Commentary

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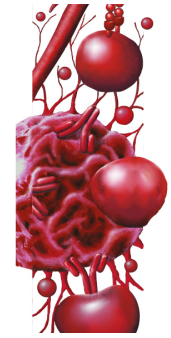

Can we just kick-and-kill HIV: possible challenges posed by the epigenetically controlled interplay between HIV and host immunity

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"How current vaccination strategies and combined LRA candidates achieve restoration of dysregulated methylation profiles is entirely unknown and the field still awaits the identification of specific immune pathways and networks under tight epigenetic regulation that are associated with HIV control."

First draft submitted: 31 May 2019; Accepted for publication: 4 June 2019; Published online: 20 June 2019

Keywords: epigenetics • eradication strategies • HIV infection • host immunity • latent viral reservoir • multilayered-omics analyses • systems vaccinology

Since the discovery of the human immunodeficiency virus (HIV) in the 1980s as the causative agent of acquired immuno-deficiency syndrome (AIDS), great efforts have been devoted to the development of preventive and therapeutic strategies. Thanks to combined antiretroviral therapies (cART), the morbidity and mortality due to HIV infection has decreased enormously, but there is still no effective preventive vaccine and no widely applicable strategies to cure existing HIV infections have been established. Over the last 15 years, there have been a series of initiatives to support the development of approaches that could lead to so-called 'HIV cure and viral eradication'. Although these terms are often used freely and interchangeably, it is generally accepted that 'cure', or 'functional cure' refers to a state of persistent viral suppression without the need to take antiretroviral drugs (combined antiretroviral treatment), while eradication refers to the complete elimination of any HIV from the body of an infected individual. The discrimination is certainly more than semantics, given that HIV establishes a life-long, largely immunologically silent, latent reservoir shortly after acute infection and that, to date no effective strategies exist to reactivate and eliminate parts or the entirety of this reservoir.

In order to advance the field of immune-based therapies, there is an urgent need to improve our understanding of how HIV persists in an infected individual, what keeps the latent reservoir from reactivation and how host immune markers associated with natural control of the virus mediate their antiviral effects *in vivo*. To date, much of the information available to address these questions stems from studies in HIV infected individuals with an inherent ability to naturally control HIV without the need for cART treatment. Such patients represent 1–3% of HIV infected population (variably referred as long-term nonprogressors or elite controllers), show reduced or undetectable viral loads and maintain elevated levels of CD4 T-cell counts for a prolonged period of time [1]. The inherent particularities of long-term nonprogressor/HIV controllers encompasses virological as well as immunological processes and past studies have yielded an ever growing number of host factors associated with virus control; among them, host immune factors (innate and adaptive) such as increased polyfunctionality and alternative effector functions of HIV-specific CTL, neutralizing antibody activities, antiviral cytokines and host genetics (e.g., human leucocyte antigen and killer-cell immunoglobulin-like receptors) [1,2]. However, many markers await confirmation in independent studies and, in general, for most factors it is unclear whether they are the cause or effect of controlled HIV infection.

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In addition to studies in HIV-infected individuals, some immune parameters associated with HIV control and/or protection from infection, have emerged from clinical trials testing preventive HIV vaccines. These include signals of partial protection in the STEP trial by sieve effect analyses [3] and HIV-specific humoral immune responses of specific isotypes in the RV144 study [4].

From HIV systems biology to systems vaccinology

As in other fields, host genetics studies, gene expressing profiling and proteomics analyses have proven useful approaches in HIV infection as well. However, relatively limited insights into HIV pathogenesis and natural control mechanisms have been gained, possibly due to the highly variable disease course of HIV, the genetic variability of the host and the virus, and the fact that often, individual factors are being assessed in isolation. To overcome some of these limitations, systems biology has shown its effectiveness as a powerful approach to improve our understanding of the complex and multifactorial processes involved in HIV pathogenesis [5]. In general, systems biology describes the integration of massive data from different –omics approaches and refined statistical approaches such as multivariate analyses and multiparametric regression models as a strategy to unravel relevant signals from different integrated datasets in order to understand the mechanisms involved in HIV infection [6].

