

Nonpathogenic Simian Immunodeficiency Virus Infection of Sooty Mangabeys Is Not Associated with High Levels of Autologous Neutralizing Antibodies[∇]

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Simian immunodeficiency virus (SIV) infection of natural-host species, such as sooty mangabeys (SMs), is characterized by a high level of viral replication and a low level of generalized immune activation, despite evidence of an adaptive immune response. Here the ability of SIV-infected SMs to mount neutralizing antibodies (Nab) against autologous virus was compared to that of human immunodeficiency virus type 1 (HIV-1) subtype C-infected subjects. While high levels of Nab were observed in HIV-1 infection, samples obtained at comparable time points from SM exhibited relatively low titers of autologous Nab. Nevertheless, SM plasma with higher Nab titers also contained elevated peripheral CD4⁺ T-cell levels, suggesting a potential immunologic benefit for SMs. These data indicate that AIDS resistance in these primates is not due to high Nab titers and raise the possibility that low levels of Nab might be an inherent feature of natural-host SIV infections.

More than 40 species of African nonhuman primates (NHPs) naturally harbor CD4⁺-tropic lentiviruses that are collectively known as simian immunodeficiency viruses (SIVs) and represent the ancestors of the human pathogens human immunodeficiency virus type 1 (HIV-1) and HIV-2. Interestingly, African NHPs infected with their cognate SIV generally do not progress to AIDS, despite high levels of sustained virus replication, with the only known exception being chimpanzee SIV (SIVcpz)-infected chimpanzees (16). Among the natural hosts for SIV infection, the sooty mangabey ([SM] *Cercocebus atys*) is of particular interest, because cross-species transmission of SM SIV (SIVsm) from this natural host into humans initiated the HIV-2 epidemic in West Africa (17). In addition, SIVsm (herein referred to as SIV) is the ancestor of the rhesus macaque SIV (SIVmac) viruses that are used in disease pathogenesis and vaccination studies in the rhesus macaque model (17). Both naturally infected and experimentally inoculated SMs remain healthy, maintain CD4⁺ T cells, and do not progress to AIDS-like disease, despite sustained high levels of virus replication (31).

Nonpathogenic infection of SMs is characterized by low levels of immune activation during the chronic phase of infection, which are reached after a transient immune activation

that occurs during primary infection (reviewed in reference 31). These findings have led to the hypothesis that the absence of generalized immune activation in SIV-infected SMs during the chronic phase of infection is an important feature that favors the preservation of CD4⁺ T-cell homeostasis, thereby avoiding disease progression (31). However, most of these earlier studies focused on T cells and innate immune cells, with a significant gap existing in our understanding of whether humoral immunity might also differ between pathogenic and nonpathogenic infections. In HIV-1-infected patients, B cells produce neutralizing antibodies against the infecting (autologous) virus, which drives viral escape, continuous *de novo* antibody production (26–28, 32), and B-cell dysfunction (24). The striking differences in both the clinical outcomes of infection and the levels of immune activation between SIV-infected SMs and HIV-1-infected humans prompted us to compare the neutralizing antibody (Nab) response against the autologous virus in these two populations. To this end, we utilized a pseudovirus assay that has been used extensively by our group and others to evaluate Nab against HIV-1 and SIV envelope (Env) glycoproteins (15, 19, 22, 26, 28, 32, 33; also unpublished data). All SMs were housed at the Yerkes National Primate Research Center (Atlanta, GA) and maintained in accordance with National Institutes of Health guidelines. The Emory University Animal Care and Use Committee approved these studies. Details of the Zambia Emory HIV Research Project (ZEHRP) have been described elsewhere (2, 10, 21). The Emory University Institutional Review Board and the University of Zambia School of Medicine Research Ethics Committee approved informed-consent and human subject protocols. None of the

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TABLE 1. Autologous Nab activity in experimentally SIV-infected SM and acutely HIV-1-infected humans

Subject ID ^a	Virus	No. of mo postinfection Nab activity was evaluated	No. of days postinfection Envs were cloned from plasma	No. of Envs tested	% neutralization at a 1:100 dilution of plasma
Fuv	SIVsm-Fuo	6	14	4	16.3
FSs	SIVsm-Fuo	6	14	3	10.6
FWv	SIVsm-Fuo	6	14	5	10.5
FFs	SIVsm-Fuo	6	14	2	10.3
FRs	SIVsm-Fuo	6	14	3	9.3
185F	HIV-1	5	33	4	94.6
153M	HIV-1	9	88	5	94.3
221M	HIV-1	6	31	6	91.5
205F	HIV-1	2	48	5	87.1

^a ID, identification.

subjects received antiretroviral therapy during the evaluation period.

