JOURNAL OF VIROLOGY, July 1997, p. 4981–4984 Vol. 71, No. 7
0022-538X/97/$04.00+0

Protection of Human Immunodeficiency Virus Type 2-Exposed Seronegative Macaques from Mucosal Simian Immunodeficiency Virus Transmission

PER PUTKONEN,[1]* BARBRO MÄKITALO,[1] DISA BÖTTIGER,[2] GUNNEL BIBERFELD,[1] AND RIGMOR THORSTENSSON[1]

Swedish Institute for Infectious Disease Control, Karolinska Institute, S-105 21 Stockholm,[1] and Microbiology and Tumorbiology Center, Karolinska Institute, S-171 77 Stockholm,[2] Sweden

Received 19 February 1997/Accepted 10 April 1997

At present it is not known which form of immunity would be most effective against infection with human immunodeficiency virus (HIV). To evaluate the possible role of cellular immunity, we examined whether four HIV type 2-exposed but seronegative macaques developed cellular immune responses and determined whether these exposed macaques were resistant to mucosal transmission of simian immunodeficiency virus (SIV). Following intrarectal challenge with SIV, 2 monkeys were protected against detectable SIV replication and another showed suppressed viral replication compared to 14 persistently infected controls. The two protected monkeys demonstrated SIV-specific cytotoxic T lymphocytes before as well as after SIV challenge. Here we provide evidence that activation of the cell-mediated arm of the immune system only, without antibody formation, can control SIV replication in macaques. The results imply that vaccines that stimulate a strong and broad cellular immune response could prevent mucosal HIV transmission.

Human immunodeficiency virus (HIV) is transmitted predominantly by sexual contact involving exposure of genital and rectal surfaces to cell-free and cell-associated virus. Experimental infection of macaques with simian immunodeficiency virus (SIV) or HIV type 2 (HIV-2) (22) is an important animal model for testing vaccine strategies to prevent HIV infection and AIDS in humans. This model allows controlled studies of the immune responses involved in protection against lentivirus infections. We (16) and others (10) have previously shown that passive transfer of antibodies from HIV-2-vaccinated or healthy, SIV-infected macaques prevents infection of other macaques after intravenous challenge with the homologous virus. However, the relative contributions of cell-mediated mechanisms to protective immunity against HIV and SIV infection in humans and primate models are not well understood. It has been speculated that the induction of cell-mediated immunity in the absence of antibody is important for protection against certain virus infections such as HIV (21).

Several studies have described specific cellular immune responses to HIV in individuals who, despite documented exposure to the virus, remain seronegative and uninfected. Such HIV-exposed but uninfected individuals produce HIV-specific cytotoxic T lymphocytes (CTL) directed against HIV (4, 9, 15, 19, 20), indicating that exposure to the virus and possibly transient infection have occurred. It is not known whether such immune responses in exposed seronegative individuals confer protection against HIV infection in the future. The objective of the present study was to determine whether four HIV-2-exposed but seronegative macaques developed cellular immune responses and to evaluate whether these animals could resist mucosal infection with a heterologous challenge virus.

* Corresponding author. Mailing address: Swedish Institute for Infectious Disease Control, Karolinska Institute, S-105 21 Stockholm, Sweden. Phone: 46 8 735 1490. Fax: 46 8 735 4136. E-mail: per.putkonen@smi.ki.se.

MATERIALS AND METHODS

Animals. Four cynomolgus monkeys (*Macaca fascicularis*) were selected for the present study following demonstration of protection from infectious HIV-2. Four of 19 animals enrolled in three separate experiments were either passively immunized with anti-HIV-2 serum or treated with antiviral drugs and subsequently shown to be protected from intravenous HIV-2 challenge. Two animals (monkeys B22 and B52) were selected from an experiment examining the prophylactic effect of passive immunization against HIV-2 challenge (16). Monkey B22 received a low dose (3 ml/kg of body weight) and monkey B52 received a high dose (9 ml/kg) of anti-HIV-2 serum intravenously, and both were challenged 6 h later with 10 50% monkey infective doses (MID_{50}) of HIV-2. Both animals were protected, whereas seven parallel macaques became infected. The third animal (monkey S6) was selected from an experiment assessing whether 3′-fluorothymidine (FLT) could prevent HIV-2 replication (2). The monkeys were treated with FLT, three times a day at 15 mg/kg for 10 days, beginning 8 h before inoculation of 10 MID_{50} of live HIV-2. Three of eight animals resisted the intravenous HIV-2 challenge, compared to eight controls which became infected with HIV-2. Only one of the three protected monkeys, S6, was available for this study. The fourth animal (monkey 75-2) was treated with 3′-azido-3′-deoxythymidine, three times a day at 20 mg/kg for 1 day and three times a day at 10 mg/kg for four days (3). Treatment started 10 min after intravenous inoculation with 30 MID_{50} of HIV-2. Four controls became infected, and the one animal demonstrating protection, monkey 75-2, was studied.

