J. Med. Primatol. 19:251–267 (1990)

Comparison of SIV/SMM Replication in CD4$^+$ T Cell and Monocyte/Macrophage Cultures From Rhesus Macaques and Sooty Mangabeys

Tamar Yehuda-Cohen,[1] Jonathan D. Powell,[1] Francois Villinger,[1,2] Harold M. McClure,[1,2] Kenneth W. Sell,[1] Aftab Ahmed-Ansari[2]

[1]*Department of Pathology and Laboratory Medicine, Winship Cancer Center, and* [2]*Division of Pathobiology and Immunobiology, Yerkes Regional Primate Research Center, Emory University, Atlanta, GA, USA*

Monocytes from SIV/SMM infected sooty mangabeys and rhesus macaques were incubated in vitro with live SIV/SMM. The reverse transcriptase (RT) activity in the supernatant fluids of the monocyte cultures of the former species was higher than the RT activity in the latter species. No differences were found in the supernatant fluid of similar cultures of CD4$^+$ T cells from both these species. Autologous (but not allogeneic) CD8$^+$ T cells from SIV infected mangabeys and rhesus macaques inhibited SIV replication in vitro. The suppression appeared more marked in monocytes from the mangabey species. These in vitro differences may relate to the clinically asymptomatic state of the sooty mangabeys and the disease-susceptible state of the rhesus macaques.

Key words: SIV infection • AIDS

INTRODUCTION

While a majority of sooty mangabeys (*Cerocebus atys*) at the Yerkes Regional Primate Research Center (YRPRC) are naturally infected with a simian immunodeficiency virus [5] and have, to date, remained clinically asymptomatic, experimental infection of rhesus macaques (*Macaca mulatta*) and pig-tailed macaques (*Macaca nemestrina*) with the SIV isolated from sooty mangabeys (SIV/SMM) results in clinical symptoms and pathology

Accepted for publication January 24, 1990.

© 1990 Munksgaard

similar to that seen with HIV-1 infection in humans [10–12]. The observation that SIV/SMM infects both sooty mangabeys and macaques, but results in clinical disease only in the latter species, provides a unique model to examine host virus relationships for the delineation of pathogenic mechanisms. Thus, it was reasoned that studies of the target cells which are susceptible to virus infection and mechanisms that control replication of the virus may provide some insight into the differential pathogenesis of SIV/SMM in these two species. Both HIV-1 and SIV share a tropism for $CD4^+$ T cells [2,11] and for cells of the monocyte/macrophage lineage [14,15,22]. While HIV and SIV induce cytopathic effects on $CD4^+$ T cells [8], it is currently believed that monocytes and macrophages most likely serve as a reservoir for these retroviruses [17]. There is also evidence that the mechanism of release of mature viruses from infected $CD4^+$ T cells and monocytes/macrophages may be distinct [6,15,16]. While both HIV and SIV can be readily isolated from $CD4^+$ T cells from infected peripheral blood and shown to replicate in a variety of $CD4^+$ T cell lines [11], it is also known that these retroviruses can replicate efficiently in monocytes and monocytic cell lines such as the U937 [4,15]. It has been suggested that there may be variants of HIV and SIV, some of which may have tropism for $CD4^+$ T cells and others for cells of the monocyte/macrophage lineage [1]. These data prompted us to examine the relative rate of replication and the control of SIV replication in enriched populations of $CD4^+$ T cells and monocytes from the two species. The data demonstrate that SIV/SMM replicates at a three to ten times higher rate in in vitro cultures of monocytes/macrophages of sooty mangabeys than in similar cultures of rhesus macaques and replicates equally well in $CD4^+$ T cells of the two species. Of interest was the finding that while $CD8^+$ T cells from sooty mangabeys inhibit SIV replication to a larger extent in autologous cultures of monocytes/macrophages than in $CD4^+$ T cell cultures, $CD8^+$ T cells from rhesus macaques inhibit SIV replication to the same extent in these two cell types. Whether such quantitative and qualitative differences of virus replication and control contribute to the differential pathogenic mechanisms seen in these two species is currently not known. Results of these studies constitute the basis of this report.

MATERIALS AND METHODS

Subjects

Adult rhesus macaques (*Macacca mulatta*) and sooty mangabeys (*Cercocebus atys*) from the Yerkes Regional Primate Research Center were the

source of blood samples for this study. All animals were maintained in accordance with the instructions of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, and the PHS guidelines, "Guide for the Care and Use of Laboratory Animals." All animals used in this study were free of retrovirus D infection and were seronegative for STLV-I at the time of these studies.

