Title
Role of CD8+ cells in controlling replication of nonpathogenic simian immunodeficiency virus SIVmac1AII

Permalink
https://escholarship.org/uc/item/3sb3x36p

Journal
Virology Journal, 3(1)

ISSN
1743-422X

Authors
Van Rompay, Koen K
Blackwood, Emily J.
Landucci, Gary
et al.

Publication Date
2006-04-03

DOI
10.1186/1743-422X-3-22

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, available at https://creativecommons.org/licenses/by/4.0/

Peer reviewed
Role of CD8+ cells in controlling replication of nonpathogenic Simian Immunodeficiency Virus SIVmac1A11

Koen KA Van Rompay*1, Emily J Blackwood1, Gary Landucci2, Don Forthal2 and Marta L Marthas1,3

Address: 1California National Primate Research Center, University of California, Davis, California, USA, 2Division of Infectious Diseases, Department of Medicine, University of California, Irvine School of Medicine, Irvine, California, USA and 3Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California, USA

Email: Koen KA Van Rompay* - kkvanrompay@ucdavis.edu; Emily J Blackwood - ejblackwood@ucdavis.edu; Gary Landucci - glanducc@uci.edu; Don Forthal - dnfortha@uci.edu; Marta L Marthas - mlmarthas@ucdavis.edu

* Corresponding author

Abstract

Infection of macaques with the avirulent molecular clone SIVmac1A11 results in transient low viremia and no disease. To investigate if this low viremia is solely due to intrinsic poor replication fitness or is mediated by efficient immune-mediated control, 5 macaques were inoculated intravenously with SIVmac1A11. Three animals that were depleted of CD8+ cells at the start of infection had more prolonged viremia with peak virus levels 1 to 2 logs higher than those of 2 animals that received a non-depleting control antibody. Thus, CD8+ cell-mediated immune responses play an important role in controlling SIVmac1A11 replication during acute viremia.

Simian immunodeficiency virus (SIV) infection of macaques has proven useful for modeling HIV disease pathogenesis and intervention strategies [1-3]. While infection of macaques with most SIV isolates results eventually in a AIDS-like disease, there are also attenuated isolates and clones. SIVmac1A11 is a molecular clone originally derived from a virus isolate from an SIV-infected macaque that was also the source of virulent uncloned SIVmac251 isolates [4,5]. Although the kinetics are slower than for other isolates, SIVmac1A11 replicates well in vitro and is highly cytopathogenic (with induction of syncytia) in T-cell lines and rhesus macaque peripheral blood mononuclear cells (PBMC); SIVmac1A11 replicates well in macrophage cultures [6]. In early studies, it was observed that SIVmac1A11 inoculation of juvenile macaques resulted in transient viremia and no disease, even after prolonged follow-up for more than 12 years ([4]; unpublished observations). Subsequent studies documented that SIVmac1A11 inoculation of fetal and newborn macaques also resulted in transient viremia and no disease [7,8]. SIVmac1A11 has a tissue distribution distinct from that of virulent isolates [9].

Because of these unique properties, SIVmac1A11 has proven useful to study determinants of viral virulence. The genome of SIVmac1A11 has been sequenced, and recombination experiments revealed that differences in more than one region of the viral genome were responsible for the lack of virulence [5,10]. SIVmac1A11 has also shown promise as a live-attenuated vaccine in both infant and juvenile/adult macaques [10-13].

The transient low-level viremia (peak levels ≤ 4 to 5 log RNA copies per ml plasma) that results from SIVmac1A11
infection suggests either poor intrinsic replication fitness in vivo and/or relatively effective immune control. CD8+ cell depletion experiments (via administration of monoclonal antibody) have demonstrated the important role of CD8+ cell-mediated immune responses in controlling acute and chronic viremia with virulent SIV isolates (such as SIVmac251; [14]) and chronic viremia with the attenuated clone SIVmac239Δ nef [15]; however, CD8+ cell depletion had no detectable effect on viremia in animals chronically infected with the more attenuated clone SIVmac239A3 [16] or with SIVmac1A11 (unpublished data). To our knowledge, no CD8+ cell depletion experiments have been performed during acute infection with nonpathogenic SIV isolates.

Accordingly, we sought to determine the role of CD8+ cell-mediated immune responses on acute SIVmac1A11 viremia. Animals in this study were juvenile rhesus macaques (Macaca mulatta; ~1 year of age), housed in accordance with American Association for Accreditation of Laboratory Animal Care Standards with strict adherence to the "Guide for the Care and Use of Laboratory Animals" [17]. When necessary, the animals were immobilized with ketamine HCL (Parke-Davis, Morris Plains, New Jersey) 10 mg/kg injected intramuscularly.

