6-10-2014

New clues to understanding HIV nonprogressors: Low cholesterol blocks HIV trans infection

Vinayaka R. Prasad
Yale University

Michael I. Bukrinsky
George Washington University

New Clues to Understanding HIV Nonprogressors: Low Cholesterol Blocks HIV *Trans* Infection

Vinayaka R. Prasad and Michael I. Bukrinsky

2014. New Clues to Understanding HIV Nonprogressors: Low Cholesterol Blocks HIV *Trans* Infection. mBio 5(3): . doi:10.1128/mBio.01396-14.

COMMENTARY

New Clues to Understanding HIV Nonprogressors: Low Cholesterol Blocks HIV *Trans* Infection

Vinayaka R. Prasad,[a] Michael I. Bukrinsky[b]

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, USA[a]; Department of Microbiology, Immunology, and Tropical Medicine, The George Washington University School of Medicine and Health Sciences, Washington, DC, USA[b]

ABSTRACT A small percentage of HIV-infected subjects (2 to 15%) are able to control disease progression for many years without antiretroviral therapy. Years of intense studies of virologic and immunologic mechanisms of disease control in such individuals yielded a number of possible host genes that could be responsible for the preservation of immune functions, from immune surveillance genes, chemokines, or their receptors to anti-HIV restriction factors. A recent *mBio* paper by Rappocciolo et al. (G. Rappocciolo, M. Jais, P. Piazza, T. A. Reinhart, S. J. Berendam, L. Garcia-Exposito, P. Gupta, and C. R. Rinaldo, mBio 5:e01031-13, 2014) describes another potential factor controlling disease progression: cholesterol levels in antigen-presenting cells. In this commentary, we provide a brief background of the role of cholesterol in HIV infection, discuss the results of the study by Rappocciolo et al., and present the implications of their findings.

Understanding the genetic basis of natural resistance to HIV-1 is a major goal in the effort to control HIV. Previous studies to identify and delineate host genetic variants responsible for complete or partial resistance to HIV infection or disease progression have pointed to host genes involved in the HIV-1 replication cycle (CCR5, CCR2b, chemokines), immune surveillance (major histocompatibility complex class I) or restriction factors (members of the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like, family; tripartite motif-containing protein 5α; and tetherin) (recently reviewed in reference 1). Such studies are valuable, as they provide important clues to identifying the viral and host targets for developing more efficacious therapies. The recent report in *mBio* by Rappocciolo et al. (2) throws new light on a molecule whose role in aiding and abetting HIV replication is already known—cholesterol. That study shows that the level of cholesterol in immune cells may be a determinant of HIV spread within the body. While the underlying genetic basis of this is still unknown, the study provides some important hints.

Cholesterol has been shown to be required for the entry or fusion of many different viruses (3–5), and the list continues to expand (6). The role of cholesterol in HIV replication has been studied for over 10 years. Early reports demonstrated that cholesterol depletion from virus-producing cells suppresses virus production (7), whereas depletion of cholesterol from mature virions or target cells inhibits virus-cell fusion and infection (8–11). This dependence of HIV on host cell cholesterol was related to viral assembly on cholesterol-rich lipid rafts of the plasma membrane and the role of cholesterol and lipid rafts in membrane fluidity (7, 12, 13). Thus, reports that HIV actively controls the cholesterol metabolism of the host cell came as little surprise. HIV, via its protein Nef, stimulates cholesterol uptake and biosynthesis by activating the transcription of sterol-responsive element binding factor 2 (SREBF-2) and SREBF-2-regulated genes (14), and Nef also inhibits the activity of the cellular cholesterol transporter ATP-binding cassette A1 (ABCA1), thus reducing cholesterol efflux from cells (15). In addition, Nef binds cholesterol and delivers it to lipid rafts (16). Together, these effects lead to an increase in intracellular cholesterol, an increase in lipid raft abundance, and an increase in viral production and infectivity (17). Conversely, depletion of cellular cholesterol by ABCA1 activation potently inhibits HIV replication (18–20).

