Antibodies That Neutralize SIV$_{mac}$251 in T Lymphocytes Cause Interruption of the Viral Life Cycle in Macrophages by Preventing Nuclear Import of Viral DNA

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Previous reports from our lab had shown that sera obtained from SIV$_{mac}$-infected animals neutralized SIV$_{mac}$ infectivity in CD4$^+$ T cells but failed to protect monkey primary macrophages from infection with the virus. However, the antibodies could inhibit completion of the viral life cycle in the macrophages at the postentry stage(s). In this report we examined the mechanisms of the late effect of the antibodies. Using monoclonal antibodies (mAbs), we demonstrated that only antibodies to the SIV envelope protein (KK17 and KK42) but not antibody to the viral core protein (FA2) had the same inhibitory effect as that of the anti-SIV sera. To identify the stage of the viral replication cycle that was inhibited by anti-SIV antibodies in macrophages, we used various PCR techniques to study viral entry/reverse transcription (by amplifying the viral gag gene), viral genome nuclear transport (by amplifying 2-LTR circular forms), viral integration (by Alu-PCR assay), and viral protein expression (by RIPA). We found that in macrophage cultures inoculated with SIV$_{mac}$251 that were preincubated with antienv mAbs, viral DNA was detected at 8 h postinoculation but the 2-LTR circular forms and integrated viral DNAs were undetectable, and viral proteins were not expressed in these infected macrophages. These results strongly suggested that anti-SIV antibodies inhibited SIV$_{mac}$ replication in macrophages by blocking nuclear transport of viral genomes since viral DNA could not be detected in the nuclei of treated cultures. Furthermore, we showed that although viral replication in macrophages was interrupted by the antibodies, when cocultured with permissive T cells, the viral genomes presented in the cytoplasm of the macrophages could readily transfer to T cells during cell-cell contact. Importantly, this transfer could not be prevented by the antibodies. These results might explain the failure of passive antibody immunization against SIV$_{mac}$251—a critical obstacle in AIDS vaccine development.

Key Words: SIV; HIV; AIDS; antibody; neutralization; nuclear transport; macrophage.

INTRODUCTION

Neutralizing antibodies that develop during viral infections usually coincide with the end of the infection and are important for the prevention of further episodes of infection. The antibodies are important players in nearly all successful antiviral vaccines. However, the role of neutralizing antibodies in modulating the pathogenesis of lentivirus infection is less clear. In HIV infections, the presence of such antibodies does not provide prognostic or predictive indications on the rate of disease progression. The only definitive correlation is that antiviral immune responses are not produced during rapid-onset disease. In more slowly progressive infections, neutralizing antibodies developing after infection coexist with productive virus replication (Burton and Montefiori, 1997; Montefiori et al., 1996). One explanation for this contradictory phenomenon is that viruses under these conditions tend to be neutralizing antibody-escape variants of the viral quasispecies (Homsy et al., 1990; Joag et al., 1993; Nara et al., 1990; Albert et al., 1990).

The discovery that SIV infection in macaques causes HIV-like disease led to the utilization of the SIV/macaque model systems to study HIV pathogenesis and host protective immunological mechanisms (Benveniste et al., 1988; Kestler et al., 1990). The SIV/macaque system, however, yielded conflicting results in prophylactic passive immunization experiments that examined the role of neutralizing antibodies in protective immunity. Whereas the passive immunization failed to prevent infection with different strains of SIV$_{mac}$ (251, 239, and J5M) (Gardner et al., 1995; Kent et al., 1994; Almond et al., 1997), the strategy was successful when progeny of SIV$_{SM}$ strains (SMM-3, B670, etc.) or SIV$_{mac}$ were used (Biberfeld et al., 1992; Putkonen et al., 1991; Clements et al., 1995; Lewis et al., 1993; Haigwood et al., 1996). These conflicting passive immunization results become a major problem in understanding the protective immunities against HIV and in the AIDS vaccine development. The question that needs to be answered is, therefore, what are the determinants of the success or failure of prophylactic passive immunization?