All 5 macaques were inoculated intravenously with a high dose of SIVmac1A11 (5 \times 10^5 50% tissue culture infectious doses, grown in CEMx174 cells). Immediately before virus inoculation, 3 animals were depleted of CD8+ cells via administration of the anti-CD8 antibody cM-T807 at a dose of 50 mg/kg body weight (administered slowly intravenously); the same dose was repeated 3 weeks later. This dosage regimen, which is higher than the regimen used in previous CD8+ cell depletion studies [14,18], was selected because it gives more prolonged depletion of CD8+ cells (K. Reimann, personal communication). In the current study, CD8+ cells (both CD8+CD3+ T lymphocytes and CD8+CD3- NK cells) in peripheral blood were undetectable or low (< 1% of lymphocytes; ≤ 40 cells per µl blood) for 21 to 35 days after treatment (Fig. 1B,C). The remaining 2 animals received a control (i.e., non-depleting) human immunoglobulin preparation (Aventis Gammar-P I.V.) at the same dosage regimen (50 mg/kg at 0 and 3 weeks).

The 2 control-antibody treated animals had peak plasma viral RNA levels of 4 to 7 \times 10^3 copies/ml at 3 days after virus inoculation (Fig. 1A). For one animal (number 35391), a second smaller peak of viremia was observed on day 17. The levels of viremia in these 2 control animals are thus similar to those described previously for SIVmac1A11-infected juvenile macaques [19]. The 3 CD8+ cell-depleted animals had viral RNA levels during the first 7 days that were indistinguishable from those of the control animals, suggesting that during these early stages, CD8+ cells had no detectable role in controlling SIVmac1A11 replication. However, after an initial decline, viral RNA levels in the CD8+ cell-depleted animals increased from day 10 onwards and reached peak levels of 45,000 to 790,000 on day 17; these values were 1–2 log higher than those of the control animals (p = 0.015, two-tailed t-test comparing day 17 values, and area-under-the curve values for day 0 to 35) and only ~1–2 log lower than peak viremia levels observed with the pathogenic molecular clone SIVmac239 [20-22]. Despite this higher viremia in the CD8+ cell-depleted animals, there were no significant changes in CD4+CD3+ T lymphocyte counts in peripheral blood (Fig. 1D): this study was not designed to monitor CD4+CD3+ T lymphocyte levels in gut-associated lymphoid tissue. Plasma viral RNA levels declined upon the return of CD8+ cells and became undetectable from 28 to 35 days of infection onwards throughout the rest of the observation period (> 6 months). These results indicate that CD8+ cells play a major role in controlling SIVmac1A11 replication because in their absence, peak viremia was higher and the acute viremia phase was significantly prolonged.