The paper by Rappocciolo and colleagues describes another role that cholesterol plays in HIV infection (2). Investigating the mechanistic basis underlying the ability of a small group of HIV-infected persons to control HIV infection for many years without antiretroviral treatment, the authors compared antigen-presenting cells (APCs) from nonprogressors (NPs) and progressors (PRs) for the ability to *trans* infect susceptible CD4$^+$ T cells. *Trans* infection is the process by which APCs such as dendritic cells (DCs) or certain B cells can take up HIV-1 and participate in enabling the infection of CD4$^+$ T cells (21). It is different from the standard cell-to-cell infection (*cis* infection) in that the APCs are not productively infected by HIV, so the virus they transduce to target cells is the virus they have captured and preserved. However, *trans* infection is similar to *cis* infection in that the target cells must express CD4 and coreceptor and the formation of a virological synapse is involved. Previous study by Viglianti's group demonstrated that *trans* infection by DCs depends on cholesterol levels and can be suppressed or stimulated by manipulating cellular cholesterol content via stimulation of the nuclear receptors LXR and PPARγ or the targeted knockdown of ABCA1, respectively (22). Rappocciolo et al. found that APCs (DCs and B cells) from NPs, when pulsed with HIV *in vitro*, did not *trans* infect autologous or heterologous CD4$^+$ T cells, whereas cells from PRs efficiently transferred the virus to susceptible cells. Importantly, the defect was localized to APCs, as T cells from both NPs and PRs were equally efficiently infected by cell-free virus.

In the authors' search for a mechanistic basis for this finding, they made a startling observation—the ability of APCs to *trans*

Published 10 June 2014

Citation Prasad VR, Bukrinsky MI. 2014. New clues to understanding HIV nonprogressors: low cholesterol blocks HIV *trans* infection. mBio 5(3):e01396-14. doi:10.1128/mBio.01396-14.

Address correspondence to Vinayaka R. Prasad, vinayaka.prasad@einstein.yu.edu, or Michael Bukrinsky, mbukrins@gwu.edu.

