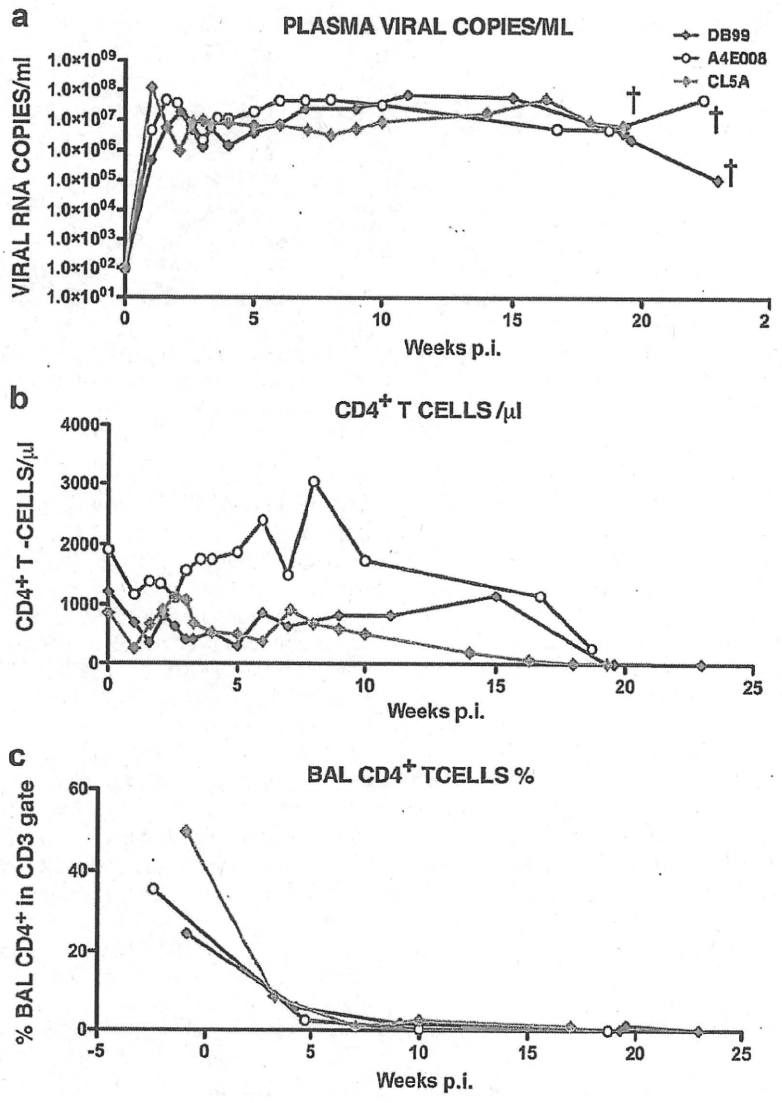


FIG. 7. Patterns of virus replication and CD4+T cell dynamics in $SHIV_{AD8}$ rapid progressors. The levels of plasma viremia (a), absolute numbers of peripheral $CD4^+$ T cells (b), and percentages of BAL fluid $CD4^+$ T cells (c) are shown. †, euthanized animals.

$>1 \times 10^7$ RNA copies/ml; rapid and irreversible loss of memory $CD4^+$ T cells in the blood and at an effector site (BAL); and intractable diarrhea, anorexia, and weight loss requiring euthanasia between weeks 19 and 23 p.i.

DISCUSSION

The results presented clearly show that the generation of a pathogenic R5-SHIV was not a trivial undertaking. Animal-to-animal passaging eventually gave rise to $SHIV_{AD8\#2}$, possessing greatly augmented infectivity for rhesus PBMC compared to the starting $SHIV_{AD8}$ construct. Although it was not appreciated at the time, $SHIV_{AD8\#2}$ had also acquired improved *in vivo* properties, as evidenced by its and its immediate derivatives' capacity to cause fatal immunodeficiency in 8 of 13 inoculated rhesus monkeys (Fig. 4 and Table 2). The most consistent and distinguishing property of the passaged $SHIV_{AD8}$ family of viruses during infections of rhesus macaques was the slow and unremitting loss of both memory and naïve $CD4^+$ T cells (Fig. 6), a pattern of depletion observed in all 10 NPs. Surprisingly, and in contrast to both SIVmac and SIVsmE lineages, the pace of $CD4^+$ T lymphocyte decline was not correlated with plasma virus loads. Although the geometric mean plasma viral-RNA level at week 50 in the $SHIV_{AD8}$-infected monkey cohort was 1.7×10^3 RNA copies/ml, the set-point virus loads varied widely in the 10 infected animals