Based on the fact that all SIV$_{mac}$ strains were derived from a common origin (Schultz and Hu, 1993), we hypothesized that the failure of passive immunization against
strains of SIVmac might stem from unique neutralization characteristics that are peculiar to SIVmac. We attempted to answer this question by using two strains of SIVmac (251 and 239). Proceeding on the background that the prophylactic passive immunization against these viruses failed, we asked whether the antiserum would neutralize the viruses in both of the target cell types (i.e., CD4+ T lymphocytes and macrophages) used by the virus in vivo. These experiments showed that CD4+ T cells (either immortalized T cell lines or macaque PBMCs) could indeed be protected from SIVmac infection by neutralizing antibodies (Zhuge et al., 1997, 1998). When virus, preincubated with the neutralizing antibodies, was inoculated into macrophage cultures, productive replication of the virus was also prevented, as judged by lack of viral p27 production (Zhuge et al., 1997). This led to the early conclusion that neutralization was successful. However, later studies showed that macrophages inoculated with preincubated virus–antibody mixtures developed PCR signals for viral DNA. Even more disturbing, although the antibodies prevented infection in CD4+ T cells and inhibited viral replication in macrophages, they failed to prevent the spread of infection from the macrophages to CD4+ T cells when the latter were added to the “latently” infected macrophages in the presence of the antibodies (Zhuge et al., 1998). The infection spread rapidly to the T cells in this coculture environment. Thus, whereas the antibodies prevented infection in T cells with cell-free virus, they could not prevent infection mediated by “latently” infected macrophages. The pseudo neutralization of SIVmac (251 and 239) in macrophages has not been observed before and its mechanisms are poorly understood. In two previous reports on this phenomenon, polyclonal antiviral sera obtained from infected macaques had been used in all neutralization experiments. Whether the antibody-mediated inhibition of SIVmac replication in macrophages was due to antiviral or anticellular antibodies was not clear.

In this study, by using various anti-SIV monoclonal antibodies, we investigated the mechanism(s) by which neutralizing antibodies inhibit SIVmac replication in macrophages. Our data suggested that antiviral envelope monoclonal antibodies, after binding to the virus, did not prevent the entry of virus into macrophages. Instead, the antibodies apparently blocked nuclear import of the viral DNA in the infected macrophages.

RESULTS

Antibody-mediated inhibition of SIVmac251 replication in macrophages is due to antiviral envelope antibodies

In previous reports we showed a phenomenon of differential neutralization of SIVmac in lymphocytes and macrophages by using polyclonal anti-SIV sera obtained from infected macaques (Zhuge et al., 1997). Whether the antibody-mediated inhibition of SIVmac replication in macrophages was due to antiviral or anticellular antibodies was not clear. In this experiment, two anti-SIV envelope neutralizing monoclonal antibodies (MAbs), KK17 and KK42, were used in the neutralization assays. It has been reported that KK42 binds to V3 (aa 321–340) of the SIVmac envelope and has a low neutralizing titer against SIVmac251 in T cells, while KK17 binds to a conformational epitope on gp120 of the SIVmac envelope and has strong neutralizing activity against SIVmac251 (Kent et al., 1992, 1991; Choi et al., 1994). In preliminary experiments, we found that both MAb neutralized the infectivity of SIVmac251 effectively at a concentration as low as 0.1 μg of MAb in 200 μl of reaction mixture when tested on normal rhesus macaque macrophage cultures (data not shown). A tested nonneutralizing anti-Gag MAb, FA2, was used as a control. To investigate whether these MAbs prevent infection of SIVmac251, we used 100, 10, 1, or 0.1 TCID50 of DNase-treated SIVmac251 to preincubate with 10 μg of either KK17, KK42, or FA2 in a total volume of 200 μl for 1.5 h at 37°C and then inoculated the mixtures into cultures of T cells (CEM × 174) or monkey primary macrophages. Twenty-four hours later, cultures were washed and total cellular DNA was extracted. A PCR-based assay to amplify the viral gag gene was performed to determine whether the cells were infected. Results showed that both KK17 and KK42 blocked SIVmac251 infection in T cells, while FA2 did not (Fig. 1A). When macrophage cultures were used as the target cell, however, the two MAbs were unable to block infection of SIVmac251 in macrophages as judged by the presence of viral DNA in the cultures (Fig. 1B). However, as indicated in radio-immunoprecipitation assays (RIPA), no viral proteins were produced at 7 days postinoculation in the infected macrophages that were inoculated with virus preincubated with KK17 or KK42 (Fig. 2, lanes 1 and 2). These results suggested that “neutralization of SIVmac251” in macrophages occurred at a post viral entry stage instead of blocking viral entry. These results confirmed our previous reports where polyclonal antiviral sera were used and demonstrated further that the phenomenon was mediated by anti-SIV envelope antibodies.

