Remarks on the Conference HIV pathogenesis, virus versus host*, Alberta, Canada, March 9-14, 2014*

REPORT

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ABSTRACT

Combined anti-retroviral therapy has been really efficient in suppressing HIV replication, but it does not cure the infection. This is due to the permanency of integrated viral DNA in the infected cells. To achieve the HIV cure, the interaction between virus and its host needs to be extremely understood. Because of this, scientific community persists in dedicating efforts to profoundly comprehend the pathogenesis of this virus. The present work summaries important aspects discussed in the last meeting HIV-Pathogenesis, Virus vs. Host. It was held in Fairmont Banff Springs, Banff, Alberta, Canada, March 9-14. The conference debated the latest advances in the biology of HIV-1. Topics as virus entry into the cell, into the host, virus exit, virus–host genetics and co-evolution, host-virus interactions and responses, reservoirs, latency, reactivation, HIV and central nervous system, animal models and HIV and the microbiome at the mucosa were updated and deeply discussed. In parallel HIV vaccines: Adaptive Immunity and Beyond meeting was celebrated. The event, part of the Keystone Symposia Global Health Series, was a singular opportunity to analyze the state of HIV research and the new challenges in the battle against HIV infection.

Keywords: HIV-1, pathogenesis, latency, reservoirs, cure, eradication

Biotecnología Aplicada 2014;31:323-327

RESUMEN

Observaciones sobre el congreso La patogénesis del VIH, virus contra hospedero, Alberta, Canadá, marzo 9-14, 2014. La terapia antirretroviral combinada ha sido muy eficiente en suprimir la replicación del VIH, pero no cura la infección. Esto se debe a la presencia de ADN viral integrado en las células infectadas. Para lograr la cura del VIH, debe entenderse profundamente la interacción entre el virus y su hospedero. Es por ello que la comunidad científica dedica esfuerzos a comprender en detalle la patogénesis de este virus. El presente trabajo resume aspectos relevantes discutidos en la Conferencia Patogénesis del VIH, Virus contra Hospedero, la cual se celebró en Fairmont Banff Springs, Banff, Alberta, Canadá, entre el 9 y el 14 de marzo del año 2014. En ella se discutieron los últimos avances en la biología del VIH como: la entrada del virus a la célula, entrada al hospedero, la salida del virus, genética virus-hospedero y co-evolución, interacciones virus-hospedero y respuestas, reservorios, latencia, reactivación, VIH y sistema nervioso central, modelos animales así como la microbioma en la mucosa. El evento se celebró de manera simultánea con la Conferencia Vacunas VIH: Más allá de la inmunidad adaptativa. Ambos escenarios constituyeron una oportunidad magnífica para analizar el estado de la investigación del VIH y los nuevos retos en la batalla contra la infección.

Palabras clave: VIH-1, patogénesis, latencia viral, reservorios virales, cura, erradicación

*I*ntroduction

Combined antiretroviral therapy (c-ART) has significantly impacted in the reduction of morbidity and mortality, transforming the Acquired Immunodeficiency Syndrome (AIDS) from a deadly to a treatable chronic disease [1]. However, replication competent viruses persist in patients despite therapy, leading to viral rebound upon treatment interruption [2-5]. Moreover, lifelong therapy has adverse effects, develops virus resistance, does not completely restore the immune system of infected patients and constitutes a great economic challenge for less-developed countries with high incidence of the epidemic [6-9].

In the last years, evidences of a cure known as "functional cure" have been presented by the scientific community. Its scope, comprising the reduction of the reservoirs, bringing about a drug-free control of infection [10, 11] has been observed in a rare population of individuals known as Elite controllers who naturally control HIV-1 infection without c-ART. Later, a virus control was also observed in the VISCONTI cohort where 14 HIV patients following discontinuation of c-ART maintained long lasting control of viremia [12]. Hopes about a new case of HIV functional cure (the "Mississippi baby") were arisen; signs of viral replication were not seen for two years in a child born from an HIV-infected mother not in therapy that received antiretroviral drugs within the first 30 h postpartum and was treated for the first 15 months before therapy interruption [13]. But a recent report informed on the viral rebound in the child [14].