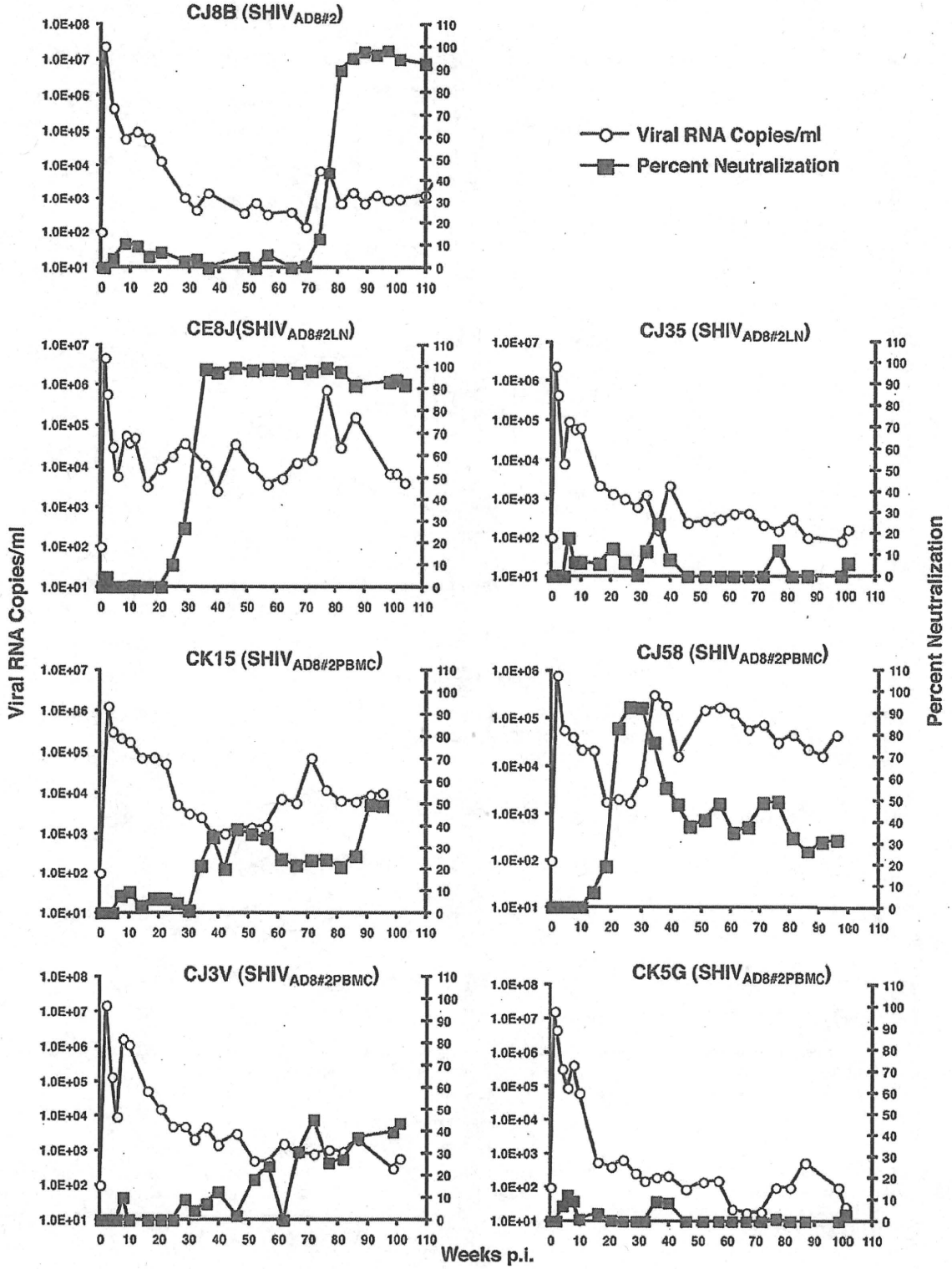
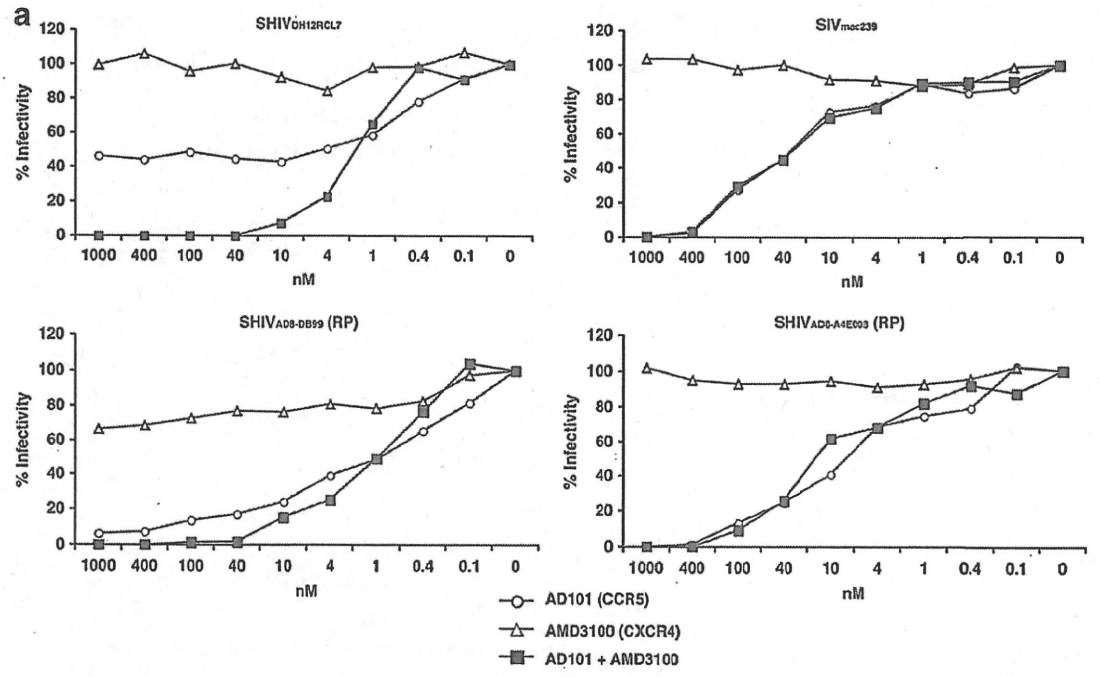