The HIV-2 challenge virus inoculated intravenously was obtained from a cell-free $HIV\text{-}2_{SBL\text{-}6669/H5}$ stock that had been grown in cynomolgus monkey (*M. fascicularis*) peripheral blood mononuclear cells (PBMC), and the infectious dose was determined in cynomolgus monkeys (17). SIV_{sm} challenge was carried out by topical application to the rectal mucosa of 10 MID_{50} of a cell-free SIV_{sm} virus pool grown in cynomolgus monkey PBMC, as previously described (18). Experimental protocols for the animals used in this study were reviewed and approved by institutional committees for the care and use of animals in research. Animals were anesthetized with ketamine (10 mg/kg intramuscularly) prior to all procedures that required the removal of animals from their cages.

Virus isolation, PCR, and serology. Virus isolation was performed as previously described (13). Virus was isolated by coculture of 2×10^6 monkey PBMC with 5×10^6 phytohemagglutinin-stimulated human PBMC from at least two different blood donors. Cultures were tested for production of viral antigen by a capture enzyme-linked immunosorbent assay. Proviral DNA was detected by nested PCR in PBMC and lymph node mononuclear cells with SIV long terminal repeat and *env* primers as previously described (18, 25). To address the possibility that virus was sequestered in lymphoid organs, lymph nodes were surgically removed from the monkeys from which virus could not be cultured. Cell suspensions were prepared, and the cells were used for virus isolation and PCR analysis as described above. Endpoint immunoglobulin G titers were investigated by enzyme-linked immunosorbent assay as previously described (13).

The CTL assay. The CTL response was determined with a standard chromium release assay as previously described (1, 18). Briefly, purified monkey PBMC

TABLE 1. Treatment and SIV_{sm} challenge of four HIV-2-exposed seronegative macaques[a]

Animal no.	Treatment regimen	Date of exposure			Pattern of viral replication after SIV_{sm} challenge in February 1995
		1st	2nd	3rd	
B22	Serum	June 1990	November 1990	November 1991	Limited
S6	FLT	June 1990	November 1990	November 1991	Inhibited
B52	Serum	November 1990	November 1991	ND	Inhibited
75-2	3′-Azido-3′-deoxythymidine	December 1992	March 1993	ND	Persistent

[a] The four cynomolgus macaques were either passively immunized with anti-HIV-2 serum or treated with antiviral drugs and subsequently shown to be protected from intravenous HIV-2 challenge. The four monkeys which showed no evidence of infection following the first HIV-2 challenge were reinoculated with the original HIV-2 inoculum without further treatment. All four monkeys continued to be seronegative and negative for virus isolation and by PCR following the second and third inoculations with HIV-2. In February 1995, the four macaques protected against HIV-2 were intrarectally challenged with 10 MID_{50} of SIV_{sm}. Further details of the treatment regimen are described in Materials and Methods. ND, not done.

were stimulated with 10 μg of concavalin A (Sigma, St. Louis, Mo.) per ml. After 3 to 4 days of culture, 20 U of recombinant human interleukin-2 (Amersham International, Amersham, England) per ml was added. The cytolytic activity was tested after 14 to 21 days in culture. Target cells for the cytotoxicity assay were autologous B-cell lines generated by transformation with herpesvirus papio produced in the S594 cell line (kindly provided by K. H. G. Mills, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, England). Target cells were infected with wild-type vaccinia virus or recombinant vaccinia virus expressing SIV_{mac32H} (J5) *nef* and reverse transcriptase (RT) genes (donated by E. W. Rud, Ottawa, Ontario, Canada) and thereafter labelled with ^{51}Cr. Various effector/target cell ratios were used. $CD8^+$-depleted cell populations were obtained by using anti-CD8 antibody-coated magnetic beads (Dynal, Skøjen, Norway). Percent specific lysis was calculated by the equation [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100. The criteria for a positive CTL value were based on the results obtained from 20 naive control monkeys. The approximate lower limits for a positive CTL value were 11.8% (5.4% + 2 standard deviations) for *nef* and 7.0% (2.5% + 2 standard deviations) for the RT gene. However, the trend in each animal was always considered, and a single positive value was not accepted unless it was confirmed on another occasion.

