JOURNAL OF VIROLOGY, Oct. 2005, p. 13186–13189 Vol. 79, No. 20
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.20.13186–13189.2005

Mucosal and Systemic Antibody Responses in Humans Infected with Simian Foamy Virus

James E. Cummins, Jr.,[1]* Roumiana S. Boneva,[1] William M. Switzer,[1] Logan L. Christensen,[1] Paul Sandstrom,[2] Walid Heneine,[1] Louisa E. Chapman,[1] and Charlene S. Dezzutti[1]

HIV and Retrovirology Branch, Division of AIDS, STD, and TB Laboratory Research, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia 30333,[1] and Bureau of HIV/AIDS, STD, and TB, Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa, Ontario, Canada[2]

Received 7 March 2005/Accepted 26 July 2005

Simian foamy virus (SFV) infection and the subsequent immune response are not well characterized. Blood plasma, saliva, and urine were obtained from four humans and nine chimpanzees persistently infected with chimpanzee-type SFV for an unknown length of time. SFV-specific immunoglobulin G (IgG) antibodies, but not IgA antibodies, against the Gag and Bet proteins were detected, by Western blotting, in all sample types from infected humans and chimpanzees. Overall, chimpanzee samples had higher anti-SFV IgG titers than humans. These results provide a first comparative evaluation of SFV-specific host mucosal humoral immunity in infected humans and chimpanzees that is characterized by a predominant IgG response and a virtually absent IgA response.

Cross-species transmission of simian foamy viruses (SFVs) to humans by nonhuman primates (NHPs) or by exposure to their body fluids has been documented (4, 10, 21, 24–26, 29). There is currently no evidence of human-to-human transmission of SFV; however, only a few cases ($n = 6$) have had a short clinical follow-up (3, 10, 26). Although SFV is more easily transmitted among captive NHPs than among humans, questions remain regarding the epidemiology and the natural history of these infections. While a variety of viral and host factors may contribute to the lack of pathogenicity or transmissibility of SFVs in natural hosts and infected humans, the host immune response may play a role in keeping these viruses persistent yet benign (17, 18).

Even though seroreactivity to SFV proteins has been documented in natural hosts (2, 8) and infected humans (4, 10, 19, 21, 26), SFV-specific immunity has not been characterized. A strong plasma antibody response primarily against the SFV Gag doublet and the nonstructural Bet viral proteins was documented in infected primates (8, 17, 19, 25) and humans (4, 9, 10, 21, 26, 29). Seroreactivity to the Gag doublet is consistently detected in plasma and considered to be a diagnostic marker of infection (8, 11, 25, 26). Although seroreactivity to SFV proteins is persistent, it is unknown whether differences in the nature and type of antibody responses in NHPs and humans play a role in virus persistence or in modulating virus transmission.

In the present study, the mucosal and systemic immunoglobulin G (IgG) and IgA immune responses in humans infected with SFV from chimpanzees (SFV_{cpz}) (cases 6, 7, 9, and 10) were evaluated and compared to those of naturally infected chimpanzees. The cases were enrolled in a Centers for Disease Control and Prevention long-term follow-up study to characterize the clinical course of SFV infection (26). The duration of first seropositivity predates the current study by 10 to 24 years; therefore, their dates of infection could not be determined (26). Matched blood plasma, parotid saliva, and urine samples were collected at defined intervals during the study. Longitudinal samples, obtained 27 to 45 months apart, were available from cases 6, 7, and 10.

For comparison, blood plasma and saliva were collected on an opportunistic basis from four naturally infected chimpanzees (CPZ 1 to 4) (26). Blood plasma, whole saliva, and urine samples were collected from five additional chimpanzees (CPZ 5 to 9) (Yerkes Primate Research Center; Emory University, Atlanta, GA). SFV_{cpz}-specific seroreactivity was confirmed in these five chimpanzees by using a previously described Western blotting (WB) protocol (11). No information was available regarding the length of infection for these chimpanzees.

The WB protocol (11) was modified to detect SFV_{cpz}-specific human or chimpanzee IgG and IgA in plasma and mucosal secretions by using horseradish peroxidase-labeled anti-human IgG or IgA (Jackson ImmunoResearch Laboratories, West Grove, PA). Samples were simultaneously screened for immunoreactivity against proteins in either uninfected or SFV_{cpz}-infected Cf2Th cell lysates. Samples with seroreactivity to the Gag doublet were considered seropositive. All samples containing SFV_{cpz}-specific antibodies were nonreactive against uninfected Cf2Th cell lysates (data not shown).