FIG. 8. Neutralizing-antibody activities detected in normal-progressor macaques following infection with SHIV$_{AD8\#2}$ or its immediate derivatives. Plasma samples (1:20 dilution) from the indicated SHIV$_{AD8}$-infected macaques were incubated in quadruplicate for 1 h at 37°C with the virus isolates shown in parentheses and then used as an inoculum to infect TZM-bl cells. The luciferase activity present in cell lysates at 28 h p.i. was measured, and the average percent neutralization activity in plasma at each time point was determined. Prechallenge plasma samples served as negative controls and baselines for zero neutralizing-antibody activity.



b

VIRUS		N-TERMINAL V3 STEM	CORECEPTOR USAGE
$SHIV_{AD8}$		NNTRKSI---HI--GPGR	CCR5
$SHIV_{AD8\text{-}DB99}$	Rapid Progressor	NNTRKSIRIGHI--GPGR	CXCR4/CCR5
$SHIV_{AD8\text{-}A4E008}$	Rapid Progressor	NNTRKSIHIGHI--GPGR	CCR5
$SHIV_{AD8\text{-}CL5A}$	Rapid Progressor	NNTRKSI---HI--GPGR	(Undetermined)
$SHIV_{162P3}$		NNTRKSI---TI--GPGR	CCR5
$SHIV_{162\text{-}BR24N}$	Rapid Progressor	NNTRKSI---TIHRGPGR	CXCR4

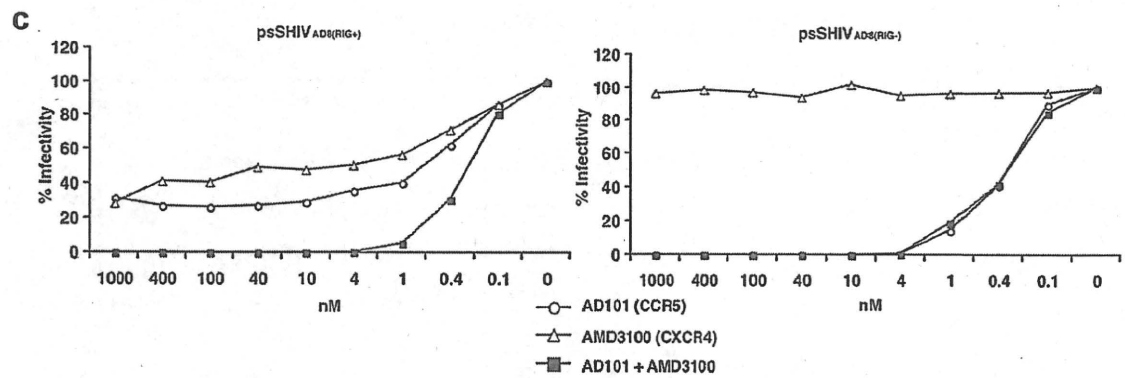


FIG. 9. Coreceptor utilization of $SHIV_{AD8}$ derivatives isolated from rapid progressors. (a) TZM-bl cells were infected in quadruplicate with viruses ($SHIV_{AD8\text{-}DB99}$ and $SHIV_{AD8\text{-}A4E008}$) recovered from rapid progressors DB99 and A4E008, respectively, in the presence of the indicated amounts of the small-molecule coreceptor inhibitors AD101 (CCR5), AMD 3100 (CXCR4), or both. SIV_{mac239} and $SHIV_{DH12RCL\text{-}7}$ were also analyzed as representative R5-tropic and dual-tropic viruses, respectively. The luciferase activities present in cell lysates 24 h p.i. were measured, and percent infectivities were determined in the absence or presence of coreceptor inhibitors. (b) gp120 sequences from the N-terminal V3 regions of $SHIV_{AD8}$ variants, recovered from three RP animals, were aligned with the starting $SHIV_{AD8}$ V3 loop. The V3 regions of the R5-tropic $SHIV_{SF162P3}$ and its $SHIV_{SF162\text{-}BR24N}$ derivative, which also emerged in an RP, are included in the alignment. (c) Coreceptor utilization of virus, pseudotyped with Envs present in RP DB99 at the time of necropsy, containing or lacking the 3-aa RIG V3 loop insertion.

