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OPINION ARTICLE

REVISED Clues to finding correlates of risk/protection for HIV-1 vaccines [version 2; peer review: 2 approved with reservations]

Marian P. Laderoute

Immune System Management Clinic & Lab, Ottawa, ON, K1S 5R5, Canada

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Abstract

Based on outcomes of informative HIV-1 vaccine prevention trials and the literature, it seems protection against HIV-1 acquisition more likely pertains to innate rather than adaptive immunity mechanisms. The proposed innate mechanism appears to be launched by alternatively activated macrophages in response to viral vectors and might be enhanced by natural female hormones. It was also suggested this novel immune mechanism was not likely amenable to discovery using standard or traditional approaches and is unlikely to be present in non-human models. A plausible, candidate innate mechanism with these characteristics pertains to the induction and production of human endogenous retrovirus-K102 (HERV-K102) particles by viruses which occurs in and generates foamy macrophages. HERV-K102 has salient features of non-pathogenic foamy retroviruses and its activation is part of the human-specific HERV-K HML-2 protective response described in HIV-1 patients. HERV-K102 particle production may be a novel and phylogenetically more recent form of innate immunity remarkably involving a viral anti-viral response.

Accumulating clinical, biological and phylogenetic evidence supports a role of the HERV-K102 virus along with HML-2 responses, in the antagonism of HIV-1 replication and/or in the prevention of HIV-1 acquisition. Thus, it will be important to test the hypothesis that HERV-K HML-2 activation, HERV-K102 particle production and antibodies and T cell responses to select HERV-K102 or HML-2 antigens might comprise correlates of protection in HIV-1 vaccine prevention trials. Other proposed work would be to expand existing studies in HIV-1 highly exposed seronegative cohorts (HESN) and elite controllers to further evaluate a role of HERV-K102 and HML-2 in protection against HIV-1 replication and acquisition. The results of such inquiries may have important ramifications for the HIV-1 cure in addition to vaccines.

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1. **Patricia E Fast** , International AIDS Vaccine Initiative-New York, New York, USA
Stanford University School of Medicine, Stanford, USA
2. **Robert Gallo**, University of Maryland, Baltimore, USA

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

HIV-1, novel, correlates of protection, HIV-1 vaccines, HERV-K HML-2, HERV-K102, foamy macrophages, innate immunity

Corresponding author: Marian P. Laderoute (hervk102@bell.net)

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REVISED Amendments from Version 1

The extensive revision now discusses in depth why generally adaptive immune response mechanisms are unlikely to significantly affect HIV-1 acquisition rates, and thus why HIV-1 vaccine prevention trials have had poor outcomes. It also expands the discussion of these outcomes with more citations, and why the proposed correlates of protection might be rejected. The introduction of HERV-K102 as a viral anti-virus response and as part of the innate immune system is strengthened in the abstract and opening comments, and the sections describing HERV-K102 attributes in defending against HIV-1 replication and acquisition are presented in a more logical order. As well newer evidence for HERV-K HML-2 (HERV-K102 is a group member) in playing a role in the control of HIV-1 replication is cited. The revision also calls into question whether VE for female gender as reported for the RV144 trial may or may not have been statistically significant as discrepancies in reporting VEs for all participants versus gender were noted. Hopefully the revision clarifies that HERV-K102 is not an exogenously acquired virus, but an endogenous, foamy-like (protector), retrovirus which works in tandem with HERV-K HML-2 activation to reduce the likelihood of integration of a lentivirus/orthoretrovirus (HIV-1). Just like the old adage to fight fire with fire, here it is suggested HERV-K102 as the only known naturally replication competent HERV-K HML-2 group member, may represent a virus-anti-virus response selected for when adaptive immune mechanisms poorly handled lentivirus exposures following the divergence of chimpanzees from humans. HERV-K102 particle production which generates foamy macrophages, along with HML-2 activation, appears to be a new dimension of human innate immunity inversely correlated with triggering adaptive immune mechanisms, which is hypothesized, partially based on clues and on some initial data, to prevent HIV-1 acquisition.

See referee reports

Introduction

It has been recently stated that immune correlates of protection for HIV-1 prevention vaccines must be complex and/or reliant on the right combination of multiple types of immune responses as a 'true' correlate of protection has not yet been characterized (Kuri-Cervantes *et al.*, 2016; Tomaras & Plotkin, 2017). In particular, the V1V2 IgG response as characterized as a primary correlate in the RV144 trial the latter which showed vaccine efficacy (VE) for reduced risk of HIV-1 acquisition (Haynes *et al.*, 2012), by itself is unlikely to signify an immune correlate of protection that can be used to predict vaccine efficacy (VE) outcomes because it was also present in the VAX003 trial (Perez *et al.*, 2017; Yates *et al.*, 2014) that lacked VE (reviewed in Tomaras & Plotkin, 2017). Other objections that can be made include the observation that in the case-control analysis performed among vaccine recipients as reported by Haynes *et al.* (2012), the error bars for V1V2 IgG overlapped for the comparison of participants who acquired HIV-1 versus those who did not. Accordingly, it was not surprising that the V1V2 IgG response was not significant in the univariate analysis, although it was marginally so in the multivariate analysis. Technically, to be useful as a predictor for vaccine trial outcomes and vaccine development, neither a causal relationship nor protective mechanism needs to be elucidated (Rolland & Gilbert, 2012) as the biomarker may be a surrogate marker of the 'true' correlate of protection (Plotkin & Gilbert, 2012). However, despite the clinical testing of over 200 candidate

vaccines since 1986 and also reflecting the fact that immune correlates of protection have not been conclusively identified for HIV-1 exposed seronegative (HESN) populations (Safrit & Koff, 2016), the discovery of 'true' correlate(s) of protection would be expected to expedite the achievement of HIV-1 vaccines as well as cures. To help this quest along, there are clues that can be derived from the literature as well as from the existing HIV-1 prevention vaccine trials which allude to the nature of the 'true' correlate(s) of protection against HIV-1 acquisition. As a result of this inquiry, a novel and plausible candidate innate immune protection mechanism against HIV-1 acquisition has been identified and will be presented. Unexpectedly and quite remarkably, this response appears to be a virus anti-virus response which may have evolved in chimpanzees during exposures to lentiviruses and subsequently acquired by humans less than 2 million years ago.

Trials and Tribulations of HIV-1 Vaccine Prevention Trials

In terms of randomized clinical trials for vaccine prevention of HIV-1 acquisition, there have been 6 trials, only 3 of 6 which were determined to be informative for correlates of risk (increased or decreased) (reviewed in Stephenson *et al.*, 2016; Tomaras & Plotkin, 2017). They were the HVTN 502 STEP trial (Buchbinder *et al.*, 2008) with follow-up data in HVTN 504 (Duerr *et al.*, 2012), the HVTN 503 Phambili trial (Gray *et al.*, 2011; Gray *et al.*, 2014) with follow-up data in Phambili HVTN 503-S (Moodie *et al.*, 2015) and the RV144 trial (Rerks-Ngarm *et al.*, 2009).

What these three trials had in common was that they all employed viral vectors for the priming/immunization whereas 2 others (Vax004 and Vax003) used proteins with an alum adjuvant and one (HVTN 505) used circular plasmids for priming. Even though the latter HVTN 505 trial used an Ad5 viral vector, it was only used for the boosting (Hammer *et al.*, 2013) and no vaccine efficacy was found. In these informative trials, waning of negative or positive VE (reviewed in Stephenson *et al.*, 2016; Tomaras & Plotkin, 2017) might imply the effects on VE in part related to non-specific effects attributable to use of viral vectors. In addition, in the RV144 trial which uniquely included HIV-1 envelope (Env), evidence of postacquisition selection pressure at 42 months by adaptive immunity may have also contributed to a positive VE (Gartland *et al.*, 2014).

In support of the notion of non-specific effects of the viral vectors employed, Huang *et al.* (2014) found that while HIV-specific responses measured at 4 weeks after the second vaccination in the STEP trial were not associated with risk of HIV-1 infection, levels of IFN-gamma by ELISpot assay in the mock responses performed in the absence of HIV-1 antigens showed a hazard ratio (HR) of 1.62 (95% CI: 1.28 to 2.04, $p < 0.001$) which was strengthened among participants with pre-existing anti-Ad5 antibodies. Consistent with this finding, IFN-gamma levels are higher in HIV-1 positive over negative individuals (Yong *et al.*, 2016) and are lower in HESN than HIV-1 unexposed controls (Jaumdally *et al.*, 2017). This raises the possibility that induction of innate rather than adaptive immunity by viral vectors may

play a more significant role in protection/risk against HIV-1 acquisition than what is currently appreciated. Unfortunately, in none of the informative clinical trials was there a placebo arm which included an empty vector as a control for HIV-1 non-specific or innate immunity effects.

Problematic issues confronting clinical trial interpretation of outcomes include the concern that disparate statistical significance can be demonstrated depending on the statistical and analytical methods employed. This is why it is essential to decide statistical and analytical methods in advance to avoid bias. For the interpretative meaning of finding correlates of risk in randomized, HIV-1 prevention clinical trials which show vaccine efficacy (VE), as alluded to above, the association does not necessarily mean that there was protection against acquisition, as it could also reflect post-acquisition control of replication of HIV-1. For example, while the RV144 trial showed 31.2% vaccine efficacy at 42 months (Rerks-Ngarm *et al.*, 2009), vaccine efficacy (VE) was 54% versus 3% ($p=0.05$) in HLA A*02 individuals versus participants lacking this Class I HLA allele in this subgroup analysis. Moreover, VE increased in the HLA A*02 individuals (74% versus 15%, $p=0.02$) when the subgroup analysis was restricted to acquired HIV-1 strains bearing the lysine residue at site 169 in the V2 domain of the immunizing scaffold epitope (Gartland *et al.*, 2014). However, these authors suggested that this enhanced VE related to post-acquisition selection pressure by adaptive immune mechanisms rather than a genuine blocking of HIV-1 acquisition. Nevertheless, this was the first time an HLA allele was associated with VE against HIV-1 acquisition.

