Notes on HIV pathogenesis, therapy and eradication

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ABSTRACT

This report summarizes the main topics delivered as lectures at the meeting Frontiers in HIV pathogenesis, Therapy and Eradication, held at the Whistler Conference Centre, in Whistler British Columbia, Canada, in March 26-31. The meeting was organized as part of the Keystone Symposia on Molecular and Cell Biology joined with the conference Cell Biology of Virus Entry. The current status on research for eradicating HIV-1 latent reservoirs and ways to reactivate them for destruction with current or novel antiretroviral therapies were the key topics. Targeted inhibition of virus entry, new classes of inhibitors, animal models for testing antiretroviral therapies and virus cell-to-cell contact-mediated transmission, together with structural analysis of HIV-1 proteins and replication processes, were also presented. The useful discussions at both the oral and poster sessions paved the way for understanding processes essential for future therapeutic attempts to effectively inhibit virus replication and transmission, including its eradication.

Keywords: HIV-1, antiretroviral therapy, viral latency, viral entry, HIV persistence

RESUMEN

Apuntes sobre la patogénesis del VIH, su terapia y eliminación. Se resumen los temas fundamentales de las conferencias del congreso Fronteras en la patogénesis del VIH, su terapia y erradicación, celebrado en el Centro de Congresos de Whistler, Whistler British Columbia, Canadá, del 26 al 31 de marzo de este año. Este se organizó junto con el evento Biología Celular de la Entrada Viral, como parte de los Simposios de Keystone, sobre Biología Molecular y Celular. Se expusieron el estado de las investigaciones para erradicar los reservorios latentes del VIH-1 y las estrategias para reactivarlos mediante terapia antirretroviral convencional o de nueva generación, y de esa forma promover su destrucción. Además se discutieron temas relacionados con la entrada viral como blanco terapéutico, nuevas clases de inhibidores, los modelos animales disponibles para evaluar las terapias antirretrovirales y la transmisión del VIH-1 mediante el contacto entre células, así como los análisis estructurales de proteínas del VIH-1 y los procesos que ocurren durante la replicación. Las discusiones orales y presentaciones en carteles favorecieron una mejor comprensión de los procesos esenciales para desarrollar terapias que no solo inhiban la replicación y transmisión del VIH-1, sino también que lo eliminen.

Palabras clave: VIH-1, terapia antirretroviral, latencia viral, entrada viral, persistencia del VIH

Introduction

Up to now, antiretroviral therapy (ART) has been unable to eradicate the human immunodeficiency virus type 1 (HIV-1) infection [1], while providing sustained suppression of plasma viral load in most HIV-infected individuals. This is currently considered by the research community as dependent on persistent viral reservoirs [2, 3]. Therefore, considerable efforts have been made on therapies to reactivate the latent cellular viral reservoirs by using various agents, such as cytokines, histone deacetylase inhibitors (HDAC) and mitogens, assuming that those cells would die of HIV-induced cytopathic effects and antiretroviral drugs would prevent the spread of infection [4].

The best studied reservoir of latent HIV-1 is resting CD4+ T cells [5, 6]. Disrupting latency via the induction HIV-1 expression in these cells is a potential strategy to facilitate the clearance of this reservoir. This has been attained *in vitro* with HDACs, but still not proven in patients [7, 8] and this is only the first step down an uncertain path towards viable eradication therapies. Regimens, safety and doses required to fully purge persistent viral genomes remain to be established, without exceeding the capability of ART to contain the spread of infection [9, 10]. Nevertheless, agents that disrupt latency may not be sufficient to

clear the replication-competent virus, and strategies to enhance the immune response or to induce death in infected cells death need to be developed. Others reservoirs such as the long term, non-replicating myeloid or astroglial cells may require different novel approaches beyond.

On the other hand, assessing and optimizing therapies that activate the latent virus requires animal models. The most advanced models available are the simian immunodeficiency virus non-human primates models and the humanized bone marrow-liver-thymus (BLT) mouse [11]. In this sense, validated animals models that recapitulate key aspects of the human condition are critical to develop and enforce novel approaches for treatment, prevention and an eventual cure for HIV-1 infection [12].