Isolation of Mononuclear Cells

Heparinized peripheral blood was layered on a freshly prepared 60% isotonic Percoll gradient and then centrifuged at 450 × g for 30 minutes at 20°C. The cells at the interface were collected, washed twice with media, and cell counts/viability determined using the trypan blue dye exclusion technique.

Enrichment for Monocytes

Adherent and nonadherent cells were separated by incubation of PBMC on sterile (7 × 7 cm) plastic culture dishes (Costar, Cambridge, MA) in the presence of 10% fetal bovine serum for one hour at 37°C. Nonadherent cells were aspirated, the plates were washed twice, and the cells were collected and pooled. Adherent cells were scraped off the plate, using a sterile cell scraper after incubation for 15 minutes on ice. Flow microfluorometric (FMF) analysis of the nonadherent and adherent cell populations showed that the adherent cells were 85–95% monocytes and that the remaining cells were B cells. The nonadherent cells were mostly T cells (70–80%), with some NK cells (5%), B cells, and others (10–20%).

Preparation of Enriched $CD4^+$ and $CD8^+$ Cells From Nonadherent Cells

The nonadherent cells were centrifuged at 150 × g and the supernatant fluid discarded. The cell pellet was disassociated by gentle tapping, and based on the cell count, the appropriate volume of undiluted monoclonal anti CD8 (anti Leu 2a) antibody was added. (A total of 0.1 ml of undiluted monoclonal antibody was added to 1×10^7 viable cells). The cell suspension was incubated on ice for 40 minutes, and then 2 ml of media were added and the cell suspension centrifuged at 150 × g for ten minutes. The supernatant fluid was discarded, and the cells were washed with media and then incubated with immunobeads (magnetic beads, conjugated with goat anti mouse Ig) at a concentration of 10–50 beads/cell or about 2 ml of the immunobeads (10^8 beads/ml). The cells with the immunobeads were in-

cubated at 4°C for 40 minutes and the test tube subjected to a magnetic field. The cells in the supernatant fluid were collected and used as a source of enriched $CD4^+$ (or $CD8^+$ depleted) cell population. The beads were incubated with 2 ml of media containing 10% fetal calf serum overnight at 37°C in a 7% CO_2 humidified atmosphere. The cell suspension was again exposed to a magnetic field, and the supernatant fluid containing the cells that became detached from the immunobeads were collected and used as a source of $CD8^+$ enriched cell populations. The relative efficiency of depletion or enrichment of these cells was determined by FMF analysis to be 95% and the viability determined to be 99% for depleted cells and about 90% for enriched cells.

Monitoring of SIV Replication

Unfractionated and $CD4^+$ or monocyte/macrophage enriched populations of cells were cultured in vitro at 1×10^6/ml in a volume of 2 ml. To triplicate cultures was added media (control) or SIV/SMM to achieve a final concentration of 1 ×. Cultures were incubated at 37°C in a 7% CO_2 humidified atmosphere for 24 hours and then washed once with media, and supernatant fluids were collected every 3–4 days and replaced by fresh media. Reverse transcriptase (RT) activity in the supernatant fluids was used as a method to monitor SIV infection and replication by the appropriate target cells in culture. RT was assayed by the micromethod [19]. Briefly, virus was pelleted from culture supernatant by ultracentrifugation at 100,000 × g for 30 minutes. It was solubilized and incubated with poly(rA)-oligo(dT) templated primer, ^{3}H-TTP, and the necessary reaction mixture conditioned for two hours at 37°C. The reaction was then precipitated with tRNA and TCA and harvested with a semiautomatic cell harvestor. Virus preparations from each supernatant fluid were aliquoted and assayed in duplicate and the mean counts per minute (cpm) of this triplicate determined. In addition, each experimental culture was performed in triplicate. Thus, a mean cpm of the triplicate cultures was derived from the mean cpm of each RT assay that was performed. The data, therefore, reflect the mean cpm of the triplicate samples. Finally, before the initial mean cpm values were derived, the mean cpm of the background (negative control) was deducted; therefore, the RT values reflect the mean of mean net cpm.

Media

Media at all times refers to RPMI 1640 supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% heat inac-

tivated fetal bovine serum (all from GIBCO, Grand Island, NY). For washing purposes, RPMI 1640 alone was used.