TABLE 2. Clinical and pathological findings in rhesus monkeys infected with $SHIV_{AD8\#2}$ and its immediate derivatives

Animal	Clinical data/pathological findings
CJ8B	Euthanized (wk 199); uncontrolled diarrhea; wt loss
CK15	Euthanized (wk 112); *P. carinii* pneumonia
CJ58	Total $CD4^+$ T cells, 154/mm^3 (wk 111)
CE8J	Euthanized (wk 117); uncontrolled diarrhea, *C. coli* enteritis
CJ35	Total $CD4^+$ T cells, 270/mm^3 (wk 129)
CJ3V	Euthanized (wk 135); uncontrolled diarrhea; typhlocolitis
CK5G	Total $CD4^+$ T cells: 101/mm^3 (wk 101)
DB99	Euthanized (wk 23); rapid progressor
DA1Z	Total $CD4^+$ T cells, 545/mm^3 (wk 65)
A4E008	Euthanized (wk 20); rapid progressor
DA4W	Total $CD4^+$ T cells, 92/mm^3 (wk 64)
CL5A	Euthanized (wk 19); rapid progressor
CL98	Euthanized (wk 100); disseminated *M. avium*

(1.6×10^2 to 1.5×10^5 RNA copies/ml). This variability was also observed in pairs of animals inoculated with identical $SHIV_{AD8\#2}$ derivatives (*viz.* CK15 and CJ58, and DB99 and DA1Z). An extreme example of the nonlinkage between viral-RNA levels and $CD4^+$ T cell loss with $SHIV_{AD8}$ occurred with animal CK5G, which had 43 and 42 circulating naïve and memory $CD4^+$ T cells/μl, respectively, at week 86 p.i. and a plasma viral load of only 5.4×10^2 RNA copies/ml. During the chronic phase of $SHIV_{AD8}$ infections, the loss of naïve $CD4^+$ T cells was more rapid and more marked than the depletion of the memory subset, as was previously observed in SIVsmE543-infected animals (35) (Fig. 6). By week 80, for example, NPs had sustained an 87 to 93% loss of naïve $CD4^+$ T cells from their preinoculation levels, whereas the depletion of memory cells was significant, but not as pronounced. The dissociation of plasma virus loads and $CD4^+$ T cell loss is reminiscent of the previously reported infection of pig-tailed macaques with SIVl'hoest and SIVsun (4). In that study, 8 of 12 infected animals developed immunodeficiency over a 5-year period while maintaining set-point viremia between 10^2 and 10^3 RNA copies/ml.

We do not presently understand why naïve $CD4^+$ T lymphocytes are lost in $SHIV_{AD8}$ NPs. Based on coreceptor expression, this T cell subset expresses CXCR4, not CCR5, on its surface and should therefore be refractory to infection by R5-tropic SHIVs and virus-induced cell killing. An assessment of the coreceptor utilization status of late-stage viruses recovered from $SHIV_{AD8}$ NPs, in fact, revealed that a coreceptor switch had not occurred in these animals (see Fig. S2 in the supplemental material). Although a dissociation between viral-RNA levels and memory/naïve $CD4^+$ T cell loss was observed, the NPs did experience increased memory $CD4^+$ T lymphocyte turnover (see Fig. S1 in the supplemental material), even in animals with very low plasma virus loads. Activation-induced proliferation and killing of memory $CD4^+$ T cells during the lengthy chronic $SHIV_{AD8}$ infection might therefore be responsible for driving the differentiation of naïve $CD4^+$ lymphocytes into memory cells and impose an unsustainable drain on this $CD4^+$ T cell subset. It is also possible that $SHIV_{AD8}$ infection of rhesus macaques negatively affects naïve $CD4^+$ T lymphocyte homeostasis in the thymus, thereby impeding the differentiation or emigration of this T cell subset. It has also recently been reported that the loss of naive $CD4^+$ T cells during SIVsmE543 infections was associated with the presence of autoreactive antibodies to $CD4^+$ T lymphocytes, platelets, double-stranded DNA, and phospholipid (27). Increased numbers of circulating IgG-coated $CD4^+$ T cells were observed in that study, and the levels of autoreactive antibodies were correlated with the extent of naïve $CD4^+$ T cell depletion.

Approximately 20% of rhesus monkeys infected with SIVmac/SIVsm lineage viruses become RPs, experiencing persistently high virus set points, rapid and complete losses of memory $CD4^+$ T cells, undetectable or transient antiviral antibody responses, and early onset (3 to 6 months p.i.) of symptomatic disease (6). Despite losing virtually all of their memory $CD4^+$ T lymphocytes, SIV RPs, at the time of death, usually maintain preinoculation levels of naïve $CD4^+$ T cells (35). This was not the case for $SHIV_{AD8}$ RPs. Although all three experienced early and massive depletions of memory $CD4^+$ T cells, two of the infected macaques had lost virtually all of their naïve $CD4^+$ T cells at the time of euthanasia. In one of these animals (DB99), the virus recovered at the time of euthanasia, as well as a virus pseudotyped with an Env possessing the RIG insertion in the V3 loop, had acquired the capacity to infect cells expressing CXCR4 (Fig. 9a and c). Interestingly, coreceptor switching has been previously reported to occur during RP infections of macaques inoculated with a different R5-tropic SHIV, $SHIV_{SF162P3}$ (18, 19, 47). In one of the $SHIV_{SF162P3}$ coreceptor-switching events, the insertion of two positively charged amino acids (HR) immediately upstream of the V3 loop GPGR crown (Fig. 9b) was shown to confer X4 tropism (18). In the case of $SHIV_{AD8\text{-}DB99}$, a 3-aa (RIG) insertion, also located in the N-terminal V3 stem and which increased the net charge of the V3 loop from +3 to +5, was responsible for the acquisition of CXCR4 usage. The insertion of HIG at the same location of the $SHIV_{AD8\text{-}A4E008}$ V3 region did not affect the net charge and did not confer tropism for CXCR4-expressing cells.