Anti-SIV envelope MAb delayed SIVmac251 entry into macrophages

To compare the time course of entry of various anti-body-treated SIVmac251 into macrophages, we inoculated macrophages with 10 TCID50 of SIVmac251 preincubated with 1 μg of either KK17 or FA2. At 1, 2, 4, 8, and 24 h postinoculation, macrophage cultures were washed and DNAs extracted and subjected to PCR amplification of the viral gag gene. As shown in Fig. 3, viral DNA readily became detectable in macrophages at 2 h postinoculation with SIVmac251 preincubated with nonneutralizing
MAb FA2. In the cultures inoculated with SIVmac251 preincubated with MAb KK17, however, viral DNA was not detected until 8 h postinoculation. There are two possible explanations for this result. One is that the viral entry or reverse transcription process was interrupted and/or altered by KK17 so the appearance of viral DNA was delayed. The other was that some viral particles became dissociated from the neutralizing MAb KK17, entered the cells, and initiated infection. Given either of the two possibilities, it was remarkable that the later stages of the viral life cycle were blocked by the neutralizing antibodies.

Anti-SIV MAb blocked nuclear import of viral genomes

Since the neutralizing MAbs were unable to block SIVmac251 entry into macrophages but inhibited its replication, which is similar to the effect of polyclonal anti-SIV sera, we sought to identify the stage of the viral life cycle in macrophages that was blocked by the antibodies. Since we amplified the viral gag gene, a product of late stage viral reverse transcription, this suggested that viral reverse transcription had occurred after the virus entered the macrophage. The next question was whether viral DNA had been transported into the nucleus. We used nested PCR to detect the 2-LTR circular form of SIVmac DNA, which is an indication of the presence of viral genomes in the nucleus (Stevenson et al., 1990). Macrophage cultures inoculated with 10 TCID50 of SIVmac251 preincubated with serially 10-fold-diluted anti-Env KK17, KK42, or anti-Gag MAb FA2 (10, 1, 0.1, and 0.01 μg of MAb in 200 μl, respectively) were washed and subjected to DNA extraction at 24 h pi. Nested PCRs amplifying either SIV gag or 2-LTR circular viral DNA from 200 ng of total DNA were performed as described under Materials and Methods. Results showed that viral gag was amplified from all the macrophage cultures inoculated with SIVmac251 preincubated with FA2, KK17, or KK42 antibodies regardless of the MAb concentrations (Fig. 4A). The 2-LTR circular form of viral DNA was detected in the macrophage cultures inoculated with SIVmac251 preincubated with FA2 (Fig. 4B, lanes 1–3), but not in the cultures inoculated with virus that was preincubated with 10, 1, or 0.1 μg of neutralizing MAb KK17 or KK42, respectively (Fig. 4B, lanes 4–6 and 8–10). These results suggested that the viral genomes had not been transported into the nucleus in the presence of the neutralizing MAbs.