In order to take full advantage of vaccines trials and the data and knowledge generated in the process, systems biology analysis has been applied giving rise to what is referred to as systems vaccinology. In HIV, applying systems vaccinology aims to better understand the immunologic processes induced by novel vaccine candidates, adjuvants and vaccination regimens and to define the correlates associated with protection or control of virus infection. However, in the absence of relevant signals from clinical trials testing therapeutic HIV vaccines, the available data from systems vaccinology studies is limited to preventive HIV vaccines and vaccines targeting other pathogens. A recent systems vaccinology study applied to the RV144 preventive HIV vaccine trial identified interferon signaling pathways that activate antiviral responses through IRF7 as well as interferon stimulated genes involved in antigen presentation as correlates of protection from HIV infection [7]. Similarly, systems vaccinology has also allowed to define specific innate immune signatures that discriminate between humoral and cellular immune responses in influenza vaccination [8]. These studies highlight the power of systems vaccinology to identify critical innate immune mechanisms that can modulate adaptive immunity in response to different vaccine adjuvants, vaccine vectors and/or route of administration [5].

Application of multilayered-omics analysis in HIV vaccines studies

The complexity of HIV infection on its own and its variable outcome between individual, is further accentuated by differences in the interplay between HIV and host immune system, general immune status, other co-infections, microbiota and possibly a large number of additional other factors that could impact the outcome of HIV infection by acting on different biological layers. This scenario gets even more complicated when different vaccine regimens are introduced and their effects being compared between them. The use of integrated -omics data, and systems biology and systems vaccinology approaches paired with rigorous statistical methods may offer an approach to identify relevant signals, biomarkers and biological processes that mediate the desired physiological effect. This is supported by results from other pathologies, where the integration of multilayered –omics datasets proved to be an effective and accurate tool to explore fundamental connections between disease trigger, immune response and therapeutic intervention [9]. Indeed, in the recent past and in addition to full genome transcriptome and proteome screenings, analyses of the microbiome and epigenome have been applied to HIV infection, exploring novel biological levels of immune regulation and control of HIV infection [10].

While many studies assess gene expression profiles through genome-wide transcriptomics analyses or include proteomics and genome-wide association studies, the underlying mechanisms that govern gene expression and proteomics are less frequently integrated into system biology studies. Of these, measures of genome-wide epigenetic control mechanisms may be of special interest as they may provide clues to the underlying mechanisms of differences observed with transcriptomics and proteomics analyses. But the potential lessons learned from epigenetic assessments have further implications than just explaining transcriptome data. To date, clinical epigenetic analyses are predominately exploited in oncology; and only starting to be used in infectious diseases. In HIV infection, the application of epigenetics has been mostly focused on virus latency but it is clear that immune responses to HIV and other viral infections are also subjects to epigenetic regulation [11]. Thus, HIV infection and other chronic diseases may induce profound epigenetically controlled signatures on gene expression profiles that any therapeutic interventions may need to deal with and correct in order to induce lasting therapeutic effects. Indeed, over the last

decade there has been a sharp increase in the development of so called 'epi-drugs' that aim to change gene expression profiles to expose cancer or virally infected cells visible to the immune system. While in the cancer field, these advances have shown great clinical applicability, the use of epi-drugs in HIV infection has not yielded comparable progress.

