Suppression of Acute Viremia by Short-Term Postexposure Prophylaxis of Simian/Human Immunodeficiency Virus SHIV-RT-Infected Monkeys with a Novel Reverse Transcriptase Inhibitor (GW420867) Allows for Development of Potent Antiviral Immune Responses Resulting in Efficient Containment of Infection

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A nonnucleoside reverse transcriptase (RT) inhibitor, GW420867, was tested for postexposure prophylaxis (PEP) in rhesus macaques experimentally infected with 100 50% tissue culture infective doses of a chimeric simian/human immunodeficiency virus (SHIV) containing the RT gene of HIV-1 (SHIV-RT). Animals were either mock treated, or treated for 4 weeks starting at 8 or 24 h postinfection (p.i.) with GW420867. While such therapy led to undetectable plasma viremia in three of six monkeys, a transient plasma viremia was noted in the other three treated animals at 2 to 4 weeks following cessation of therapy. Following this transient viremia all drug-treated animals showed low or undetectable levels of plasma viremia up to the last sample examined at 90 weeks p.i. Despite low and/or undetectable viremia, virus-specific cytotoxic T lymphocyte and viral Env-specific proliferative responses were seen in the peripheral blood mononuclear cells of both mock- and drug-treated animals as early as 3 weeks p.i. Such virus-specific cellular responses, however, were better maintained in the drug-treated animals than the mock-treated animals. In contrast to the virus-specific cellular response, the magnitude and kinetics of virus specific humoral responses appeared to correlate with the detection of viremia. These data support the view that a short-term PEP with GW420867 permits the generation and maintenance of long-lasting virus-specific cell-mediated immune responses while markedly reducing viral loads to undetectable levels for a prolonged period of time (90 weeks) and leads to long-term disease protection. This model provides a unique means to define mechanisms and correlates of disease protection.

A dramatic decrease in viral load coincident with a recovery of CD4+ T-cell counts has been noted in human immunodeficiency virus (HIV)-infected patients following highly active antiretroviral therapy (HAART) (2, 10, 11). However, it has been noted that while such therapy does lead to recovery of CD4+ T-cell levels, it fails to establish effective anti-HIV immune responses, as evidenced by a rebound of viremia which has been noted among HIV-infected individuals in whom HAART was discontinued (31). Of great concern is the finding that latent HIV infection may persist for more than several decades even in successful HAART cases (7, 27, 30). These findings indicate a need for defining therapeutic modalities that will continue to maintain low viral loads and gradually either eliminate latent virus-infected cells and/or reduce and/or eliminate the likelihood of disease. However, the precise nature of the host immune response(s) that is capable of containing viral load and/or preventing disease remains to be defined. Some insights have been gained on this issue by the study of the host antivirus-specific immune responses in HIV type 1 (HIV-1)-infected long-term nonprogressors and in nonhuman primates infected with attenuated simian immunodeficiency virus (SIV) viral constructs (13, 15). However, to date, there have been limited if any studies of the use of chemotherapeutic drugs that reduce viral loads yet allow for the development of the quality and quantity of antiviral immune responses outlined above.

Our laboratory has been studying a nonnucleosidic class of the quinoxalines as inhibitors of HIV-1 reverse transcriptase (RT), using HBY 97 as the first clinical candidate (16, 19, 20). A search for a compound with improved pharmacokinetics led to the discovery of HBY 1293A (now GW420867), which allows a single daily dosing to reach and maintain therapeutic levels in humans (data not shown). Toward the goal of determining the efficacy of this drug, we utilized rhesus macaques...
infected intravenously with a relatively high dose (100 50% tissue culture infective doses [TCID₅₀]) of a pathogenic chimeric simian/human immunodeficiency virus (SHIV) containing the RT gene of HIV-1 (SHIV-RT) (23, 25). Groups of animals were either mock treated (control) or treated for 4 weeks with GW420867 starting at 8 or 24 h postinfection (p.i.). Whereas the mock-treated animals showed typical acute and then persistent productive infection, besides a transient viremia in three of six animals at 2 to 4 weeks postcession of therapy, all six drug-treated animals showed suppression of viral replication to undetectable levels until the last sample analyzed at 90 weeks p.i. Of interest was the finding that despite such marked reduction in viral loads, the drug-treated animals showed readily detectable antiviral cellular immune responses as early as 3 weeks p.i., which were maintained for a prolonged period, in contrast to fading virus-specific cellular response in the mock-treated animals. Such a model for the first time allows for the use of a chemotherapeutic agent to potentially identify disease-protective immune responses. Results of the studies performed constitute the basis of this report.