Plasma from cases 6 and 9 had SFV-specific IgG that reacted equally well to the Gag doublet and Bet proteins, and plasma from cases 7 and 10 had predominant reactivity to the Bet protein (Fig. 1A). Plasma from CPZ 1 and 2 had SFV-specific IgG with predominant reactivity to the Gag doublet, plasma from CPZ 4 had predominant reactivity to the Bet protein, and plasma from CPZ 3 had equivalent reactivity to the Gag doublet and Bet proteins (Fig. 1B).

* Corresponding author. Present address: Southern Research Institute, 431 Aviation Way, Frederick, MD 21701. Phone: (301) 228-2186. Fax: (301) 694-7223. E-mail: cummins@sri.org.

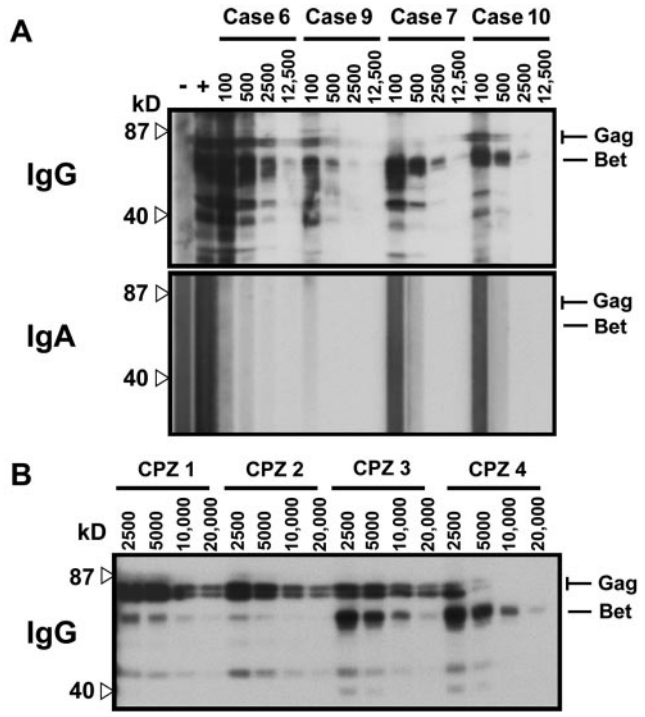

FIG. 1. SFV_{cpz}-specific immunoreactivity, by Western blot analysis, in human (A) and chimpanzee (B) plasma samples. IgG reactivity in both human (A, upper panel) and chimpanzee (B) samples is shown. IgA reactivity in the human samples is also shown (A, lower panel). Plasma samples (1:500 dilutions) from an uninfected human (−) and an SFV_{cpz}-infected chimpanzee (+), as controls, are shown. Dilution values are indicated above each lane for the plasma samples.

Saliva from cases 6 and 10 had SFV-specific IgG with predominant reactivity to the Gag doublet, and saliva from case 7 had predominant reactivity to the Bet protein (Fig. 2A). Since a limited amount of saliva from case 7 precluded testing at lower dilutions, WB analysis at higher dilutions (≥1:16) may have missed reactivity to other SFV_{cpz} proteins. Saliva from CPZ 5 and 6 had SFV-specific IgG with equivalent reactivity to the Gag and Bet proteins, and saliva from CPZ 7 had predominant reactivity to the Gag doublet (Fig. 2B).

Urine from cases 6 and 10 had SFV-specific IgG with equivalent reactivity to the Gag doublet and Bet proteins (Fig. 2C). As observed with saliva, urine from case 7 had reactivity only to the Bet protein (Fig. 2C). Both chimpanzee urine samples (CPZ 8 and 9) had SFV-specific IgG with similar reactivity to the Gag doublet and Bet proteins (Fig. 2D).

In general, human samples (plasma, saliva, and urine) from longitudinal time points had a similar pattern of immunoreactivity to SFV proteins, indicating a persistent humoral response (data not shown). Semiquantitative serial dilutions of plasma and secretions indicated that SFV-specific IgG titers were approximately two- to fourfold higher in chimpanzees than in humans (Fig. 1 and 2).