SIV/SMM Virus

SIV/SMM was originally isolated from sooty mangabeys [5] from the colony at the YRPRC. The virus was grown in the HT cell line on contract (Advanced Biotechnology, Inc., Silver Spring, MD), ultracentrifuged, and purified by sucrose gradient centrifugation. The 1000× stock virus contained 10^{10} to 10^{11} virus particles/ml and was used at a 1 × concentration for infection of the target cells in this study.

RESULTS

In Vitro Replication of Exogenously Added SIV/SMM

Unfractionated PBMC from SIV seropositive and seronegative sooty mangabeys and from uninfected and experimentally infected rhesus macaques were cultured in vitro with media (control) or 1 × of SIV/SMM stock, as described in the Materials and Methods section. Supernatant fluid was collected every four days up to day 21 and assayed for RT activity. Representative data from one experiment are shown in Fig. 1. The mean RT activity in the supernatant fluid of PBMC cultures over the entire time period from seropositive mangabeys and experimentally infected rhesus macaques was essentially the same. Major differences were not observed in the RT activity in supernatant fluids of cultures of PBMC from uninfected and experimentally infected rhesus macaques. However, SIV/SMM replicated to a relatively lesser extent in PBMC from SIV/SMM seronegative sooty mangabeys, at least as measured by the RT assay; this difference was statistically meaningful in repeated experiments.

Study of the Kinetics of SIV/SMM Replication in Enriched Populations of $CD4^+$ and Monocyte/Macrophage Cultures

The knowledge that SIV replicates in both $CD4^+$ T cells and monocytes/macrophages prompted us to examine the relative replication of SIV and the kinetics of its replication in these enriched cell populations. $CD4^+$ cells and monocyte/macrophage populations were enriched and cultured with SIV/SMM, as described in the Materials and Methods section. Supernatant fluid was collected every four days and assayed for RT activity. As seen in Fig. 2, peak RT activity was seen on day 14 in the monocyte/macrophage cultures