The only clinical strategy implemented to cure the HIV-1 infection derives from the single documented case of HIV-1 cure to date [13]. It comprises subsequent myeloablative chemotherapy, followed by total body irradiation, anti-thymocyte globulin infusion and allogenic hematopoietic stem cell transplantation (HSCT) from a CCR5Δ32 homozygous donor. It is unclear which components of the many and complex therapies that patient went through will be required

1. Lewin SR, Evans VA, Elliott JH, Spire B, Chomont N. Finding a cure for HIV: will it ever be achievable? J Int AIDS Soc. 2011;14:4.

2. Trono D, Van Lint C, Rouzioux C, Verdin E, Barre-Sinoussi F, Chun TW, et al. HIV persistence and the prospect of long-term drug-free remissions for HIV-infected individuals. Science. 2010;329(5988): 174-80.

3. Palmer S, Josefsson L, Coffin JM. HIV reservoirs and the possibility of a cure for HIV infection. J Intern Med. 2011;270(6):550-60.

4. Chun TW, Murray D, Justement JS, Hallahan CW, Moir S, Kovacs C, et al. Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. J Infect Dis. 2011;204(1):135-8.

5. McNamara LA, Ganesh JA, Collins KL. Latent HIV-1 infection occurs in multiple subsets of hematopoietic progenitor cells and is reversed by NF-κB activation. J Virol. 2012; Forthcoming.

6. McNamara LA, Collins KL. Hematopoietic stem/precursor cells as HIV reservoirs. Curr Opin HIV AIDS. 2011;6(1):43-8.

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to eradicate HIV-1 reservoirs in others and whether a similar outcome can be achieved [14]. Clinical trials are planned to further elucidate these issues. Other groups propose engineering cells to resist HIV-1 infection, by modifying them as CCR5$^{-/-}$. T cells modified with a zinc finger nuclease targeting CCR5 are already being evaluated in clinical trials for such purpose. The ability to modify hematopoietic stem/progenitor cells may allow a longer lasting therapy that would also protect non-T cell targets such as macrophages from HIV-1 infection [15, 16].

All these topics were concern of the Frontiers in HIV Pathogenesis, Therapy and Eradication conference, held at the Whistler Conference Centre, in Whistler, Canada, last March 26-31.

Frontiers in HIV pathogenesis, therapy and eradication

The meeting was organized as part of the Keystone Symposia on Molecular and Cell Biology joined with the conference Cell Biology of Virus Entry. The convention was structured in several sessions: Keynote session, Retroviral entry mechanisms, Inhibition of virus entry, Mechanisms of HIV latency, HIV therapy: State of the art, Targeting latency for eradication, Cell biology of HIV infection, New approaches to antiviral treatment, Models for therapy and eradication, and Host factors and HIV replication. Alan N. Engelman, Eric O. Freed and John M. Coffin were the scientific organizers of the meeting.

A pre-meeting workshop was organized for those granted the Global Health Travel Award. Lectures on Molecular virology of HIV-1 replication, Molecular mechanisms of HIV latency and Clinical aspects of highly active ART therapy were dictated by Eric Freed from the National Center Cancer, USA; Melanie Ott from the University of California (San Francisco, USA), and Robert F. Siliciano from the Johns Hopkins University School of Medicine, USA respectively. The workshop was moderated by Jeff Lehman, Scholarship coordinator of the Keystone Symposia. It was an opportunity to debate with these prestigious researchers in the field of HIV.

Robert F. Siliciano delivered a remarkable lecture on how antiretroviral therapy for HIV infection really works. He stated that residual viremia after ART intensification is not due to ongoing replication but rather to release from stable reservoirs. Some classes of drugs have steep, highly cooperative dose response curves, reflecting a unique form of intermolecular cooperation. A biophysical model for the cooperative inhibition of infectivity was described, also explaining the effects of resistance mutations that alter not only the half maximal inhibitory concentration (IC50) but also the shape of dose response curves. Dr. Siliciano also explained on methods for calculating the combined inhibitory potential of drug combinations, permitting a comparison of antiviral activity of different drug regimes. This quantitative approach to understand ART may be useful for developing simpler ART regimes for patients [17-20].