WBs employing IgA-specific detection reagents were used on the same plasma, saliva, and urine samples that were tested for SFV-specific IgG. No detectable SFV-specific IgA reactivity against the SFV Gag doublet was observed in any of the tested plasma, saliva, and urine samples (Fig. 1A and 2A, human plasma and saliva, respectively; chimpanzee and urine samples not shown). In the few instances in which WB reactivity was observed with the IgA-specific reagents, this reactiv-

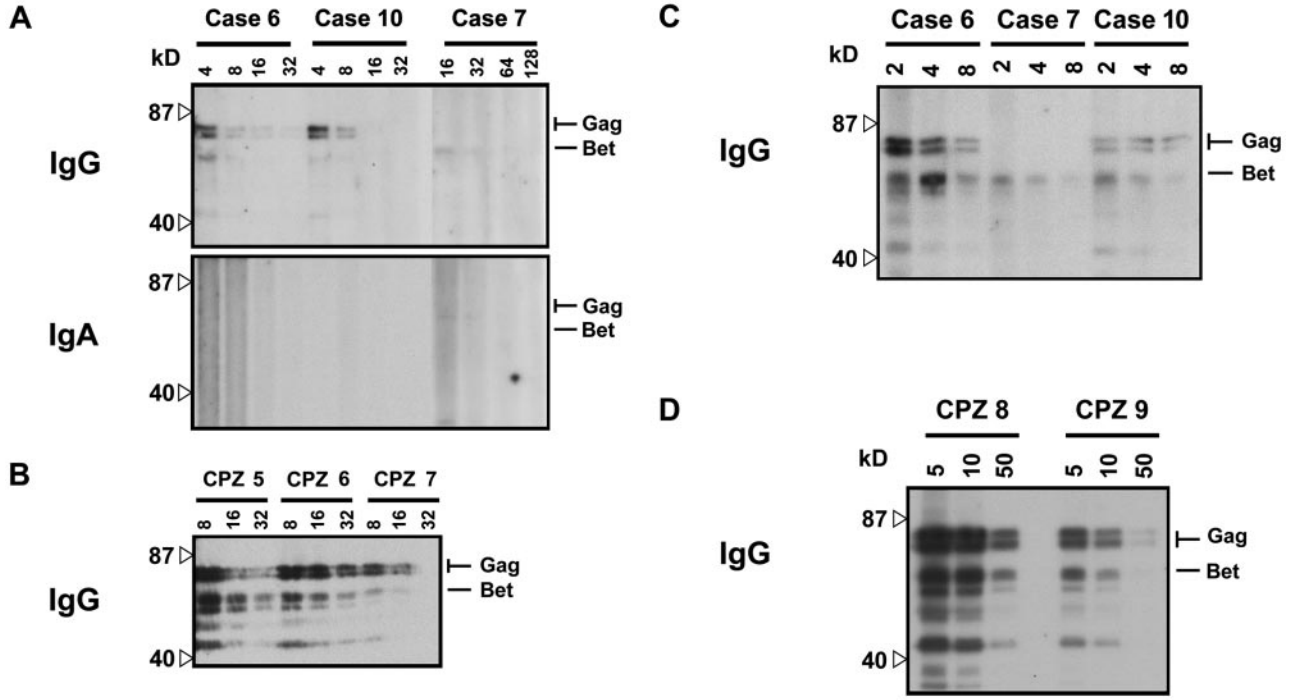

FIG. 2. SFV_{cpz}-specific immunoreactivity, by Western blot analysis, in human and chimpanzee saliva (A and B, respectively) and urine (C and D, respectively) samples. IgA reactivity in the human saliva samples is shown (A, lower panel). Dilution values are indicated above each lane for the saliva samples. Due to a limited amount of saliva, the minimal starting dilution of saliva for case 7 was 1:16.