Because the cM-T807 antibody depletes both CD8+ T lymphocytes as well as NK cells, the relative contribution of each cell type could not be determined. CD8+ T cells and NK cells inhibit virus replication in vitro through a variety of mechanisms, including cytolytic and non-cytolytic pathways [23-25]. Most NK cells also have the low-affinity Fc-gamma III receptor (CD16), which triggers antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell-mediated virus inhibition (ADCVI). ADCVI is similar to ADCC but is a measure of virus inhibition, rather than target cell cytotoxicity. ADCVI has been observed ex vivo with serum and effector cells from HIV-infected humans and SIV-infected macaques [26](Forthal et al, manuscript submitted). Forthal et al. have also demonstrated that ADCVI-mediating antibodies can be found early during HIV-1 infection and reduce HIV-1 yield both by lysis of infected target cells and by the release of beta-chemokines from NK effector cells [27,28]. In the current study, the SIVmac1A11-infected animals had detectable antiviral IgG antibodies (as measured by whole SIV ELISA techniques; [29]) at ~2 weeks of infection, and the CD8+ cell-depleted animals had a more rapid increase in antibody titers, possibly due to more antigenic stimulation (Fig. 1E). Early plasma samples were also tested for ADCVI activity; pronounced inhibition (> 70%) was observed in plasma collected at 17 days of infection at a 1:100 dilution in all animals (Fig. 1F). Thus, some of the loss of viremia control following CD8+ cell depletion could be due to the loss of CD8+ NK cells that would likely serve as ADCVI effector cells.
Figure 1
Effect of CD8+ cell depletion on SIVmac1A11 infection: viral and immunologic parameters. Five animals were inoculated with SIVmac1A11 at time zero. Three animals were CD8+ cell depleted via administration of cM-T807 while the other 2 animals received control antibody. (A) Viral RNA levels in plasma (measured by bDNA assay, with a limit of detection of 125 copies/ml; [18]). Results from virus isolation from 1 million PBMC, using CEMx174 cells and p27 measurement [34] are given as positive (+) or negative (–). The absolute counts of CD8+CD3+ T lymphocytes, CD8+CD3- NK cells and CD4+CD3+ T lymphocytes were measured according to flow cytometry techniques described previously [18], and are presented in graphs B through D, respectively. (E) SIV-specific IgG titers measured by a whole SIV ELISA [29]; although the CD8+ cell depleted animals made a faster response than the undepleted animals, from week 6 onwards, both animal groups had similar antiviral IgG titers (1: 6,400 to 1: 25,600). (F) Antiviral activity of plasma collected at 17 days after SIVmac1A11 inoculation as measured in an ADCVI assay, described in detail elsewhere (Forthal et al., submitted for publication). Briefly, CEMx174 cells were infected with SIVmac1A11 at a MOI of 0.01; 48 hours later, cells were plated in 96-well plates at 50,000 cells per well. Plasma samples (including negative and positive control samples) were added at a 1:100 dilution and human PBMC effector cells were added to obtain an effector:target cell ratio of 10:1. Five days later, SIV p27 was measured in supernatant fluid using a commercially available ELISA (Zeptometrix Corporation, Buffalo, NY). Percent inhibition by the plasma samples collected on day 17 was calculated relative to the level of virus replication in the presence of plasma collected on day zero (before SIVmac1A11 inoculation); the presented values represent mean +/- SEM of 4 separate assays (with effector PBMC of 4 different donors). In the absence of effector cells, no significant inhibition (≤ 11%) was observed (data not shown).
Levels of interleukin-12 and interferon-α were measured in plasma using commercial ELISA-kits (monkey IL-12 ELISA, U-CyTech, Utrecht, the Netherlands; human interferon-α ELISA kit, PBL Biomedical Laboratories, Piscataway, NJ). Although variable levels were detected for both cytokines, there was no correlation with virus levels (data not shown).

In conclusion, this experiment demonstrated that the acute low-level viremia of SIVmac1A11 which is observed following inoculation of untreated animals cannot be explained solely by poor intrinsic replication fitness of the virus; instead, immune responses that are dependent on CD8+ cells limit the magnitude and duration of acute viremia. Viremia of SIVmac1A11 has always been observed to be transient (~2–6 weeks), even following inoculation of fetal and newborn macaques [7,8]. This indicates that these antiviral immune responses are not abrogated or prevented from emerging during acute SIVmac1A11 viremia and suggests that there is relatively little or no virus-induced immunosuppression. Rather, the anti-SIVmac1A11 immune responses appear able to induce a long-term asymptomatic infection [10]. This is in contrast to infection with virulent SIV isolates, for which irreversible damage to the immune system appears to occur early during the course of infection [9,30-32]. Accordingly, further experiments that combine avirulent strains such as SIVmac1A11 infection with selective deletions of immune cell populations may prove to be a useful and sensitive model to further unravel precisely the immune responses that are important to control viremia, but that may be difficult to detect during infection with virulent isolates. Attempts to boost or preserve such immune responses may lead to immunotherapeutic strategies that are more effective in achieving long-term control on viremia of virulent virus isolates, including HIV-1.

Competing interests
The author(s) declare that they have no competing interest.

Authors’ contributions
KVR designed and coordinated the study, and drafted the manuscript; EB performed and analyzed viral and immunological assays; GL and DF performed and analyzed the ADCVI assays; MM participated in the design and interpretation of the study. All authors helped with and approved the final manuscript.

Acknowledgements
We thank I. Cazares, T. Dearman, L. Hirst, A. Spinner, W. von Morgenland, the Veterinary Staff, Colony Services, and Clinical Laboratory of the California National Primate Research Center, for expert technical assistance; the Bayer Reference Testing Laboratory (Emeryville, California) for bDNA analysis; K. Reimann (Harvard Medical School, Boston) for assistance and useful discussions. This research was supported by NIH/NIAID grants AI58056 (K.V.R.) and Public Science Health grant RR00169 from the National Center for Research Resources. Reagents used in this work were provided by the NIH Nonhuman Primate Reagent Resource (RR016001 and AI040101) and produced by the National Cell Culture Center.