MATERIALS AND METHODS

Virus. The chimeric SHIV-RT consists of a SIVmac239 virus backbone in which the SIV RT gene was replaced by the HIV-1 HxB2 RT gene (17). The parent viral stocks were prepared by DNA transfection of proviral DNA into COS-1 cells. The virus stock used in the studies infected rhesus monkeys (23, 25). The parent viral stocks were prepared by DNA which the SIV RT gene was replaced by the HIV-1 HxB2 RT gene as previously described (26). The sensitivity of plasma viral RNA detection by this technique was determined to be 1,000 copies per ml of plasma.

Proviral DNA load. PBMC samples were purified from heparinized blood utilizing standard Ficol-Hypaque gradient centrifugation. Cellular DNA was purified from 10⁶ cells with a commercial DNA PCR kit (27) with Tq DNA polymerase (Takara). Measurement of amplified RNA was achieved utilizing a fluorescence imaging analyzer (FLA2000, Fuji Film, Tokyo, Japan), and the viral RNA load was calculated using an Excel spreadsheet (Microsoft, Tokyo, Japan) as previously described (26).

Viral replication. Fourfold dilutions of PBMC (starting with 10⁶ in 0.5 ml) were cocultured with 2.5 x 10⁵ C8166 cells (0.5 ml) in duplicate wells of 24-well plates. The cocultures were incubated for 3 to 4 weeks, and incubation media was collected at intervals as specified for each of the assays described herein. Biopsies of inguinal lymph nodes from one animal in the control group and from one animal in each of the drug-treated groups were performed at 8 weeks.

Plasma viral load. Viral RNA was purified from 0.2 ml of plasma with a commercial viral RNA isolation kit (Boehringer Mannheim, Tokyo, Japan) and was dissolved with 50 μl of the elution buffer included in the kit. Aliquots of the viral RNA were stored at −80°C. Plasma viral RNA load was measured initially by a commercially available RT-PCR kit (Boehringer Mannheim) and with the use of a competitive RT-PCR assay which served as a standard. The values were then confirmed by using the quantitative competitive RT-PCR method previously described (26). The sequence of the gag primers utilized included SG05 (5'-AGCTGTTCAAGATGCAAC-3') and SG06 (5'-CTACTGCTTCCTCAAGAGGAGATG-3'), which were designed to amplify most SIVmac and SIVsmm gag sequences. The competitor RNA fragment was generated by amplification of SIVmac239 gag sequences with SG05i and SG06i and cloning of the 59-bp fragment into a pGEM-T vector (Promega, Madison, Wis.). The insert was then digested with SalI and XcmI, blunted, and religated in order to remove a 133-bp fragment. The plasmid was then linearized downstream of the insert using SalI, and a competitor RNA was generated by using T7 RNA polymerase. The competitor RNA was purified by RNase-free isolation kit (Boehringer Mannheim, Tokyo, Japan) and was stored at −80°C until use. Serially diluted (1:5) viral RNA mixed with the competitor RNA (usually 100 or 200 copies) was amplified utilizing a single-step RNA PCR kit with the specific primers and an RNase inhibitor (Takara). The amplified viral gag RNA and competitor RNA were separated on a 2% agarose gel by electrophoresis and stained with SYBR green (Takara). Measurement of amplified RNA was achieved utilizing a fluorescence imaging analyzer (FLA2000, Fuji Film, Tokyo, Japan), and the viral RNA load was calculated using an Excel spreadsheet (Microsoft, Tokyo, Japan) as previously described (26). The sensitivity of plasma viral RNA detection by this technique was determined to be 1,000 copies per ml of plasma.