TABLE 1. Depletion of IgG from plasma and saliva

Plasma or saliva sample from indicated case or chimpanzee	IgG		% IgG depletion	IgA		% IgA depletion
	Pre	Post		Pre	Post	
Plasma (mg/ml)[a]						
Case						
9	5.66	0.497	91	0.817	0.610	25
7	10.2	1.73	83	2.11	1.39	34
10	5.30	0.392	93	0.900	0.945	0
CPZ						
1	31.3	18.8	40	4.56	3.75	18
2	16.9	4.05	76	6.38	4.45	30
4	14.3	1.40	90	3.00	3.15	0
Saliva (μg/ml)[a]						
Case						
6	0.560	0.004	99	153	118	23
7	1.53	0.004	99	64.5	38.5	40
10	2.16	0.040	98	23.3	12.1	48
CPZ						
7	4.57	0.200	96	31.3	34.6	11
5	5.25	0.311	94	62.2	50.3	19
6	8.08	0.294	96	94.9	84.1	11

[a] IgG and IgA levels before (Pre) and after (Post) removal of IgG.

ity was weak and inconsistent between samples, indicating nonspecific reactivity.

To rule out selective IgA deficiency as a cause for undetectable SFV-specific IgA, levels of total IgA or IgG in plasma and saliva samples were measured by enzyme-linked immunosorbent assay as previously described (15) with the following modifications: unlabeled anti-human IgA- or IgG-coating antibodies (BioSource International, Camarillo, CA), a pooled human serum control (The Binding Site, San Diego, CA), and biotinylated anti-human IgA or IgG antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). All tested samples from SFV_{cpz}-infected humans and chimpanzees contained normal levels of IgA in plasma and saliva (Table 1). IgG levels in chimpanzees were 1.4- to 5.9-fold higher in plasma and 2.1 to 9.4-fold higher in saliva than in human samples (Table 1).

Since physiologic concentrations of plasma IgG may "mask" the activity of IgA (1, 14, 27), GammaBind G Sepharose was used (16) to remove IgG in selected plasma and saliva samples (median depletion of 86.5%) (Table 1). There was minimal loss of IgA in human and chimpanzee plasma (median depletion of 21.5%). Despite minimal loss of IgA in chimpanzee saliva (median depletion of 21%), there was a twofold greater loss of IgA in human saliva (Table 1). Depleted samples were run simultaneously with matched untreated samples in the modified WB protocol. Removal of IgG resulted in a marked decrease in the detection of SFV proteins in depleted plasma (Fig. 3) and saliva (not shown). Importantly, increased detection of SFV-specific IgA was not observed in depleted plasma (Fig. 3) and saliva (data not shown), indicating that IgG was not masking IgA.

This is the first report comparing the presence and type of antibodies in plasma and mucosal secretions from occupationally SFV-infected humans and naturally infected chimpanzees. SFV-specific WB reactivity was restricted to IgG antibodies

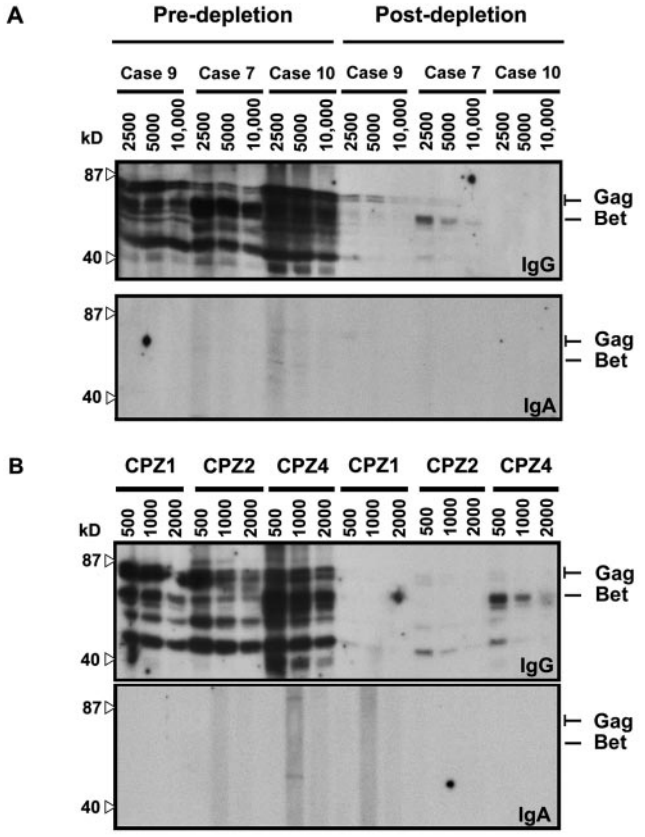

FIG. 3. SFV_{cpz}-specific immunoreactivity, by Western blot analysis, in human (A) and chimpanzee (B) plasma samples before (Pre-depletion) and after (Post-depletion) IgG depletion. IgG reactivity in both human (A, upper panel) and chimpanzee (B, upper panel) samples is shown. IgA reactivity in both human (A, lower panel) and chimpanzee (B, lower panel) samples is shown. Dilution values are indicated above each lane for the plasma samples.