Independent and unrecognized cross-species transmissions and spread of SIVsm at different U.S. primate facilities during the 1970s contributed to the emergence of SIVmac and SIVsmE660 lineages with distinctive replicative and pathogenic phenotypes. The serial passaging of $SHIV_{AD8}$ in rhesus monkeys described here also resulted in an AIDS-inducing primate lentivirus with its own characteristic properties. First, in contrast to commonly used pathogenic SIVs, $SHIV_{AD8\#2}$ and its immediate derivatives generated sustained but, as previously noted, highly variable set-point virus loads in NPs. Similarly variable viral loads were also observed in eight rhesus monkeys inoculated with four independent $SHIV_{AD8}$ stocks prepared from macaques CK15, CE8J, CL98, and CJ58 at the time of their euthanasia (data not shown). Profound depletions of both memory and naïve $CD4^+$ T cells, which accompany relatively low virus set points (geometric mean level, 1.7×10^3 RNA copies/ml) in NPs, is a second property that distinguishes the R5-tropic $SHIV_{AD8}$ from pathogenic SIVs. Finally, unlike SIVs, $SHIV_{AD8}$ RPs experience an initial loss of memory $CD4^+$ T lymphocytes and a later rapid deletion of naïve $CD4^+$ T cells prior to death, which in one animal occurred following a CCR5-to-CXCR4 coreceptor switch. Based on the results shown in Fig. 4 and Table 2, we plan to use and distribute

$SHIV_{AD8\#2LN}$, $SHIV_{AD8\#2PBMC}$, or the SHIVs recovered from NPs at the time of euthanasia ($SHIV_{AD8-CL98}$, $SHIV_{AD8-CK15}$, or $SHIV_{AD8-CE8J}$) as challenge viruses in vaccine experiments. Animals inoculated with cell-free preparations of the last group of viruses have experienced variable but sustained plasma viremia associated with a gradual but significant $CD4^+$ T cell loss during 30 weeks of infection. Some of these macaques have developed a rapid-progressor clinical course.

ACKNOWLEDGMENTS

We are indebted to Keith Reimann and the NIH Nonhuman Primate Reagent Resource for providing cM-T807; to the NIH AIDS Research and Reference Reagent Program for providing AMD3100; and to Julie Strizki, Schering-Plough, for providing AD101. We thank John Mascola for TZM-bl cells and instructions for performing virus neutralization assays, Robin Kruthers and Ranjini Iyengar for determining viral RNA levels, and Vanessa Hirsch for critical comments during the preparation of this paper. We appreciate the contributions of Boris Skopits in diligently assisting in the care and maintenance of our animals.