Although the presence or the absence of the 2-LTR circular form of viral DNA has been used by most investigators as an indicator of the nuclear import of viral genome (Stevenson et al., 1990; Pancio et al., 2000; Schmidtmayerova et al., 1998), we extended the study to determine whether integration of the viral genome had occurred. We employed an Alu-PCR assay to detect integrated viral DNA. Results showed that integrated viral DNA was undetectable in macrophages inoculated with SIVmac251 preincubated with down to 0.1 and 0.01 μg of KK17 or KK42 (Fig. 4C, lanes 4–6 and 8–10), but was detectable in macrophage cultures inoculated with virus preincubated with FA2 (Fig. 4C, lanes 1–3).

Viral genomes harbored in the macrophages could be transferred to permissive T cells by cocultivation but require cell–cell contact

We had reported previously that macrophages harboring SIVmac239 could disseminate the virus to T cells upon cocultivation even in the presence of neutralizing antibody (Zhuge et al., 1998). But it is unclear how the process occurred. In this experiment, we used the transwell culture system to dissect the phenomenon of spread. SIVmac251 (100 TCID50) was preincubated with 1 μg of KK17 or FA2 at 37°C for 1.5 h, and the virus/antibody mixtures were inoculated into rhesus macrophage cultures in 6-well plates. After overnight incubation, the cultures were washed five times to remove inocula, and the cultures reincubated with fresh medium.

**FIG. 1.** Neutralizing anti-Env MAbs failed to block SIVmac251 entry into macrophages. Various concentrations of DNase-treated SIVmac251 (100, 10, 1, 0.1 TCID50) were preincubated with 10 μg of anti-Gag MAb FA2, anti-Env MAb KK17, or anti-Env MAb KK42 at 37°C for 1.5 h and then the mixtures were inoculated onto T cells (CEM × 174) or primary macrophages. Twenty-four hours later, cultures were washed and the DNA was extracted for PCR assay to amplify the viral gag gene. Panel A shows the results from T cells and panel B macrophage cultures. Pos, positive control; Neg, negative control.
containing same amount of MAb. One day later, the cultures were washed again and replenished with the same medium/MAb. One million T cells (CEM × 174 cells) were then added either directly into each well containing macrophage cultures (permitting cell–cell contact) or indirectly into 1-μm transwell chambers inserted into the macrophage cultures to prevent cell–cell contact. The cultures were monitored for cytopathic effect (CPE) and culture supernatants were collected at sequential time points for RT assay. CPE and RT activities developed after CEM × 174 cells were added directly or indirectly into macrophage cultures that were inoculated with SIVmac251 preincubated with FA2. In the macrophage cultures that were inoculated with SIVmac251 preincubated with neutralizing MAb KK17, CPE and RT activities developed only after CEM × 174 cells were added directly into the macrophage cultures but not into the transwell chambers in which the two cell types were separated. The RT activities reached similar extension in both macrophage cultures that had CEM × 174 cells added directly into the wells regardless of which MAb was used (Fig. 5). We then asked whether a similar macrophage–T-cell transfer of SIVmac251 would occur if a coculture of these macrophages with primary rhesus PBMCs instead of cell line T cells (CEM × 174) was done. To address this question, similarly treated macrophage cultures as described above were set up. After extensive wash as described, the same amounts of MAbs were added back to the culture medium, and 2 × 10⁶ PHA-activated rhesus PBMCs were then added to each well to coculture with the macrophages. Culture supernatants were collected for RT assay. As shown in Fig. 6, primary rhesus PBMCs, similar to CEM × 174 cells, were readily infected if cocultured with macrophages that were inoculated with either antibody (FA2 or KK17)-treated SIVmac251, and this process is not prevented by the presence of neutralizing antibody (KK17) in the culture medium. These macrophage–T-cell transfer results gave additional evidence showing that neutralizing MAb KK17 failed to block SIVmac251 entry into macrophages and further demonstrated that viral genomes harbored in such antibody-treated macrophages were infectious and could be transferred to permissive T lymphocytes via cell–cell contact.