and IgG titers were generally lower in humans than in chimpanzees. Although normal IgA levels were detected, SFV-specific IgA against the Gag doublet or Bet proteins was not detected in mucosal secretions or plasma samples. Unlike studies in which removal of IgG from plasma or serum resulted in elevated titers of human immunodeficiency virus (HIV)-specific IgA (1, 14, 27), no enhancement of SFV-specific IgA reactivity was observed in IgG-depleted plasma and saliva samples. The lack of an SFV-specific IgA response in the mucosa suggests that an IgG-mediated systemic humoral response predominates in infected humans and chimpanzees. Similar WB profiles between saliva, urine, and plasma in matched human samples suggest that SFV_{cpz}-specific IgG antibodies transude from plasma into mucosal secretions.

It is unknown whether these findings with SFV_{cpz}-infected humans are representative of what occurs in individuals infected with other SFV variants. HIV type 1 and simian immunodeficiency virus, retroviruses that infect across the mucosa, are also inefficient at inducing an IgA response but induce a strong IgG response in the genitourinary (28) and gastrointestinal tracts (12, 22, 23, 30). In humans lacking HIV-specific IgA, normal influenza-specific IgA levels were detected, suggesting an intact common mucosal immune system (30). Al-

though other IgA-specific responses were not tested in this study, it is likely that these individuals have intact IgA immunity to other mucosal pathogens. While poor HIV-specific IgA responses may be due to low-dose tolerance (7) or HIV-specific T-helper-cell anergy (5), it remains unclear why virus-specific IgA responses are not induced. Since a lack of HIV-specific IgA antibodies in the mucosa may facilitate HIV replication in mucosal lymphoid tissues (30), a similar paucity of SFV-specific mucosal IgA antibodies may explain SFV shedding into saliva (2) and replication in the oral mucosa (6, 13). However, differences in plasma and mucosal viral loads could be more important determinants of virus transmission than the presence of SFV-specific antibodies, as is the case for HIV type 1 (20). Further, mechanisms of transmission in humans differ substantially from those of chimpanzees that involve biting, scratching, and fighting behaviors.

The results from this study provide an initial comparison of host mucosal humoral immunity and SFV-host interactions in humans and chimpanzees. Further study of SFV-specific host mucosal immunity is necessary to determine the contribution to viral persistence in the infected host and differences in transmission between chimpanzees and humans.

We thank Vinod Bhullar and Althaf Hussain for kindly providing the SFV_{cpz} WB lysates. We are grateful to the veterinary and administrative staffs at the New Iberia Research Center and the Yerkes Primate Research Center for providing the chimpanzee specimens. We thank James Gathany for his expertise in graphic design for editing the WB figures. We also thank Brigitte Beer for review of the manuscript.

J.E.C. was supported by an American Society for Microbiology/National Centers for Infectious Diseases Postdoctoral Research Associate fellowship and a National Research Service Award 5 F32 HD40727 from the National Institutes of Health, National Institute of Child Health and Human Development.

Use of trade names is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention.