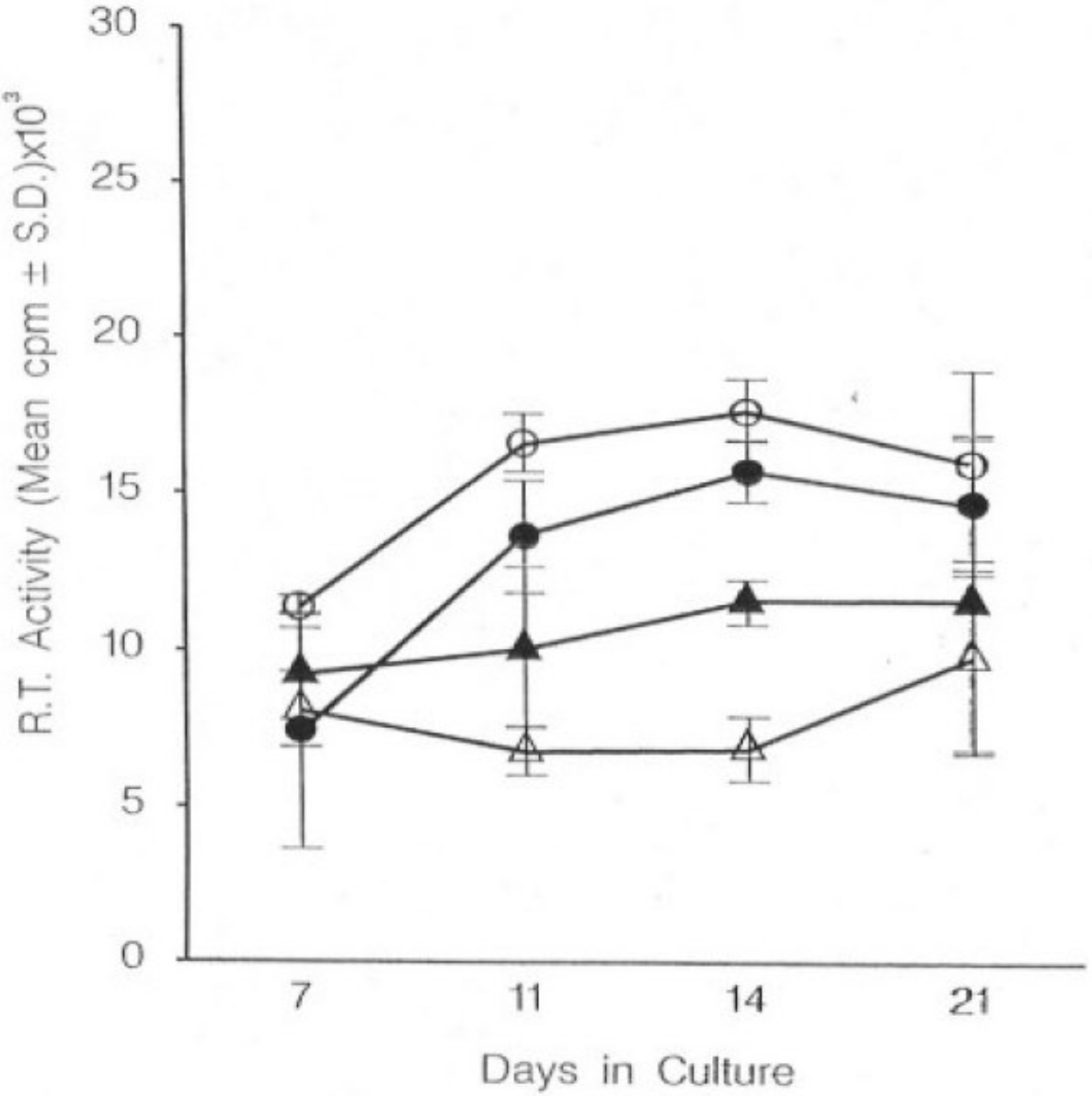

Fig. 1. Kinetics of in vitro replication of exogenously added SIV/SMM in peripheral blood mononuclear cell cultures from uninfected (○–○) and experimentally infected (●–●) rhesus macaques and from seronegative (△–△) and seropositive (▲–▲) sooty mangabeys. On days 7, 11, 14, and 21, supernatant fluids of triplicate cultures were each assayed in duplicate for the level of reverse transcriptase activity (RT) as described in the Materials and Methods section, and the mean cpm ± SD was calculated. Each data point reflects the mean of means of each triplicate culture. The experiment was repeated three times, and the data showed similar profiles each time.