**DISCUSSION**

In the present study, by using anti-SIV monoclonal antibodies, we have extended our previous reports that antisera obtained from SIV-infected animals were unable to block the entry of SIVmac into macrophages but inhibited its replication in the cells. We have demonstrated here that (1) anti-envelope antibody contributes to the phenomenon of antibody-mediated inhibition of SIVmac replication in macrophages; (2) this inhibiting effect is due to the ability of antibody to block nuclear import of the viral genome in infected macrophages; and (3) the macrophage-harbored viral genome could be transferred to T cells but requires cell–cell contact, and this macrophage–T-cell transfer process cannot be prevented by neutralizing antibody.

We tested three MAbs in this study: two anti-envelope MAbs (KK17 and KK42) and one anti-Gag MAb (FA2). We found that only anti-envelope MAbs have the ability to
inhibit the SIVmac replication in macrophages, while the anti-Gag MAb has no neutralizing effect. It has been reported that KK42 binds to V3 (aa 321–340) of the SIVmac envelope and has a low neutralizing titer against SIVmac251 in T cells, while KK17 binds to a conformational epitope on gp120 of the SIVmac envelope and has strong neutralizing activity against SIVmac251 (Kent et al., 1992, 1991; Choi et al., 1994). Consistent with these reports, we also demonstrated that both KK17 and KK42 blocked the entry of SIVmac251 into T cells. In the contrast, when the same neutralization assay was tested on macrophages, we found that both anti-Env MAbs failed to block viral entry into macrophages but nevertheless inhibited viral replication at high titers.

Proceeding on the conclusion that antibodies blocked the SIVmac251 replication cycle at a postentry stage in macrophages, we next attempted to identify the stage of the viral life cycle where the blockage occurred. We addressed the question in this study by investigating various stages of the viral life cycle, including viral reverse transcription, transportation of the viral preintegration complex into the nucleus, viral DNA integration, and production of viral proteins in the infected macrophage cultures. Our results showed that, after incubation with neutralizing MAbs, SIVmac251 entered macrophages and completed reverse transcription, but the 2-LTR circular forms of viral DNA, which are a hallmark of the presence of the viral genome in the nucleus, were absent. Further, there was no evidence of viral DNA integration or production of viral proteins. These data strongly suggested that antibodies blocked the nuclear transport of viral DNA from the cytoplasm of infected macrophages.

The question of why the neutralizing antibodies were unable to block SIVmac251 entry into macrophages while they completely blocked entry of the same virus into T cells remains unsolved. In a previous report, we had excluded the obvious suspicion that infection in macrophages was mediated by Fc receptor-mediated endocytosis of antibody-bound virus because the identical sequence of events occurred when the Fab was used instead of the intact neutralizing IgG (Zhuge et al., 1997).

A further question is how could antienvelope antibodies block nuclear import of the viral genome in macrophages? We speculate that antibodies might have interfered with signal transduction upon binding of the viral envelope to its receptors (CD4 and CCR5 for SIVmac). It has been reported that upon binding to receptors, envelopes derived from M-tropic HIV-1 and SIVmac triggered a calcium influx into macrophages, while envelopes derived from non-M-tropic viruses did not.

![Figure 3](image1.png)

**FIG. 3.** Anti-Env MAb delayed SIVmac251 entry or the reverse transcription process. Macrophage cultures were inoculated with 10 TCID₅₀ of SIVmac251 that was preincubated with 1 μg of either anti-Gag MAb FA2 or anti-Env MAb KK17. At 1, 2, 4, 8, and 24 h postinoculation, macrophage cultures were washed and DNAs extracted and subjected to PCR amplification of the viral gag gene.