Another lecture was delivered by Thomas J. Hope from the Northwestern University of Chicago, USA, on how early events of HIV-1 infection can influence late outcomes. After fusion of HIV-1 with the plasma membrane of a target cell, it must complete a series of steps before its genome can enter the nucleus and integrate into the cell´s genome. Hope's group demonstrated that reverse transcription facilitates the process of uncoating. They showed that Q63/67A, E45A and N74D mutations in the p24^{CA} protein slow the rate of uncoating of the viral capsid. Viruses showing these three mutations were also shown to use an alternative nuclear import pathway and influence integration site selection. They also studied the mechanism of the tripartite motif-containing protein 5 (TRIM5) alpha-mediated restriction by using green fluorescent protein as a fluid phase marker incorporated into mature virions. The authors stated that early steps of HIV-1 replication are intertwined to a great extent and that the process of uncoating can influence later events such as nuclear import and even integration site preference [21-23].

Michel J. Root from the Thomas Jefferson University, Philadelphia, USA spoke about the HIV-1 gp41 structural changes associated with viral membrane fusion. He explained that despite the wide disparity in the native state structures of fusion glycoproteins of enveloped viruses, the final conformational transitions that drive membrane fusion show a remarkable degree of topological conservation. These proteins assume a homotrimeric extended intermediate state, bridging the viral and cellular membranes before collapsing into a symmetric trimer of hairpin conformation that juxtaposes the two membranes. Dr. Root's group determined that the HIV-1 fusion glycoprotein Env (gp120/gp41) function requires folding of all three hairpins per gp41 trimer and that these hairpins fold in a highly cooperative manner. Their results suggested that the function of a single Env trimer is sufficient to promote viral entry. That is difficult to reconcile with the current N-ethylmaleimide–sensitive factor attachment protein receptor-like model of viral membrane fusion and suggest an alternate mechanism for the entry of HIV-1 [24].

Dr. Clare Jolly from the University College of London, UK, dictated a lecture in the Retroviral Entry Mechanisms Session on cell-cell transfer of HIV-1. This transmission takes place in virological synapses between an HIV-1 infected cell and a neighboring receptor-expressing target cells. The active, cytoskeleton-driven recruitment of viral and cellular proteins and the dynamic nature of virological synapses suggest that these structures are highly regulated, although the molecular effectors are mostly unknown [25]. This group showed that T cell-T cell HIV-1 virological synapses polarize the microtubule organizing center and associated organelles within the HIV-1 infected cells towards the engaged target T cell. Currently, they are identifying factors that regulate the cell-cell spread at the virological synapses as potential therapeutic targets for antiviral therapy [26].

Peter D. Kwong from the National Institute of Allergy and Infectious Diseases in Bethesda, MD, USA, talked on antibody-mediated inhibition of HIV-1 entry. He mentioned that monoclonal antibodies from HIV-1 infected individuals (15-25% of HIV-1 infected individuals generate neutralizing antibodies) fall into two distinct categories for an effective neutralization of the HIV-1 gp120 regarding the target site: 1) at the site of CD4 receptor binding; and 2) at conserved gly-

7. Archin NM, Espeseth A, Parker D, Cheema M, Hazuda D, Margolis DM. Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. AIDS Res Human Retrovir. 2009; 25(2): 207-12.

8. Margolis DM. Histone deacetylase inhibitors and HIV latency. Curr Opin HIV AIDS. 2011;6(1):25-9.

9. Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomerantz RJ. The challenge of finding a cure for HIV infection. Science. 2009;323(5919):1304-7.

10. Bowman MC, Archin NM, Margolis DM. Pharmaceutical approaches to eradication of persistent HIV infection. Expert Rev Mol Med. 2009;11:e6.