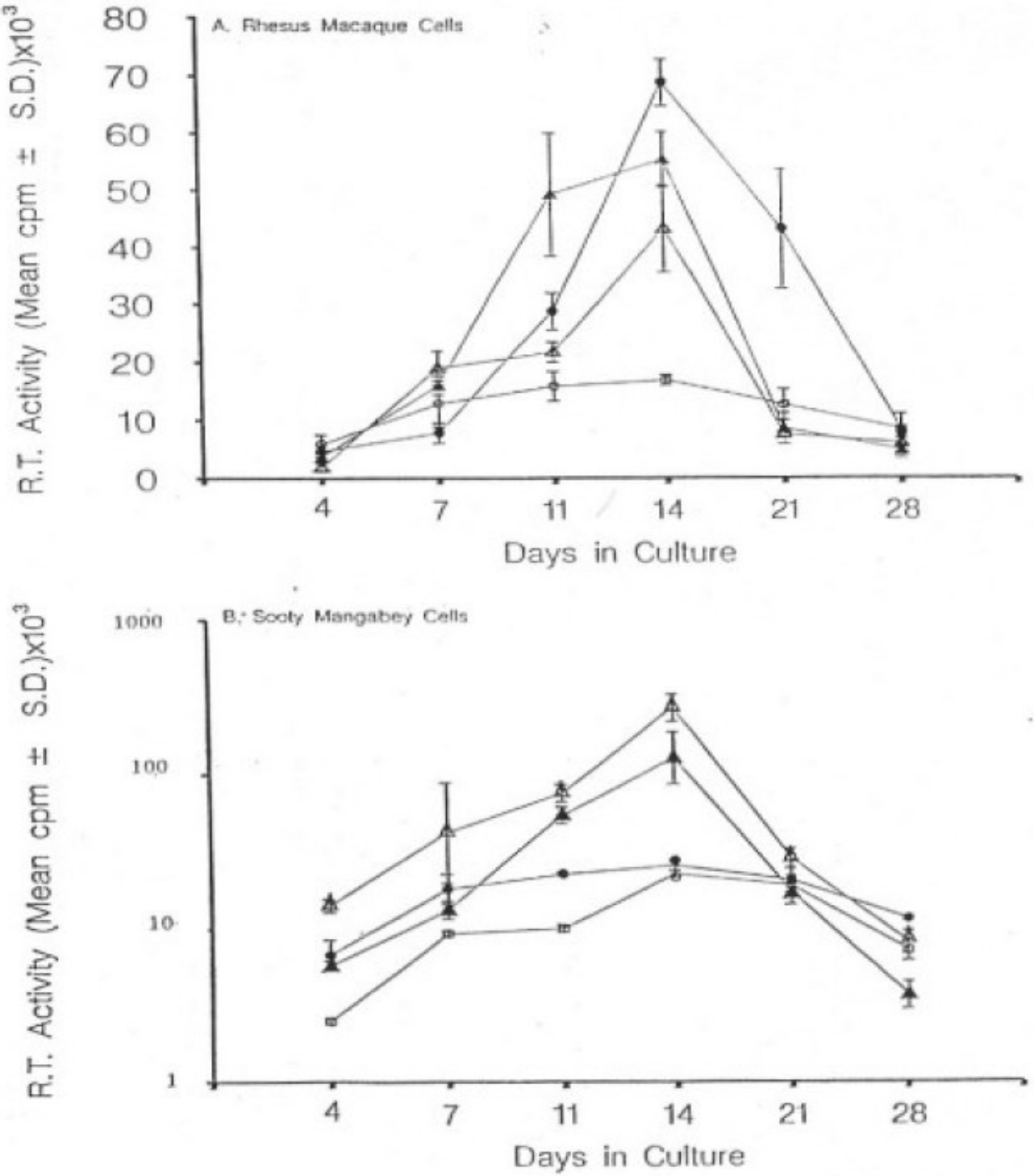

Fig. 2. Kinetics of in vitro replication of exogenously added SIV/SMM in enriched populations of $CD4^+$ T cells and monocyte/macrophage cultures established from the peripheral blood mononuclear cells of (A) rhesus macque and (B) sooty mangabey. SIV^-CD4^+ T cells, ○–○; SIV^+ $CD4^+$ T cells, ●–●; SIV^- monocytes, △–△; SIV^+ monocytes, ▲–▲. On days 7, 11, 14, and 21, supernatant fluids were collected from triplicate cultures and assayed each in triplicate for RT activity, and the mean ± SD was calculated. Each data point reflects the mean of means of each triplicate culture.

and the $CD4^+$ T cell cultures of both the seropositive and seronegative mangabey and the uninfected and experimentally infected rhesus macaques.

These preliminary data allowed us to use day 14 to examine the differences in SIV replication in enriched populations of $CD4^+$ cells and monocytes/macrophages in a larger number of monkeys. As seen in Fig. 3A, no significant differences in the replication (RT activity) of SIV were seen in enriched populations of $CD4^+$ cells from both rhesus macaques and sooty mangabeys. There was, in addition, no difference in SIV replication regardless of whether the $CD4^+$ cells were from SIV seropositive or seronegative mangabeys or from SIV infected or uninfected rhesus macaques. However, as seen in Fig. 3B, marked differences were noted in the relative replication (RT activity) of SIV/SMM in monocyte/macrophage cultures from sooty mangabeys and rhesus macaques. First, SIV replicated much more efficiently in monocyte/macrophage cultures of sooty mangabeys than in either SIV infected or uninfected rhesus macaques. Second, of great interest was the finding that SIV replicated with the highest efficiency (RT activity) in monocyte/macrophage cultures of seronegative sooty mangabeys (see Fig. 3B). These differences were not secondary to differences in kinetics of SIV replication in the various cell populations in vitro. This was true not only from our preliminary kinetic studies (see Fig. 2) but also in some cases in which we monitored RT activity in the supernatant fluid of cultures; as described in Fig. 3, the peak activity still occurred on day 14. Further, the differences were still marked at each time interval tested.

It was reasoned that such differences in the relative replication of exogenously added SIV to these cultures could be due to the presence of replication defective endogenous SIV or an as yet unknown molecular mechanism present in monocytes/macrophages of SIV seropositive sooty mangabeys. To address this issue, supernatant fluid from monocyte/macrophage cultures derived from SIV seropositive sooty mangabeys and rhesus macaques were monitored for RT activity every four days for a total of 21 days. Highest RT activity was seen on days 10 and 15, as depicted in Fig. 4. As seen, there was no striking difference in the level of RT activity in these culture supernatant fluids. Similarly, $CD4^+$ T cell cultures from SIV^+ mangabeys and macaques showed no difference in endogenous levels of SIV replication (RT activity), data not shown. These data suggest that the only significant difference in the replication of SIV in vitro is that monocytes/macrophages of sooty mangabeys, as compared to rhesus macaques, are more efficient for SIV replication in vitro. In addition, monocytes/macrophages from seronegative sooty