References
1. Staprans SI, Feinberg MB: The role of nonhuman primates in the preclinical evaluation of candidate AIDS vaccines. Expert Rev Vaccines 2004, 3(4 Suppl):SS-32.
2. Haigwood NL: Predictive value of primate models for AIDS. AIDS Reviews 2004, 6(4):187-198.
3. Van Rompay KKA: Antiretroviral drug studies in non-human primates: a valid animal model for innovative drug efficacy studies? Pathogenesis of simian immunodeficiency virus. AIDS Reviews 2005, 7:67-83.
4. Marthas ML, Banapour B, Surjito S, Siegel ME, Marx PA, Gardner MB, Pedersen NC, Luciw PA: Rhesus macaques inoculated with molecularly cloned simian immunodeficiency virus. Journal of Medical Primatology 1989, 18:311-319.
5. Luciw PA, Shaw KE, Lusinskas V, Stout MW, Lackner JE, Pratt-Lowe E, Leung NJ, Banapour B, Marthas ML: Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIV-mac). AIDS Res Hum Retroviruses 1992, 8(2):395-402.
6. Banapour B, Marthas ML, Murn M, Luciw PJ, Marx PA: In vitro macrophage tropism of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus. Virology 1991, 183:1-12.
7. Tarantal AF, Marthas ML, Gargosky SE, Otsyula M, McIntosh MB, Miller CJ, Hendrickx AG: Effects of viral virulence on intrauterine growth in SIV-infected fetal rhesus macaques Macaca mulatta. J Acquir Immune Defic Syndr Hum Retrovirology 1995, 10(2):129-138. Oct 1
8. Marthas ML, Van Rompay KKA, Otsyula M, Miller CJ, Canfield D, Pedersen NC, McIntosh MB: Viral factors determine progression to AIDS in simian immunodeficiency virus-infected newborn rhesus macaques. Journal of Virology 1995, 69(7):4198-4205.
9. Lackner AA, Vogel P, Ramos RA, J. D. Kluge JD, Marthas M: Early events in tissues during infection with pathogenic (SIVmac239) and non-pathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus. American Journal of Pathology 1994, 145(2):428-439.
10. Marthas ML, Ramos RA, Lohman BL, Van Rompay KKA, Unger RE, Miller CJ, Banapour B, Pedersen NC, Luciw PA: Viral determinants of simian immunodeficiency virus (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. J Virol 1993, 67(10):6047-6055.
11. Marthas ML, Surjito S, Higgins J, Lohman B, Toret J, Luciw PA, Marx PA, Pedersen NC: Immunization with a live, attenuated simian immunodeficiency virus (SIV) prevents early disease but does not infection in rhesus macaques challenged with pathogenic SIV. J Virol 1990, 64(8):3693-3700.
12. Van Rompay KKA, Greenier JL, Cole KS, Earl P, Moss B, Steckbeck JD, Pahar B, Rourke T, Montefiori DC, Tarara RP, Miller CJ, McIntosh MB, Marthas ML: Immunization of newborn rhesus macaques with simian immunodeficiency virus (SIV) vaccines prolongs survival after oral challenge with virulent SIVmac251. Journal of Virology 2003, 77:179-190.
13. Otsyula MG, Miller CJ, Tarantal AF, Marthas ML, Greene TP, Collins JL, Van Rompay KKA, McChesney MB: Fetal or neonatal infection with attenuated simian immunodeficiency virus results in protective immunity against oral challenge with pathogenic SIVmac251. J Virol 1996, 70(10):6275-6278.
14. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Khunkhun RS, McEvers KJ, Gillis J, Pataki M, Lifton MA, Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus. AIDS Reviews 2005, 7:67-83.
Reichel K, Gelman RS, Letvin NL, Montefiori DC, Ruprecht RM, Desrosiers RC, Reimann KA: Effect of CD8+ lymphocyte depletion on virus containment after simian immunodeficiency virus SIVmac251 challenge of live attenuated SIVmac239delta3-vaccinated rhesus macaques. J Virol 2005, 79(13):8131-8141.

17. National Research Council: Guide for the care and use of laboratory animals. Washington, D.C., National Academy Press; 1996.

18. Van Rompay KKA, Singh RP, Pahar B, Sodora DL, Wingfield C, Lawson JR, Marxhas ML, Bischofberger N: CD8+ cell-mediated suppression of virulent simian immunodeficiency virus during tenofovir treatment. Journal of Virology 2004, 78:5324-5337.