11. Ling B, Mohan M, Lackner AA, Green LC, Marx PA, Doyle LA, et al. The large intestine as a major reservoir for simian immunodeficiency virus in macaques with long-term, nonprogressing infection. J Infect Dis. 2010;202(12):1846-54.

12. Denton PW, Garcia JV. Humanized mouse models of HIV infection. AIDS Rev. 2011;13(3):135-48.

13. Allers K, Hutter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E, et al. Evidence for the cure of HIV infection by CCR5Delta32/Delta32 stem cell transplantation. Blood. 2011;117(10):2791-9.

14. Hutter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med. 2009;360(7):692-8.

15. Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, et al. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol. 2010;28(8):839-47.

16. Taaffe JE, Bosinger SE, Del Prete GQ, Else JG, Ratcliffe S, Ward CD, et al. CCR5 blockade is well tolerated and induces changes in the tissue distribution of CCR5+ and CD25+ T cells in healthy, SIV-uninfected rhesus macaques. J Med Primatol. 2012;41(1):24-42.

17. Shen L, Rabi SA, Sedaghat AR, Shan L, Lai J, Xing S, et al. A critical subset model provides a conceptual basis for the high antiviral activity of major HIV drugs. Sci Transl Med. 2011;3(91):91ra63.

18. Shen L, Rabi SA, Siliciano RF. A novel method for determining the inhibitory potential of anti-HIV drugs. Trends Pharmacol Sci. 2009;30(12):610-6.

19. Sedaghat AR, Dinoso JB, Shen L, Wilke CO, Siliciano RF. Decay dynamics of HIV-1 depend on the inhibited stages of the viral life cycle. Proc Natl Acad Sci USA. 2008;105(12):4832-7.

20. Jilek BL, Zarr M, Sampah ME, Rabi SA, Bullen CK, Lai J, et al. A quantitative basis for antiretroviral therapy for HIV-1 infection. Nat Med. 2012;18(3):446-51.

21. Hulme AE, Perez O, Hope TJ. Complementary assays reveal a relationship between HIV-1 uncoating and reverse transcription. Proc Natl Acad Sci USA. 2011;108(24):9975-80.

22. Lee K, Ambrose Z, Martin TD, Oztop I, Mulky A, Julias JG, et al. Flexible use of nuclear import pathways by HIV-1. Cell Host Microbe. 2010;7(3):221-33.

cans. He proposes the use CD4-binding-site-directed and glycan-directed monoclonal antibodies as templates to design immunogens to re-elicit such antibodies [27]. This also reinforces a possible active role of glycans for immune control of the virus, beyond the glycan-shield theory.

Another interesting lecture was delivered by Michael Farzan from the Harvard Medical School, Massachusetts, USA. He focused on sulfotyrosine-binding pockets of gp120 as a therapeutic target. HIV-1 co-receptors are modified at their amino-terminal tyrosines by sulfate groups. These sulfotyrosines directly contact HIV-1 gp120 and contribute significantly to the binding energy required for the interaction of gp120 and the CCR5 co-receptor. The sulfotyrosine-binding pockets are cross-clade conserved and bear co-receptor preference. The author elaborated that sulfopeptides derived from neutralizing antibodies function as coreceptor-mimetics and can induce or stabilize the CD4-bound conformation of gp120. Fusions of these peptides with CD4-Ig or CD4-mimetic peptides are highly potent at neutralizing a range of HIV-1 isolates [28].

Melanie Ott from the University of California (San Francisco, USA), delivered an outstanding lecture on regulation of HIV-1 transcription by Tat post-translational modifications. The HIV-1 Tat protein is a critical activator of transcription elongation via its interactions with TAR RNA and the positive transcription elongation factor b (P-TEFb). Tat also interacts with many chromatin-modifying enzymes, being also targeted by some of them. The authors focused on two critical modifications within Tat: monomethylation of lysine 51 (K51) and acetylation of lysine 50 (K50). They identified the lysine-specific demethylase 1 (LSD1/KDM1) as targeting Tat K51 and as new co-activator of HIV transcription in latently infected T cells [29].