RESULTS

CTL studies in HIV-2-exposed seronegative macaques. The four cynomolgus macaques were initially either passively immunized or treated with antiviral drugs and shown to be protected against intravenous HIV-2 challenge, as described in Table 1. The monkeys also resisted a second and third HIV-2 rechallenge without further treatment. The monkeys were considered protected if they did not seroconvert to HIV-2 positivity and remained free of any detectable virus in PBMC as demonstrated by coculture and by nested PCR.

The four HIV-2-exposed uninfected monkeys had no demonstrable serum antibodies, and their blood lymphocytes did not show any virus-specific proliferative response to HIV-2 or SIV. However, $CD8^+$-dependent *nef*- and RT gene-specific CTL responses were detected in two monkeys, B52 and S6 (Fig. 1). Monkey B22 had no detectable CTL. Monkey 75-2 was not investigated for CTL due to difficulties in establishing an autologous B-cell line.

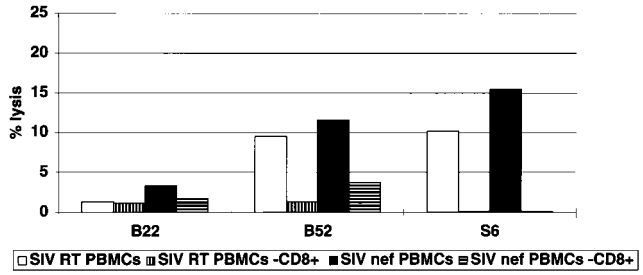

FIG. 1. SIV-specific CTL responses against RT and Nef in HIV-2-exposed seronegative monkeys.

Outcome of mucosal SIV_{sm} challenge. Twenty-three to 39 months following the last HIV-2 exposure, the four exposed macaques and four naive monkeys were challenged with a heterologous SIV_{sm} administered by a mucosal route to mimic the natural transmission of virus in humans. After the pathogenic SIV_{sm} challenge, all four control monkeys were positive for virus culture during the 1-year observation period (Table 2). Similar results were obtained with 10 historical controls, where the intrarectal challenge dose caused high persistent viremia in all 10 animals. Two monkeys previously exposed to HIV-2 resisted the SIV_{sm} challenge, as shown by an inability to isolate virus from PBMC and by negative SIV DNA PCR (Table 2). The aviremic animals were also negative for virus isolation and by SIV DNA PCR from lymph node cells taken 6 and 12 months after SIV_{sm} challenge. The two protected animals were further tested for the presence of SIV by culture of $CD8^+$ cell-depleted PBMC, since $CD8^+$ cell depletion has been shown to increase the sensitivity for detection of HIV and SIV (7). However, the $CD8^+$ cell-depleted cultures did not yield any detectable virus (data not shown). In the remaining two monkeys, virus was isolated from PBMC after SIV_{sm} challenge. However, in one of these animals (monkey B22) virus was isolated only during the first month following SIV_{sm} inoculation (Table 2). Animals B22, B52, and S6 had demonstrable CTL responses to SIV Nef and RT at 3 weeks after SIV_{sm} challenge (data not shown).

Viral antibodies appeared in the 14 control animals within 3 to 4 weeks of SIV_{sm} challenge. Monkey B52 remained seronegative, but viral antibodies appeared in the remaining three

TABLE 2. Results of virus isolation and PCR after SIV_{sm} challenge[a]