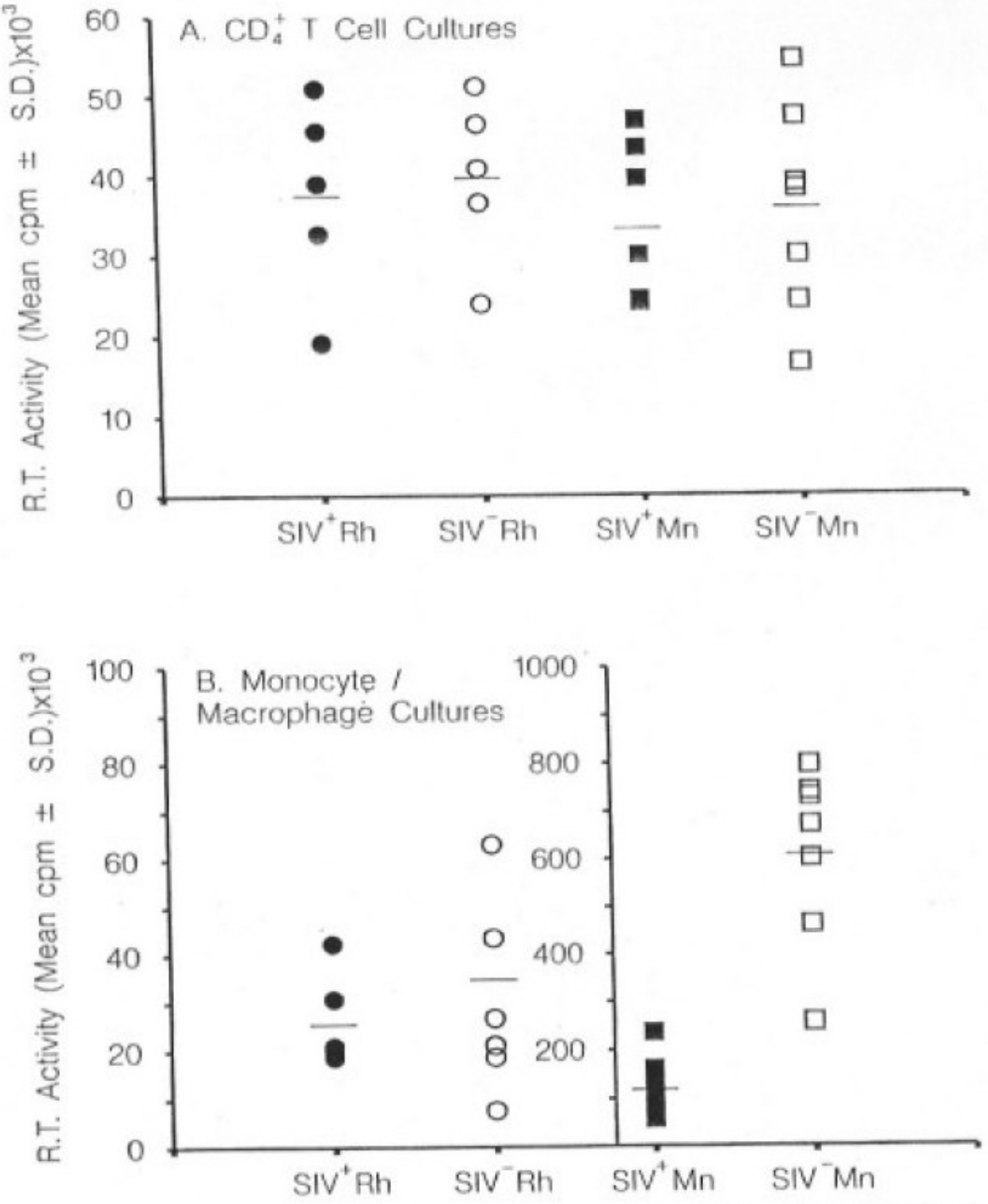

Fig. 3. Day 14 RT activity in in vitro cultures of enriched populations of (A) $CD4^+$ T cells and (B) monocytes and macrophages (adherent cells) from uninfected (SIV^- Rh, ○) and experimentally infected (SIV^+Rh, ●) rhesus macaques and from SIV seronegative (SIV^-Mn, □) and seropositive (SIV^+Mn, ■) sooty mangabeys pulsed with 1 × concentration of SIV/SMM. Enriched populations were prepared as described in the Materials and Methods section. Data represent peak RT activity in $CD4^+$ T cells from five SIV^- and SIV^+ rhesus macaques each, seven SIV^- and SIV^+ sooty mangabeys each, and monocyte/macrophage cultures from six SIV^- and SIV^+ rhesus macaques, nine SIV^+ and seven SIV^- sooty mangabeys. The mean ± SD was calculated from the mean of means of each triplicate.