In HIV-1 infection, autologous Nabs develop to relatively high titers against the newly transmitted virus within the first few months (15, 19, 26–28, 32). Here we sought to test whether a similar increase in Nab titer occurs during nonpathogenic SIV infection of SMs. Samples were obtained from five animals that were inoculated intravenously with plasma from a naturally infected SM as part of a previous study (30). Multiple, biologically functional Envs were cloned from plasma collected at day 14 postinoculation (Table 1), and Nab activity was evaluated in plasma collected at 6 months postinoculation. To facilitate comparison with early HIV-1 infection, Nab activity in plasma was also evaluated between 2 and 9 months against Envs that were cloned between 31 and 88 estimated days after infection from four subtype C HIV-1-infected seroconverters in Zambia (Table 1). Figure 1A demonstrates that Nab activity in plasma diluted 1:100 was readily detectable in all HIV-1-infected subjects at levels approaching 100% neutralization. However, Nab activity in the SM plasma was significantly lower than in the human subjects (median, 10% versus 93%, respectively; $P = 0.02$). Binding antibody was detected in all five SMs at titers greater than 1:51,200 by enzyme-linked immunosorbent assay (ELISA), demonstrating that all monkeys had seroconverted by 6 months and maintained high titers of binding antibody throughout the evaluation period (Fig. 1B). Thus, the low level of Nab was not due to a diminished humoral immune response.

The low level of Nab activity observed in the five experimentally inoculated SMs persisted for 16 months and did not exceed 50% at a 1:100 dilution of plasma at any time point tested (Fig. 1C). In contrast, the high levels of Nab activity in the HIV-1-infected subjects persisted for over 2 years, often exceeding 50% inhibitory titers of 1:3,000 against the early virus, as is characteristic of early subtype C HIV-1 infection (15, 19, 26, 28). Figure 1D demonstrates that a transient increase in proliferating B cells, as measured by positive Ki-67 staining (12), occurred in the SMs and peaked around day 30 postinfection and then declined to a level just above baseline by day 60. Analysis using a Wilcoxon signed-rank test for paired samples showed that the percentages of Ki-67-positive (Ki-67⁺) B cells were higher at days 21 and 30 than at day -5, reaching borderline significance at both time points ($P = 0.06$). In contrast, the percentages of Ki-67⁺ B cells on days 60 and 475

were not significantly different from that on day -5 ($P = 0.8$ and 0.3, respectively). An early but transient decrease in the percentage of circulating CD20⁺ B cells was also observed during the initial 20 days of infection (Fig. 1E). Thus, the B-cell compartment within the SM underwent changes consistent with immune activation followed by resolution. Based on these results, it does not appear that a global defect in the B-cell response in the SM can account for the low-level Nab response elicited.

To investigate Nab responses during established infection, we extended this analysis to a panel of 11 naturally SIV-infected SMs in the Yerkes colony and 5 chronically HIV-1-infected subjects in Zambia. Envs were cloned from these monkeys and human subjects using peripheral blood mononuclear cell (PBMC) DNA or plasma samples, and sensitivity to Nab was evaluated. Because Nab activity against contemporaneous Env is often low or undetectable in HIV-1 infection (1, 5, 14, 25, 27, 28, 32), we evaluated plasma collected between 6 and 55 months after the Envs were cloned from each individual. Table 2 shows that the SM Envs reflected the four SIV subtypes that circulate in the Yerkes colony (3). Figure 2A demonstrates that Nab activity in the chronically HIV-1-infected subjects was high (median, 91%), whereas in the naturally SIV-infected SMs it was again significantly lower (median, 14%; $P = 0.003$). Nevertheless, Nab activity in the naturally infected SMs exhibited a considerable range, from undetectable to 84% neutralization (Fig. 2A). This observation prompted us to investigate whether parameters associated with disease progression in HIV-1 infection were correlated with the level of Nab activity. Figure 2B demonstrates that the number of CD4⁺ T cells was positively correlated with the potency of neutralization ($r = 0.69$; $P = 0.02$), while the plasma viral load showed a trend toward an inverse correlation with neutralization (Fig. 2C) ($r = -0.54$; $P = 0.08$). A correlation between plasma viral load and autologous Nab titer in established HIV-1 infection has not been observed (9).