Pharmacokinetics of GW420697. A quinoxaline class of the nonnucleoside reverse transcriptase inhibitor GW420867 (formerly HBY 1293A) was assayed for the presence of SIV-specific antibodies using standard ELISA techniques. The 96-well microtiter plates were precoated with a 1:100 dilution of each plasma sample in phosphate-buffered saline (PBS) and then incubated at 4°C for 1 h. Plates were washed five times with PBS containing 0.1% Tween 20 and blocked with 1% BSA in PBS for 1 h at 37°C. The plates were then washed five times with PBS containing 0.1% Tween 20 and incubated at 37°C for 1 h with a rabbit anti-SIV polyclonal antibody (SIV33 neutralization transgenic mice ascites fluid) diluted 1:200 in PBS containing 1% BSA. Plates were then washed five times with PBS containing 0.1% Tween 20 and incubated for 1 h at 37°C with peroxidase-conjugated goat anti-rabbit IgG (Zymed Laboratories, South San Francisco, Calif.) diluted 1:200 in PBS containing 1% BSA. After washing, the plates were developed with 1,2,3-triphenyltetrazolium chloride (Sigma), dissolved with 50 μl of the elution buffer included in the kit. Aliquots of the viral RNA were stored at −80°C. Plasma viral RNA load was measured initially by a commercially available RT-PCR kit (Boehringer Mannheim) and with the use of a competitive RT-PCR assay which served as a standard. The values were then confirmed by using the quantitative competitive RT-PCR method previously described (26). The sequence of the gag primers utilized included SG05 (5'-AGCTGTTCAAGATGCAAC-3') and SG06 (5'-CTACTGCTTCCTCAAGAGGAGATG-3'), which were designed to amplify most SIVmac and SIVsmm gag sequences. The competitor RNA fragment was generated by amplification of SIVmac239 gag sequences with SG05i and SG06i and cloning of the 59-bp fragment into a pGEM-T vector (Promega, Madison, Wis.). The insert was then digested with SalI and XcmI, blunted, and religated in order to remove a 133-bp fragment. The plasmid was then linearized downstream of the insert using SalI, and a competitor RNA was generated by using T7 RNA polymerase. The competitor RNA was purified by RNase-free isolation kit (Boehringer Mannheim, Tokyo, Japan) and was stored at −80°C until use. Serially diluted (1:5) viral RNA mixed with the competitor RNA (usually 100 or 20 copies) was amplified utilizing a single-step RNA PCR kit with the specific primers and an RNase inhibitor (Takara). The amplified viral gag RNA and competitor RNA were separated on a 2% agarose gel by electrophoresis and stained with SYBR green (Takara). Measurement of amplified RNA was achieved utilizing a fluorescence imaging analyzer (FLA2000, Fuji Film, Tokyo, Japan), and the viral RNA load was calculated using an Excel spreadsheet (Microsoft, Tokyo, Japan) as previously described (26). The sensitivity of plasma viral RNA detection by this technique was determined to be 1,000 copies per ml of plasma.

Anti-SIV ELISA. A 1:100 dilution of each plasma sample in phosphate-buffered saline (PBS) was contained in 96-well microtiter plates (Costar, Cambridge, Mass.) coated with SIV Gag antigen (Dainabot, Japan) and was assayed for the presence of SIV-specific antibodies utilizing standard ELISA techniques. The 96-well microtiter plates were precoated with a SIVmac239 virion lysate as previously described (14). The OD₉₀ was recorded and utilized as a relative measure of antibody titer.