A very comprehensive lecture was delivered by Tae-Wook Chun from the National Institute of Allergy and Infectious Diseases in Bethesda, MD, USA. He analyzed the perspectives for eradicating the cellular HIV-1 reservoirs and the development of therapeutic strategies. The existence of latently-infected, resting CD4+ T cells carrying replication-competent HIV-1 provirus has posed one of the greatest challenges to the long-term control or eradication of HIV-1 in infected individuals on ART. There has been considerable work on therapies to reactivate the latent viral reservoir. The author pinpointed that such approaches have shown no clinical benefit to date and that it has been suggested that low levels of HIV-1 replication may persist in subsets of CD4+ T cells in blood and lymphoid tissues of infected individuals receiving ART but showing no detectable plasma viral load. He explained the potential mechanisms of HIV persistence and prospects for eradication and new therapeutic approaches in HIV-infected individuals under effective ART [4].

David M. Margolis (University of North Carolina at Chapel Hill, USA) subsequently focused on disrupting latency for eradication via the induction HIV-1 expression in latently infected resting CD4+ T cells. He pointed out the *in vitro* effectiveness of HDAC inhibitors such as suberoylanilide hydroxamic acid (vorinostat, VOR) for this purpose, pending to be tested in patients [30]. A larger and more complex scenario needs to be developed for eradication studies than that resembled by current *in vitro* models. Dr. Margolis mentioned that although the challenges can scarcely be underestimated, exciting emerging tolls and advances suggest that focused and collaborative efforts may succeed in eradicating HIV infection in mid-to near term [7].

Zeger Debyser from the Catholic University of Leuven, Belgium, elaborated on a novel class of integration inhibitors (LEDGF/p75 inhibitors, LEDG-INs). He pointed that unlike clinically approved integrase strand transfer inhibitors, LEDGINs do not bind to the catalytic site of the HIV-1 integrase but to its LEDGF/p75 binding pocket. A broad activity spectrum against multiple clades and clinical isolates, and the synergism between LEDGINs and integrase strand transfer inhibitors in combination reveal the potential of these compounds for clinical use [31].

J. Víctor García-Martínez from the University of North Carolina at Chapel Hill, USA, examined the humanized BLT mice as an outstanding *in vivo* model to study HIV infection. His team demonstrated that a combination of well-characterized human retroviral drugs is capable of effectively controlling viral replication in BLT mice, as well as the presence of latently-infected resting human CD4+ T cells in ART-treated BLT which can be induced *ex vivo* to produce HIV-1. These authors observed a frequency of infected resting human CD4+ T cells in tissues from BLT mice in the same range observed in patients undergoing suppressive ART [12].

Alan Engelman from the Dana-Farber Cancer Institute in Boston, USA talked on HIV-host interactions in nuclear import and integration. They have investigated HIV-host interactions relevant for preintegration complex (PIC) nuclear import and integration. Genetic analyses identified cellular transportin 3 (TNPO3) and nucleoporin 153 (NUP153) and HIV-1 capsid (CA) at the PIC nuclear import though, interestingly, TNPO3 and NUP153 each bind to the viral integrase *in vitro*. They have determined that TNPO3 and NUP153 can separately bind to the CA protein *in vitro*, the latter preferentially binding to CA over IN [32, 33].

At the end of the talk, Eric O. Freed from the National Cancer Institute in Frederick, USA, delivered a remarkable lecture on host cell factors relevant for retrovirus assembly and release. The HIV-1 Gag polyprotein precursor (Pr55Gag) domains responsible for the assembly/release process are largely known but the role of host cell factors and pathways for particle production remain to be fully unraveled. The authors identified novel candidate cellular cofactors that are potentially involved in the late retroviral replication events [34-37]. Growth arrest-specific 7 (GAS7) and proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) are sequence-related proteins that harbor the conserved F-BAR domain that deforms membranes. It was shown that GAS7 interacts directly with the tumor susceptibility gene 101 (Tsg101) and the capsid region of HIV-1Gag via its F-BAR domain, being incorporated into virions and further cleaved by the viral protease.