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

REFERENCES

1. **Amara, R. R., F. Villinger, J. D. Altman, S. L. Lydy, S. P. O'Neil, S. I. Staprans, D. C. Montefiori, Y. Xu, J. G. Herndon, L. S. Wyatt, M. A. Candido, N. L. Kozyr, P. L. Earl, J. M. Smith, H. L. Ma, B. D. Grimm, M. L. Hulsey, J. Miller, H. M. McClure, J. M. McNicholl, B. Moss, and H. L. Robinson.** 2001. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science **292**:69–74.
2. **Barnett, S. W., I. K. Srivastava, E. Kan, F. Zhou, A. Goodsell, A. D. Cristillo, M. G. Ferrai, D. E. Weiss, N. L. Letvin, D. Montefiori, R. Pal, and M. Vajdy.** 2008. Protection of macaques against vaginal SHIV challenge by systemic or mucosal and systemic vaccinations with HIV-envelope. AIDS **22**:339–348.
3. **Barouch, D. H., S. Santra, J. E. Schmitz, M. J. Kuroda, T. M. Fu, W. Wagner, M. Bilska, A. Craiu, X. X. Zheng, G. R. Krivulka, K. Beaudry, M. A. Lifton, C. E. Nickerson, W. L. Trigona, K. Punt, D. C. Freed, L. Guan, S. Dubey, D. Casimiro, A. Simon, M. E. Davies, M. Chastain, T. B. Strom, R. S. Gelman, D. C. Montefiori, M. G. Lewis, E. A. Emini, J. W. Shiver, and N. L. Letvin.** 2000. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science **290**:486–492.
4. **Beer, B. E., C. R. Brown, S. Whitted, S. Goldstein, R. Goeken, R. Plishka, A. Buckler-White, and V. M. Hirsch.** 2005. Immunodeficiency in the absence of high viral load in pig-tailed macaques infected with simian immunodeficiency virus SIVsun or SIVlhoest. J. Virol. **79**:14044–14056.
5. **Brenchley, J. M., T. W. Schacker, L. E. Ruff, D. A. Price, J. H. Taylor, G. J. Beilman, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek.** 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J. Exp. Med. **200**:749–759.
6. **Brown, C. R., M. Czapiga, J. Kabat, Q. Dang, I. Ourmanov, Y. Nishimura, M. A. Martin, and V. M. Hirsch.** 2007. Unique pathology in simian immunodeficiency virus-infected rapid progressor macaques is consistent with a pathogenesis distinct from that of classical AIDS. J. Virol. **81**:5594–5606.
7. **Chen, Z., X. Zhao, Y. Huang, A. Gettie, L. Ba, J. Blanchard, and D. D. Ho.** 2002. CD4+ lymphocytopenia in acute infection of Asian macaques by a vaginally transmissible subtype-C, CCR5-tropic simian/human immunodeficiency virus (SHIV). J. Acquir. Immune Defic. Syndr. **30**:133–145.
8. **Collman, R., N. F. Hassan, R. Walker, B. Godfrey, J. Cutilli, J. C. Hastings, H. Friedman, S. D. Douglas, and N. Nathanson.** 1989. Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1). Monocyte-tropic and lymphocyte-tropic strains of HIV-1 show distinctive patterns of replication in a panel of cell types. J. Exp. Med. **170**:1149–1163.
9. **Committee on the Care and Use of Laboratory Animals.** 1985. Guide for the care and use of laboratory animals. Department of Health and Human Services publication no. NIH 85-23. National Institutes of Health, Bethesda, MD.
10. **Dudley, D. M., J. L. Wentzel, M. S. Lalonde, R. S. Veazey, and E. J. Arts.** 2009. Selection of a simian-human immunodeficiency virus strain resistant to a vaginal microbicide in macaques. J. Virol. **83**:5067–5076.
11. **Endo, Y., T. Igarashi, Y. Nishimura, C. Buckler, A. Buckler-White, R. Plishka, D. S. Dimitrov, and M. A. Martin.** 2000. Short- and long-term clinical outcomes in rhesus monkeys inoculated with a highly pathogenic chimeric simian/human immunodeficiency virus. J. Virol. **74**:6935–6945.
12. **Fauci, A. S., M. I. Johnston, C. W. Dieffenbach, D. R. Burton, S. M. Hammer, J. A. Hoxie, M. Martin, J. Overbaugh, D. I. Watkins, A. Mahmoud, and W. C. Greene.** 2008. HIV vaccine research: the way forward. Science **321**: 530–532.
13. **Feinberg, M. B., and J. P. Moore.** 2002. AIDS vaccine models: challenging challenge viruses. Nat. Med. **8**:207–210.
14. **Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, and D. S. Burke, et al.** 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J. Exp. Med. **167**:1428–1441.
15. **Harouse, J. M., A. Gettie, R. C. Tan, J. Blanchard, and C. Cheng-Mayer.** 1999. Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. Science **284**:816–819.
16. **Hessell, A. J., P. Poignard, M. Hunter, L. Hangartner, D. M. Tehrani, W. K. Bleeker, P. W. Parren, P. A. Marx, and D. R. Burton.** 2009. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat. Med. **15**:951–954.