This study is the first to directly compare the Nab response against the autologous virus in nonpathogenic SIV versus HIV-1 infection, including evaluation of both the early, developing Nab response in acute infection and the mature response in chronic infection. A significant difference in the magnitude of Nab activity was apparent during both early and later time points, with relatively strong but ultimately ineffective neutralization activity developing and persisting into chronic infection

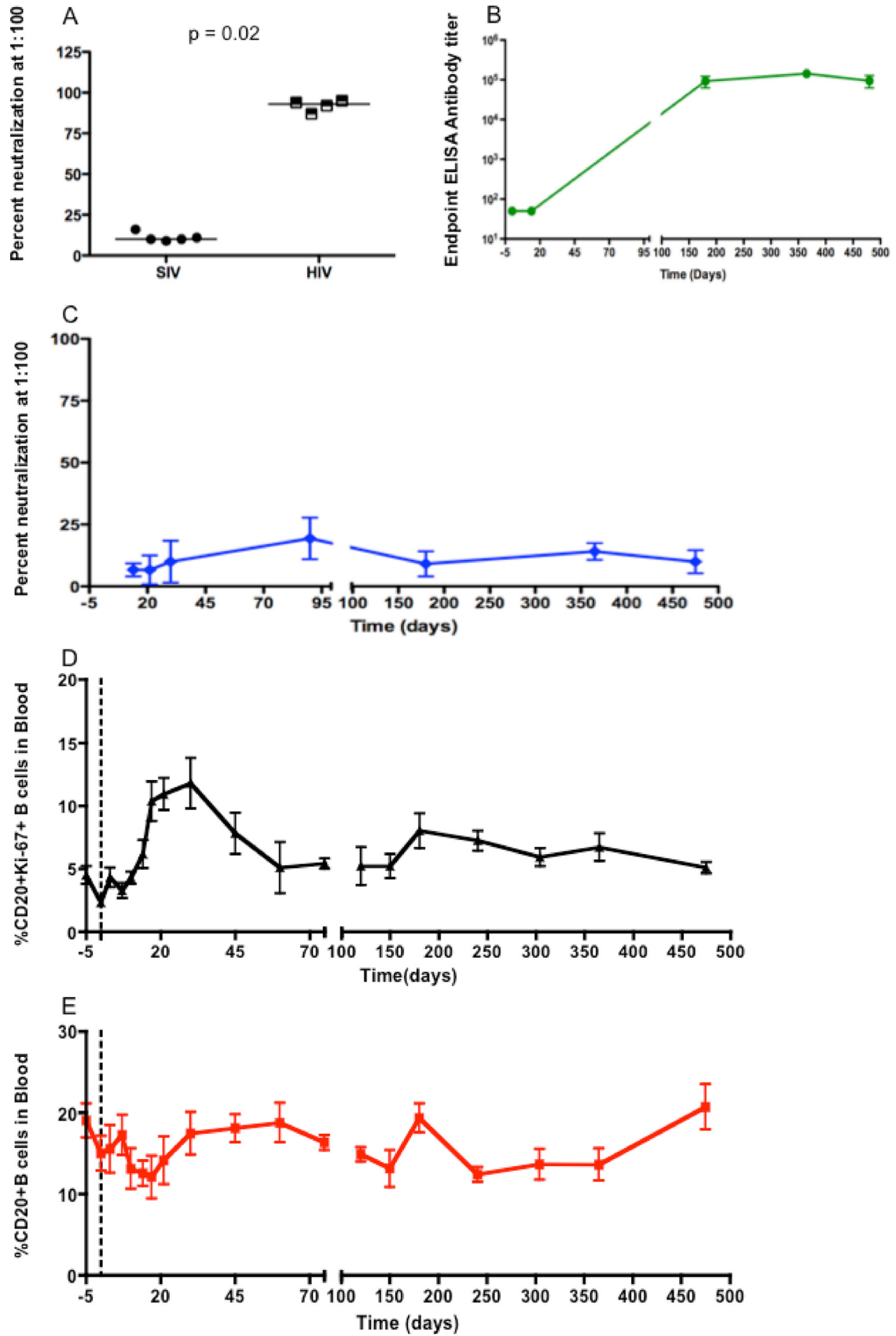


TABLE 2. Autologous Nab activity in naturally SIV-infected SMs and HIV-1-infected humans with established infections