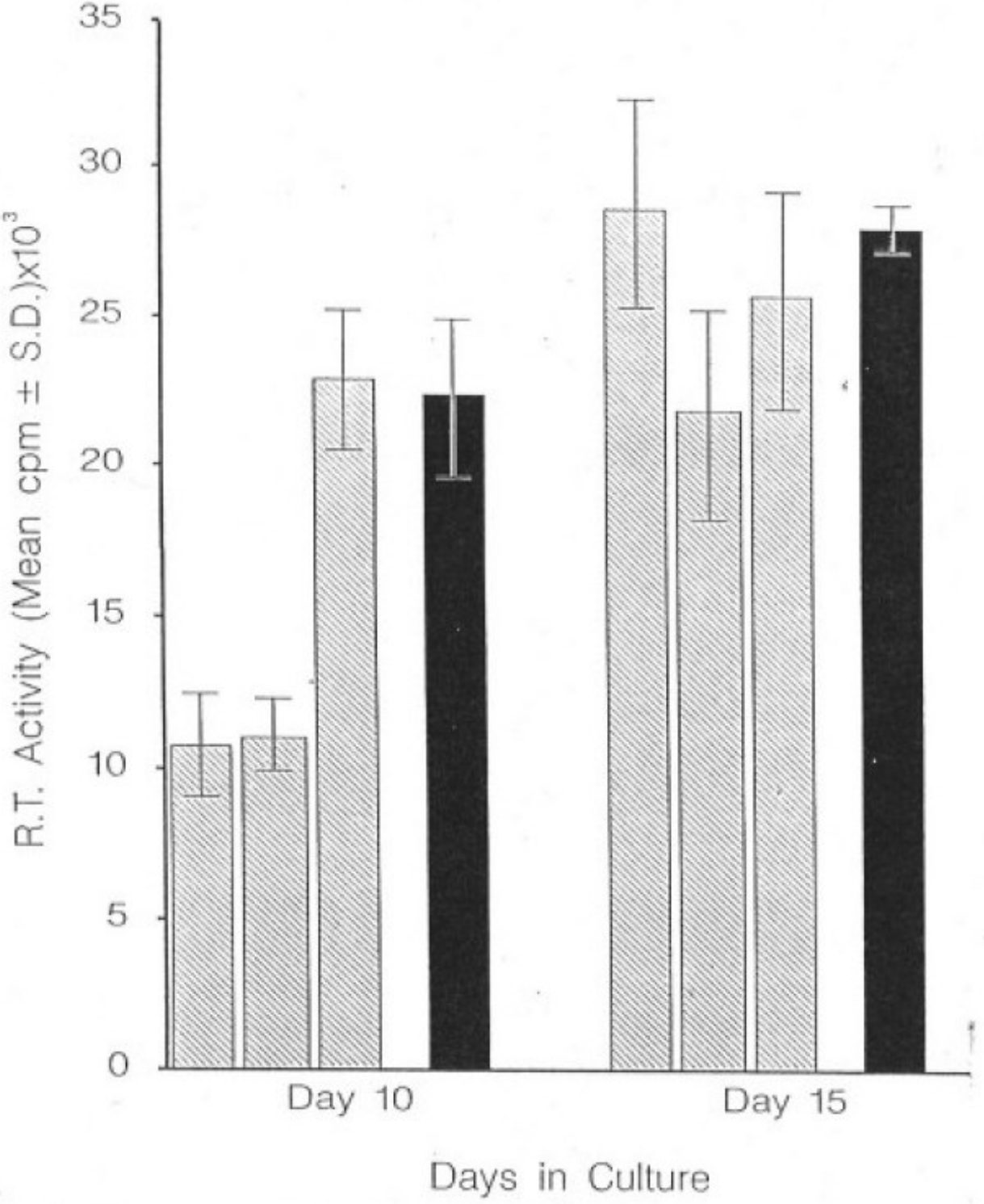

Fig. 4. Endogenous replication of SIV/SMM in peripheral blood mononuclear cells from three seropositive sooty mangabeys (▨) and one experimentally infected rhesus macaque (■). Supernatant fluids were collected from triplicate cultures on days 10 and 15 and assayed each in triplicate for RT activity as described in the Materials and Methods section. The mean ± SD of the triplicate culture supernatant fluid assayed each in triplicate was calculated. Similar results were obtained in three separate experiments.

mangabeys are better than seropositive mangabeys as targets for SIV replication in vitro.