Indeed, genome wide association studies have only established CCR5 and certain HLA alleles as being associated with protection against HIV-1 replication and/or acquisition (reviewed in Naranbhai & Carrington, 2017). These alleles, such as HLA-B*27 are also associated with protection against hepatitis C and curiously also associate with autoimmunity (Crux & Elahis, 2017; Khan, 2017). The finding that natural killer alleles appear to interact with HLA-B*57 in control of HIV-1 replication (Tallon *et al.*, 2014), may argue that the HLA-B supertypes which confer protection against HIV or HCV may be through innate mechanisms. In the STEP trial, analysis of host genetics associations identified that the protector HLA alleles (B*27, B*57, B*58:01) known to be associated with HIV-1 control had a lower viral load (Fitzgerald *et al.*, 2011) and showed robust CD8+ T cell activity (Migueles *et al.*, 2011). However, the HIV-1 specific T cell responses measured in the trial did not correspond with the sieve findings on the HIV-1 strains that were acquired and were not associated with lower risk of infection in the vaccine recipients (reviewed in Tomaras & Plotkin, 2017).

The above discussion raises the important but thorny issue of whether or not adaptive immunity per se to HIV-1 specific antigens can actually prevent HIV-1 acquisition. The premise that adaptive immunity can prevent infection which has been clearly shown for a wide variety of pathogens and resulted in successful vaccines, may not be valid for HIV-1. This is because HIV-1 strains show hypervariability in protein sequences, antigen drift and are

vulnerable to pseudotyping of its particles by envelope derived from HERV-K retroviruses particularly certain HERV-K HML-2 group members as will be discussed later. Thus, in theory, preformed recognition of HIV-1 specific envelope (Env) epitopes proffered by vaccines whether by antibody or T cell receptors might be expected to only provide postacquisition selection pressure and/or weak correlates of protection. For example, in open label studies involving anti-retroviral treatment interruption, administration of VRC01, a broadly neutralizing antibody targeting the HIV-1 CD4-binding site found in Env, was shown to significantly delay viral rebound in many persons with HIV-1 infection as measured at 4 weeks when compared with historical controls, but the difference was not significant at week 8 (Bar *et al.*, 2016). HIV-1 is also more complicated than many pathogens as it targets activated immune cells which are generated during immunization. Moreover, adaptive cytokines like IL-1 and IL-2 enhance HIV-1 replication in contrast to the innate cytokines interferon alpha and beta which instead inhibit HIV-1 replication (reviewed in Naif, 2013). These considerations question the fundamental basis for the conduct of prevention HIV-1 vaccine efficacy trials that have the goals of stimulating adaptive immunity.

As discussed by Haynes *et al.* (2012), no direct evidence in humans was offered for suspecting adaptive immunity may be a strong correlate of protection in preventing HIV-1 infection; only that adaptive immunity modulated progression (postacquisition selection pressure) in humans. In fact, accumulating evidence suggests lowering adaptive responses but without overt immunosuppression may be key to preventing HIV-1 acquisition as determined in HIV-1 exposed seronegative (HESN) cohorts (reviewed in Lajoie *et al.*, 2017). Indeed, the presence of adaptive immunity to HIV-1 antigens does not alter superinfection in high risk individuals (Ronen *et al.*, 2017) providing direct evidence in humans that adaptive responses may not significantly alter HIV-1 acquisition rates. The strongest data taken as a basis for conducting randomized clinical trials to generate adaptive immunity for the prevention of HIV-1 acquisition were derived instead from studies conducted in simians with SIV or SHIV (Haynes *et al.*, 2012). As will be detailed below, a proposed candidate 'true' correlate of protection based on innate immunity is notably absent from these models implying simian (macaques) models might not necessarily be predictive for human correlates of risk/protection for HIV-1 prevention vaccines.

Overview of the Trial Outcomes

The RV144 trial (Rerks-Ngarm *et al.*, 2009) was the only trial that showed vaccine efficacy for prevention of HIV-1 acquisition (reviewed in Haynes *et al.*, 2012; Kim *et al.*, 2015; Kuri-Cervantes *et al.*, 2016; McMichael & Haynes, 2012; Perez *et al.*, 2017; Stephenson *et al.*, 2016; Tomaras & Plotkin, 2017). For this trial conducted in Thailand in heterosexual low risk individuals, the VE of about 60% at one year (6 months after the last immunization) fell to 31.2% at 42 months (Rerks-Ngarm *et al.*, 2009). Although vaccination did not have a significant effect on viral load ($p=0.24$), the placebo group had 1.26 times better control of viral load over vaccines. This may be consistent with the notion that vaccination might have increased the

number of targets, and thereby enhanced HIV-1 replication upon HIV-1 acquisition.

When broken down by gender, females (38.6% VE) had about a 1.5 better VE over males (25.8% VE) in the modified intention-to treat (MITT) analyses, but where the p values were not provided. The provided 95% confidence intervals at -8.6 to 65.3 % for females and -7.3 to 53 % for males in Table 2 on VE appeared to be anomalous given that in total, the VE in Table 2 was given as 31.2 % with 95% CI of 1.7 to 51.8% (ie., if the two components have a negative lower CI how is it possible that the total has a positive lower boundary of CI and the upper boundary lower than the components). Moreover the numbers in Table 2 (MITT) for the calculation of VE (n=15,948) did not match that reported in the abstract for MITT, which instead indicated n was 16,395 and the VE was 31.2% with 95% CI, 1.1 to 52.1; (p=0.04). A quick analysis revealed there were relatively more men removed from the analysis in Table 2 when the MITT based VE for the total population was calculated. The supplemental information did not discuss these discrepancies. Accordingly it is possible but not for certain that females exhibited up to a 1.5 better VE over men in the RV144 trial suggesting a possible female hormone influence on the correlate of risk reduction. The rates of HIV-1 acquisition for females were 0.185 per person-year (pp-y) in the vaccine arm and 0.301 pp-y in the placebo. The corresponding rates for males were 0.197 pp-y in the vaccine arm and 0.266 pp-y in the placebo arm.

With respect to female hormones exhibiting an influence on HIV-1 susceptibility or VE, it should be remembered that in each trial which only enrolled women of child-bearing ages but who were not pregnant nor breast-feeding, women were counseled to practice effective contraception (RV144, [Rerks-Ngarm et al., 2009](#)) or required to use hormonal contraceptives and a barrier method (Phambilli HVTN 503, [Gray et al., 2011](#)) during the immunization period and for one to several months thereafter. In the STEP trial, no restrictions applied ([Buchbinder et al., 2008](#)). In a recent meta-analysis, while the use of various progestins for oral contraception were associated with a significantly increased adjusted hazard ratio of HIV-1 acquisition over women who did not use contraceptives, the combined oral contraceptive was not ([Morrison et al., 2015](#)). Thus, a potential confounder of trials involving women is the type and timing of steroid contraception used which should be taken into consideration at the time of trial design, used as a parameter to validate trial randomization, and for checking bias at later time points of analyses. The other two trials which used the same Ad5 vector were stopped early due to an increased risk of HIV-1 acquisition in males discovered in the interim analysis of the STEP trial ([Buchbinder et al., 2008](#)). In the extended analysis, in the STEP trial (HVTN 504), there were 172 HIV-1 infections in 1836 males providing a follow-up HR of 1.40 (CI: 1.03 to 1.92, p=0.03) but where there were too few infections in females for HR analysis. The impact of the baseline Ad5 antibodies or lack of circumcision on VE was present in the STEP trial only during the first 18 months ([Duerr et al., 2012](#)). In the follow-up for the Phambilli recall trial (HVTN 503-S) with an

additional 1286 person-years of follow-up, the total HR was 1.52 (95% CI 1.08 to 2.15, p=0.02). Interestingly in males, the HR was 2.75 (95% CI 1.49 to 5.06, p=0.001) whereas for females it was 1.12 (95% CI 0.73 to 1.72, p=0.62) and non-significant. This trial did not identify Ad5 pre-existing immunity issues possibly due to high seroprevalence at 81%, and similarly did not find circumcision to have any effects in contrast to the STEP trial which focused on high risk men having sex with men. They concluded that significant predictors of HIV-1 acquisition were vaccine treatment, male gender and baseline HSV-2 status ([Moodie et al., 2015](#)). Thus of the two trials which were informative for gender effects, females may have exhibited less risk of HIV-1 acquisition related to vaccination or tended towards having higher VE although the discrepancies in the reported analyses of RV144 may need clarification. Taken together with the aforementioned meta-analysis, the issue is raised that estrogen plus progesterone but not progestins alone, might enhance a protector effect against HIV-1 acquisition promoted by viral vectors, which may warrant further investigation.

Studies on Correlates of Risk

A number of groups have suggested partial correlates of risk reduction as demonstrated from the RV144 HIV prevention trial, particularly, V1-V2-specific, polyfunctional IgG antibody responses (reviewed in [Kim et al., 2015](#); [McMichael & Haynes, 2012](#); [Rolland et al., 2012](#); [Stephenson et al., 2016](#)).