Group and monkey	Isolation of virus on day:									PCR on day:			
	0	14	28	58	91	183	240	295	365	14	28	183	365
HIV-2 exposed													
B22	−	+	+	−	−	−	−	−	−	+	+	−	−
B52	−	−	−	−	−	−	−	−	−	−	−	−	−
S6	−	−	−	−	−	−	−	−	−	−	−	−	−
75-2	−	+	+	+	+	+	+	+	+	+	+	+	+
Control													
C54	−	+	+	+	+	+	+	+	+	+	+	+	+
C57	−	+	+	+	+	+	+	+	+	+	+	+	+
C68	−	+	+	+	+	+	+	+	+	+	+	+	+
C73	−	+	+	+	+	+	+	+	+	+	+	+	+

[a] The results of virus isolation and PCR after intrarectal SIV_{sm} challenge of HIV-2-exposed but seronegative macaques and control monkeys challenged only with SIV_{sm}. Challenge was done on day 0. +, virus detected by virus isolation or by PCR; −, no virus detected.

monkeys. Surprisingly, monkey S6 developed a low antibody titer after SIV_{sm} challenge in spite of the fact that virus could not be isolated by coculture, demonstrated by PCR, or transmitted by blood transfusion.

Transfusion experiment. The two protected animals (B52 and S6) and the partially protected monkey B22 were further tested for the presence of SIV_{sm} by the transfusion of blood to untreated recipients. Four untreated monkeys were each transfused with 5 ml of heparinized blood from monkeys B52, S6, B22, and C57 (infected control). The blood was taken 6 months after challenge with SIV_{sm}. Transfused monkeys were monitored for signs of SIV infection during 6 months of follow-up by virus isolation, SIV-specific antibody response, and PCR. Transfer of blood from the aviremic monkeys B52 and S6 revealed no evidence of SIV infection in the two recipients. However, the two monkeys that received blood from B22 and C57 became productively infected.

DISCUSSION

This is the first demonstration that highly exposed macaques show virus-specific CTL responses in the absence of antibodies and that the presence of CTL is correlated with protection against mucosal SIV challenge. Here we report cross-protection against detectable SIV replication in 2 of 4 monkeys and suppression of viral replication in a third animal compared with 14 control animals which were repeatedly positive for virus isolation. HIV-specific CTL activity normally requires actively replicating virus for stimulation, implying that the monkeys were exposed to HIV-2 at a level sufficient to prime T-cell immunity but insufficient to induce antibody production. The lack of measurable antibodies prior to SIV_{sm} challenge and the presence of cytotoxic T cells before and after challenge suggest that cellular immunity was responsible for the cross-protection observed. Since CTLs cannot neutralize cell-free virus, the mechanism of protection was probably clearance of early infection. The CTL may have tipped the balance in favor of the host and made it possible to clear the early infection while the number of infected cells was still low. However, it is also possible that other cellular mechanisms such as $CD8^+$ T-cell-mediated, noncytotoxic, antiviral activity (24) and $CD8^+$ cell-produced chemokines (6) accounted for or contributed to the protection observed.

This study is consistent with previous clinical observations indicating that naturally occurring immunity to HIV may exist and may have helped to protect some individuals who remain seronegative and uninfected despite documented exposure. Specific CTL have been detected in infants born of infected mothers (20), long-time partners of HIV-infected men or women (9), some female prostitutes in Africa (19), and some health-care workers exposed to body fluids of HIV-infected individuals (15). Hom and collaborators (8) have demonstrated in a mouse model that transfer of specific immune T cells can mediate protective immunity against live Rauscher murine leukemia virus challenge. These findings, together with evidence from the present study, suggest that cellular immunity alone is sufficient for protection against a retrovirus.

Another possible explanation is that the resistance to infection observed in two of the monkeys in the present study is due to genetic factors rather than to virus-induced immunity. Earlier Paxton et al. (14) described a relative resistance to HIV-1 infection of $CD4^+$ lymphocytes from persons who remained uninfected despite multiple high-risk sexual exposures. Liu et al. (11) showed that these two individuals were homozygous for a defect in the gene encoding CKR-5, a major coreceptor for macrophage-tropic HIV-1 isolates. It is not known whether the protected monkeys in our study lacked this coreceptor. However, we found that cells from the protected animals were easily infected in vitro by the SIV_{sm} strain used for challenge (data not shown).

Clerici et al. (5) have reported that macaques exposed to low doses of SIV mount cellular responses to the virus in the absence of antibody and that some macaques were protected from subsequent homologous rectal challenge. However, CTL responses were not examined in these experiments. Furthermore, the SIV challenge stock was grown on a human T-cell line, and immunity to nonmonkey HLA antigens could have been involved in the protection (23). It should be noted that our challenge stocks were free from human HLA antigens since our HIV-2 and SIV stocks were grown on macaque PBMC.