Subject ID ^a	Virus	Env subtype	No. of mo between plasma collection and Env cloning	No. of Envs tested	% neutralization at 1:100 dilution of plasma
FWk	SIVsm	2	28	5	84.4
FNn	SIVsm	1	31	4	63.5
FFv	SIVsm	1	50	4	59.7
FFm	SIVsm	1	30	4	42.0
FNg	SIVsm	5	48	4	28.0
FBn	SIVsm	3	49	2	13.9
FDo	SIVsm	3	6	5	12.0
FZo	SIVsm	1	28	3	8.8
FOh	SIVsm	1	6	2	4.7
FPn	SIVsm	1	32	5	0.6
FFj	SIVsm	1	54	2	0.0
109M	HIV-1	C	6	5	91.4
55M	HIV-1	C	15	8	91.3
135F	HIV-1	C	16	4	97.4
106M	HIV-1	C	17	5	79.4
153F	HIV-1	C	55	5	99.0

^a ID, identification.

in humans but not in SMs. Although the SIV and HIV-1 samples were obtained during similar stages of infection, the disparity in the magnitude of autologous Nab activity during early infection could in part reflect differences such as the route of infection (intravenous versus mucosal) or the complexity of the founder virus (a single variant in HIV-1 versus multiple variants in SIV). In addition, the production of SIV Env pseudoviruses in human 293T cells could have altered the glycosylation pattern or the proteins that are embedded within the virion, decreasing the neutralization susceptibility of the SIV Env pseudoviruses. However, production of a subset of these pseudoviruses in an African green monkey-derived cell line (COS-1) did not alter their Nab sensitivity (data not shown).

Despite the lack of potent autologous Nab, both naturally and experimentally SIV-infected SMs produce antibodies that bind Env in ELISAs or Western blotting (4, 6, 13, 18, 23). It is possible that the SIV Env glycoproteins elicit a different profile of Nab than does HIV-1 Env. The potential for structural and biological differences between SIV and HIV-1 Envs has not been thoroughly investigated, although they would not be unexpected due to the low level of amino acid sequence conser-

vation between them. SIVsm/HIV-2 lineage-derived Envs (i.e., the SIVmac series) show a “wide evolutionary distance” and lack of cross-reactivity with SIVcpz/HIV-1-derived Envs, with an overall sequence identity in gp120 of ~25% across HIV-1, HIV-2, and SIVsm (7, 8). Clear biological differences in immunogenicity have been described for HIV-1 group M subtypes, which all derive from a common SIV ancestor (reviewed in reference 20). Furthermore, SM IgG antibody molecules have less flexibility in the hinge region than human IgG, which could lead to a failure of the SM antibodies to recognize recessed neutralization targets such as the receptor binding domains (29). Thus, HIV-1 Env could elicit neutralizing antibodies that are qualitatively different from those induced by SIV Env.

Early resolution of immune activation could be a key feature that distinguishes nonpathogenic from pathogenic infection (12, 31). The data presented here are consistent with that hypothesis, in that signs of early B-cell proliferation were present in the experimentally infected SMs but were resolved and did not result in potent neutralizing activity. However, later in infection, the naturally infected SMs did develop low-to-moderate levels of Nab activity, and these levels were positively correlated with the number of peripheral CD4⁺ T cells. This finding suggests that synergy between CD4⁺ T cells and B cells is maintained in this nonpathogenic setting. Other biologic factors could contribute to this correlation; however, differences in age and viral subtype in this cohort of SMs could not explain this finding (data not shown).

Taken together, these results indicate that a low level of autologous Nab activity is a novel and previously unappreciated feature of nonpathogenic SIV infection of SMs. The fact that high-titer Nabs are not necessary to avoid disease progression during SIV infection of SMs is consistent with the notion that the apathogenicity of natural SIV infections is not the result of particularly effective adaptive immune responses against the virus (11). It is possible that this low level of autologous Nab activity in SMs stems in part from antibody recognition of targets that are poorly exposed on the native SIV Env glycoproteins. A low level of neutralizing activity in SM may therefore have a protective effect because it does not drive viral escape or induce chronic immune activation in the B-cell compartment. Moreover, a low level of immune activation in B cells and/or preservation of CD4⁺ T cells could enhance the quality of the neutralizing antibody response. It