In the RV144 initial analysis, a case-control analysis on samples collected two weeks following the last immunization, it was designed to identify factors predicting HIV-1 infection through 42 months of follow-up in the vaccinated group. It compared 41 vaccinees who became infected with 205 uninfected vaccinees and involved 6 preselected antibody and T cell tests ([Haynes et al., 2012](#)). However, since the comparison involved vaccination, there was considerable overlap in the box plots meaning a significant proportion of both those infected or not had the factor being assayed and frequently at the same concentration. This suggested a priori that such a factor may not be a strong candidate for a true correlate of protection. It is unclear if analyses of samples taken 2 to 4 weeks prior to HIV-1 acquisition would have been more informative but this would have presented logistical issues including increased labor and costs for collecting monthly samples.

As reported in the initial study of the potential correlates of protection for the RV144 vaccine trial, it was suggested that there was no role of IgG avidity, ADCC, neutralizing antibody or Env-specific CD4 + T cells in the protective response detected ([Haynes et al., 2012](#)). On the other hand, while IgA antibody binding to HIV-1 envelope (Env) directly correlated with acquisition risk in both univariate and multivariate analyses, scaffolded V1-V2 Env IgG antibodies while marginally significant in multivariate analysis, failed to achieve significance in univariate analysis for correlation of protection against HIV-1 acquisition ([Haynes et al., 2012](#)). However, secondary analysis suggested that Env-specific IgA antibodies may have interfered with the effects of the protective IgG V1-V2 antibodies.

These findings were subsequently confirmed in different laboratories with different assays and reagents (Zolla-Pazner *et al.*, 2014). Follow-up studies suggested the pertinent Env V1V2 antibodies were IgG3 (Yates *et al.*, 2014), or dependent upon complement activation within the IgG V1V2 population of antibodies (Perez *et al.*, 2017). In the latter case the response rate and magnitudes were higher in RV144 than in VAX003 and VAX004.

In the study by Yates *et al.* (2014), the IgG or IgG3 response rates (i.e., number of participants with the factor) to the mutated V169K V1V2 strain significantly dropped from testing at 26 weeks to 52 weeks (97 to 11% for IgG and 50 to 3% for IgG3) and the authors suggested a correlation with VE waning shown for the RV144 trial. However, at 52 weeks the VE was about 60% at a time when these antibody responses reached background which instead implied there was no temporal correlation with VE waning. As well, the response rates were not significantly different when compared with visit 5 VAX003 participants where VE was not shown. Moreover, the magnitude of the response was higher in VAX003 visit 5 vaccine recipients for the same epitope. The IgG3 response rates to any of the V1V2 strains tested did not correlate with reduced HIV-1 acquisition except when tested on a V169K mutated V1V2 strain epitope which was significant in both univariate and IgA adjusted analysis. On the other hand, the magnitude of the response to this epitope did not significantly correlate with a reduced risk with or without adjustment for the IgA interference. Interestingly, the response magnitude did reach significance when adjusted for the influence of IgA interference for a number of V1V2 strains but not for the mutated V169K epitope. Taken all together, it seems these findings would not support the contention that IgG3 antibodies induced to V1V2 Env epitopes through vaccination mediated the protection observed.

It should be noted as reviewed in Corey *et al.* (2015), that the prevalence of K169 in the HIV-1 Env V1V2 region is about 85% in Thailand, while in the RV144 clinical trial, the HIV-1 strains acquired in the placebo-treated group were 83% with K169, while in the vaccine treated it was 66%. This showed that the strain that emerged in the vaccine arm did not match the vaccine, as would be expected. Indeed Rolland *et al.* (2012) estimated that the vaccine efficacy against viruses matching the vaccine at position K169 was 48% (p value of 0.0036; 95% CI 18–60%) while the vaccine efficacy for mismatched was not significant. This latter finding contradicts the finding of a correlation of the response rate for a mismatched V1V2 epitope, namely the V169K mutation with protection in the RV144 vaccine trial as studied and reported by Yates *et al.* (2014).

For a more intensive discussion of the various risk and protection factors discovered in HIV-1 vaccine prevention trials, the reader is referred to more comprehensive reviews (Stephenson *et al.*, 2016; Tomaras & Plotkin, 2017).

Clues to True Correlates of Protection

The relatively low rates of acquisition of HIV-1 per exposure at less than 1 in 1000 for heterosexual transmission (Becerra *et al.*, 2016), might argue that defense against HIV-1 in humans possibly involves a simpler and more potent mechanism than

what has yet been elucidated or appreciated. Indeed, that a new scientific paradigm may be needed to advance the development of the HIV-1 vaccine has been proclaimed (Esparza, 2015). What then can we currently deduce about the characteristics of this unknown correlate of risk/protection, taking also into account the results in the three informative HIV-1 prevention vaccine trials?

The elusive correlate is likely launched by activated macrophages

In any immune response, innate and/or adaptive, activated macrophages control the response. Accordingly, it follows that any risk or protection associated with HIV-1 vaccines must then relate to a key and so far, ill-defined macrophage activation pathway. Moreover, in HIV-1 acquisition, the transmitting/founder strains are generally CCR5-tropic and target macrophages (reviewed in Borggren & Jansson, 2015) as originally shown as early as 1986 (Gartner *et al.*, 1986). Together these findings point to the likelihood that there exists a novel macrophage-based ‘gate-keeper’ defense mechanism that itself determines whether HIV-1 will be acquired, although a role of vaginal myeloid dendritic cells in capturing and disseminating HIV-1 may also play a role in the gatekeeping function (Shen *et al.*, 2014).

The Ad5 vector used in the STEP trial, highly targets liver Kupffer cells, which represent about 80–90% of the macrophages in the body (Khare *et al.*, 2011). Kupffer cells are infected by HIV-1 (Mosoian *et al.*, 2017). In the STEP trial, male uncircumcised participants with Ad5 antibodies were significantly at increased risk of transmission compared with those without vector antibodies (Buchbinder *et al.*, 2008). In contrast, no increased risk of HIV-1 transmission was found in the placebo arm for participants with Ad 5 antibodies. This suggests that at the interface of the Ad5 vector with macrophages, the presence of bound Ad5 antibodies enriched in the local milieu may have somehow blocked the induction of the putative, gatekeeper/defense mechanism of macrophages. With time the levels of the Ad5 vector would have cleared accounting for the waning observed and also since Ad5 seropositivity had no effects in the placebo arm (Duerr *et al.*, 2012). A possible candidate mechanism for this interference by antibody is tuftsin. Tuftsin is a short peptide consisting of the sequence TKPR and is released from bound IgG. Tuftsin is known to inhibit macrophage activation at higher concentrations, while at lower levels, it augments macrophage activation (Siemion & Kluczyk, 1999). Thus, it may be plausible that higher risk participants (uncircumcised men) with pre-existing Ad5 antibodies injected with an Ad5 vector could have experienced inhibition of the putative, novel macrophage defense mechanism, through the local generation of tuftsin, thereby explaining their increased risk. Clearly, this conjecture may warrant further investigation.

Other salient clues to the characteristics of the novel host protection mechanism revealed from the informative HIV-1 vaccine trials

Another important clue relates to gender. As discussed above, in the STEP trial (Buchbinder *et al.*, 2008), there were insufficient female participants and HIV-1 infections to address vaccine efficacy in comparison to men. In a follow-up study of the Phambili trial (Moodie *et al.*, 2015), males continued to show

heightened risk related to vaccination but which was not found for females. The hazard ratio for men was 2.75 ($p=0.001$) and for women it was not significant 1.12 ($p=0.62$) despite high rates of HIV-1 acquisition which implied there was sufficient power for the statistical assessment. In contrast, in the RV144 trial, vaccine induced protection against HIV-1 acquisition was more evident in females than males (about 1.5-fold better) (Rerks-Ngarm *et al.*, 2009), but as mentioned, the statistical significance values reported need re-examination and clarification as discrepancies were noted. All together these findings support the notion that the novel, macrophage based protection mechanism induced by viral vectored vaccines might be enhanced by female hormones.

That the risk enhancement/reduction was only temporary in either trial, generally lasting 6 to 12 months after the last immunization (Buchbinder *et al.*, 2008; Rerks-Ngarm *et al.*, 2009), was consistent with an innate rather than adaptive immunity mechanism.

Since both risk enhancement (Buchbinder *et al.*, 2008) and risk reduction (Rerks-Ngarm *et al.*, 2009) were found with viral vectored vaccines, and not in the VAX003 and VAX004 HIV-1 vaccine trials, which instead involved proteins and adjuvants (reviewed in Shin, 2016), this raises the likelihood that viruses in general may preferentially activate the macrophage-based defense mechanism and/or that adjuvants diminish it.

Overall, these observations point to the existence of a novel, potent innate HIV-1 protection mechanism induced by viruses

and enhanced with female hormones, which is launched by alternatively activated macrophages, and that this activation may be sensitive to inhibition by locally bound antibodies, for example as might be the case through tuftsin. Moreover, despite a considerable effort both inside and outside of the trials, convincing immune correlates of risk or prevention against HIV-1 have yet to be revealed. This failure raises the following notions about the mechanism. It is likely not addressed by traditional *in vitro* culture methods, not discoverable using conventional detection methods on participant samples, such as by examining plasma RNA, microarray and genome wide association studies, nor is it likely present in the rhesus macaque. The critical question becomes, has such a novel, innate, potent, defense mechanism unique to human activated macrophages and difficult to study under standard conditions, been previously described?

Surprisingly, the answer to this may be, yes.