19. Miller CJ, Mathews M, Greenier J, Lu D, Deley P, Lu Y. In vivo replication capacity rather than in vitro macrophage tropism predicts efficiency of vaginal transmission of simian immunodeficiency virus or simian/human immunodeficiency virus in rhesus macaques. Journal of Virology 1998, 72(4):3248-3258.

20. Compton L, Rourke T, Montefiori D, Lu D, Rothuesler K, Fritts L, Bost K, Miller CJ: Simian-human immunodeficiency virus SHIV89.6-induced protection against intravaginal challenge with pathogenic SIVmac239 is independent of the route of immunization and is associated with a combination of cytotoxic T-lymphocyte and alpha interferon responses. Journal of Virology 2003, 77(5):3099-3118.

21. Horton H, Vogel TU, Carter DK, Vielhuber K, Fuller DH, Shipley T, Fuller JT, Kunsmann K, Sutter G, Montefiori DC, Erfle V, Desrosiers RC, Wilson N, Picker LJ, Wolinsky SM, Wang C, Allison DB, Watkins DJ: Immunization of rhesus macaques with a DNA prime-modified vaccinia virus Ankara boost regimen induces broad simian immunodeficiency virus (SIV)-specific T-cell responses and reduces initial viral replication but does not prevent disease progression following challenge with pathogenic SIVmac239. J Virol 2002, 76(4):1877-1822.

22. Lifson JD, Platak M, Cline AN, Rossio JL, Purcell J, Pandre D, Bischofberger N, Blanchard J, Veazey RS: Transient early post-inoculation anti-retroviral treatment facilitates controlled infection with sparing of CD4+ T cells in gut-associated lymphoid tissues in SIVmac239-infected rhesus macaques, but not resistance to rechallenge. J Med Primatol 2003, 32(4-5):201-210.

23. Bernstein HB, Kinter AL, Jackson R, Fauci AS: Neonatal natural killer cells produce chemokines and suppress HIV replication in vitro. AIDS Res Hum Retroviruses 2004, 20(11):1189-1195.

24. Oliva A, Kinter AL, Vaccarezza M, Rubbert A, Catanzaor A, Moir S, Monaco J, Ehler L, Mizell S, Jackson R, Li Y, Romano JW, Fauci AS: Natural killer cells from human immunodeficiency virus (HIV)-infected individuals are an important source of CC-chemokines and suppress HIV-1 entry and replication in vitro. Journal of Clinical Investigation 1998, 102(1):223-231.

25. Dines I, Rumpenat VM, Persechini PM: What is going on with natural killer cells in HIV infection? Int Arch Allergy Immunol 2004, 133(4):330-339.

26. Brenner BG, Gryllis C, Wainberg MA: Role of antibody-dependent cellular cytotoxicity and lymphokine-activated killer cells in AIDS and related diseases. J Leukoc Biol 1991, 50(6):628-640.

27. Forthal DN, Landucci G, Daar ES: Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural killer effector cells. Journal of Virology 2001, 75(15):6953-6961.

28. Forthal DN, Landucci G, Pan TH, Becerra J: Interactions between natural killer cells and antibody Fc result in enhanced antibody neutralization of human immunodeficiency virus type 1. J Virol 2005, 79(4):2042-2049.

29. Van Rompay KKA, Singh R, Brignolo L, Lawson JR, Schmidt KA, Pahar B, Canfield DR, Tarara RP, Bischofberger N, Marthas ML, Theran M: The clinical benefits of tenofovir for simian immunodeficiency virus-infected macaques are larger than predicted by its effects on standard viral and immunological parameters. Journal of Acquired Immune Deficiency Syndromes 2004, 36(4):900-914.

30. Veazey RS, Lackner AA: Getting to the guts of HIV pathogenesis. J Exp Med 2004, 200(6):697-700.

31. Li Q, Duan L, Estes JD, Ma ZM, Rourke T, Wang Y, Reilly C, Carlis J, Miller CJ, Haase AT: Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 2005, 434(7037):1148-1152.

32. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M: Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 2005, 434(7037):1093-1097.

33. Van Rompay KKA, Marthas ML, Ramos RA, Mandelli CP, McGowan EK, Jowe SM, Pedersen NC: Simian immunodeficiency virus (SIV) infection of infant rhesus macaques as a model to test antiretroviral drug prophylaxis and therapy: oral 3'-azido-3'-deoxythymidine prevents SIV infection. Antimicrob Agents Chemother 1992, 36(11):2381-2386.