REFERENCES

1. **Black, K. P., P. N. Fultz, M. Girard, and S. Jackson.** 1997. IgA immunity in HIV type 1-infected chimpanzees. I. Systemic immunity. AIDS Res. Hum. Retrovir. **13:**1263–1272.
2. **Blewett, E. L., D. H. Black, N. W. Lerche, G. White, and R. Eberle.** 2000. Simian foamy virus infections in a baboon breeding colony. Virology **278:** 183–193.
3. **Boneva, R. S., A. J. Grindon, S. L. Orton, W. M. Switzer, V. Shanmugam, A. I. Hussain, V. B. Bhullar, M. E. Chamberland, W. Heneine, T. M. Folks, and L. E. Chapman.** 2002. Simian foamy virus infection in a blood donor. Transfusion **42:**886–891.
4. **Brooks, J. I., E. W. Rud, R. G. Pilon, J. M. Smith, W. M. Switzer, and P. A. Sandstrom.** 2002. Cross-species retroviral transmission from macaques to human beings. Lancet **360:**387–388.
5. **Dybul, M., G. Mercier, M. Belson, C. W. Hallahan, S. Liu, C. Perry, B. Herpin, L. Ehler, R. T. Davey, J. A. Metcalf, J. M. Mican, R. A. Seder, and A. S. Fauci.** 2000. CD40 ligand trimer and IL-12 enhance peripheral blood mononuclear cells and CD4+ T cell proliferation and production of IFN-γ in response to p24 antigen in HIV-infected individuals: potential contribution of anergy to HIV-specific unresponsiveness. J. Immunol. **165:**1685–1691.
6. **Falcone, V., J. Leupold, J. Clotten, E. Urbanyi, O. Herchenroder, W. Spatz, B. Volk, N. Bohm, A. Toniolo, D. Neumann-Haefelin, and M. Schweizer.** 1999. Sites of simian foamy virus persistence in naturally infected African green monkeys: latent provirus is ubiquitous, whereas viral replication is restricted to the oral mucosa. Virology **257:**7–14.
7. **Friedman, A., and H. L. Weiner.** 1994. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc. Natl. Acad. Sci. USA **91:**6688–6692.
8. **Hahn, H., G. Baunach, S. Brautigam, A. Mergia, D. Neumann-Haefelin, M. D. Daniel, M. O. McClure, and A. Rethwilm.** 1994. Reactivity of primate sera to foamy virus Gag and Bet proteins. J. Gen. Virol. **75:**2635–2644.
9. **Heneine, W., M. Schweizer, P. Sandstrom, and T. M. Folks.** 2003. Human infection with foamy viruses. Curr. Top. Microbiol. Immunol. **277:**181–196.
10. **Heneine, W., W. M. Switzer, P. Sandstrom, J. Brown, S. Vedapuri, C. A. Schable, A. S. Khan, N. W. Lerche, M. Schweizer, D. Neumann-Haefelin, L. E. Chapman, and T. M. Folks.** 1998. Identification of a human population infected with simian foamy viruses. Nat. Med. **4:**403–407.
11. **Hussain, A. I., V. Shanmugam, V. B. Bhullar, B. E. Beer, D. Vallet, A. Gautier-Hion, N. D. Wolfe, W. B. Karesh, A. M. Kilbourn, Z. Tooze, W. Heneine, and W. M. Switzer.** 2003. Screening for simian foamy virus infection by using a combined antigen Western blot assay: evidence for a wide distribution among Old World primates and identification of four new divergent viruses. Virology **309:**248–257.
12. **Jackson, S., Z. Moldoveanu, J. Mestecky, A. M. Pitts, J. H. Eldridge, J. R. McGhee, C. J. Miller, and P. A. Marx.** 1995. Decreased IgA-producing cells in the gut of SIV-infected rhesus monkeys. Adv. Exp. Med. Biol. **371B:**1035–1038.
13. **Johnston, P. B.** 1961. A second immunologic type of simian foamy virus: monkey throat infections and unmasking of both types. J. Infect. Dis. **109:** 1–9.
14. **Kozlowski, P. A., K. P. Black, L. Shen, and S. Jackson.** 1995. High prevalence of serum IgA HIV-1 infection-enhancing antibodies in HIV-infected persons. Masking by IgG. J. Immunol. **154:**6163–6173.
15. **Kozlowski, P. A., D. Chen, J. H. Eldridge, and S. Jackson.** 1994. Contrasting IgA and IgG neutralization capacities and responses to HIV type 1 gp120 V3 loop in HIV-infected individuals. AIDS Res. Hum. Retrovir. **10:**813–822.