HERV-K102: a virus-anti-virus immune mechanism

A novel, innate, viral anti-viral defense mechanism unique to humans, associated with the production of foamy macrophages (Figure 1) was serendipitously discovered by scientists working at the Public Health Agency of Canada about 10 years ago, and fulfills all the above criteria for a novel defense mechanism launched by alternatively activated macrophages in response to viruses (Laderoute *et al.*, 2007; Laderoute *et al.*, 2015; Laderoute, 2015). The term alternatively activated macrophages refers to the generation of foamy macrophages which is not observed within studies of adaptive immunity. Moreover, under permissive conditions (culture of cord blood mononuclear cells in IMDM media rather than RPMI) the addition of PHA and

Figure 1. Spontaneous foamy macrophage generation associated with HERV-K102 particle production in cord blood mononuclear cells (CB) when cultured in IMDM. Left panel, H and E stain of day 11 cultured CB prepared by cytopsin. Right panels, electron micrographs show vacuoles in the foamy macrophages contain large numbers of immature particles with envelope spikes. Blue arrow points to the cytoplasmic capsid assembly outside of the vacuole, typical of foamy retroviruses (Hütter *et al.*, 2013). Left panel reproduced under a CC BY-NC 4.0 license (Laderoute *et al.*, 2015). Right panels reproduced with permission from the AIDS journal (Laderoute *et al.*, 2007).

IL-2 (various protocols) completely blocked HERV-K102 replication, particle production and the generation of foamy macrophages implying foamy macrophage generation is an alternative to adaptive immunity. HERV-K102 is a non-pathogenic retrovirus with foamy retroviral properties whereby particles accumulate in vacuoles (Laderoute *et al.*, 2015). As expected for foamy retroviruses, capsid assembly occurs in the cytoplasm adjacent to vacuoles (Hütter *et al.*, 2013, and see Figure 1 blue arrow). In these foamy macrophages, HERV-K102 particles are not released through the cell surface and release is instead dependent upon lysis of the foamy macrophages (Laderoute *et al.*, 2015). It is the only human specific HERV-K HML-2 group member (Subramanian *et al.*, 2011) shown to be naturally replication competent *in vitro* and *in vivo* (Laderoute *et al.*, 2007; Laderoute *et al.*, 2015).

Expression and activity of HERV-K HML-2 and HERV-K102 with HIV-1 infection

HERV-K102 activation was demonstrated in about 96% of HIV-1 patients which includes particles (75.7%) and/or HERV-K102 surface unit envelope specific antibodies (70 to 80%) (post hoc analysis, Laderoute *et al.*, 2007).

HERV-K102 particles have cDNA genomes rather than RNA due to its foamy retrovirus properties and reversed life cycle to orthoretroviruses (Laderoute *et al.*, 2007; Laderoute *et al.*, 2015). It can be estimated from previously reported data that in HIV-1 patients, there may only be on average about 8,200 HERV-K102 pol cDNA containing particles per ml of plasma with a response rate of about 72% (post hoc analysis of Laderoute *et al.*, 2007). This coincides with estimates of about 8,300 HERV-K HML-2 DNA (transmembrane) env containing particles with a response rate of about 73% from the data of Bhardwaj *et al.* (2014). It should be noted that two groups have reported the absence of HERV-K HML-2 RNA containing particles in plasma samples from HIV-1 patients (Bhardwaj *et al.*, 2014; Karamitros *et al.*, 2016), suggesting earlier reports of HML-2 RNA in plasma from HIV-1 patients likely were due to cellular contamination. Taken together these results might suggest the only HML-2 particles in HIV-1 patients might be HERV-K102 particles with cDNA genomes.

Several research groups have confirmed that HERV-K102, is induced by HIV-1 *in vitro* (Brinzevich *et al.*, 2014; Vincendeau *et al.*, 2015). Moreover, HERV-K102 may be the only full length, human specific, HML-2 element induced by HIV-1 and/or Tat (Gonzalez-Hernandez *et al.*, 2012). While the envelope of HERV-K18 and a consensus sequence for HERV-K HML-2 were able to pseudotype HIV-1 virions, interestingly HERV-K102 Env did not (Brinzevich *et al.*, 2014; Lee & Bieniasz, 2007). That HIV-1 may be pseudotyped by HML-2 envelope raises the notion that such pseudotyped particles could help explain, in part, the altered tropism for macrophages bearing the CCR5 coreceptor, which is commonly used by transmitting/founder strains (Borggren & Jansson, 2015). If this is indeed the case, it would also help explain why vaccination against HIV-1 envelope generally fails to prevent HIV-1 acquisition (reviewed

in Shin, 2016), or why passive immunization with HIV-1 envelope specific, broadly neutralizing envelope antibodies failed to significantly control viremia upon antiretroviral treatment interruption (Bar *et al.*, 2016; Caskey *et al.*, 2017).

In potential substantiation of an important role of HERV-K HML-2 in the control of HIV-1 replication, HERV-K HML-2 gag and envelope RNA expression in peripheral blood mononuclear cells (PBMCs) in HIV-1 patients were shown to be inversely correlated with T cell activation markers (Ormsby *et al.*, 2012). Since it is known that activated T cells correlate with HIV-1 progression (Deeks *et al.*, 2004), this implies HML-2 expression generally, and by proxy HERV-K102 activation, may antagonize HIV-1 replication and/or the HML-2 response is an alternative to adaptive immunity. Indeed, recent evidence suggests that the phylogenetically newer HERV-K HML-2 elements containing LTR5Hs (which include HERV-K102) were upregulated in CD11c+ myeloid dendritic cells isolated from HIV-1 patients, whereas, in normal healthy controls, the older LTR5A and LTR5B bearing HML-2 elements prevailed (Young *et al.*, 2014). Thus, the HML-2 response to HIV-1 involves primarily human specific HML-2 group members which would and does involve HERV-K102 (Laderoute *et al.*, 2007).

Both antibodies and T cell responses to HERV-K HML-2 and/or HERV-K102 envelope have been demonstrated in HIV-1 and breast cancer patients (reviewed in Laderoute *et al.*, 2015). A T cell clone isolated from an elite controller, which recognized a peptide identical to HERV-K102 envelope, was shown *in vitro* to specifically eliminate cells infected with various HIV and SIV strains (Jones *et al.*, 2012). More recent evidence from the same group showed elite controllers have stronger antibody and T cell responses (rates and magnitude) to HERV-K HML-2 gag than viremic controllers and normal healthy controls (de Mulder *et al.*, 2017). Altogether these results imply HERV-K HML-2 activation which includes HERV-K102 particle production (Laderoute *et al.*, 2007), may play an important role in controlling HIV-1 replication.

Remarkably, a monoclonal antibody made to HERV-K102 surface unit of envelope could directly provoke apoptosis *in vitro* and *in vivo* of breast cancer cells (Wang-Johanning *et al.*, 2012). This might suggest that the expression of HML-2 envelope on the surface of virally infected or transformed cells, but which is not found on normal cells, plays a more active role in innate host protection than merely as a surrogate marker. These findings may also further document the unexpected potency of this innate protector mechanism against HIV-1, which unlike adaptive immunity, functions irrespective of the hypervariability of HIV-1, quasi-species and/or strains of HIV-1 or even lentivirus involved (Jones *et al.*, 2012).

HERV-K102 particle activity correlates with protection against HIV-1 acquisition in an HESN cohort

In preliminary experiments, increased HERV-K102 integration (mean about 5 fold) over normal healthy controls was associated with protection against HIV-1 acquisition in a female, HIV-1 highly

exposed seronegative cohort (HESN), at the level of about 80% of the tested cohort which was not detected in HIV-1 infected individuals irrespective of their use of anti-virals (Laderoute *et al.*, 2015). This is consistent with high integration levels reported for foamy viruses in hematopoietic cell lines *in vitro* (Meiering *et al.*, 2000). It should be noted that the analysis was performed on genomic DNA isolated from plasma (where cDNA was digested to avoid interference) which in all likelihood may have favored analysis of recently lysed cells, such as macrophages releasing HERV-K102 particles. Increased integration of HERV-K102 was also found *in vitro* associated with HERV-K102 replication (Laderoute *et al.*, 2015).

It should also be noted that increased integration *in vivo* provides direct evidence that HERV-K102 particles are infectious. Dube *et al.* (2014) have shown with a resuscitated HERV-K HML-2 virus construct, that both viruses with DNA and RNA genomes are infectious.

Moreover, protection in the infamous Nairobi HESN cohort (Fowke *et al.*, 1996) is known to be temporary, as resistance to HIV-1 acquisition dwindled as early as 6 months to a year following abstinence from sex trade work (Kaul *et al.*, 2001). Thus, HERV-K102 which might be implicated in the Nairobi HESN cohort, potentially, may meet a key criterion of temporary activity and waning after 6 to 12 months. This would also be expected for innate immune mechanisms more generally.

HERV-K HML-2 activity has been phylogenetically associated with decreased integration of orthoretroviruses in hominins

HERV-K102 is a member of the HERV-K human specific HML-2 group and has hallmark features of foamy viruses (Laderoute *et al.*, 2015). This raises the notion that HERV-K102 may be a candidate foamy virus of humans which has alluded investigators. Foamy viruses are unconventional retroviruses with a reversed life cycle to orthoretroviruses (Linial, 1999; Rethwilm & Bodem, 2013). They are not pathogenic and co-speciated in primates over the past 30 years (Switzer *et al.*, 2005). Accordingly, foamy viruses likely serve a beneficial role to the host. In this regard, accumulating phylogenetic evidence is consistent with a potential role of HERV-K HML-2 in limiting invasion by orthoretroviruses (Magiorkinis *et al.*, 2015). Ancestral HML-2 elements emerged about 10.3 million years ago (Mya) (Subramanian *et al.*, 2011). There has been a striking decline of insertions of ERVs in the last 10 My in the genomes of all sequenced hominids (great apes and gibbons), but not in old world monkeys (baboons and macaques), particularly regarding HERV-H (Magiorkinis *et al.*, 2015). HERV-H makes up 88% of all the ERV integrations into the human genome in the last 30 My and became extinct over the past 10 My. HERV-H is a gammaretrovirus, which integrated around 45 to 60 Mya and has about 962 copies in the human genome (Chuong *et al.*, 2016). HERV-K, with 10 groups in the clade, only one of which is HML-2, on the other hand, entered the genome of ancestral catarrhines about 32 to 44 Mya, after the split from New World monkeys and before the split of hominids from the Old World monkeys (Kim & Han, 2015). The sister lineages

of HERV-K in most other catarrhines appear to have become extinct. Most remarkably, the HERV-K HML-2 group in humans is the only HERV-K that has continued to replicate since the origin of the catarrhines (Magiorkinis *et al.*, 2015). HERV-K102 is a member of the bioactive HERV-K HML-2 group and appears to be the only known replication competent member both *in vitro* and *in vivo* (Laderoute *et al.*, 2007; Laderoute *et al.*, 2015).