17. **Hessell, A. J., E. G. Rakasz, P. Poignard, L. Hangartner, G. Landucci, D. N. Forthal, W. C. Koff, D. I. Watkins, and D. R. Burton.** 2009. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog. **5**:e1000433.
18. **Ho, S. H., S. Tasca, L. Shek, A. Li, A. Gettie, J. Blanchard, D. Boden, and C. Cheng-Mayer.** 2007. Coreceptor switch in R5-tropic simian/human immunodeficiency virus-infected macaques. J. Virol. **81**:8621–8633.
19. **Ho, S. H., N. Trunova, A. Gettie, J. Blanchard, and C. Cheng-Mayer.** 2008. Different mutational pathways to CXCR4 coreceptor switch of CCR5-using simian-human immunodeficiency virus. J. Virol. **82**:5653–5656.
20. **Hsu, M., J. M. Harouse, A. Gettie, C. Buckner, J. Blanchard, and C. Cheng-Mayer.** 2003. Increased mucosal transmission but not enhanced pathogenicity of the CCR5-tropic, simian AIDS-inducing simian/human immunodeficiency virus SHIV(SF162P3) maps to envelope gp120. J. Virol. **77**:989–998.
21. **Humbert, M., R. A. Rasmussen, R. Song, H. Ong, P. Sharma, A. L. Chenine, V. G. Kramer, N. B. Siddappa, W. Xu, J. G. Else, F. J. Novembre, E. Strobert, S. P. O'Neil, and R. M. Ruprecht.** 2008. SHIV-1157i and passaged progeny viruses encoding R5 HIV-1 clade C env cause AIDS in rhesus monkeys. Retrovirology **5**:94.
22. **Igarashi, T., O. K. Donau, H. Imamichi, M. J. Dumaurier, R. Sadjadpour, R. J. Plishka, A. Buckler-White, C. Buckler, A. F. Suffredini, H. C. Lane, J. P. Moore, and M. A. Martin.** 2003. Macrophage-tropic simian/human immunodeficiency virus chimeras use CXCR4, not CCR5, for infections of rhesus macaque peripheral blood mononuclear cells and alveolar macrophages. J. Virol. **77**:13042–13052.
23. **Igarashi, T., Y. Endo, G. Englund, R. Sadjadpour, T. Matano, C. Buckler, A. Buckler-White, R. Plishka, T. Theodore, R. Shibata, and M. Martin.** 1999. Emergence of a highly pathogenic simian/human immunodeficiency virus in a rhesus macaque treated with anti-CD8 mAb during a primary infection with a nonpathogenic virus. Proc. Natl. Acad. Sci. U. S. A. **96**:14049–14054.
24. **Igarashi, T., Y. Endo, Y. Nishimura, C. Buckler, R. Sadjadpour, O. K. Donau, M. J. Dumaurier, R. J. Plishka, A. Buckler-White, and M. A. Martin.** 2003. Early control of highly pathogenic simian immunodeficiency virus/human immunodeficiency virus chimeric virus infections in rhesus monkeys usually results in long-lasting asymptomatic clinical outcomes. J. Virol. **77**: 10829–10840.
25. **Imamichi, H., T. Igarashi, T. Imamichi, O. K. Donau, Y. Endo, Y. Nishimura, R. L. Willey, A. F. Suffredini, H. C. Lane, and M. A. Martin.** 2002. Amino acid deletions are introduced into the V2 region of gp120 during independent pathogenic simian immunodeficiency virus/HIV chimeric virus (SHIV) infections of rhesus monkeys generating variants that are macrophage tropic. Proc. Natl. Acad. Sci. U. S. A. **99**:13813–13818.
26. **Joag, S. V., Z. Li, L. Foresman, E. B. Stephens, L. J. Zhao, I. Adany, D. M. Pinson, H. M. McClure, and O. Narayan.** 1996. Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4+ T cells and AIDS in pig-tailed macaques. J. Virol. **70**:3189–3197.
27. **Kuwata, T., Y. Nishimura, S. Whitted, I. Ourmanov, C. R. Brown, Q. Dang, A. Buckler-White, R. Iyengar, J. M. Brenchley, and V. M. Hirsch.** 2009. Association of progressive CD4(+) T cell decline in SIV infection with the induction of autoreactive antibodies. PLoS Pathog. **5**:e1000372.
28. **Li, J. T., M. Halloran, C. I. Lord, A. Watson, J. Ranchalis, M. Fung, N. L. Letvin, and J. G. Sodroski.** 1995. Persistent infection of macaques with simian-human immunodeficiency viruses. J. Virol. **69**:7061–7067.
29. **Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase.** 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature **434**:1148–1152.
30. **Luciw, P. A., E. Pratt-Lowe, K. E. Shaw, J. A. Levy, and C. Cheng-Mayer.** 1995. Persistent infection of rhesus macaques with T-cell-line-tropic and macrophage-tropic clones of simian/human immunodeficiency viruses (SHIV). Proc. Natl. Acad. Sci. U. S. A. **92**:7490–7494.
31. **Lusso, P., F. Cocchi, C. Balotta, P. D. Markham, A. Louie, P. Farci, R. Pal, R. C. Gallo, and M. S. Reitz, Jr.** 1995. Growth of macrophage-tropic and