CTL assay. The cytotoxic T-lymphocyte (CTL) assay method used has been previously described (30). In brief, PBMC samples stored at −150°C were thawed and cultured in RPMI 1640 medium with concanavalin A (5 μg per ml) at 10⁶ PBMC per ml for 3 days, washed and then maintained for another 3 days in medium supplemented with human interleukin 2 (2 U per ml). These effector cells were then cocultured for 7 days with autologous herpesvirus papain-transformed B-lymphoblastoid cell lines (B-LLC), which were previously either infected with recombinant vaccinia virus (rVV) expressing the SIVmac239 gag-pol, SIVmac239 env, or the parental VV (NYCBH strain) for 16 h at 37°C. The VV constructs were obtained via the courtesy of D. Panicali (Therion Corp., Cambridge, Mass.). For the CTL assay, similarly infected autologous (B-LLC) target cells were labeled with ⁵¹Cr and then incubated at 10⁶ cells/well with various concentrations of effector cells for 5 h. Supernatant fluids from each of the target cells (10⁶ cells per well) incubated alone with medium was used to calculate spontaneous release, and supernatant fluid from each of the target cells (10⁶ cells per well) incubated 3 days after infection was used to calculate specific release. The effector-to-target cell combination was performed in triplicate. Specific net lysis was calculated as the percentage of SIV Env- or Gag-Pol-specific lysis minus the percentage of lysis obtained using the control VV (NYCBH-infected target cells) as the target. CTL control lysis was always 8%.

SIV Env-specific cell proliferation. The method utilized has been previously described (13). In brief, PBMC samples were thawed and triplicate cultures of
PBMC (4 × 10^6 per well) were incubated with irradiated autologous B-LCL (1 × 10^5 per well) that had been previously infected (for 16 h) with either VV containing SIVmac239 env or control VV. Cultures were performed in triplicate, and [3H]thymidine was added to each well 8 h prior to harvest on day 5. The incorporation of [3H]thymidine was measured by standard liquid scintillation counting. The standard deviation of the triplicate samples was <10%. The stimulation index was calculated by dividing the mean uptake of [3H]thymidine values of the PBMC cocultured with the VV SIV env-infected autologous cells by the mean uptake of [3H]thymidine values obtained by coculture of an aliquot of the same PBMC with the VV (NYCBH)-infected autologous cells. Because depletion of CD4^+ cells from PBMC at 8 weeks p.i. with monoclonal antibody 19b/5D7 (17) in the presence of complement resulted in the loss of the env-specific proliferative response in the positive PBMC specimen, the majority of the cells stimulated with Env proteins were assumed to be CD4^+ T cells.

RESULTS

Containment of SHIV infection by short-term post-exposure prophylaxis with GW420867. We used GW420867 to treat monkeys that were infected with a high dose (100 TCID_50) of SHIV-RT. Treatment was initiated at 8 or 24 h p.i., with the drug or vehicle (in control animals) being administered twice a day for a total of 4 weeks. The effect of GW420867 therapy on plasma and cellular viral load was examined in samples obtained from each of the nine animals at various times p.i. As seen in Fig. 1, all three mock-treated animals showed significant plasma viremia, which reached set points in two of three animals shortly following the acute viremia phase. The third mock-treated animal showed transient plasma viremia following the acute phase but thereafter showed low levels of plasma viremia. In contrast, all six drug-treated animals showed undetectable levels (below the limits of RT-PCR detection, which was determined to be 1,000 copies/ml) of plasma viremia up to 3 weeks postcessation of therapy, regardless of the difference in the time of drug therapy initiation. Thereafter, three of six animals showed transient plasma viremia at about 4 weeks following cessation of drug therapy, which subsequently returned to undetectable levels. Studies of the cellular viral loads as determined by QVI showed a significant number of virus-infected cells in the mock-treated animals at various time points following infection. The frequency of virus replication-competent infected cells decreased with time in the control animals, presumably due to trapping of such cells in regional lymph nodes. In comparison, low but detectable levels of infected cells were noted in three of six drug-treated animals between 6 and 11 weeks p.i., a low level was detected at a single time point in two of six drug-treated animals, and undetectable levels were noted in all the other remaining samples tested (Table 1).