![Figure 4](image2.png)

**FIG. 4.** Anti-Env MAbs blocked nuclear import of SIVmac251 DNA in macrophages. Ten TCID₅₀ of DNase-treated SIVmac251 was incubated with 10, 1, 0.1, or 0.01 μg of anti-Gag MAb FA2, or anti-Env MAb KK17, or KK42 at 37°C for 1.5 h. The mixtures were then inoculated into macrophage cultures. At 24 h postinoculation, the macrophage cultures were washed and subjected to DNA extraction. Nested PCRs amplifying either SIV gag, 2-LTR circular viral DNA, or integrated viral DNA (Alu-PCR) from 200 ng of total DNA were performed as described under Materials and Methods. PCR results show in A the viral gag gene; in B, the 2-LTR circular form of viral DNA; and in C, the integrated viral DNA by Alu-PCR.
It is more intriguing that although CCR5-utilizing non-M-tropic HIV-1 was capable of entering macrophages, its replication cycle was blocked due to lack of signaling. However, productive replication of this non-M-tropic HIV-1 occurred in macrophages after the addition of MIP-1α, indicating that a CC chemokine-mediated signal through CCR5 provided the necessary stimulus to allow the virus to complete its life cycle (Arthos et al., 2000). Based on those findings, we hypothesize that after binding to virus, an antibody may block the CC chemokine-mediated signal so although virus gets into the cells, its life cycle at the stage of nuclear import of viral DNA is blocked. The other possibility is that antibodies were internalized with bound virus, prevented subsequent viral uncoating, and interfered with the formation of the preintegration complex or transportation of the preintegration complex to the nucleus.

In humans, infection is presumed to be mediated by an M-tropic HIV-1 (Schuitemaker et al., 1992; Zhu et al., 1993). It is well known that most HIV-1 patient isolates utilize CCR5 as coreceptor for viral entry (Zhang et al., 1998), and they are more resistant to neutralizing antibodies than laboratory-adapted strains (Moore et al., 1995; Hioe et al., 1997; Mascola et al., 1997; Parren et al., 1998; Cecilia et al., 1998). We reported previously that entries of both HIV-1 Bal and 89.6 strains into either T cells or macrophages were blocked by neutralizing antibodies (Zhuge et al., 1998). Although both strains of HIV-1 were considered primary isolates by many investigators, they were passed many times in various laboratories so they might behave more like lab-adapted strains. Therefore we still want to ask, does neutralization of patient isolates of HIV-1 in human macrophages behave similarly to the pseudo-neutralization of SIVmac251?

We ask this because Ruppach et al. recently reported that antisera obtained shortly after HIV-1 infection inhibited the output of autologous HIV-1 primary isolates differently in inoculated human macrophages than in T cells (Ruppach et al., 2000), a phenomenon very similar to the neutralization of SIVmac251 in monkey macrophages we had reported. In this recent report, Ruppach et al. tested 10 strains of primary HIV-1 isolates, including 6 strains of subtype B, 1 strain of subtype C, and 3 strains of subtype E. It will be very interesting to investigate whether antibodies could block the entry of those HIV-1 primary isolates into human macrophages and whether virus could be rescued by coculture with T cells, as seen in the SIV studies.

It has been a puzzle for many years why passive administration of neutralizing antibodies failed to protect animals from SIVmac251 infection (Gardner et al., 1995; Kent et al., 1994), while the same strategy was very successful in preventing infections with SIVSM, SIVmac, SHIVKU, SHIV89.6P, and some HIV-1 strains (Biberfeld et al., 1992; Lewis et al., 1993; Foresman et al., 1995).
1998; Mascola et al., 1999, 2000; Eichberg et al., 1992; Emini et al., 1992). These conflicting results in passive immunization trials raised a serious question regarding the role of neutralizing antibody in the host protective immunities against HIV and the strategy of AIDS vaccine development. One explanation for the failure of passive immunization against SIVmac251 is that SIV mac251 primary stocks grown in PBMCs were more resistant to neutralization (Langlois et al., 1998; Means et al., 1997). Our results presented here and reported previously could offer another explanation. We think, due to the failure of neutralizing antibodies to protect macrophages from infection with SIVmac251 and because of the failure of neutralizing antibodies to prevent transfer of the viral genomes from macrophages to CD4$^+$ T cells, SIVmac251 could infect macrophage-lineage cells in the bloodstream following inoculation even in the presence of circulating neutralizing antibodies, and the virus could spread rapidly from infected macrophages to permissive CD4$^+$ T cells. In contrast to SIVmac251, it is noteworthy that passive admission of neutralizing antibodies successfully protected animals from infection with either SHIVkg or SHIV89.6P in various animal studies (Foresman et al., 1998; Mascola et al., 1999, 2000), and our earlier report coincidentally showed that neutralizing antibodies protected both T cells and macrophages from infection with SHIVkg and HIV-1 89.6 (from which the envelope of SHIV89.6P derived) (Zhuge et al., 1998).