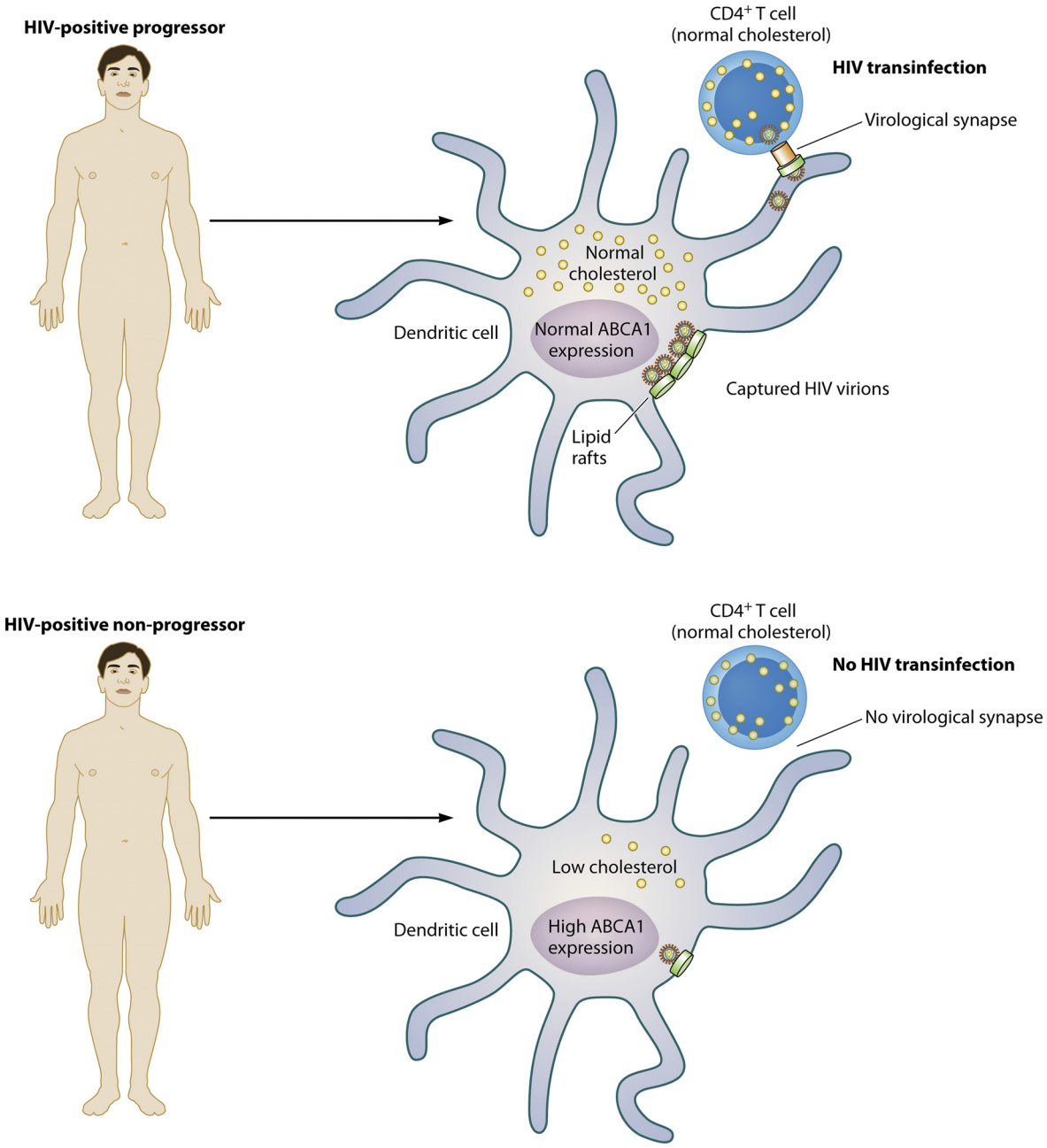

FIG 1 Schematic depicting the role of *ABCA1* expression levels in APCs during HIV-1 *trans* infection. Individuals with genetically determined high levels of *ABCA1* gene expression have lower cholesterol levels than normal subjects in cells expressing *ABCA1*, such as DCs, whereas the cholesterol levels in cells where *ABCA1* is not expressed, such as T cells, are similar in these two groups. The paper by Rappocciolo et al. suggests that HIV disease in individuals with high *ABCA1* expression progresses more slowly than in normal individuals. They demonstrate an absence of *trans* infection of $CD4^+$ T cells by DCs and B cells derived from NPs (individuals with high *ABCA1* expression levels). Cholesterol depletion has been shown to inhibit HIV uptake by DCs. Therefore, high *ABCA1* expression is expected to reduce HIV uptake and consequently *trans* infection. *Trans* infection proceeds through a virological synapse, which is formed with the participation of lipid rafts. Lipid rafts are reduced when *ABCA1* expression increases. See text for details.

infect $CD4^+$ T cells was directly correlated with APC cholesterol content. In other words, cells from NPs had lower cholesterol than cells from PRs. Replenishment of cholesterol in APCs from NPs reversed the block to *trans* infection, and depletion of cholesterol or inhibition of cholesterol biosynthesis with statins blocked the ability of APCs from PRs to transfer HIV to susceptible cells. Of note, the difference in APC cholesterol content between PRs and NPs is likely due, at least in part, to genetic or epigenetic regulation, as ABCA1 expression was higher in cells from NPs. These findings support a previously proposed role for ABCA1 as an innate anti-HIV-1 (and probably a more general antiviral) factor (17). In this case, the decreased cholesterol levels controlled by ABCA1 affect the ability of DCs and B cells to capture HIV and transfer it to susceptible cells in a process that requires tight cell-