The implication of our findings is that individuals who are exposed to HIV and develop virus-specific CTL without antibody production may build up a truly protective immunity. Additionally, we show that attenuation of a primate lentivirus infection by chemotherapy or passive immunization may stimulate the development of a protective cellular immune response. Our evidence for cell-mediated protection implies that vaccine strategies favoring the induction of a stable and strong cellular immune response could protect against subsequent mucosal transmission of HIV. It has recently been demonstrated that antiviral CTL are present in the vaginal epithelium (12), which indicates that an appropriate immunization regimen may be able to generate anti-HIV CTL in the mucosal immune system of the genital tract. Taken together, our observations support the notion that stimulation of a CTL response is an important goal in the development of an effective prophylactic vaccine against HIV infection and AIDS.

ACKNOWLEDGMENTS

We thank Reinhold Benthin, Kerstin Hild, and Katarina Karlén for expert technical assistance.

This work was supported by grants from the Swedish International Cooperation Agency (SIDA), Department for Research Cooperation (SAREC), Swedish National Board for Industrial and Technical Development (NUTEC), and Swedish Medical Research Council.

REFERENCES

1. **Andersson, S., B. Mäkitalo, R. Thorstensson, G. Franchini, J. Tartaglia, K. Limbach, E. Paoletti, P. Putkonen, and G. Biberfeld.** 1996. Immunogenicity and protective efficacy of a human immunodeficiency virus type 2 recombinant canarypox (ALVAC) vaccine candidate in cynomolgus monkeys. J. Infect. Dis. **174:**977–985.
2. **Böttiger, D., P. Putkonen, and B. Öberg.** 1992. Prevention of HIV-2 and SIV infections in cynomolgus monkeys by prophylactic treatment with 3′-fluorthymidine. AIDS Res. Hum. Retroviruses **8:**1235–1238.
3. **Böttiger, D.** Personal communication.
4. **Cheynier, R., P. Langlade-Demoyen, M.-R. Marescot, S. Blanche, G. Blondin, S. Wain-Hobson, C. Griselli, E. Vilmer, and F. Plata.** 1992. Cytotoxic T lymphocytes responses in the peripheral blood of children born to human immunodeficiency virus-1-infected mothers. Eur. J. Immunol. **22:** 2211–2217.
5. **Clerici, M., E. A. Clark, P. Polacino, I. Axberg, L. Kuller, N. I. Casey, W. R. Morton, G. M. Shearer, and R. E. Benveniste.** 1994. T-cell proliferation to subinfectious SIV correlates with lack of infection after challenge of macaques. AIDS **8:**1391–1395.
6. **Cocchi, F., A. L. DeVico, A. Garzino-Demo, S. K. Arya, R. C. Gallo, and P. Lusso.** 1995. Identification of RANTES, MIP-1-α, and MIP-1β as the major HIV-suppressive factors produced by $CD8^+$ T cells. Science **270:**1811–1815.
7. **Desrosiers, R. C., M. Wyand, T. Kodama, D. J. Ringler, L. O. Arthur, P. K. Sehgal, N. L. Letvin, and M. D. Daniel.** 1989. Vaccine protection against simian immunodeficiency virus infection. Proc. Natl. Acad. Sci. USA **86:** 6353–6357.
8. **Hom, R. W., R. W. Finberg, S. Mullaney, and R. M. Ruprecht.** 1991. Protective cellular immunity requires both $CD4^+$ and $CD8^+$ immune cells. J. Virol. **65:**220–224.
9. **Langlade-Demoyen, P., N. Ngo-Giang-Huong, F. Ferchal, and E. Oksenhendler.** 1994. Human immunodeficiency virus (HIV) *nef*-specific cytotoxic T