FIG. 1. Autologous Nab activity and B-cell proliferation during experimental infection of SMs. (A) Neutralization activity levels in plasma from five SMs (filled black circles), which were experimentally inoculated with plasma from a naturally SIV-infected SM, and four HIV-1-infected Zambian subjects (half-filled squares), who were recently infected through heterosexual contact, are shown. The horizontal bars represent the median for each group. To assess neutralizing activity, pseudoviruses were created by expressing each cloned Env with an HIV-1 *env*-deficient backbone (Δ SG3). JC53-BL (Tzm-bl) cells were infected with each pseudovirus in the presence or absence of serially diluted autologous plasma. Each point represents the average level of neutralization at a 1:100 dilution of plasma for at least two Env clones (see Table 1 for number of Envs tested). Each neutralization assay was performed twice independently, using duplicate wells. Statistical significance between the groups was determined by a Mann-Whitney test, using GraphPad Prism 5. Longitudinal measurements of endpoint antibody ELISA titers in plasma (filled green circles) (23) (B), autologous neutralization activity in plasma (filled blue diamonds) (C), percentages of Ki-67⁺ CD20⁺ cells in blood (filled black triangles) (D), and percentages of CD20⁺ cells in blood (filled red squares) (E) are shown for the five experimentally inoculated SMs combined. In panel C, each point represents average neutralization at a 1:100 dilution of plasma over time for at least two day 14 Env clones from each SM. For panels D and E, PBMCs were gated by forward and side scatter, and the CD3⁻ CD20⁺ population was assessed for Ki-67 staining (D) by flow cytometry. SP34-2 was used to stain CD3, L27 was used for CD20, and B56 was used for Ki-67 (all from BD Biosciences). Error bars represent the standard errors of the means (SEMs). Plasma viral load peaked at day 14 (data not shown). Filled symbols in panels A through E indicate data generated from experimentally infected SMs.

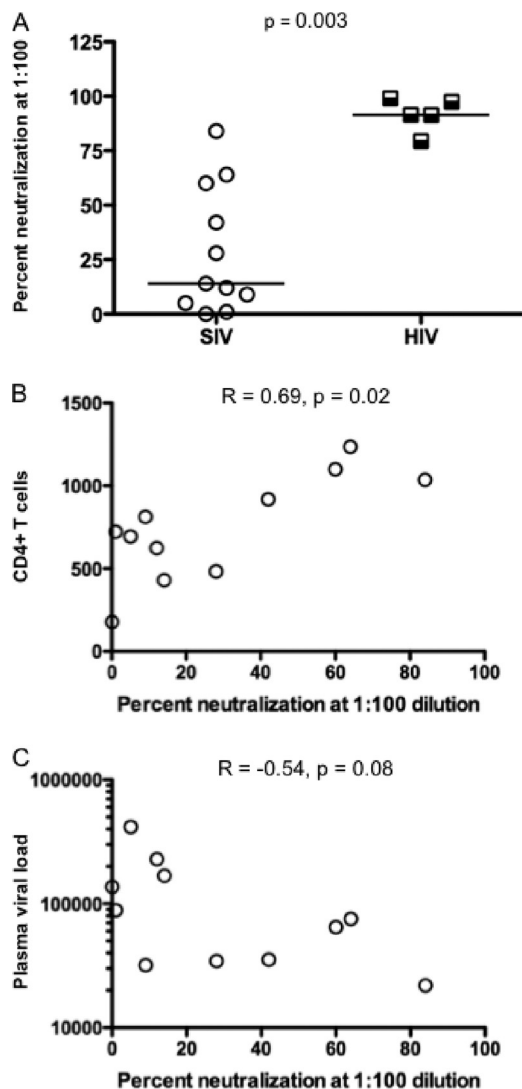


FIG. 2. Autologous Nab activity and its correlation with CD4⁺ count and plasma viral load during established natural infection of SMs. (A) Neutralization activity levels in plasma from 11 naturally SIV-infected SMs in the Yerkes colony (open circles) and 5 chronically HIV-1-infected human subjects from Zambia (half-filled squares) are shown. Statistical significance between groups was determined by a Mann-Whitney test using GraphPad Prism 5. Correlation between Nab activity and CD3⁺ CD4⁺ T cell counts or plasma viral load in naturally infected SMs (open circles) is shown in panels (B) and (C), respectively. The percent neutralization at a 1:100 dilution of plasma (shown in panel A) is plotted along the x axis. Each CD4⁺ T cell count and viral load value represents the average of three measurements from samples collected from the 11 SMs approximately 1 year apart. The significance of each correlation was determined using a nonparametric Spearman test. Open circles indicate data from naturally infected SMs.

will be important, in future work, to assess how this low level of autologous Nab activity in SIV-infected SMs meshes with the lower levels of immune activation and dysregulation observed in these animals. Understanding the qualitative and quantitative differences in the Nab response during pathogenic versus nonpathogenic infection could provide critical information regarding protection from AIDS.

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