to-cell contact to form a virological synapse (Fig. 1). Jolly and Sattentau previously showed that the formation of virological synapses between HIV-infected and uninfected T cells requires the integrity of lipid rafts (23). It is likely that these are the same membrane structures as those involved in the assembly of infectious HIV-1 particles. Thus, the mechanism of anti-HIV-1 activity of ABCA1 may be the same in all cells and comes down to reduction of lipid raft abundance. The authors examined the ability of B cells, isolated from PRs and NPs both before and after seroconversion, to participate in *trans* infection and found that the inability to *trans* infect existed prior to seroconversion, thus suggesting that the inability of APC to *trans* infect is an innate property of NPs. The specific genetic or epigenetic mechanisms underlying the high *ABCA1* expression in APCs from nonprogressors remain to be uncovered. It is not unlikely that this is due to single nucleotide polymorphism in *ABCA1* or genes encoding transcription factors that specifically regulate *ABCA1* expression in APCs.

Results of this study, while provocative and exciting, are only the first step in understanding the mechanisms that operate in the ability of some individuals to control HIV replication. It is most likely that several mechanisms that engage different molecular players are involved, and careful studies are needed to pinpoint the contribution of cholesterol to each mechanism. It will be important to determine whether cholesterol levels of APCs can explain the differences between elite controllers (undetectable viral loads), viremic controllers (plasma HIV-1 levels of 50 to 2,000 copies/ml), and viremic nonprogressors (variable viral loads but stable $CD4^+$ T cell counts). Although the sample size in the study by Rappocciolo et al. was small ($n = 8$), it is striking that the APCs from all 8 NP individuals were unable to effect *trans* infection.

Some questions remain unanswered. The authors demonstrated the dependence of *trans* infection on cholesterol by repleting APCs with cholesterol prior to infection. However, since repletion was performed prior to loading of the APCs with HIV, it is unclear if cholesterol is required for the process of HIV-1 capture by APCs, for the actual *trans* infection process, or for both. *Trans* infection of $CD4^+$ T cells by APCs, in particular DCs, is known to play a significant role in the mucosal transmission of HIV (24), but its contribution to the posttransmission stage of disease progression remains unknown. It is possible that B cells play a role at this step (25), but evidence for this remains scarce. It is also intriguing that the cholesterol levels in the recipient $CD4^+$ T cells did not matter; rather, the cholesterol levels in the APCs controlled *trans* infection. This conclusion was based on experiments where the authors used 20% serum to culture T cells; given low *ABCA1* expression in T cells, such culture conditions could overload the cells with cholesterol, masking differences between cells from PRs and those from NPs. High *ABCA1* expression in nonprogressors may also be responsible for the restricted infection of macrophages and other myeloid cells that are productively infected with HIV. This may limit the viral reservoir (26), contributing to nonprogression. In any case, the possibility that levels of *ABCA1* expression may determine the control of HIV infection deserves further study. The implications and translational opportunities are colossal, from preexposure prophylaxis to microbicides to clinical maintenance of HIV-infected subjects.

ACKNOWLEDGMENTS

This work was supported by NIH grants R01 HL093818 and R01 HL101274 to M.I.B. and R37 AI030861 to V.R.P., by the District of Columbia Developmental Center for AIDS Research (DC D-CFAR) (P30 AI087714), and by Einstein-Montefiore CFAR (P30 AI051519).

We thank Kartik Chandran for critical reading of the manuscript.