Up to now, three different strategies have been considered to achieve the functional cure: 1) initiation of c-ART during the very early primary stage of acute HIV-1 infection [13]; 2) selective depletion of discrete CD4+ T cell subsets carrying integrated the HIV-1 DNA without viral reactivation [15]; and 3) the

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socalled "shock/kick and kill" strategy consisting in inducing, through drugs, transcription of quiescent, replication-competent HIV-1 provirus (the shock/kick phase) in the presence of c-ART (to block viral spread), making virus reactivating cells susceptible to immune clearance, cytopathic effects and/or the effects of *ad hoc* therapeutics (the kill phase) [16].

This last strategy has rise considerable enthusiasm, with histone diacetilase inhibitors (HDACis) as the most advanced intervention as HIV-1 antilatency agents in the clinical testing. Results of a pilot single-dose trial in few aviremic patients on c-ART with the suberoylanilidehydroxamic acid, SAHA (vorinostat) have shown that this drug can disrupt latent infection in a detectable proportion of the reservoir of latent resting CD4+ T cells [17]. Nevertheless, the wide effectiveness of the treatment at clearing the viral reservoir is still unknown. Besides, SAHA is a mutagen *in vitro* in standard bacterial assays and, consequently, its use in humans has been accepted only for short-term exposure [18]. Novel HDACis are also being studied in order to reduce toxicity and increase specificity. These include givinostat, belinostat [19], panobinostat [20] oxamflatin [21], NCH-51 [22], romidepsin [23] and droxinostat [24]. Histone methyltransferase inhibitors, chaetocin inhibitors [25, 26], inducers of protein kinase C and NF-κB/NF-AT pathways and P-TEFb stimulators have also been considered [27]. Disulfiram, a zinc-chelating agent approved by the Food and Drug Administration to treat alcoholism, has been recently reported to turn on HIV-1 transcription without global T cell activation or cytokine release in a primary cell model of latent HIV infection [28]. Nevertheless, initial results of an ongoing single-arm pilot clinical trial (NCT01286259) did not provide clear evidence of any positive effect, showing a rapid and modest increase in plasma HIV-1 RNA in all participants, but a very modest reduction in the size of the latent reservoir [29]. Two classes of quinoline derivatives from quinoline-8-ol, a bivalent cationchelator, adducts of 5-ch5-chloroquinolin-8-ol, and quinolin-8-yl carbamates, induce HIV-1 expression without cell activation [30].

Several immunologic strategies have been proposed in combination with reactivating compounds ,to enhance the clearance of reactivated latently-infected cells and to improve host immune responses. These strategies include therapeutic vaccines, neutralizing monoclonal antibodies and immune modulating drugs.

The fact that potent CD8+ T lymphocytes can control HIV-1 by selectively killing virus-producing cells in Elite controllers [31] has led to a renewed interest in therapeutic vaccines, but this time with the goal of increasing virus-specific immune responses, either accelerating the decay of the reservoir during c-ART or improving the control of viral rebound after interruption of c-ART. Such interventions include CMV vectors; Ad26 prime, modified vaccinia Ankara (MVA) boost regimens; and lymph node-targeted amphiphilic peptide vaccines [27, 32].

A new generation of monoclonal antibodies (mAbs) with improved potency and breadth has also encouraged new therapeutic approaches. Particularly, two studies in rhesus monkeys demonstrated that infusion of mAbs cocktails or individual mAbs in chronically SHIV-infected rhesus monkeys resulted in substantial, although transient, suppression of viremia [33, 34], based on previous studies in humanized mice [35].

The blockade of immune checkpoint molecules PD-1, CTLA-4, LAG-3, TIM-3, TIGIT, and 2B4 may also prove effectiveness, with clinical trials of PD-1 inhibitors in treated HIV-1 disease been initiated [32].

Another approach, the use of cytokines, is being analyzed to stimulate HIV-1 replication and to interfere with mechanisms responsible for HIV-1 latency in individuals on c-ART [36-38]. Currently, the ERAIMMUNE 01 clinical trial is ongoing to test IL-7 in combination with an anti-HIV-1 vaccine and raltegravir plus maraviroc, to inhibit proliferation or reseeding of the HIV latent reservoir [27].

The ultime goal, the sterilizing cure, has been also considered. It is aimed to eradicate the latent virus reservoirs. This intervention has been only successfully achieved in the "Berlin patient", a man receving an allogenic hematopoietic stem cells transplant (HSCT) from a homozygous CCR5Δ32 donor for acute myelogeneous leukemia and who appeared to be free of both cancer and HIV-1 for over 5 years, without c-ART [39-41]. A similar case comprised two HIV-1 patients in 2010, known as the "Boston patients" who received HSCT for treatment of refractory lymphoma and being also covered with c-ART for two or more years after intervention [42], but unfortunately, viremia rebound within two weeks post-interruption. Phylogenetic analysis demonstrated that viral rebound was caused by the presence of one or few latent proviruses, being consistent with the persistence of a minimal reservoir of infected cells despite HSCT [43].