primary human immunodeficiency virus type 1 (HIV-1) isolates in a unique CD4+ T-cell clone (PM1): failure to downregulate CD4 and to interfere with cell-line-tropic HIV-1. J. Virol. 69:3712–3720.

32. **Mehandru, S., M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Hogan, D. Boden, P. Racz, and M. Markowitz.** 2004. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J. Exp. Med. 200:761–770.
33. **Nehete, P. N., S. Chitta, M. M. Hossain, L. Hill, B. J. Bernacky, W. Baze, R. B. Arlinghaus, and K. J. Sastry.** 2001. Protection against chronic infection and AIDS by an HIV envelope peptide-cocktail vaccine in a pathogenic SHIV-rhesus model. Vaccine 20:813–825.
34. **Nishimura, Y., C. R. Brown, J. J. Mattapallil, T. Igarashi, A. Buckler-White, B. A. Lafont, V. M. Hirsch, M. Roederer, and M. A. Martin.** 2005. Resting naive CD4+ T cells are massively infected and eliminated by X4-tropic simian-human immunodeficiency viruses in macaques. Proc. Natl. Acad. Sci. U. S. A. 102:8000–8005.
35. **Nishimura, Y., T. Igarashi, A. Buckler-White, C. Buckler, H. Imamichi, R. M. Goeken, W. R. Lee, B. A. Lafont, R. Byrum, H. C. Lane, V. M. Hirsch, and M. A. Martin.** 2007. Loss of naive cells accompanies memory CD4+ T-cell depletion during long-term progression to AIDS in simian immunodeficiency virus-infected macaques. J. Virol. 81:893–902.
36. **Nishimura, Y., T. Igarashi, O. K. Donau, A. Buckler-White, C. Buckler, B. A. Lafont, R. M. Goeken, S. Goldstein, V. M. Hirsch, and M. A. Martin.** 2004. Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. Proc. Natl. Acad. Sci. U. S. A. 101:12324–12329.
37. **O'Doherty, U., W. J. Swiggard, and M. H. Malim.** 2000. Human immunodeficiency virus type 1 spinoculation enhances infection through virus binding. J. Virol. 74:10074–10080.
38. **Pal, R., B. Taylor, J. S. Foulke, R. Woodward, M. Merges, R. Praschunus, A. Gibson, and M. Reitz.** 2003. Characterization of a simian human immunodeficiency virus encoding the envelope gene from the CCR5-tropic HIV-1 Ba-L. J. Acquir. Immune. Defic. Syndr. 33:300–307.
39. **Pitcher, C. J., S. I. Hagen, J. M. Walker, R. Lum, B. L. Mitchell, V. C. Maino, M. K. Axthelm, and L. J. Picker.** 2002. Development and homeostasis of T cell memory in rhesus macaque. J. Immunol. 168:29–43.
40. **Pitcher, C. J., C. Quittner, D. M. Peterson, M. Connors, R. A. Koup, V. C. Maino, and L. J. Picker.** 1999. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. 5:518–525.
41. **Reimann, K. A., J. T. Li, R. Veazey, M. Halloran, I. W. Park, G. B. Karlsson, J. Sodroski, and N. L. Letvin.** 1996. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. J. Virol. 70:5922–6928.
42. **Rose, N. F., P. A. Marx, A. Luckay, D. F. Nixon, W. J. Moretto, S. M. Donahoe, D. Montefiori, A. Roberts, L. Buonocore, and J. K. Rose.** 2001. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. Cell 106:539–549.
43. **Schubert, U., and K. Strebel.** 1994. Differential activities of the human immunodeficiency virus type 1-encoded Vpu protein are regulated by phosphorylation and occur in different cellular compartments. J. Virol. 68:2260–2271.
44. **Shibata, R., M. Kawamura, H. Sakai, M. Hayami, A. Ishimoto, and A. Adachi.** 1991. Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. J. Virol. 65:3514–3520.
45. **Shibata, R., F. Maldarelli, C. Siemon, T. Matano, M. Parta, G. Miller, T. Fredrickson, and M. A. Martin.** 1997. Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing. J. Infect. Dis. 176:362–373.
46. **Shiver, J. W., T. M. Fu, L. Chen, D. R. Casimiro, M. E. Davies, R. K. Evans, Z. Q. Zhang, A. J. Simon, W. L. Trigona, S. A. Dubey, L. Huang, V. A. Harris, R. S. Long, X. Liang, L. Handt, W. A. Schleif, L. Zhu, D. C. Freed, N. V. Persaud, L. Guan, K. S. Punt, A. Tang, M. Chen, K. A. Wilson, K. B. Collins, G. J. Heidecker, V. R. Fernandez, H. C. Perry, J. G. Joyce, K. M. Grimm, J. C. Cook, P. M. Keller, D. S. Kresock, H. Mach, R. D. Troutman, L. A. Isopi, D. M. Williams, Z. Xu, K. E. Bohannon, D. B. Volkin, D. C. Montefiori, A. Miura, G. R. Krivulka, M. A. Lifton, M. J. Kuroda, J. E. Schmitz, N. L. Letvin, M. J. Caulfield, A. J. Bett, R. Youil, D. C. Kaslow, and E. A. Emini.** 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature 415:331–335.
47. **Tasca, S., S. H. Ho, and C. Cheng-Mayer.** 2008. R5X4 viruses are evolutionary, functional, and antigenic intermediates in the pathway of a simian-human immunodeficiency virus coreceptor switch. J. Virol. 82:7089–7099.
48. **Theodore, T. S., G. Englund, A. Buckler-White, C. E. Buckler, M. A. Martin, and K. W. Peden.** 1996. Construction and characterization of a stable full-length macrophage-tropic HIV type 1 molecular clone that directs the production of high titers of progeny virions. AIDS Res. Hum. Retroviruses 12:191–194.
49. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science 280:427–431.
50. **Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw.** 2003. Antibody neutralization and escape by HIV-1. Nature 422:307–312.
51. **Willey, R., M. C. Nason, Y. Nishimura, D. A. Follmann, and M. A. Martin.** 2010. Neutralizing antibody titers conferring protection to macaques from a simian/human immunodeficiency virus challenge using the TZB-bl assay. AIDS Res. Hum. Retroviruses 26:89–98.
52. **Willey, R. L., T. Klimkait, D. M. Frucht, J. S. Bonifacino, and M. A. Martin.** 1991. Mutations within the human immunodeficiency virus type 1 gp160 envelope glycoprotein alter its intracellular transport and processing. Virology 184:319–329.
53. **Wu, L., G. LaRosa, N. Kassam, C. J. Gordon, H. Heath, N. Ruffing, H. Chen, J. Humblias, M. Samson, M. Parmentier, J. P. Moore, and C. R. Mackay.** 1997. Interaction of chemokine receptor CCR5 with its ligands: multiple domains for HIV-1 gp120 binding and a single domain for chemokine binding. J. Exp. Med. 186:1373–1381.