Accordingly, since phylogenetic evidence supports an association of HERV-K HML-2 activity with protection against integration of orthoretroviruses (i.e., acquisition), this may help substantiate the notion that modern day HERV-K102 particles, along with expression of proteins from other HML-2 elements, might prevent HIV-1 acquisition.

The origins of HERV-K102 in humans

Somewhat ironically, humans apparently acquired the HERV-K102 defense mechanism from the same source of the modern HIV-1 pandemic strain; namely, chimpanzees, possibly between 500,000 and up to 2 Mya (Romano *et al.*, 2006; Subramanian *et al.*, 2011).

The Homo-Pan split has been estimated at 6.6 Mya (Magiorkinis *et al.*, 2015) or earlier at 7-8 Mya (Langergraber *et al.*, 2012). As mentioned, the HERV-K HML-2 elements originated in primates about 10.3 Mya and the CERV-K102 sequence (DQ112149), which is 97% identical to HERV-K102, was estimated to have integrated into chimpanzees at a non-orthologous position about 10 (+/- 3.3) Mya (Romano *et al.*, 2006). Lentiviruses may have been active in primates since the divergence of chimpanzees and humans (Katzourakis *et al.*, 2007; Sawyer *et al.*, 2004). Moreover, it has been suggested the ancestor to HIV-1 may have arisen in chimpanzees about 4 Mya (Gifford, 2012). Since it has been reported that subsets of chimpanzees with chronic HIV-1 infection showed progression analogous to humans, including greater expression of CD38 in CD8+ HLA-DR+ T cells (O'Neil *et al.*, 2000), this raises the notion that an HERV-K102 ancestor, as a potential antidote for HIV-1 infection may have been selected through evolution in chimpanzees before it was acquired by humans. Accordingly, it is possible over about a 2 million-year window or longer, the HERV-K102 ancestor may have adapted to HIV-1 like ancestor lentiviruses in chimpanzees prior to its acquisition by humans. Thus, the phylogenetic evidence raises the notion that HERV-K102 as a replication competent HERV-K HML-2 retrovirus, may have evolved to limit HIV/lentivirus replication and genome invasion. Testing of this hypothesis could be done by examining the bioactivity of HERV-K102 and CERV-K102 particles on HIV-1 infected human and chimpanzee cells under *in vitro* conditions permissive for HERV-K102 versus HIV-1 replication.

Other points relevant to HERV-K102 protection against HIV-1 acquisition

When cord blood mononuclear cells (CB) were cultured in IMDM rather than RPMI media, HERV-K102 spontaneously replicated, generating high levels of foamy macrophages (Laderoute *et al.*, 2007; Laderoute *et al.*, 2015). Others have similarly reported the induction of foamy macrophages when CB was cultured

in IMDM (Stec *et al.*, 2007). From Stec *et al.* (2007) the foamy macrophages produced under these conditions were identified as CD14 ++/CD16+, whereas HIV-1 is known to also preferentially infect these macrophages as opposed to other macrophage subpopulations (Ellery *et al.*, 2007). Indeed vacuoles in HIV-1 infected macrophages were previously observed (Gartner *et al.*, 1986). It may be critical to HIV-1 acquisition that HIV-1 first infects macrophages which are the ones which produce HERV-K102 particles to inhibit this protector response.

It is notable that HERV-K102 can be quickly and strongly induced *in vivo* increasing from no particles detected in plasma to 2.55×10^{11} cDNA containing particles per ml of plasma within 84 hours (unpublished study; Marian Laderoute). This rapid and intense induction is likely to be critical to whether HIV-1 is acquired or not. While high levels of particles at 10^{10} to 10^{12} per ml of plasma were frequently found in patients viremic for various bloodborne pathogens, the maximum levels in HIV-1 patients were notably 7 to 8 log-fold downmodulated in comparison (Laderoute *et al.*, 2007). These results are consistent with the notion that upon HIV-1 acquisition, HERV-K102 particle production is strongly inhibited. This contrasts with HESN where 5 fold elevated integrated levels of HERV-K102 *pol* were demonstrated in genomic DNA isolated from plasma (Laderoute *et al.*, 2015) which was suggestive of previous high levels of particle production.

HERV-K102 particles released by freeze-thaw cycles of cultured CB cells, induced rapid and complete cell lysis of MRC-5 cells at 24 hours, which was not demonstrated for other cell lines tested in parallel (unpublished study; Marian Laderoute). This was expected as foamy viruses are well known to produce rapid cell lysis of some, but interestingly, not all fibroblastic cell lines (Linial, 2001). However, it importantly remains to be determined if HERV-K102 particles might similarly rapidly lyse HIV-1 infected cells.

In terms of other characteristics of the defense mechanism deduced earlier from the informative HIV-1 vaccine clinical trials, combination female steroid hormones (estrogen then progesterone) have been shown to stimulate the expression of HERV-K HML-2 (Ono *et al.*, 1987). As mentioned, in a recent meta-analysis, while the use of various progestins for oral contraception were associated with a significantly increased adjusted hazard ratio of HIV-1 acquisition over women who did not use contraceptives, the combined oral contraceptive was not (Morrison *et al.*, 2015). Thus, HERV-K HML-2 activity may be enhanced by female combination hormones, whether in response to viral vectors, viruses or not.

Relevant to the increased risk of HIV-1 acquisition related to Ad5 antibodies in the STEP trial (Buchbinder *et al.*, 2008), at a high concentration (2 mg/ml), tuftsin inhibited the production of HERV-K102 DNA in cultured cord blood mononuclear cells (CB) by 53%, while at a lower concentration (200 ng/ml),

tuftsin enhanced the replication of HERV-K102 *pol* containing DNA over normal genomic levels by 237% (unpublished study; Marian Laderoute). Thus, it seems as a protector mechanism launched by alternatively activated macrophages, HERV-K102 particle production might be subject to modulation by tuftsin and thus possibly relevant to the adverse outcomes of the STEP trial. Clearly, further investigation of the mechanisms of how pre-existing antibodies were associated with adverse outcomes in the STEP trial appears to be warranted and should include studies on levels of tuftsin.

HERV-K102 particle production is not addressed by standard methodology

The identification and elucidation of correlates of protection against HIV-1 have been challenging. Overall the failure to identify HERV-K102 particles pertains largely to the notion that its presence is, more often than not, overlooked or not addressed by standard methodological approaches. For example, because HERV-K102 is unique to humans (Subramanian *et al.*, 2011), it is absent from animal models, such as macaques and rodents, which are commonly used for vaccine or immunological investigations. In addition to HERV-K102 replication being inhibited when PBMCs or CBs are cultured in the more traditional RPMI media invariably used by immunologists (Argaw-Denboba *et al.*, 2017; Laderoute *et al.*, 2015), HERV-K102 activation is also blocked by the depletion of CD14 + cells from PBMC, and also by the addition of PHA and IL-2 to cultures performed in IMDM (unpublished studies; Marian Laderoute). It should be noted that the latter also raises the important notion that adjuvants might inhibit HML-2 activation and HERV-K102 particle production whereas viral vectors may preferentially activate this alternative innate mechanism. Accordingly, it may not be a co-incidence that the conditions that block HERV-K102 particle production *in vitro* are those that instead are commonly employed to demonstrate HIV-1 infectivity, such as purified T cells activated with PHA and IL-2 cultured in RPMI. Indeed, these observations would be consistent with the possibility that HERV-K102 particles may antagonize HIV-1 replication *in vitro*. Importantly, while this needs to be directly examined for HERV-K102 particles, interference of HIV-1 replication/infectivity by HML-2 elements has been previously reported (Monde *et al.*, 2012; Padow *et al.*, 2000).

The detection of the presence of HERV-K102 particles also eludes other common approaches utilized for investigations. For example, detection of particles in plasma requires an alternative isolation strategy seldom employed by retrovirologists. It requires DNA and not RNA isolation from plasma (Laderoute *et al.*, 2007), where the common use of DNase would be contraindicated. As well, genome wide association studies and microarray analysis typically exclude highly repetitive sequences (Baranzini *et al.*, 2010; Held *et al.*, 2003, respectively) to which this element belongs. Accordingly, HERV-K102 particle production appears to have eluded the field due to the difficulty in demonstrating its presence using standard or traditional approaches.

Conclusion

This inquiry has led to the notion that HERV-K102 particle production, which alternatively generates foamy macrophages as part of innate immunity, appears to fulfil the requirements of a deduced candidate correlate of protection against HIV-1 acquisition. HERV-K102 appears to be a 'virus anti-virus' response which may have evolved in chimpanzees during exposures to lentiviruses and subsequently acquired by humans less than 2 million years ago. HIV-1 is a challenge for adaptive immunity due to the high levels of antigenic heterogeneity, potential pseudotyping such as by HML-2 Env, that some adaptive cytokines enhance HIV-1 replication, and that HIV-1 infects activated immune target cells. In contrast, the HERV-K102/HML-2 paradigm of defence relies upon recognition of surrogate HML-2 surface markers on HIV-1 infected by HML-2 antibodies or T cells, and that HML-2 elements interfere with HIV-1 infectivity and/or replication. The HERV-K102 virus, in theory, may undergo lytic replication within HIV-1 infected cells where high levels of pre-formed HERV-K102 particles in the host may potentially block acquisition. Accordingly, it will be very important to study lytic infections of HERV-K102 in HIV-1 infected cells and Tat transformed cells to confirm or disprove this hypothesis.