Bone marrow transplant was also considered, but its associated risks have limited its applicability in HIV patients. Related attempts comprise transforming the patient's cells by generating a CCR5 deletion cells resistant to infection or the direct deletion of the virus from infected cells, followed by an autologous transplant. In fact, HIV co-receptors and pro-viral sequences can be targeted through the use of DNA-editing enzymes [44], such as: zinc-finger nucleases (ZNFs) [45], transcription activator-like effector nucleases and homing endonucleases [44, 46, 47].

One completed [48] and two ongoing (NCT01044654, NCT01252641) [49, 50] clinical trial explore the use of ZFNs to manipulate CCR5 expression in CD4+ T cells and precursor cells [45, 51]. They bring evidences on the safety and efficacy of CCR5-ZFN-treated autologous cells in patients with chronic aviremic HIV infection under c-ART. Promising results were obtained, with increased CD4+ T cell counts, reduced viral load, improved ability of cells to localize to anatomical reservoirs, and decreased reservoir size. Moreover, engineered cells were detected to persist in the body, their populations declining at slower rates than non-engineered cells after c-ART interruption [27]. Nevertheless, these cells do not prevent the infection by CXCR4-tropic viruses and may drive selection for either X4-specific or dual-tropic HIV-1 viruses [52, 53]. Recent preclinical studies in cell lines and humanized mice with ZFNs targeting

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CXCR4 in CCR5Δ32 CD4+ T cells indicate protection from both R5- and X4-tropic HIV viruses [54, 55]. Otherwise, the physiological impact of lacking both receptors needs to be characterized. Although all these interventions seem in general to be safe, their efficacy in humans has yet to be proven [27].

Many of these issues, together with other relevant aspects on the wide spectrum of the virus-host interaction were discussed in the *HIV Pathogenesis, virus versus host* meeting, as part of the Keystone Symposia Global Health Series, held at Fairmont Banff Springs, in Banff, Alberta, Canada, on March 9 to 14, 2014.

The *HIV pathogenesis, virus versus host* meeting

The meeting focused on the latest advances in basic and translational HIV research with emphasis on mechanistic models. Key aspects of HIV biology from infection to AIDS were presented. The program included: 1) Virus entry/exit into/from the cell; 2) Entry into the host; 3) Virus-host genetics and co-evolution; 4) Virus-host interactions and responses; 5) Pathogenesis; 6) Reservoirs, latency and reactivation; and 7) HIV and the microbiome at the mucosa. Scientific environment was enriched with the simultaneous meeting on "HIV Vaccines: Adaptive Immunity and Beyond", which shared opening and closing keynote addresses and two plenary sessions with this meeting.

The organizers presented a Pre-meeting Workshop for the Global Health Travel Award winners. Prominent lectures on hot topics were delivered by leaders in the HIV field: Dr. Stephen DeRosa from the University of Washington, USA, talking on the HIV immunity; Dr. Penny Moore from the National Institute for Communicable Diseases, South Africa, humoral HIV immunity; Dr. Michael Emerman from the Fred Hutchinson Cancer Research Center, USA, on HIV restriction factors; and Dr. David Margolis from the University of North Carolina, Chapel Hill, USA, on the HIV cure perspectives.

During the congress, Dr. Victor Garcia Martinez, Professor of Medicine at the Center for AIDS Research, USA, dictated an outstanding conference about the contribution of humanized bone marrow/liver/thymus (BLT) mice to the elucidation of the molecular basis of vaginal HIV transmission and prevention. BLT mice develop a functional human immune system and offer a rapid, reliable and reproducible experimental system for HIV research. Very relevant to HIV transmission is the extensive reconstitution of the female reproductive tract of BLT mice with human hematopoietic cells. Dr. Garcia Martinez and his group have evaluated the vaginal transmission of highly relevant transmitted/founder viruses, the role of semen on HIV transmission, the effect of semen on the efficacy of topical microbicides and the role of cell-associated virus on vaginal transmission. The results have been crucial in providing novel insight into mucosal HIV transmission and the effectiveness of different prevention strategies.