REFERENCES

1. **Walker BD, Yu XG.** 2013. Unravelling the mechanisms of durable control of HIV-1. Nat. Rev. Immunol. **13:**487–498. http://dx.doi.org/10.1038/nri3478.
2. **Rappocciolo G, Jais M, Piazza P, Reinhart TA, Berendam SJ, Garcia-Exposito L, Gupta P, Rinaldo CR.** 2014. Alterations in cholesterol metabolism restrict HIV-1 *trans* infection in nonprogressors. mBio **5**(3): e01031-13. http://dx.doi.org/10.1128/mBio.01031-13.
3. **Daya M, Cervin M, Anderson R.** 1988. Cholesterol enhances mouse hepatitis virus-mediated cell fusion. Virology **163:**276–283. http://dx.doi.org/10.1016/0042-6822(88)90267-X.
4. **Vashishtha M, Phalen T, Marquardt MT, Ryu JS, Ng AC, Kielian M.** 1998. A single point mutation controls the cholesterol dependence of Semliki Forest virus entry and exit. J. Cell Biol. **140:**91–99. http://dx.doi.org/10.1083/jcb.140.1.91.
5. **Danthi P, Chow M.** 2004. Cholesterol removal by methyl-beta-cyclodextrin inhibits poliovirus entry. J. Virol. **78:**33–41. http://dx.doi.org/10.1128/JVI.78.1.33-41.2004.
6. **Petersen J, Drake MJ, Bruce EA, Riblett AM, Didigu CA, Wilen CB, Malani N, Male F, Lee FH, Bushman FD, Cherry S, Doms RW, Bates P, Briley K, Jr..** 2014. The major cellular sterol regulatory pathway is required for Andes virus infection. PLoS Pathog. **10**(2):e1003911. http://dx.doi.org/10.1371/journal.ppat.1003911.
7. **Ono A, Freed EO.** 2001. Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc. Natl. Acad. Sci. U. S. A. **98:** 13925–13930. http://dx.doi.org/10.1073/pnas.241320298.
8. **Mañes S, del Real G, Lacalle RA, Lucas P, Gómez-Moutón C, Sánchez-Palomino S, Delgado R, Alcamí J, Mira E, Martinez AC.** 2000. Membrane raft microdomains mediate lateral assemblies required for HIV-1 infection. EMBO Rep. **1:**190–196. http://dx.doi.org/10.1093/embo-reports/kvd025.
9. **Guyader M, Kiyokawa E, Abrami L, Turelli P, Trono D.** 2002. Role for human immunodeficiency virus type 1 membrane cholesterol in viral internalization. J. Virol. **76:**10356–10364. http://dx.doi.org/10.1128/JVI.76.20.10356-10364.2002.
10. **Viard M, Parolini I, Sargiacomo M, Fecchi K, Ramoni C, Ablan S, Ruscetti FW, Wang JM, Blumenthal R.** 2002. Role of cholesterol in human immunodeficiency virus type 1 envelope protein-mediated fusion with host cells. J. Virol. **76:**11584–11595. http://dx.doi.org/10.1128/JVI.76.22.11584-11595.2002.
11. **Liao Z, Cimakasky LM, Hampton R, Nguyen DH, Hildreth JE.** 2001. Lipid rafts and HIV pathogenesis: host membrane cholesterol is required for infection by HIV type 1. AIDS Res. Hum. Retroviruses **17:**1009–1019. http://dx.doi.org/10.1089/088922201300343690.
12. **Nguyen DH, Hildreth JE.** 2000. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. J. Virol. **74:**3264–3272. http://dx.doi.org/10.1128/JVI.74.7.3264-3272.2000.
13. **Blanchette CD, Lin WC, Ratto TV, Longo ML.** 2006. Galactosylceramide domain microstructure: impact of cholesterol and nucleation/growth conditions. Biophys. J. **90:**4466–4478. http://dx.doi.org/10.1529/biophysj.105.072744.
14. **van 't Wout AB, Swain JV, Schindler M, Rao U, Pathmajeyan MS, Mullins JI, Kirchhoff F.** 2005. Nef induces multiple genes involved in cholesterol synthesis and uptake in human immunodeficiency virus type 1-infected T cells. J. Virol. **79:**10053–10058. http://dx.doi.org/10.1128/JVI.79.15.10053-10058.2005.
15. **Mujawar Z, Rose H, Morrow MP, Pushkarsky T, Dubrovsky L, Mukhamedova N, Fu Y, Dart A, Orenstein JM, Bobryshev YV, Bukrinsky M, Sviridov D.** 2006. Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. PLoS Biol. **4**(11):e365. http://dx.doi.org/10.1371/journal.pbio.0040365.
16. **Zheng YH, Plemenitas A, Fielding CJ, Peterlin BM.** 2003. Nef increases the synthesis of and transports cholesterol to lipid rafts and HIV-1 progeny virions. Proc. Natl. Acad. Sci. U. S. A. **100:**8460–8465. http://dx.doi.org/10.1073/pnas.1437453100.
17. **Cui HL, Grant A, Mukhamedova N, Pushkarsky T, Jennelle L, Dubrovsky L, Gaus K, Fitzgerald ML, Sviridov D, Bukrinsky M.** 2012.

