may not involve normal T cell activation). These T and B cell autoimmune responses kill HIV-1 infected cells where HERV-K102 antigens might be abnormally expressed at the cell surface. Finally, similar mechanisms of protection (mechanisms 2 through 4) may apply to generally to viruses and other intracellular pathogens as well as against tumors.

patients in contradiction of earlier reports (Contreras-Galindo *et al.*, 2006; 2008; 2012), their published data instead provided clear evidence for HML-2 "DNA" bearing particles. For more details on HERV-K HML-2 expression in disease and in HIV-1 infection, the reader is referred to review articles (Bannert and Kurth, 2004; Christensen, 2000; Van der Kuyl, 2012; Hohn *et al.*, 2013).

Summary

It has been suggested that age-related immune dysfunction may at least in part explain the aging process (Fulop *et al.*, 2013; 2014). However, no single theory explains all aspects of aging. Indeed, the causes of immunosenescence which involves immunosuppression and inflammation, remain to be deciphered (Jenny, 2012).

This treatise was not an attempt to address aging per se, but was to try to explain how the age-related changes in the DHEA/cortisol level might be associated with the age-associated risk of chronic disease involving immunosenescence. The salient ideas behind this paradigm are that the induction of HERV-K102 particles and AFP expression is mediated by cortisol. Due to stress on the system and/or immune reactivity; tumors, infectious agents, toxins or trauma, may also indirectly increase cortisol levels. In order to explain the link with aging, the blocked release of HERV-K102 particles from foamy macrophages may be favored when DHEA levels may be insufficient to inactivate AFP. DHEA levels are known to diminish with aging and are depressed in patients with chronic disease. In aged or non-healthy adults, insufficient DHEA allows for the persistence of foamy macrophages as there is less DHEA in the system to inactivate AFP. These persistent foamy macrophages presumably release inflammatory mediators as well as immunosuppressive molecules like active AFP, although this remains to be directly shown. This then is postulated to directly contribute to immunosenescence and the dysfunction of the immune system.

It is known that dietary antioxidants such as flavonoids and vitamin C which may also behave as nutraceuticals, may delay or ameliorate symptoms of aging (Peng *et al.*, 2014). Accordingly, plausible paradigms to explain immunosenescence would need to have the role of flavonoids addressed which was achieved here. It is suggested flavonoids may lower AFP activity and/or expression and there is some evidence in the literature to support this (Saleem *et al.*, 2013). It is also notable that various flavonoids may serve to down-modulate cortisol activity by preventing the conversion of inactive cortisone to cortisol (Hintzpeter *et al.*, 2014; Tagawa *et al.*, 2015).

As a new paradigm, it would be anticipated that conflicts with prevailing notions would surface. The most notable of these would be the idea that foamy macrophages may be more commonly reflective of the induction of a human foamy virus in macrophages such as by cortisol, by viruses, and/or other intracellular pathogens, rather than by being directly induced by elevations in cholesterol. Indeed myocardial infarction events are known to peak with peak flu season but this association is only found in populations 65 years of age or older (Foster *et al.*, 2013). This is consistent with the new paradigm presented here about induction of HERV-K102 particles by viruses and blocked release associated with aging. On the other hand, seasonal variability of cholesterol levels have not been reported and over 75 % of patients suffering heart attacks do not have elevated LDL (Sachdeva *et al.*, 2009).

Conclusions

The paradigm presented here of cortisol-mediated HERV-K102 blocked particle release in foamy macrophages as well as the association of a role of AFP in immunosenescence may be invaluable to understanding the risk of chronic illnesses with age. If correct, it is expected to drive rational prevention and treatment strategies, for example as suggested by Fantidis (2010). On the other hand, the current approach of using immunosuppressive therapies to treat age-associated autoimmune diseases and cancer, or to prevent atherosclerosis and heart disease such as by statins, may need to be revisited. It will therefore be of high priority to determine whether or not HERV-K102 particles are oncolytic or virolytic at least under permissive conditions. It will be equally important to address whether these particles in plasma are associated with remission of chronic diseases as has been shown in a limited number of patients addressed so far. It would also be useful to develop better clinical methods for unveiling the presence of active, immunosuppressive AFP in serum in patients with chronic or active illnesses to help determine whether AFP is implicated or not, in a wide variety of age-associated chronic diseases.

It is interesting that there are two camps of medical opinion on how cardiovascular risks could be minimized. One camp represented by the Canadian Heart and Stroke Foundation favors reduction of cholesterol such as by statins. On the other hand, Hypertension Canada favors healthier diets, smoking cessation, weight loss, more exercise, the avoidance or minimization of stress, better carbohydrate balance, and other

lifestyle changes in order to lower blood pressure. It is very clear that the new paradigm presented here favors the latter approach. If followed, one might enjoy the added benefit of amelioration of immunosenescence, and thus the risk of other age-associated chronic disease.

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Disclosure

M.P.L. is an inventor on patents related to the discovery of HERV-K102 particles assigned to the Public Health Agency of Canada for which no benefits are provided to inventors. M.P.L. retired from Immune System Management Clinic and Lab during the writing of this manuscript.

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