An excellent lecture on the host and HIV exit was delivered by Dr. Fadila Bouamr from the National Institute of Health, USA. Her group investigated how viruses interact with hosts to promote their replication. The nucleocapsid (NC) domain of the HIV-1 structural protein Gag was found to accomplish a surprinsingly complex set of functions during the HIV replication cycle. In addition to the role of this protein in genome integration, Gag-Gag multimerization, and reverse transcription, Dr. Fadila and colleagues reported the first evidence of NC involvement in HIV-1 budding and exit. NC mutant virions remained tethered to the surface of CD4+ T cells, indicating the failure to recruit or utilize members of the host machinery that catalizes membrane scission and virus exit. Alix Bro 1, a member of the endosomal sorting complexes required for transport (ESCRT) binds NC, establishing a direct role for NC in mediating interactions with ESCRT necessary for virus release, with the involvement of RNA for molecule recruitment.

Dr. Janice Clements from the Johns Hopkins University School of Medicine, USA, spoke about the macrophages contribution to the latent viral reservoir in tissues of c-ART-suppressed SIV-infected macaques. HIV latency has been well established in resting CD4+ T cells and their longevity provides a lifelong reservoir in HIV infected individuals on c-ART. It is known that HIV and SIV infect CD4+ T cells, monocytes, macrophages and microglia. Nevertheless, viral latency has not been well studied in tissues of HIV and SIV infected individuals and viral assays are needed for quantitating infected and latent monocytes and macrophages. Clements and colleagues used an SIV macaque model suppressed by c-ART to less than 10 copies per milliliter of plasma. Monocytes and macrophages from SIV-infected macaques, either suppressed or not, were quantified. The study demonstrated that monocytes and macrophages support SIV infection and the suppression of SIV by c-ART leads to low level virus replication in tissues. SIV was reactivated from monocytes and tissue macrophages using the Quantitative Viral Outgrowth Assay. This demonstrates that macrophages lineage cells can provide a latent reservoir for SIV.

Results about APOBEC3 genes family (A3), an interferon-stimulated genes family, were showed by Dr. Richard T. D'Aquila from Northwestern University Feinberg School of Medicine, USA. He and his group posit that early in the infection, high A3 levels/activities are one of the innate defenses lost during acute infection in non-controllers and preserved by the anti-HIV CTL activity in controllers. They tried to unravel how to favor A3 control of Vif-positive HIV-1 virus by boosting cellular A3s. Dr. D'Aquila presented lead compounds unrelated to interferons that induce some A3s, and block early steps of Vif-positive HIV replication *in vitro*. They also considered that knowing the A3F virion core localization mechanisms may contribute to enhance anti-HIV activity of other virion-packaged A3s.

Dr. Andrea Cimarelli from the International Center for Infectology Research, USA, explored the complexity of infection of myeloid cells by primate lentiviruses during the early phases of infection. Myeloid cells support viral replication at low levels due to a major restriction in reverse transcription. An important factor of this restriction is SAMHD1, a triphosphohydrolase that limits dNTPs. Dr. Cimarelli suggested that the slow accumulation of viral DNA (vDNA) as orchestrated by SAMHD1 leads to the exposure of vDNA

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to the protracted action of cytoplasmic effectors. Evidences indicate that one such factor is the cytidine deaminase APOBEC3A which restricts lentiviral infection by decreasing vDNA accumulation in myeloid cells. On the other hand, based on experiments with Vpx (a protein that degrades SAMHD1) and HIV-2/SIVSM and HIV-1 viruses, it seems like the low levels of replication in myeloid cells may be a common goal for primate lentiviruses that has been resolved through different evolutionary strategies. This and other results suggest that another restriction may differentially target primate lentiviruses in myeloid cells.

Regarding the natural resistance to HIV infection, Dr. Reuben S. Harris from the University of Minnesota, USA, spoke on the role of select APOBEC3H haplotypes. They demonstrated that naturally occurring variants of APOBEC3H are stably expressed and are able to encapsidate and restrict HIV replication in primary CD4+ T cells. Weak Vif alleles comprise approximately 25 % of HIV subtype B isolates, suggesting a dynamic relationship between the evolution of Vif to antagonize APOBEC3H in populations in which some individuals express an active APOBEC3H.