Subsequent analyses of samples obtained at 50, 62, 76, and 90 weeks p.i. indicated that plasma viral RNA levels were maintained below 1,000 copies/ml in each of the six drug-treated animals. Results of the proviral DNA analysis (Table 2) correlated with the plasma viral loads. No proviral DNA was detected in PBMC samples at any time during antiviral treatment, while it was readily detectable in samples from the control group early p.i. Also at later time points, a far lower level of infection was noted in the drug-treated group relative to the control group. These data nevertheless demonstrate that despite drug therapy, all the animals in both groups became infected, as evidenced by the detection of SHIV-RT DNA sequences at least at one time point in the PBMC and/or LNMC of each of these animals.

Table 1: QVI

<table>
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<th>8^a</th>
<th>9^a</th>
<th>14^b</th>
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<th>21^a</th>
<th>30^a</th>
<th>42^a</th>
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^a Results are given as the number of infected cells/10^6 PBMC followed by the results of viral RNA PCR performed with supernatants of the cultures (+, positive; −, negative).

^b GW420867 treatment 8 h p.i. through 28 days p.i.
^c GW420867 treatment 24 h p.i. through 28 days p.i.
standard ELISA techniques with 96-well plates precoated with SIVmac239 virion lysate (14). The OD 492 was recorded and used as a relative measure of antibody titer.

Analyses of plasma samples from the carrier (control) or GW420867-treated animals obtained at 2, 4, 15, 40, and 50 weeks p.i. and LNMC obtained at 8 weeks p.i. were assayed for SHIV-RT proviral DNA as described in Materials and Methods. The limit of detection was 1 copy/10^5 cells.

**TABLE 2. SHIV-RT proviral DNA in PBMC and LNMC**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wk p.i.</th>
<th>No. of DNA copies/10^5 cells for animal no.:</th>
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<tr>
<td></td>
<td></td>
<td>8^a</td>
</tr>
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<td>PBMC</td>
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<td>50</td>
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<tr>
<td>LNMC</td>
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<td>8</td>
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</table>

^a PBMC samples from the carrier (control) or GW420867-treated animals obtained at 2, 4, 15, 40, and 50 weeks p.i. and LNMC obtained at 8 weeks p.i. were assayed for SHIV-RT proviral DNA as described in Materials and Methods. The limit of detection was 1 copy/10^5 cells.

^b Control.

^c GW420867 treatment 8 h p.i. through 28 days p.i.

^d GW420867 treatment 24 h p.i. through 28 days p.i.

^e ND, biopsy was not done.

Despite undetectable viral loads in this animal (Fig. 1 and Table 1), a lymph node biopsy done on this animal showed that this animal was indeed infected but had low levels of proviral DNA (Table 2). Another drug-treated animal (animal 11) showed an initial antiviral antibody response similar to those seen in the control animals but then showed a transient decrease, with a return to high levels by 18 to 20 weeks p.i. This presumably anamnestic response followed the detection of infectious virus in PBMC samples from this animal at 11 weeks p.i. (Table 1). The relatively early seroconversion of drug-treated animals 5 and 11 suggests that the limited viral replication that occurred in these two animals may have led to sufficient availability of antigen to induce antibody responses. Yet another drug-treated animal (animal 4) seroconverted by 11 weeks p.i. but maintained low but significant SIV-specific antibody titers relative to the other remaining three animals. The other three drug-treated animals showed a delay in the kinetics of the humoral antiviral response, with seroconversion at 11 weeks p.i., corresponding to 7 weeks postcessation of drug therapy. In general, therefore, the kinetics of antiviral antibody generation appeared to follow the levels of viremia seen in the respective animals. The antibody titers in the drug-treated animals gradually reached levels approximately similar to those seen in the mock-treated control animals. These data also suggest that there is no correlation between the antiviral humoral responses and protection in these animals. However, such data do not rule out differences in the quality of virus-specific humoral responses that may be the contributing factor in decreased viremia and disease protection.