23. Campbell EM, Perez O, Anderson JL, Hope TJ. Visualization of a proteasome-independent intermediate during restriction of HIV-1 by rhesus TRIM5alpha. J Cell Biol. 2008;180(3):549-61.

24. Root MJ, Kay MS, Kim PS. Protein design of an HIV-1 entry inhibitor. Science. 2001;291(5505):884-8.

25. Martin N, Welsch S, Jolly C, Briggs JA, Vaux D, Sattentau QJ. Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. J Virol. 2010;84(7):3516-27.

26. Jolly C, Welsch S, Michor S, Sattentau QJ. The regulated secretory pathway in CD4(+) T cells contributes to human immunodeficiency virus type-1 cell-to-cell spread at the virological synapse. PLoS Pathogens. 2011;7(9):e1002226.

27. McLellan JS, Pancera M, Carrico C, Gorman J, Julien JP, Khayat R, et al. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature. 2011;480(7377):336-43.

28. Kwong JA, Dorfman T, Quinlan BD, Chiang JJ, Ahmed AA, Choe H, et al. A tyrosine-sulfated CCR5-mimetic peptide promotes conformational transitions in the HIV-1 envelope glycoprotein. J Virol. 2011;85(15):7563-71.

29. Ott M, Geyer M, Zhou Q. The control of HIV transcription: keeping RNA polymerase II on track. Cell Host Microbe. 2011;10(5):426-35.

30. Shan L, Deng K, Shroff NS, Durand CM, Rabi SA, Yang HC, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. Immunity. 2012;36(3):491-501.

31. Taltynov O, Desimmie BA, Demeulemeester J, Christ F, Debyser Z. Cellular Cofactors of Lentiviral Integrase: From Target Validation to Drug Discovery. Mol Biol Int. 2012;2012:16.

32. Koh Y, Matreyek KA, Engelman A. Differential sensitivities of retroviruses to integrase strand transfer inhibitors. J Virol. 2011;85(7):3677-82.

33. Li X, Krishnan L, Cherepanov P, Engelman A. Structural biology of retroviral DNA integration. Virology. 2011;411(2):194-205.

34. Balasubramaniam M, Freed EO. New insights into HIV assembly and trafficking. Physiology (Bethesda). 2011;26(4):236-51.

35. Joshi A, Garg H, Ablan SD, Freed EO. Evidence of a role for soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) machinery in HIV-1 assembly and release. J Biol Chem. 2011;286(34):29861-71.

36. Ono A, Ablan SD, Lockett SJ, Nagashima K, Freed EO. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. Proc Natl Acad Sci USA. 2004;101(41):14889-94.

37. Fujii K, Hurley JH, Freed EO. Beyond Tsg101: the role of Alix in 'ESCRTing' HIV-1. Nature Rev Microbiol. 2007;5(12):912-6.

Concluding remarks

In spite of having reduced plasma viral RNA to undetectable levels and, consequently, HIV-related morbidity and mortality and its perinatal and behavior-associated transmission [38], ART is unable to eradicate the virus. A comprehensive understanding of HIV pathogenesis will be necessary to achieve a cure for HIV infection, especially its persistence and latency. The scientific community is working to develop better *in vitro* and *in vivo* models for that purpose. Some drugs are currently proposed for effective virus control and eradication. Clinical trials should be carefully designed. The HIV Pathogenesis, Therapy and Eradication Conference organized by the Keystone Symposia this year in Whistler, Canada, was a great opportunity to exchange knowledge between research groups working in all these topics. More than 50 lectures and very extensive sessions of poster discussion were carried out. Only a representative number of results discussed during the conference are included in this report.

Acknowledgements

38. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1 -infected adults and adolescents. Rockville: Department of Health and Human Services; 2012. Available from: http://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv-guidelines/0/.