STUDIES OF THE ROLE OF CD8⁺ T LYMPHOCYTES ON THE ESTABLISHMENT OF SIV RESERVOIR UNDER ART

0371

Maura Statzu[1], Andrew Wong[1], Julia McBrien[1], Jin Wang[2], Miles Davenport[2], Brandon Keele[3], Thomas H. Vanderford[1], Ann Chahroudi[4], Mirko Paiardini[1], Guido Silvestri[1], Deanna A. Kulpa[1]

[1]Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA. [2]Infection Analytics Program, Kirby Institute for Infection and Immunity, University of New South Wales, Sydney, Australia. [3]AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, MD, USA. [4]Emory University, Atlanta, GA, USA.

BACKGROUND

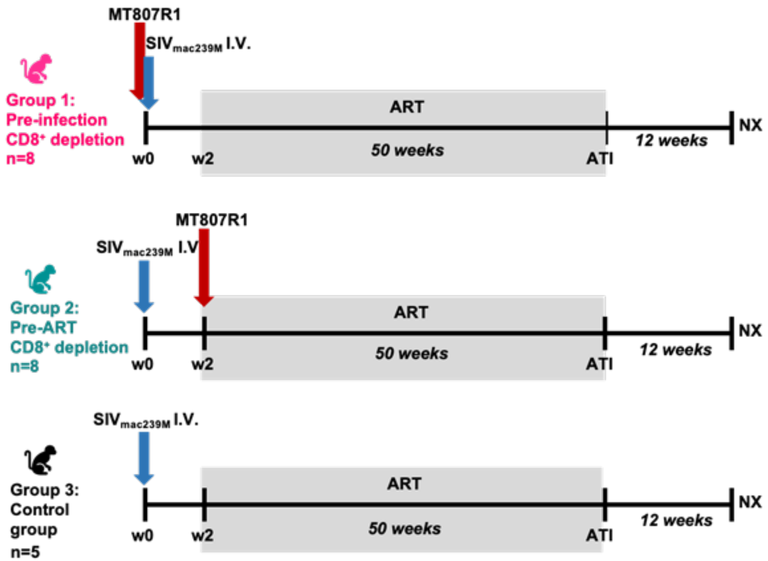
Human Immunodeficiency Virus (HIV) remains a major threat to human health, with an estimated 680,000 deaths worldwide in 2020[1]. Despite a major decline in HIV-induced morbidity and mortality which followed the introduction of antiretroviral therapy (ART), there is still neither a vaccine nor a cure for this infection. The critical barrier to an HIV cure is the presence of a population of latently-infected cells harboring integrated replication-competent virus (often referred to as "viral reservoir") which persists indefinitely in ART-treated people living with HIV. Despite significant research efforts, the mechanisms responsible for the establishment and maintenance of the persistent reservoir under ART remain incompletely understood in both HIV infection of humans and simian immunodeficiency virus (SIV) infection of rhesus macaques (*Macaca mulatta*, RMs), which represents the best validated and most used non-human primate model for HIV infection and AIDS[2]. In this regard, a key unanswered question is whether the latently-infected CD4⁺ T cells that comprise the persistent reservoir under ART are formed following a phase of productive infection or, alternatively, directly after virus integration and without any expression of virus gene products.

Many lines of evidence indicate that CD8⁺ T cell-mediated CTL activity plays an important role in controlling virus replication during the acute phase of HIV and SIV infection[3]. First, the post-peak decline of viremia only occurs after the emergence of virus-specific CD8⁺ T cells, suggesting that CD8⁺ T cells are involved in the initial control of viral replication[4,5]. Second, during the first weeks of infection viral mutants capable of escaping the CD8⁺ T cell response begin to appear and rapidly become fixed in the overall virus population, thus demonstrating a strong evolutionary pressure imposed on the virus to escape immunological recognition by CD8⁺ T cells[6-9]. Third, and more directly, depletion of CD8⁺ T cells during acute SIV infection of RM results in the abrogation of the post-peak decline of viremia, confirming a critical role of these cells in the initial resolution of viral control[10,11]. Of note, a series of recent studies also suggest that CD8⁺ T cells inhibit virus production through non-cytolytic mechanisms that act specifically by suppressing HIV and SIV transcription[12,13], thus leading to a novel paradigm according to which CD8⁺ T cells suppress virus replication and influence the size and stability of the reservoir under ART through both "canonical", virus-specific CTL and non-cytolytic suppression of virus production[3].

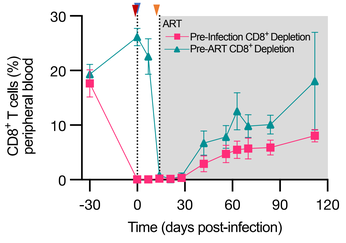
In this study, we used the previously validated *in vivo* experimental system of antibody-mediated CD8⁺ lymphocyte depletion[14,15] to directly test the two above-mentioned alternative hypotheses (i.e., direct formation of latency vs. induction of latency after of phase of productive infection) on the mechanisms responsible for the establishment of the persistent virus reservoir in SIV-infected, ART-treated RMs. The conceptual premise of this study was that CD8⁺ T-cell-mediated cytotoxic T lymphocyte (CTL) activity would clear a substantial fraction of productively infected cells during acute SIV infection, and therefore this activity would result in a net reduction of the overall reservoir size if this is indeed comprised primarily of cells that went through a phase of productive infection before entering latency. On the other hand, CD8⁺ T-cell-mediated CTL activity would not have a discernible impact on the reservoir size if this was comprised predominantly of latently-infected cells that are formed directly after virus integration. As such, we reasoned that by directly examining the effect of CD8+ lymphocyte depletion we could assess the role of these cells in the establishment of the virus reservoir during early infection and, by inference, we could determine whether and to what extent the latently infected CD4+ T cells that constitute the persistent reservoir under ART are formed after a period of active virus production that will make them sensitive to CTL activity.