**Cell-mediated immune response in SHIV-infected monkeys treated with GW420867.** The virus-specific cell-mediated immune responses were also studied in each of the nine animals. Samples assayed included PBMC obtained from the animals at 6 days before infection (baseline) and at 3, 8, 20, and 40 weeks p.i. Significant SIV Gag-Pol (Fig. 3A)- and SIV Env (Fig. 3B)-specific CTL activity was noted in samples obtained at 3 and 8 weeks p.i. from two of three control animals. These levels, however, decreased at 20 and 40 weeks p.i. Interestingly, in one of three control animals (animal 14), there was a delay in the kinetics of CTL activity despite similar levels of acute viremia in this animal (Fig. 1). In contrast to the control animals, all of the drug-treated animals showed significant SIV Gag-Pol- and Env-specific CTL activity in samples from as early as 3 weeks p.i., despite the absence of detectable viral replication during the drug treatment period. More importantly, again in contrast to the control animals, the CTL levels were maintained up to 40 weeks p.i. with minor variations (Fig. 3). It is important to note that viral load by itself does not account for such virus-specific CTL responses, but viral control may be due to other host responses in addition to competent helper T cells. These findings are reminiscent of the data that have documented the maintenance of HIV antigen-specific CTL responses seen in the PBMC of long-term nonprogressors in contrast to those individuals who progress to AIDS (13, 18). In addition to the virus-specific CTL response, the SIV env-specific proliferative responses in these animals were also studied. Results showed that the PBMC from the control animals demonstrated a transient but weak proliferative response at 3 weeks p.i. This response remained low throughout the period of assay in two of the control animals (animals 8 and 9) (Fig. 4) and increased in the remaining animal (animal 14) that showed naturally developed good long-term control of viremia (Fig. 1).
In marked contrast, PBMC samples from the drug-treated animals showed significantly higher SIV env-specific proliferative responses as early as 3 weeks p.i., and these responses were maintained throughout the period of assay. In select experiments, depletion of CD4+ T cells prior to the proliferation assay led to marked diminution of the proliferative response, indicating indeed that the proliferative response involved CD4+ T cells in these assays.

**DISCUSSION**

One of the goals of this study was to investigate whether treatment of monkeys as early as 8 to 24 h p.i. would be able to prevent the establishment of SHIV infection in vivo, as shown in previous studies using (R)-9-(2-phosphonylmethoxypropyl) adenine (PMPA) (24) and zidovudine, respectively (25). Clearly, GW420867 postexposure prophylaxis monotherapy...
was unable to prevent infection in our protocol, possibly due to the different experimental conditions. Although prevention of infection was not achieved, our results provide four main findings that may prove crucial for the delineation of protective chemotherapeutic interventions in HIV infection and may even provide a rationale for the formulation of an effective vaccine against HIV.