It will be interesting to further identify which property of SIVmac251 is responsible for the failure of neutralizing antibody in blocking viral entry into macrophages and to investigate whether a similar phenomenon occurred in HIV-1 primary isolates. Also, since neutralizing antibodies are unable to prevent the cell–cell spread of virus infection as we demonstrated here, it would be a big concern in preventing HIV infection among IV drug users that virus-infected cells are frequently the source of transmission.

In summary, we could show for the first time that anti-SIV envelope neutralizing monoclonal antibodies that prevented infection in T cells were unable to block the entry and reverse transcription of SIVmac251 in macrophages. Our data suggested strongly that antibody-mediated inhibition of the SIVmac251 life cycle occurred at the stage of nuclear transport of the viral DNA. Nevertheless, the viral genomes remained infectious in the macrophage and could readily be transferred to T cells upon contact even in the presence of the neutralizing MAbs. These results provide an explanation of why prophylactic passive immunization failed to protect animals from SIVmac251 infection. Further studies of the mechanisms of antibody-mediated inhibition of virus replication in macrophages will provide valuable information for better understanding of host protective immunities.
against HIV/SIV infections and for future AIDS vaccine development.

**MATERIALS AND METHODS**

**Cells**

CEM × 174 cells were cultured at a concentration of about 0.5–1 × 10^6/ml in RPMI 1640 supplemented with 10 mM HEPES buffer, pH 7.3, 50 μg/ml gentamicin, 5 × 10^-5 M 2-mercaptoethanol, 2 mM glutamine (together making serum-free RPMI or sRPMI), and 10% fetal bovine serum (the complete medium called R10FBS).

Normal SIV-negative rhesus monkey PBMCs were separated from buffy coat cells by centrifugation through Ficoll-Hypaque density gradients. For making activated PBMCs, the separated PBMCs were cultured at a density of 2 × 10^6/ml in R10FBS and stimulated with 1 μg/ml PHA for 2 days. After PHA stimulation, the PBMCs were washed and maintained in R10FBS supplemented with 100 μg/ml of rIL-2 (Cetus). For rhesus primary macrophage cultures, the separated PBMCs were seeded into 6- or 12-well plates (at a concentration of 6 × 10^5 or 3 × 10^5 cells/well, respectively) with macrophage differentiation medium (MDM), which consisted of sRPMI supplemented with 10% human serum, 5 units/ml of macrophage colony-stimulating factor (M-CSF, Genetics Institute), and 100 units/ml of granulocyte macrophage colony-stimulating factor (GM-CSF, Genetics Institute). Macrophages were obtained after 7–10 days of cultivation with extensive washes and three medium changes.

**Virus and antibodies**

SIV mac251 is a biologically cloned pathogenic M-tropic virus. Virus stock was obtained by inoculating the agent into CEM × 174 cells at m.o.i.s of approximate 0.01 and harvesting the culture supernatant fluids 7 days later by centrifugation at 2000 rpm for 10 min. Virus stock was aliquoted and stored at −80°C until use. The infectious titer of virus stock was measured using methods previously described (Joag et al., 1993).