HIV-1 Nef mobilizes lipid rafts in macrophages through a pathway that competes with ABCA1-dependent cholesterol efflux. J. Lipid Res. **53:** 696–708. http://dx.doi.org/10.1194/jlr.M023119.

18. **Morrow MP, Grant A, Mujawar Z, Dubrovsky L, Pushkarsky T, Kiselyeva Y, Jennelle L, Mukhamedova N, Remaley AT, Kashanchi F, Sviridov D, Bukrinsky M.** 2010. Stimulation of the liver X receptor pathway inhibits HIV-1 replication via induction of ATP-binding cassette transporter A1. Mol. Pharmacol. **78:**215–225. http://dx.doi.org/10.1124/mol.110.065029.
19. **Dubrovsky L, Van Duyne R, Senina S, Guendel I, Pushkarsky T, Sviridov D, Kashanchi F, Bukrinsky M.** 2012. Liver X receptor agonist inhibits HIV-1 replication and prevents HIV-induced reduction of plasma HDL in humanized mouse model of HIV infection. Biochem. Biophys. Res. Commun. **419:**95–98. http://dx.doi.org/10.1016/j.bbrc.2012.01.137.
20. **Jiang H, Badralmaa Y, Yang J, Lempicki R, Hazen A, Natarajan V.** 2012. Retinoic acid and liver X receptor agonist synergistically inhibit HIV infection in $CD4^+$ T cells by up-regulating ABCA1-mediated cholesterol efflux. Lipids Health Dis. **11:**69. http://dx.doi.org/10.1186/1476-511X-11-69.
21. **Rinaldo CR.** 2013. HIV-1 *trans* infection of CD4(+) T cells by professional antigen presenting cells. Scientifica (Cairo) 2013:164203. http://dx.doi.org/10.1155/2013/164203.
22. **Hanley TM, Blay Puryear W, Gummuluru S, Viglianti GA.** 2010. PPARgamma and LXR signaling inhibit dendritic cell-mediated HIV-1 capture and trans-infection. PLoS Pathog. **6:**e1000981. http://dx.doi.org/10.1371/journal.ppat.1000981.
23. **Jolly C, Sattentau QJ.** 2005. Human immunodeficiency virus type 1 virological synapse formation in T cells requires lipid raft integrity. J. Virol. **79:**12088–12094. http://dx.doi.org/10.1128/JVI.79.18.12088-12094.2005.
24. **McDonald D.** 2010. Dendritic cells and HIV-1 trans-infection. Viruses **2:**1704–1717. http://dx.doi.org/10.3390/v2081704.
25. **Rappocciolo G, Piazza P, Fuller CL, Reinhart TA, Watkins SC, Rowe DT, Jais M, Gupta P, Rinaldo CR.** 2006. DC-SIGN on B lymphocytes is required for transmission of HIV-1 to T lymphocytes. PLoS Pathog. **2**(7): e70. http://dx.doi.org/10.1371/journal.ppat.0020070.
26. **Kumar A, Abbas W, Herbein G.** 2014. HIV-1 latency in monocytes/macrophages. Viruses **6:**1837–1860. http://dx.doi.org/10.3390/v6041837.

The views expressed in this Commentary do not necessarily reflect the views of this journal or of ASM.