lymphocytes in noninfected heterosexual contact of HIV-infected patients. J. Clin. Invest. **93:**1293–1297.
10. **Lewis, M. G., W. R. Elkins, F. E. McCutchan, R. E. Benveniste, C. Y. Lai, D. C. Montefiori, D. S. Burke, G. A. Eddy, and A. Schafferman.** 1993. Passively transferred antibodies directed against conserved regions of SIV envelope protect macaques from SIV infection. Vaccine **11:**1347–1355.
11. **Liu, R., W. A. Paxton, S. Choe, D. Ceradini, S. R. Martin, R. Horuk, M. E. MacDonald, H. Stuhlmann, R. A. Koup, and N. R. Landau.** 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell **86:**367–377.
12. **Lohman, B. L., C. J. Miller, and M. B. McChesney.** 1995. Antiviral cytotoxic T lymphocytes in vaginal mucosa of simian immunodeficiency virus-infected rhesus macaques. J. Immunol. **155:**5855–5860.
13. **Nilsson, C., R. Thorstensson, G. Gilljam, S. Sjölander, K. Hild, K. Broliden, L. Åkerblom, B. Morein, G. Biberfeld, and P. Putkonen.** 1995. Protection against monkey-cell grown cell-free HIV-2 challenge in macaques immunized with native HIV-2 envelope glycoprotein gp 125. Vaccine **4:**165–175.
14. **Paxton, W. A., S. R. Martin, D. Tse, T. R. O'Brien, J. Skurnick, N. L. VanDevanter, N. Padian, J. F. Braun, D. P. Kotler, S. M. Wolinsky, and R. A. Koup.** 1996. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk exposures. Nat. Med. **2:**412–417.
15. **Pinto, L. A., J. Sullivan, J. A. Berzofsky, M. Clerici, H. A. Kessler, A. L. Landay, and G. M. Shearer.** 1995. ENV-specific cytotoxic T lymphocyte responses in HIV seronegative health care workers occupationally exposed to HIV-contaminated body fluids. J. Clin. Invest. **96:**867–876.
16. **Putkonen, P. R., Thorstensson, L. Ghavamzadeh, J. Albert, K. Hild, G. Biberfeld, and E. Norrby.** 1991. Prevention of HIV-2 and SIVsm infection by passive immunization in cynomolgus monkeys. Nature **352:**436–438.
17. **Putkonen, P., R. Thorstensson, L. Walther, J. Albert, L. Åkerblom, O. Granquist, G. Wadell, E. Norrby, and G. Biberfeld.** 1991. Vaccine protection against HIV-2 infection in cynomolgus monkeys. AIDS Res. Hum. Retroviruses **7:**271–277.
18. **Quesada-Rolander, M., B. Mäkitalo, R. Thorstensson, Y.-J. Zhang, E. Casanos-Velez, G. Biberfeld, and P. Putkonen.** 1996. Protection against mucosal SIVsm challenge in macaques infected with a chimeric SIV that expresses HIV type 1 envelope. AIDS Res. Hum. Retroviruses **12:**993–999.
19. **Rowland-Jones, S., J. Sutton, K. Ariyoshi, T. Dong, F. Gotch, S. McAdam, D. Whitby, S. Sabally, A. Gallimore, T. Corrah, M. Takuguchi, T. Schultz, A. McMichael, and H. Whittle.** 1995. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nat. Med. **1:**59–64.
20. **Rowland-Jones, S. L., D. F. Nixon, M. C. Aldhouse, F. Gotch, K. Ariyoshi, N. Hallam, J. S. Kroll, K. Froebel, and A. McMichael.** 1993. HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. Lancet **341:**860–861.
21. **Salk, J., P. A. Bretscher, P. L. Salk, M. Clerici, and G. M. Shearer.** 1993. A strategy for prophylactic vaccination against HIV. Science **260:**1270–1272.
22. **Schultz, A. M., and E. J. Stott.** 1994. Primate models for AIDS vaccines. AIDS **8**(Suppl. 1)**:**203–212.
23. **Stott, E. J., P. A. Kitchin, M. Page, B. Flanigan, L. F. Taffs, W. L. Chan, K. H. G. Mills, P. Silvera, and A. Rodgers.** 1991. Anti-cell antibody in macaques. Nature **353:**393.
24. **Walker, C. M., D. J. Moody, D. P. Stites, and J. A. Levy.** 1986. $CD8^+$ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science **234:**1563–1566.
25. **Walther, L., O. Granquist, P. Putkonen, G. Biberfeld, and R. Thorstensson.** 1996. Nested polymerase chain reaction primers that distinguish between SIVsm and HIV type 2. AIDS Res. Res. Hum. Retroviruses **12:**1077–1079.