Three monoclonal antibodies were used in this study. Two anti-SIV mac envelope MAbs, KK17 (IgG2a) and KK42 (IgG1), and an anti-SIV mac Gag MAb, FA2 (IgG2b), were obtained from the NIH AIDS Research and Reference Reagent Program. The properties of these antibodies had been described previously (Kent et al., 1992, 1991; Choi et al., 1994; Sutjipto et al., 1990).

**Virus neutralization assays**

Virus neutralization assays were performed as previously described with some modifications (Zhuge et al., 1997). Generally, unless stated specifically, twofold dilutions of antibody were incubated with 100 TCID50 of SIV mac251 in a total volume of 0.2 ml for 1.5 h at 37°C, and the virus–antibody mixtures inoculated into indicator cells (about 1 × 10^5 cells in 1 ml/well) cultured in 6-well plates. Inoculated cultures were incubated for 5–7 days. Cultures were then washed five times with 5 ml of serum-free medium, replenished with culture medium without the antibody, and reincubated at 37°C. Culture supernatant fluids were then collected after overnight incubation and examined for content of viral reverse transcriptase activities (RT assay) and the 90% neutralization end point was calculated. In some experiments, productive viral replication was examined by using RIPA as described previously (Zhuge et al., 1997).

**Detection of viral genome using PCR**

Virus stock was first filtered through a 0.22-μm filter and then treated with 136 Units of DNase I (GIBCO BRL) in a volume of 1 ml for 15 min at 25°C. DNase-treated virus stocks tested negative for viral DNA by PCR described below (data not shown). Various concentrations of DNase-treated virus were reacted with antibody in a volume of 400 μl for 1.5 h at 37°C. T cell and macrophage cultures were then inoculated with equal volumes of the virus/antibody mixtures (200 μl each) plus 1 ml of additional medium. One day later (or at stated time points), the cultures were washed five times with serum-free medium, cells were lysed in 0.5 ml of lysis buffer (TE buffer containing 0.05% of SDS), and cellular DNA was extracted; 200 ng of each DNA was used in nested PCR to seek the presence of the SIV gag gene. The oligonucleotide primers used for the SIV gag gene amplification (which amplify 240 bp of fragment) were described previously (Zhuge et al., 1997). PCR amplification was performed with an automated DNA Thermal Cycler (Perkin–Elmer Cetus) for 35 cycles using the following conditions: Denaturation at 92°C for 1 min, annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. For detecting the 2-LTR circular form of SIV DNA, 200 ng of extracted DNA was used in nested PCR under the same amplification conditions as described for gag. The first round PCR primer sets were sense, 5′-CACTAGCAGG-TAGAGCCTGGGTGT-3′; anti-sense, 5′-GTCATCCCAT-GGAAAGATTGAGC-3′, which correspond to SIV mac239 sequence 10060–10081 and 185–207, respectively (Regier and Desrosiers, 1990). The inner primer sets were sense, 5′-GCTAGACTCTCACCAGCACTTG-3′; anti-sense, 5′-CTCATCCTCTGGCCTCATCT-3′, which correspond to SIV mac239 sequence 10031–10054 and 230–207, respectively (Regier and Desrosiers, 1990). The inner primer sets were sense, 5′-GTCATCCCAT-GGAAAGATTGAGC-3′, which correspond to human Alu sequence 172–196, and the anti-sense primer was 5′-GTCATCCCAT-GGAAAGATTGAGC-3′, located in the U3 region (bp 230–207) of SIV LTR. After the first round Alu-PCR, 1 μl of PCR product was used in nested PCR with the following primer pair: sense 5′-TGAAGGG-
ATTATTACGTGCAAGAAGAC-3′ (bp 1–30 of SIVmac239), anti-sense 5′-CTCATCCTCTGCTGCTATC-3′ (bp 185–164 of SIVmac239). The amplification conditions for Alu-PCR were 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and primer extension at 72°C for 3 min. Following the second round of amplification, a 10-μl aliquot was removed and run on a 1.5% agarose gel and bands were visualized by staining with ethidium bromide.

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