This candidacy has been strengthened by biological, clinical and phylogenetic evidence, including that which implies

preformed HERV-K102 particles may be associated with protection against HIV-1 acquisition. That conversely, acquisition of HIV-1 would be associated with significantly log-lower levels of HERV-K102 particles, would be anticipated and was observed. Given also the preliminary evidence that tuftsin at higher levels could block the replication of HERV-K102 *in vitro*, suggests the blocking of the same mechanism, such as by Ad5 antibodies in the STEP trial shown in males at higher risk, could plausibly account for the increased risk observed in this informative trial. Finally, that the host source of this remarkable virus anti-virus innate protection mechanism appears to be the same as that for pandemic strains of HIV-1 would strengthen its authenticity, especially given the likelihood of millions of years of co-evolution of the HERV-K102 and the HIV-1 ancestors in chimpanzees. Overall, the available evidence substantiates that a special antagonistic relationship exists between HIV-1 and a foamy-like retrovirus, HERV-K102.

Accordingly, it will be extremely important to prioritize the testing of human endogenous retrovirus K102 (HERV-K102) particle production, integration, and/or envelope specific antibody production to prove or disprove it as a correlate of risk/protection on actual STEP, Phambili and RV144 clinical trial participants (Figure 2). Exploratory studies in other HESN cohorts, in elite controllers, and in post treatment interruption

Figure 2. Hypothesized working model of outcomes of the STEP and RV144 trials based on HERV-K102 particle blockage or induction, respectively. Adapted from Figure 1.

controllers, may also serve to further strengthen or dismiss the correlation. No less significantly, the clinical ramifications of pseudotyping of HIV-1 virions by HML-2 envelope (Brinzevich *et al.*, 2014), needs to be addressed as it may also help explain in part the failed vaccine and cure attempts and possibly co-receptor usage by transmitted/founder HIV-1 strains.

Competing interests

No competing interests were disclosed. While the author was named as one of the inventors in patent applications for the discovery of HERV-K102 as a replication competent foamy-like

virus and for envelope specific antibodies either of which may have applications in the field of infectious diseases, by policy at the Public Health Agency of Canada, public servants have no rights nor entitlements.

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Robert Gallo

Institute of Human Virology, University of Maryland, Baltimore, MD, 21201, USA

In her hypothesis Dr. Laderoute makes her interesting case for the possible central position of innate immunity as the key for prevention of HIV infection by vaccines. She specifies that this is likely mediated by expression of specific endogenous human retroviruses, namely HERV-K 102 HML-2. She builds her arguments from several directions relying on vaccine history, specific HIV vaccine trial data, in vitro virus studies, classical virology (pseudotyping), and phylogeny. Although having much experience in adaptive immunity with monkey and clinical vaccine studies the field is not unaware of the possible importance of innate immunity, and indeed several investigators are turning in that direction. Even more, Dr. Laderoute considers endogenous retroviruses origin and evolution may be to counter exogenous retrovirus infections.

Dr. Laderoute has been early in this thinking, and in general the hypothesis expressed in detail could be useful. However, there are statements that concern me, and of these the last (#5) the most troublesome.

1. Humans are not only susceptible to infection with HIV – they also can be infected with another dangerous exogenous retrovirus, HTLV-1. It would enhance the presentation of awareness if this were evident and if she could include her thinking on this for more ancient infection of man than HIV.

2. Insufficiently touched upon is the importance of T-cell activation opening doors to increased HIV infectivity due to more target cells. This may be what happened in the STEP trial that increased infections. We have argued this for many years, i.e., we need some T-cell activation for any adaptive immunity but a bit too much negates any vaccine efficacy. A second point relates to antibody longevity. The key antibodies of protection are short “lived”. That is an alternate explanation to the results of RV144 (quite good for a while but not lasting). If I may reference my two papers published in that regard some years ago are:

- Fouts TR, Bagley K, Prado JJ, Bobb K, Schwartz JA, Xu R, Zagursky RJ, Egan MA, Eldridge JH, LaBranche CC, Montefiori DC, Le Buanec H, Zagury D, Pal R, Pavlakis GN, Felber BK, Franchini G, Gordon S, Vaccari M, Lewis GK, DeVico AL, Gallo RC. Balance of Cellular and

Humoral Immunity Determines the Level of Protection by HIV Vaccines in Rhesus Macaque Models of HIV Infection. PNAS. 2015 Feb¹.

- Lewis GK, DeVico AL, Gallo RC. Antibody persistence and T-cell balance: Two key factors confronting HIV vaccine development. PNAS, vol. 111(44); pp 15614-15621, 2014².

3. Discussing HERV-K as infectious (page 9) may cause confusion. Explain what you mean because infectious will suggest to some you mean in vivo – one person to another.

4. Like Dr. Fast I think the discussion of women being more protected is a little shaky. I agree in making the case but very carefully and not several times over. For example, many statistics of male infections are in gay men and HIV penetration under those circumstances is easier than via vaginal sex.

5. Most important: I spoke with some experts in the field such as Rafael Contreras-Galindo, who reminded me that the Laderoute argument for uniqueness of the K102 type 1 strain of HERV-K HML-2 is flawed in that its env gene has deletions which cause fusion of pol-env, making it improbable that it serves as pseudotyping-HIV. In this regard the author has not clearly shown the particles she sees are really K102 and not one or more other HERV-K HML-2. Moreover, others have reported a variety of HERV-K HML-2 which could create recombinants. This is the essence of a major concern of this hypothesis: namely that the K102 plays a specific role in HIV immunity. The hypothesis needs to be broadened and with recognition of this and references to the contributors to that literature, or these points need to be definitively rebutted.

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Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Partly

Are arguments sufficiently supported by evidence from the published literature?

Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 05 April 2018

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Patricia E Fast

¹ International AIDS Vaccine Initiative-New York, New York, USA

² Pediatric Infectious Diseases, Stanford University School of Medicine, Stanford, CA, USA

Dr. Laderoute has revised the paper to make it clearer. She presents the hypothesis that HERV-K102 endogenous foamy viruses become active after vaccination of humans with HIV vaccine candidates that are based on virus vectors, and that they can mediate immunity to HIV. A number of studies have shown some level of interaction between viral transcription/replication of HERV and HIV and there is some evidence for cross-reactivity between them in the literature. Dr. Laderoute, however, postulates that vaccine effects such as protection in RV144 might be due to the K-HERV. To prove this hypothesis, evidence would have to be amassed from HIV vaccine efficacy trials with an efficacy signal. To be credible, the overall argument would have to somehow accommodate a few facts.

The hypothesis is said not to apply to non-human primates, but these animals clearly can be protected against Simian Immunodeficiency Virus or hybrid SHIV (Simian-Human Immunodeficiency Virus) by some vaccines and also by passive antibodies; likewise, immunosuppression of NHP by removal of CD8+T cells can nullify the control that they have over SIV replication early in infection. It seems highly unlikely that clear-cut adaptive immune mechanisms can protect NHP without reflecting mechanisms that also can work in humans. The SIV and SHIV models are widely accepted as valid analogs for HIV infection of humans. Hence it is difficult to imagine that the adaptive immune response cannot be harnessed, eventually, to prevent HIV.

A particular type of antibody has been identified, IgG with specificity for a V1V2 region, that is associated with lower risk of infection in the RV144 trial. The proposed mechanism of action is cell-mediated killing of HIV-infected cells, or inhibition of their ability to produce HIV. IgA antibodies against this same region, which would not mediate the cell-mediated killing, negate the apparent protective effect of the IgG¹. While this explanation remains to be proven, it is one adaptive mechanism for which evidence exists. Dr. Laderoute points out that in a different HIV vaccine efficacy trial (using the Env protein alone) VAX003/4, IgG antibodies against the same epitope can be found without an efficacy signal, however alternate explanations may exist, including subtle differences in the IgG or IgA blocking of the effect.

Dr. Laderoute's discussion on statistical analysis of efficacy trials and gender is somewhat difficult to follow and seems to have some incorrect elements. As an example, the contention that the confidence interval for both genders in RV144 could not be smaller than the CI's for each of the

two genders alone ignores the effect of sample size on confidence interval. The comment that oral contraception was not required in the men-only STEP trial is not biologically meaningful, as we have no hormonal contraceptives for men.

In summary, though the paper is much clearer, selective interpretations of data in the literature have been made to link K-HERV with protection from HIV infection, and alternate explanations are not fully explored.

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Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Partly

Are arguments sufficiently supported by evidence from the published literature?

Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vaccines, HIV epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reader Comment 06 Apr 2018

Dr. Marian Laderoute, Immune System Management Clinic & Lab, Canada

I would like to sincerely thank Dr. Fast for continuing this important discussion in an open forum, and also the approval of the manuscript (with reservations).

First I would like to clarify that the STEP trial did involve 1135 females along with 1,844 males (Table 2 from Buchbinder *et al.*, Lancet 2008). Second, I was not arguing that HERV-K antigens are cross-reactive with HIV-1 antigens and this was the primary source of protection. Instead I was saying that HERV-K HML-2 Env is present on HIV-1 infected cells but not normal cells, and is used as surrogate marker of virus infected cells for their clearance by T cells against HML-2 Env (eg., Jones *et al.*, 2012). Antibodies against HERV-K102 Env also clear breast cancer cells *in vitro* and *in vivo* (Wang-Johanning *et al.*, 2012) and remarkably, appear to directly trigger apoptosis. Moreover, the HERV-K102 particles are

postulated to undergo lytic infections in HIV-1 cells but not normal cells. Although this has not been directly shown, indirect evidence supporting the notion that the replicative activity of HERV-K102 particles may protect against HIV-1 acquisition has been shown in an HESN cohort, wherein genomic copy numbers were found to be significantly increased over normal and not found in persons already infected with HIV-1 irrespective of use of anti-retrovirals (Laderoute *et al.*, 2015).