Implication of the use of epi-drugs in HIV eradication strategies

A major roadblocks to the development of a successful therapeutic HIV vaccine that could mediate functional cure or viral eradication, is the ability of the virus to form latent viral reservoirs in various cell types and anatomical compartments [12]. Different strategies to awaken this latent reservoir by drugs that act directly on gene expression have been tested, in most cases focusing on latency reactivator agents (LRA) derived from the family of histone deacetylases inhibitors (iHDACs) [11]. In *in vitro* latency models and even in individuals under cART, some of these agents can induce robust, although short-lived viral gene transcription, which may sensitize these infected cells to elimination by virus-specific CTLs. This ability of iHDACs (e.g., romidepsin, vorinostat, paranobinostat and others) to reactivate the latent HIV reservoir has given rise to combined strategies, commonly referred to as 'kick-and-kill' strategies, that use reactivators of the viral reservoir with potent therapeutic HIV vaccines, so that the viral reservoir can be purged and the infected individual could be taken off cART without running the risk of showing rapid viral rebound [13]. A number of clinical trials have evaluated or are currently evaluating this strategy *in vivo* (RISVAC03 (NCT01571466), REDUC (NCT02092116), RIVER (NCT02336074) and BCN02 (NCT02616874) trials) [14,15]. Of these, the BCN02 trial, which combined romidepsin with one of the most immunogenic vaccine regimens available (MVA and Chimpanzee-adenovirus vectored HIVcons sequences [16]) has shown intriguing signals and an elevated percentage of individuals apparently able to control viral replication in the absence of cART [17]. Whether the observed rate of post-treatment control was influenced by vaccination and romidepsin administration or solely by early antiretroviral treatment initiation is yet to be elucidated, and multilayered -omics analysis may help to dissect the contribution of the different treatment components. An increasing number of different classes of potentially latency reversing agents are in development, acting on different cellular levels to induce and initiate viral gene expression, for example, Toll-like receptor agonists [18,19]. This will hopefully help to improve the rather limited virus reactivation potency of current LRA and render HIV infected cells susceptible to virus-specific host immunity.

However, and although approaches using more potent LRA may have great potential in the reactivation of the virus, the unspecific off-target effects of iHDAC and other LRA on host–gene transcription needs to be considered. There is currently limited knowledge of how LRA and epi-drugs may affect host–gene expression profiles, especially when looking at host genes involved in the antiviral immunity to HIV. This is of special relevance considering that chronic viral infections, such as HIV, can leave severe epigenetic signatures on the host genome and may employ these to evade a more effective immune surveillance. This also extends to other epigenetics mechanism such as methylation and de-methylation processes. In these cases, the recruitment of DNA methyltransferases that methylate/demethylate preferential positions (e.g., CpG sites) in the DNA of the host genome and which may remain with these modifications during chronic infection could severely impact gene expression levels. Indeed, our own data (5th European Congress of Immunology 2018, abstract P.E1.01.08) and a few recently published, whole host DNA methylome studies, demonstrate specific sites and methylation profiles associated with HIV infection [20]. In addition, HIV promoter long terminal repeat sequences of integrated virus, have also been found to undergo CpG methylation [21]. These findings are likely to be critical for future development of therapeutic HIV vaccine and cure/eradication strategies, as future immunotherapies may only be effective if they have the capacity to restore dysregulated methylation profiles in immune effector cells.How current vaccination strategies and combined LRA candidates achieve restoration of dysregulated methylation profiles is entirely unknown and the field still awaits the identification of specific immune pathways and networks under tight epigenetic regulation that are associated with HIV control. The subsequent challenge will then be to find ways, through vaccine vector choice, adjuvants, epi-drugs and possibly other (combined) strategies to modulate specific epigenetic profiles and thereby restore gene expression and proteomic signatures patterns with the capacity to provide sustained antiviral immune control. Thus, the integration of multilayered -omics analyses, including determinations of underlying epigenetic determinants will be critical to advance the therapeutic strategies to achieve a functional cure and eventual eradication of HIV.

Conclusion

Despite great efforts made in understanding HIV infection and developing antiretroviral treatments, there remain still mechanisms involved in HIV pathogenesis that need to be unraveled. Among them, relatively little is known about how chronic HIV infection impacts epigenetic control of genes involved in the host immune response. Such epigenetic imprints may pose considerable challenges for cure therapeutic strategies that aim at induction of virus free remission and HIV eradication. This may be especially important in light of the fact that HIV reservoir mobilizers will possibly be needed for effective virus eradication and that such latency reserving agents may depend on and impact host gene expression profiles as well. In addition, potent therapeutic interventions may need to be designed that can overcome epigenetic imprints caused by HIV infection so that adequate gene expression profiles can be restored and sustained viral control be achieved.

Financial & competing interests disclosure

C Brander is CSO at AELIX Therapeutics, Barcelona. The writing of this article was party supported by grants from NIH (P01-AI131568) and the European Union's Horizon 2020 Research and Innovation program, agreement 681137-EAVI2020. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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