HIV eradication approaches were also adressed by Dr. David M. Margolis. Most eradication strategies elicit proviral expression with the assumption that c-ART will prevent new infections while the immune system, possibly with the help of other therapeutics, will clear infected cells. There are other strategies on the use of HIV-specific recombinases to destroy proviral DNA, or HIV-dependent suicide genes to selectively kill HIV-infected cells. Several strategies are being evaluated as transcriptional activators, such as: HDACis, Disulfiram, PD-1 inhibition and vaccination. Dr. Margolis talked about the complex and multimodal nature of HIV eradication approaches, with emphasis on the results of clinical trials with vorinostat, a HDACis currently approved for oncologic clinical use which activates HIV transcription in cell-line models of latent HIV and in *ex vivo* cultured cells. Vorinostat, administered as a 400-mg single dose, induces the expression of the full-length HIV RNA in latently-infected resting CD4+ T cells. After 14 doses, ninety percent of participants had a significant increase in HIV production from the former latently-infected cells. These results prove that this drug can bring HIV out of hiding in resting T cells.

A very interesting lecture about prospects of HIV eradication was delivered by Dr. Robert Siliciano from the Johns Hopkins University School of Medicine of Baltimore, USA. The availability of assays that accurately measure the size of the latent reservoir in resting CD4+ T cells is crucial from the therapeutic perspective, with latency reversing agents (LRAs) as key triggers of reservoir activation. Dr. Siliciano discussed a recent work indicating that PCR-based assays overestimate the size of the reservoir, while the Quantitative Viral Outgrowth Assay may underestimate the reservoir by as much as 60-fold due to the failure to induce replication competent proviruses by a single round of activation. Recent studies with patients' cells in the presence of LRAs were also analyzed [56]. Remarkably, the most currently used LRAs do not reverse latency in patients' cells, but they are effective in *in vitro* models of latency. This finding suggests that LRAs proven to be effective in patients' cells must be of critical priority.

Dr. Nicholas Chomont, from the Vaccine and Gene Therapy Institute of Florida, USA, talked about what could be learned about HIV persistence during infection from CD4+ T cell homeostasis. Chomont and colleagues identified a small group of specific checkpoint blockers (including PD1) that are associated with immune dysfunction, the size of the viral reservoir, and which may induce latency in CD4+ T cells. On the other hand, it has been observed that the gamma-c cytokine IL-7 plays a crucial role in the maintenance of the viral reservoir by promoting the homeostatic proliferation of latently-infected cells. All these indicate that HIV persistence during c-ART is ensured by immunological mechanisms that are responsible for the homeostasis of the memory CD4+ T cell pool. Dr. Chomont proposed that interfering with the regulators of T cell homeostasis may disrupt HIV latency in virally-suppressed subjects under suppressive c-ART. It would also pave the way for the development of novel strategies to cure the HIV infection.

In another talk, novel means for eliminating latent HIV reservoirs were presented by Dr. Jerome A. Zack from the University of California, Los Angeles, USA. His group designed and synthesized new latency activating compounds that function via the protein kinase C (PKC) signalling pathway. They we able to produce a naturally occurring nanoparticle called "vault", by which can latency activating compounds can be more selectively introduced into the target cell type. The novel molecules were evaluated in cell line models for HIV latency, together with several primary cell assays and latently infected cells from c-ART treated patients. All the tested compounds potently activated HIV from latency, in many cases more strongly than the natural molecules. Particularly, vault nanoparticles carrying bryostatin 1 were able to activate HIV from latency and showed to be active in CD4+ splenocytes following its intravenous administration in immunocompetent mice. Dr. Zack considered that novel PKC activators and nanoparticle delivery methods may impact in activation-elimination strategies to eradicate latent HIV reservoirs.

Concluding remarks

HIV eradication is still an outstanding challenge for the scientific community [27]. ART has achieved to control viral replication and significantly decreased the AIDS-related morbidity and mortality, but without curing the infection [57]. An integral understanding of the virus-host interaction remains as a major prerequisite for the rational improvement of therapeutic strategies [58]. Two major strategies are envisaged to eradicate HIV infection: a functional cure and a sterilizing cure, both aimed to diminish and eliminate the viral reservoirs, respectively [10, 11]. The finding of reliable, fast and economic methods that can reproducibly evaluate the real effectiveness of treatments under investigation in different laboratories, still requires especial and sustained scientific efforts [27].

Acknowledgements

The authors thanks to the Global Health Travel Award for making possible the participation at the

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