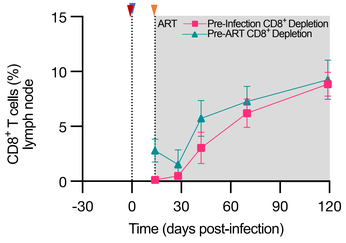
METHODS

A total of 21 RMs were infected with 10000 IU barcoded SIVmac239 I.V. and ART was initiated 2 weeks post-infection (tenofovir disoproxil fumarate + emtricitabine + dolutegravir). 8 RM received the CD8α-depleting antibody MT-807R1 before SIV infection, 8 RM were CD8 depleted just prior to ART initiation, and 5 SIV-infected ART treated RM served as the control group. After 50 weeks on ART, all RM underwent ATI and were followed for 12 weeks. SIV expression was monitored by plasma viral load.

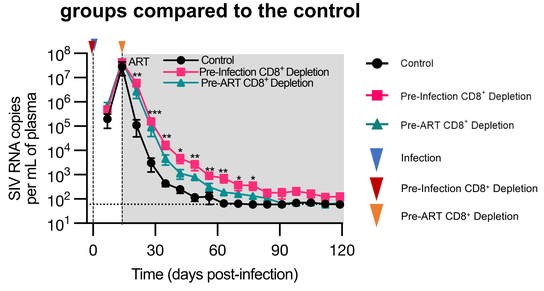


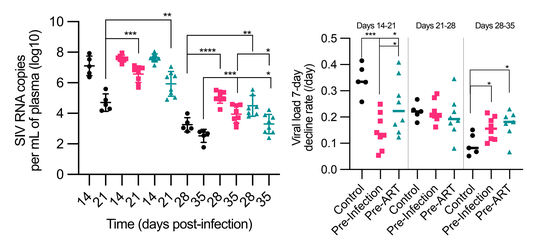
Kinetics of CD8⁺ T lymphocytes depletion





Plasma viral load decay was slower in the two intervention groups compared to the control





RESULTS

- Treatment with anti-CD8α MT807R1 depleted on average 99% of CD3⁺CD8⁺ T cells in peripheral blood and lymph nodes
- Reconstitution of CD8⁺ T cell was variable and occured by day 42 after antibody administration
- All animals showed suppression of viremia after ART, with plasma viral load below the detectable limit of our standard assay (60 copies per mL of plasma)
- Quantification of the viral load revealed a slower decline in plasma viremia in both the Pre-infection and Pre-ART initiation CD8⁺ T cell depleted animals

CONCLUSIONS

It is well established from numerous studies that CD8⁺ T cells are key players in the antiviral response to HIV and SIV during each stage of infection, including when the infection is treated with ART. As expected, the data indicates that CD8⁺ T lymphocyte depletion was followed by slower decline in virus replication. Overall, the current set of results confirms that experimental depletion of CD8⁺ T lymphocytes during SIV infection of macaques remains a valuable research tool to investigate the mechanisms by which these cells suppress virus replication *in vivo,* and represent a valuable experimental model to understand the mechanisms by which cytolytic and non-cytolytic CD8⁺ T cell functions impact the establishment and maintenance of the virus reservoir under ART.

References

1. UNAIDS. UNAIDS Data 2021. Joint United Nations; Program on HIV/AIDS (2021)
2. Evans DT, Silvestri G., Curr Opin HIV AIDS. 2013
3. McBrien JB et al., Eur J Immunol. 2018 \ \
4. Koup RA et al., J Virol. 1994
5. Borrow P et al., J Virol. 1994
6. Borrow P et al., Nat Med 1997
7. Chen ZW et al., The Journal of Immunology 2000.
8. McMichael AJ and Phillips RE, Annual Review of Immunology 1997
9. Vanderford TH et al., PLoS Pathog 2011\\\
10. Matano T et al., Journal of Virology 1998
11. Schmitz JE et al., The American Journal of Pathology 1999
12. Zanoni M et al., PLoS Pathog. 2020
13. Cartwright EK et al., Immunity. 2016
14. McBrien JB et al., Nature 2020
15. Mavigner M et al., J Virol. 2020

ADDITIONAL KEY INFORMATION

Author Contact Information
Maura Statzu, Ph.D.
Division of Microbiology & Immunology
Yerkes National Primate Research Center
Atlanta, GA 30329 USA .
Phone: 470-556-2725.
Email: maura.statzu@emory.edu

Acknowledgements

The authors would like to thank all the animal care and veterinary staff at the Yerkes National Primate Research Center; Barbara Cervasi and Kiran Gill at the Emory University Flow Cytometry Core; the Emory CFAR Virology Core for viral loads. We would also like to acknowledge Keith Reimann and Diogo Magnani with the NHP Reagent Resources for the MT807R1 antibody, Romas Geleziunas and Gilead Pharmaceuticals for providing Tenofovir and Emtricitabine, and Jim Demarest and ViiV Healthcare for providing Dolutegravir for this study.

This work is supported by UM1AI164562, co-funded by National Heart, Lung and Blood Institute, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Neurological Disorders and Stroke, National Institute on Drug Abuse and the National Institute of Allergy and Infectious Diseases, and by the National Institutes of Health's Office of the Director, Office of Research Infrastructure Programs, P51 OD011132 and U42 OD011023.