16. **Kozlowski, P. A., and S. Jackson.** 1992. Serum IgA subclasses and molecular forms in HIV infection: selective increases in monomer and apparent restriction of the antibody response to IgA1 antibodies mainly directed at env glycoproteins. AIDS Res. Hum. Retrovir. **8:**1773–1780.
17. **Linial, M.** 2000. Why aren't foamy viruses pathogenic? Trends Microbiol. **8:**284–289.
18. **Meiering, C. D., C. Rubio, C. May, and M. L. Linial.** 2001. Cell-type-specific regulation of the two foamy virus promoters. J. Virol. **75:**6547–6557.
19. **Netzer, K. O., A. Rethwilm, B. Maurer, and V. ter Meulen.** 1990. Identification of the major immunogenic structural proteins of human foamy virus. J. Gen. Virol. **71:**1237–1241.
20. **Quinn, T. C., M. J. Wawer, N. Sewankambo, D. Serwadda, C. Li, F. Wabwire-Mangen, M. O. Meehan, T. Lutalo, R. H. Gray, et al.** 2000. Viral load and heterosexual transmission of human immunodeficiency virus type 1. N. Engl. J. Med. **342:**921–929.
21. **Sandstrom, P. A., K. O. Phan, W. M. Switzer, T. Fredeking, L. Chapman, W. Heneine, and T. M. Folks.** 2000. Simian foamy virus infection among zoo keepers. Lancet **355:**551–552.
22. **Schafer, F., S. Kewenig, N. Stolte, C. Stahl-Hennig, A. Stallmach, F. J. Kaup, M. Zeitz, and T. Schneider.** 2002. Lack of simian immunodeficiency virus (SIV) specific IgA response in the intestine of SIV infected rhesus macaques. Gut **50:**608–614.
23. **Schneider, T., T. Zippel, W. Schmidt, G. Pauli, W. Heise, U. Wahnschaffe, E. O. Riecken, M. Zeitz, and R. Ullrich.** 1997. Abnormal predominance of IgG in HIV-specific antibodies produced by short-term cultured duodenal biopsy specimens from HIV-infected patients. J. Acquir. Immun. Defic. Syndr. Hum. Retrovirol. **16:**333–339.
24. **Schweizer, M., V. Falcone, J. Gange, R. Turek, and D. Neumann-Haefelin.** 1997. Simian foamy virus isolated from an accidentally infected human individual. J. Virol. **71:**4821–4824.
25. **Schweizer, M., R. Turek, H. Hahn, A. Schliephake, K. O. Netzer, G. Eder, M. Reinhardt, A. Rethwilm, and D. Neumann-Haefelin.** 1995. Markers of foamy virus infections in monkeys, apes, and accidentally infected humans: appropriate testing fails to confirm suspected foamy virus prevalence in humans. AIDS Res. Hum. Retrovir. **11:**161–170.
26. **Switzer, W. M., V. B. Bhullar, V. Shanmugam, M. E. Cong, B. Parekh, N. W. Lerche, J. L. Yee, J. J. Ely, R. S. Boneva, L. E. Chapman, T. M. Folks, and W. Heneine.** 2004. Frequent simian foamy virus infection in persons occupationally exposed to nonhuman primates. J. Virol. **78:**2780–2789.
27. **Weiblen, B. J., R. T. Schumacher, and R. Hoff.** 1990. Detection of IgM and IgA HIV antibodies after removal of IgG with recombinant protein G. J. Immunol. Methods **126:**199–204.
28. **Williams, S. B., T. P. Flanigan, S. Cu-Uvin, K. Mayer, P. Williams, C. A. Ettore, A. W. Artenstein, A. Duerr, and T. C. VanCott.** 2002. Human immunodeficiency virus (HIV)-specific antibody in cervicovaginal lavage specimens obtained from women infected with HIV type 1. Clin. Infect. Dis. **35:**611–617.
29. **Wolfe, N. D., W. M. Switzer, J. K. Carr, V. B. Bhullar, V. Shanmugam, U. Tamoufe, A. T. Prosser, J. N. Torimiro, A. Wright, E. Mpoudi-Ngole, F. E. McCutchan, D. L. Birx, T. M. Folks, D. S. Burke, and W. Heneine.** 2004. Naturally acquired simian retrovirus infections in central African hunters. Lancet **363:**932–937.
30. **Wright, P. F., P. A. Kozlowski, G. K. Rybczyk, P. Goepfert, H. F. Staats, T. C. VanCott, D. Trabattoni, E. Sannella, and J. Mestecky.** 2002. Detection of mucosal antibodies in HIV type 1-infected individuals. AIDS Res. Hum. Retrovir. **18:**1291–1300.