A main point of contention appears to be differences in opinion/perspective.

Dr. Fast cites important work in the simian model which supports adaptive immunity protection against HIV-1/SHIV replication and sometimes acquisition, and postulates that we should be able to harness the adaptive response to prevent HIV-1 acquisition. However, in humans, while clearly parts of adaptive immunity may control HIV-1 replication post-acquisition (Bar *et al.*, 2016), there is no direct evidence provided in humans that adaptive immunity protects against acquisition (Haynes *et al.*, 2012). Indeed, there is direct evidence that the presence of adaptive immunity does not alter superinfection with a different strain (Ronon *et al.*, 2017). In the STEP trial, those with protector HLA alleles, had lower viral loads and showed robust T cell activity (Migueles *et al.*, 2011) but the T cell activity was not associated with a lower risk of acquisition (reviewed in Tomaras & Plotkin, 2017). The increased VE related to the presence of HLA A*02 in the RV144 trial, was probably related to post-acquisition selection pressure by adaptive mechanisms and not a genuine blocking of HIV-1 acquisition (Gartland *et al.*, 2014). There have been hundreds of adaptive immunity vaccine trials which failed to show efficacy against HIV-1. These facts imply that adaptive immune mechanisms are not likely significantly involved in the prevention of HIV-1 acquisition in humans. Thus, these facts are accommodated in the proposal that an innate protector mechanism may instead be at play. Indeed, the proposed innate protector mechanism against HIV-1 acquisition does not exist in simians, which helps to explain the dichotomy between humans and simians and the role of adaptive immunity in HIV-1 acquisition. This does not discount at all that adaptive immunity serves to control HIV-1 post-acquisition in humans or more generally in simians.

In the human situation, we know that HERV-K HML-2 and/or HERV-K102 Env is expressed on HIV-1 infected cells (and breast cancer cells) and serves as a surrogate target for such. T cell clones against an HML-2 Env epitope identical to HERV-K102, clear HIV-1 infected cells *in vitro* (Jones *et al.*, 2012). These T and B cell responses to HML-2/HERV-K102 Env have not been well-elucidated but could be innate or T helper cell independent responses. It remains to be determined if HERV-K102 particles, the upregulation of HML-2 and/or T and B cell responses to HML-2 Env comprise '**trained (innate) immunity**' which has been reported to explain memory in the innate immunity compartment and which also provides surrogate protection against unrelated microbes.

Be that as it may, the fact is HML-2 Env (but not HERV-K102 Env) pseudotypes HIV-1 particles (Brinzevich *et al.*, 2014), and so, how would an antibody or T CTL clones to HIV-1 envelop block transmission of these particles? Productive clinical transmission of HIV-1 frequently results from a single transmitted/founder (T/F) virus (Shaw GM *et al.*, 2012) although there is newer evidence of multiple T/F strains in up to 20-60% of new acquisitions (Kijak, 2017). Accordingly, on these grounds, the prediction would be made that protection

in humans by adaptive immunity against HIV-1 acquisition would be less efficient than what is generally assumed. As simians do not have HERV-K102 or HML-2, this issue is not likely applicable to this animal model.

In terms of an alternative explanation for the existence of a potent innate protector mechanism against HIV-1 which is: induced by viruses, possibly enhanced with female hormones, launched by alternatively activated macrophages and that this activation of macrophages may be sensitive to inhibition by locally bound antibodies (for example as might be the case through tuftsin), and which has been hard to identify/study with traditional methods; unfortunately, I could not find one. Moreover, the fact that HML-2 activity has been phylogenetically associated with significantly reduced levels of orthoretroviral invasion of the human genome (Magiorkinis *et al.*, 2015) and that a HERV-K102 precursor may have co-evolved in chimpanzees alongside of lenti-viruses or HIV-1 like precursor lentiviruses over at least a few million years before humans acquired HERV-K102 (reviewed above), would be consistent with the notion that HERV-K HML-2 activity and HERV-K102 particles might play a significant role in protection against HIV-1 acquisition in humans, but not in simians.

The value of the paper is that in part, it reinforces the notion of adaptive T immunity quiescence in protection from HIV-acquisition as studied in the HIV-exposed seronegative cohorts (Card *et al.*, 2013), and highlights HERV-K102 particles and the HML-2 innate response together as a strong candidate for correlates of protection to be tested retrospectively in informative trials, or to be monitored prospectively, going forward with HIV-1 preventative vaccine trials involving viral vectors.

Competing Interests: I have no competing interests. The Public Health Agency of Canada holds patents for the discovery of HERV-K102 as a replication-competent, foamy-like protector virus, but by policy, inventors have no rights nor benefits.

Version 1

Reviewer Report 27 November 2017

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Patricia E Fast

¹ International AIDS Vaccine Initiative-New York, New York, USA

² Pediatric Infectious Diseases, Stanford University School of Medicine, Stanford, CA, USA

Dr. Laderoute has advanced a novel and interesting hypothesis, that an innate immune state

associated with the virus HERV-K 102 can, in some circumstances, prevent or reduce the risk of HIV infection by a mechanism of infection of macrophages and activation of innate immunity. Dr Laderoute also proposes that this mechanism can go wrong in such a way as to increase risk and finally that vaccination with HIV vaccine candidates can influence this interaction or vice versa. A number of facts or interpretations are marshalled to support this hypothesis.

The main weakness of the paper is that it discusses a proposed causal relationship but, at many points in the argument, mentions only one interpretation of existing data and ignores alternative explanations, even when there are reasonably strong data supporting those explanations (for example, the elegant demonstration of correlate of risks in RV144 described by Haynes, Kim, Tomaras, Zolla-Pazner and others).

The basic postulate proposed is that an endogenous retrovirus, HERV-K102 might interact with macrophages, particularly Kupffer cells, and that the innate immunity induced would temporarily (6 months) alter host susceptibility to HIV infection, in a way that could interact with HIV vaccine effects. Part of this argument is that 'macrophages' are involved in initiation or control of adaptive immune responses. However, the dendritic cells that are intimately involved in initiating immune responses, differ from Kupffer cells, and this difference is not discussed.

Hormonal influence on the HERV-K102 link to innate immune states are postulated because in efficacy trials of one HIV vaccine, there appeared to be a gender effect on the results (the difference in efficacy between women and men in RV144 was not statistically significant). In fact the exposure, risk factors and baseline incidence differed between men and women in the two trials of the Ad5-vectored vaccine, STEP and Phambili, and it cannot be concluded from those data alone that there is a hormonal influence on susceptibility, let alone that is linked to endogenous retroviruses.

The cohort of highly-exposed female sex workers in Nairobi is also cited as potentially protected by transient innate immune mechanisms related to infection of macrophages with the endogenous virus. This putative immunity fades within months after the women cease sex work—it seems unlikely that a mechanism based on co-infection with HERV-K102 would be influenced by ending sex work or that the endogenous retrovirus infection somehow depends upon continued sex work. If the HERV-K102 virus-related mechanism fades after 6 months, that should be 6 months from the time of infection with the HERV-K102 virus (which is unknown), not 6 months after ceasing sex work.

No evidence is cited to link the virus infection itself or its interaction with macrophages to vaccination with HIV vaccines (a suggestion is made that the peptide tuftsin could be involved, but the link of tuftsin to HIV vaccines is not clear to me). To Dr Laderoute's credit, she points out that a direct examination of this prediction is needed to test the hypothesis.

In summary, the endogenous retrovirus is fascinating, and its potential interactions with HIV may be worthy of study. This is clearly an opinion paper, so some latitude in the discussion is permissible, but the paper does not make a sufficient case for its hypothesis. Highly selective interpretations of data in the literature have been made to link that virus with protection from HIV infection, and alternate explanations are not explored adequately. The postulated alteration of the effects of HIV vaccines on risk of infection requires several logical leaps, each of which has alternate explanations, and the range of evidence is not discussed in sufficient detail. The paper

could be improved by limiting its scope and providing a much more comprehensive and balanced argument.

Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

No

Are arguments sufficiently supported by evidence from the published literature?

No

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vaccines, HIV epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 14 Jan 2018

Dr. Marian Laderoute, Immune System Management Clinic & Lab, Canada

First I would like to sincerely thank Dr. Patricia Fast for taking the time to read through this opinion-hypothesis paper as the concept of correlates of protection ('true' or not) against HIV-1 is vitally important for the derivation of HIV-1 prevention vaccines as well as cure. In hindsight, I wholeheartedly agree that I should have had more discussion on proposed correlates of protection from HIV-1 vaccine prevention trials and why these have been rejected by others and myself. This has been corrected in the revision and importantly includes a vigorous discussion on why adaptive immunity mechanisms are unlikely to prevent HIV-1 acquisition (i.e., the elephant in the room) substantiated directly by the finding that there is no evidence in humans to defend this approach. The concept that the HERV-K102 virus unique to humans represents a 'virus anti-virus response' (in this case a foamy retrovirus anti-lentivirus response) and is part of innate immunity which generates foamy macrophages, is hard to grasp because it is so revolutionary. So now I have made this point of it being a 'virus anti-virus response' crystal clear not only in the abstract, but in the introduction, and have also tried to present the concept of the HERV-K102 virus being anti-HIV-1 more clearly. I think this may be why publication in F1000 Research with its open review can be extremely valuable for the communication of completely novel, if not totally foreign ideas. As a writer, I have to know why readers are not 'getting it' so that I can try to explain the concepts better.