The first finding is that the inhibition of the acute initial viral replication did not appear to restrict the development of virus-specific cell-mediated responses against the virus inoculated, indicating that induction of cell-mediated responses does not require massive antigen production in vivo. Compared to the virus-specific CTL response, the virus-specific humoral response required a higher antigen load, as evidenced by delays in the kinetics for anti-SIV antibody responses in the drug-treated animals compared to that in the control animals. The second finding is that a delicate balance appeared to be required between very limited viral replication (below detectable levels) and sufficient pathways of antigen presentation to evoke long-lasting disease-protective host antiviral immune functions in the drug-treated animals. This balance appeared to have been preserved by early short-term treatment with this novel RT inhibitor. In addition, very low continuous or intermittent viral replication presumably results in a quality (viral epitope specificity) of virus-specific humoral response that may also play a role in disease protection. Thus, induction of such humoral response during the treatment period correlated with a more-effective restriction of virus replication in the treated animals. The third finding is that antiviral treatment during acute infection appeared to preserve mechanisms responsible for the development and maintenance of an antiviral host defense that included CTL effector function, which was most likely preserved by the continuous support of CD4+ T helper cell function in addition to other as yet undefined mechanisms. Because there was a more significant difference in env-specific proliferative response than in CTL response between the treated groups and the control group, virus-specific CD4+ T cells may play a more important role than CTLs. The fourth finding is that it is possible that there are as yet to be defined additional effector mechanisms other than CTLs that play important roles for the containment of the virus infection. This view is based on the results seen in one of the untreated control animals that suppressed acute viremia despite a relatively low CTL response during primary infection and another control animal that suppressed viremia weakly despite a relatively potent virus-specific CTL response. Chemokines and other cell-free factors have been identified as candidates for such undefined effector mechanisms (4, 8, 22). It is thus possible that virus infection-induced activation of CD4+ and CD8+ T cells may function to secrete high levels of cell-free factors that play a dominant role in these select animals. Thus, orchestrated antiviral host responses consisting of mechanisms that permit low levels of viral replication sufficient to induce and maintain appropriate levels of virus-specific CD4+ T helper responses, which in turn facilitate the generation and maintenance of virus-specific CTL responses, in addition to undefined mechanisms may be the basis for the protective effect observed in the drug-treated animals. Support for this view has been recently documented with the use of a live attenuated SIVΔ nef immunization protocol (9). A more detailed characterization of the precise epitopes of the virus that permit the generation of the helper CD4 and cytotoxic T-cell responses coupled with identification of the contributory role, if any, of the other effector immune mechanism(s) in such models, including the one described herein, may provide insights on the nature of the immunological responses that constitute effective antiviral immunity and disease protection.

A similar vaccination effect has been noted before in a murine retrovirus model (21). For lentiviruses, SIVΔ nef infection of macaques has been described in which low but detectable levels of viral replication were followed by CTL induction during the acute infection period (13). Virus-specific CTL induction in our drug-treated animals appeared similar to that seen in SIVΔ nef infected animals, despite apparently even lower levels of virus replication. In addition, although SIVΔ nef-induced immunity required 3 to 6 months to be fully protective (28), the data presented herein suggest that vigorous antiviral responses were already present at 4 weeks p.i., when antiviral treatment was discontinued. These differences may suggest that a relationship exists between the strength of the protective immune response and the extent of attenuation of virus as previously reported (6). Alternatively, pathogenic virus may inherently differ from attenuated virus with respect to the influence of virus infection on host immune responses. Thus, the chemotherapy-assisted containment of the pathogenic virus infection may differ quantitatively and qualitatively from the attenuated SIV infection in the context of protection from pathogenic infection. Recent data on a few HIV-1 infected patients for whom HAART was initiated early p.i. support the data reported herein, since discontinuation of HAART in these individuals did not result in viral rebound, suggesting that HAART may be able to induce immune responses capable of containing viral loads (3). In contrast, as previously noted, discontinuation of HAART therapy initiated at later stages of HIV-1 infection did not lead to the induction of immune responses sufficient to contain viral rebound (31). In the current controversy of early versus late therapy following exposure to HIV, our results strongly suggest that initiation of therapy past the acute viral infection stage may be too late and prevent the optimal development of antiviral response. If human studies come to the same conclusion, early treatment of a (presumed) HIV infection by selected well-tolerated and convenient drugs must become the rule, whenever possible.

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REFERENCES

7. Finzi, D., J. Blankson, J. D. Siliciano, J. B. Margolick, K. Chadwick, T.