1. A main weakness is the poor discussion of correlates of protection from HIV-1

vaccine prevention trials and only mentions one interpretation of existing data.

The revision now incorporates a lengthy discussion of correlates of protection from HIV-1 vaccine prevention trials and why one must conclude that a surrogate correlate of protection or a 'true' correlate of protection protecting against HIV-1 acquisition has not been demonstrated in the HIV-1 vaccine prevention trials. Indeed, a true correlate of protection has not been convincingly demonstrated not only in HIV-1 vaccine prevention trials (over 200 attempts) but also in studies of HIV-1 exposed seronegative cohorts (Safrit & Koff, 2016).

2. Macrophages such as Kupffer cells are distinct from dendritic cells (DCs) and Dr. Fast implies only DCs are involved in initiating immune responses. This difference was not discussed.

According to a recent review article, developmental studies in mice suggest DCs originate independently from monocytes and tissue macrophages, and for a long time these subsets of antigen-presenting cells were thought to overlap (Collin M *et al.*, Immunology 2013). However, both cell types are antigen-presenting cells and are still studied and are relevant in terms of activating innate and adaptive immunity as derived and studied from human blood (Sander J *et al.*, Immunity 2017; Metcalf TU *et al.*, J. Immunology 2017). Kupffer cells constitute 80-90% of the tissue macrophages in the body and are essential to innate and adaptive immunity (Li P *et al.*, Molecular Immunology 2017). The point being made in the paper is that the Ad5 vector targets Kupffer cells (which are macrophages) and the foamy macrophages which appear to produce HERV-K102 particles are most likely CD14 positive (as elimination of the CD14 positive cells from cord blood mononuclear cells abrogated HERV-K102 particle production, the production of foamy macrophages, the accumulation of HERV-K102 cDNA and the increase in HERV-K102 *pol* gene copy number in genomic DNA (unpublished data)). These foamy macrophages are morphologically distinct from all others and resemble those produced under similar conditions from cord blood mononuclear cells by Stec M *et al.*, (J. Leukocyte Biology, 2007) which were characterized as CD14++ CD16+ macrophages which showed enhanced expression of CD11b, HLA-DR and CCR5. CCR5 is critical to the HIV-1 story, and thus, foamy macrophages may be very relevant to the proposed protective innate mechanism. Gartner *et al.*, (Science, 1986) were first to elucidate that acquisition of HIV-1 first involves infection of macrophages and this has been followed by many publications regarding the R5 versus X4 strains (eg. see reviews Arif MS *et al.*, 2017; Naif HM, Inf Dis Rep 2013), wherein CCR5-tropic (R5) viruses are preferentially transmitted by all routes of infection (Shaw GM & Hunter E, Cold Spring Harb Perspect Med 2012). The CD14++CD16+ monocytes express the highest levels of CCR5 than CD14++CD16- or CD14lowCD16+ and are preferentially infected by HIV (Ellery PJ *et al.*, J Immunology 2007). In contrast it should be noted that DCs express CD4 but are poorly sensitive to HIV-1 infection (Chauveau L *et al.*, J. Virol 2017). Moreover, SAMHD1 in DCs not only impacts HIV-1 replication but also antigen presentation (Ayinde D *et al.*, J. Virol 2015). In a recent study involving humanized mice, it was the CD14+CD16+ monocytes/macrophages rather than CD14-CD16+ dendritic cells which increased during early infection and harbored integrated HIV-1 DNA such as found in spleen (Arainga M *et al.*, Sci.Reports 2016). It is the monocyte/macrophage which serves as a viral reservoir during HIV-1 infection (Arainga M *et al.*, Retrovirology 2017). Accordingly, the issue of dendritic cells versus macrophages was not discussed as DCs may not be so relevant to HIV-1 infection and

pathogenesis. However, in fairness, it has been suggested in mucosal studies that the vaginal myeloid dendritic cells may transmit founder HIV-1 (Shen *et al.*, 2014).

3. **Gender differences on VE in the HIV-1 prevention vaccine trials was only shown in one of two trials. There were insufficient HIV-1 infections in the STEP trial to calculate VE. The difference in VE between women and men in RV144 was not statistically significant.** While the authors (Rerks-Ngarm *et al.*, NEJM 2009) state that subgroup analyses revealed no significant heterogeneity in vaccine efficacy according to baseline variables (Table 2), p values were not provided for the subgroup analyses in Table 2. A discrepancy between the MITT VE 95% CI reported in Table 2 (n=15,948) and the abstract (n=16,395) was noted for “all subjects” whereby in the analyses of Table 2 there were 447 less participants (missing 237 in the vaccine arm of which 158 were male and 210 in the placebo arm of which 146 were male, from Table 1). Potential discrepancies in the lower and upper boundaries of the 95% CI reported for the two subgroup analyses by gender when compared with all subjects particularly the lower boundary (negative values for each of the sex subgroups but positive for the ‘all subjects’ category) might imply an error was made in the reported CIs. For example, it might be possible that the lower boundary for the CI of the female subgroup analysis might have been positive, suggesting statistical significance. Accordingly, a VE of 38.6% in the female subgroup when compared with 25.8 % in the male subgroup may or may not have been statistically significant and needs further clarification. Nevertheless, presently, one could refer to this finding as a trend towards potential significance. This fine point has been rectified in the revision.
4. **There is no evidence from the vaccine prevention trials that endogenous retroviruses are hormonally regulated or that susceptibility to HIV-1 infection is influenced by HERVs.** This is correct because it was not tested. The point being made is to test for HERV-K102 particles and/or integration levels by gender subgroups (and by type and use of contraceptives) to characterize if HERV-K102 might be involved as a correlate of protection.
5. **Co-infection with HERV-K102, the time of infection which is unknown, cannot explain why in an HESN cohort involving sex work this putative innate immunity could fade such as by 6 months by ending sex work.** There is no co-infection of the host by HERV-K102 as it is not an exogenous virus. The induction of HERV-K102 virus which is unique to humans, is a host ‘virus anti-virus’ response. The HERV-K102 virus located on chromosome 1 is found in all humans. It becomes active and produces particles in large numbers of foamy macrophages in response to viruses (Laderoute *et al.*, AIDS 2007; Open AIDS J, 2015). Elite controllers have strong cellular and antibody responses to HERV-K HML-2 Gag (de Mulder M *et al.*, Retrovirology 2017) not present in viremic non-controllers nor healthy controls. While particles can be made at extremely high levels in patients with infected with blood borne pathogens (commonly up to 10^{11} particles per ml of plasma), in patients with HIV-1 infection the mean particle production is around 8,300 particles per ml (maximum levels are 6-7 logs reduced) and is found in about 72 % of HIV-1 patients. In the Nairobi HESN cohort, evidence of past high levels of HERV-K102 particle production was provided by showing on DNA isolated from plasma, that the mean genomic copy number was significantly ($p=0.0005$) elevated by about 5 fold above controls (the latter shown not to have any HERV-K HML-2 particles in plasma including HERV-K102). Increased

integration was not found in HIV-1 infected patients (which were not statistically different from uninfected healthy controls). There can be a very rapid induction of large numbers of HERV-K102 particles *in vitro* and *in vivo*, the latter reaching 2.55×10^{11} particles per ml of plasma in 84 hours. Studies by others have shown that antibodies to HERV-K HML-2 antigens wane within 6 months with germ cell tumor remissions (Kleiman A *et al.*, Int J Cancer 2004). In the absence of exposure to pathogens (or cancer), the stimulus for HERV-K102 particle production and thus antibody/T cell responses might wane, consistent with findings in normal healthy controls as reported by many groups. Thus, the finding by Kaul *et al.*, 2001 that resistance to HIV-1 acquisition fades as early as 6 months following sex work interruption in the Nairobi HESN cohort could be related to decreased HERV-K102/HML-2 activation due to lack of stimulation by or exposure to intracellular microbes.

6. **No evidence is cited to link the virus infection itself (HERV-K102) or its interaction with macrophage to vaccination with HIV vaccines.** There is no exogenous infection for an endogenous retrovirus. It is proposed that vaccination with viral vectors used in the 3 informative HIV prevention trials and not those lacking vectors for the priming may be analogous to exposures to viruses which might preferentially activate HERV-K102 particle production along with HML-2 activation over adaptive immunity. There seems to be reciprocal activation of adaptive immunity with the HERV-K102/HML-2 response. For example, *in vitro*, the addition of PHA and IL-2 has been shown to completely block HERV-K102 particle production under conditions permissive for HERV-K102 particle production (i.e., culture in IMDM and not RPMI media, more than a dozen replicates unpublished data). Similarly culture in RPMI blocks HERV-K102 particle production (unpublished observations) but allows adaptive immunity mechanisms (see also Argaw-Denboba *et al.*, 2017 for HERV-K activation interference). As well, RNA for HERV-K MHL-2 *gag* and *env* was found to be inversely correlated with T cell activation markers when tested on PBMCs from HIV-1 patients, also implying an inverse correlation of HML-2 responses with adaptive immunity. Moreover as discussed in the revised manuscript, there were non-specific effects of the viral vectors unrelated to HIV-1 antigen specificities (STEP, Huang *et al.*, 2014; STEP, Migueles *et al.*, 2011, and see discussion in Tomaras & Plotkin, 2017).

Competing Interests: I am the author of the article and am responding to the objections raised by Dr. Fast. I have no competing interests except that HERV-K102 particles and HML-2 responses should be investigated in HIV-1 vaccine prevention trial participants which is why I wrote the article and now have revised it.

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