$CD8^+$ Cells Inhibit Viral Replication in Both $CD4^+$ and Monocyte/Macrophage cultures

The data above demonstrate that in both species, monocytes/macrophages support the growth of SIV much better than do the unfractionated PBMC from the same blood donor. While it is quite possible that the reason for increased virus replication is the increased number of the target cells being utilized, the previous data from Walker et al. [20] and Kannagi et al. [7], indicating that $CD8^+$ cells from both experimentally infected rhesus macaques and HIV-1 infected humans markedly suppress the replication of SIV and HIV-1 in autologous cells in vitro, prompted us to examine the regulation of SIV replication in the enriched populations of $CD4^+$ T cells and monocyte/macrophage cultures, both in the presence and absence of autologous $CD8^+$ cells. As described above, exogenous SIV/SMM was added, and the cultures were incubated overnight, washed once with medium, and then resuspended in fresh medium. The supernatant fluid was monitored for RT activity over a 28 day period. As seen in Table I, while addition of $CD8^+$ cells from uninfected rhesus macaques to autologous $CD4^+$ cells or monocyte/macrophage cultures had no effect on the rate of SIV replication, consistent with the findings of Walker et al. [21] and Kannagi et al. [7], addition of $CD8^+$ cells to autologous $CD4^+$ or monocyte/macrophage enriched cultures from SIV infected rhesus macaques and SIV seropositive sooty mangabeys showed marked suppression of RT activity. The inhibition of SIV replication was more marked in the monocyte/macrophage cultures than in the $CD4^+$ cultures of the sooty mangabeys.

In an effort to determine if the ability of $CD8^+$ cells to suppress SIV replication in vitro is MHC restricted, $CD8^+$ cells were added to autologous and allogeneic cultures of monocytes/macrophages. As seen in Table II, the inhibition of SIV production by $CD8^+$ cells occurs only when autologous cells are used. The addition of allogeneic $CD8^+$ cells, however, results in enhancement of SIV replication. This enhancement is not surprising as allogeneic effects, most likely induced by the stimulation of the $CD4^+$ T cells and the monocytes/macrophages, increased the susceptibility of these cells to SIV infection. The fact that cell activation leads to higher viral replication has been previously reported [3,9,13]. These preliminary data suggest that the $CD8^+$ cell mediated suppression of SIV replication in vitro is MHC restricted.

TABLE I. $CD8^+$ cells inhibit the replication of exogenously added SIV/SMM virus to cultures of autologous $CD4^+$ T cells and monocytes/macrophages in vitro[1]

Source of cells[2]	Virus cultured with	RT activity (mean cpm ± SD)		Inhibition (%)
		Media control	$CD8^+$ cells	
SIV^+ Rhesus	$CD4^+$ Cells	109,628 ± 6,877	63,654 ± 5,020	42
	Monocytes	19,494 ± 1,285	14,266 ± 982	27
SIV^- Rhesus	$CD4^+$ Cells	102,565 ± 8,073	103,102 ± 3,569	− 1.5
	Monocytes	18,992 ± 1,243	19,953 ± 3,350	− 10.3
SIV^+ Mangabey	$CD4^+$ Cells	90,002 ± 8,413	44,450 ± 6,007	51
	Monocytes	795,000 ± 130,000	165,000 ± 14,260	80

[1]Enriched populations of $CD4^+$ cells and monocyte/macrophage cells were obtained as described in the Materials and Methods Section. The cultures were incubated at 1×10^6/ml overnight at 37°C in a 7% CO_2 humidified atmosphere. Subsequently, enriched population of autologous $CD8^+$ cells (positively selected) were added to triplicate cultures. Control consisted of the addition of media instead of $CD8^+$ cells. SIV/SMM virus (1 × concentration) was added, and 20 hr later, the cells were rinsed and incubated with media only. Supernatant fluid was collected from each culture at days 4, 7, 11, 14, 21, and 28. Peak RT activity occurred on day 14; thus, data from day 14 only is described above. This experiment has been repeated five times, with essentially similar findings.

[2]SIV^+ rhesus refers to a rhesus macaque experimentally infected with SIV/SMM, SIV^- refers to an uninfected rhesus macaque, and SIV^+ mangabey refers to a mangabey which is serologically positive for SIV/SMM.

DISCUSSION

The experiments described above were carried out in efforts to determine differences in the in vitro virus susceptibility of PBMC from sooty mangabeys and rhesus macaques which may shed light on the disease susceptibility of the latter species and the chronically infected but clinically asymptomatic state of the former. Previous observations from other laboratories demonstrate that lentiviruses are tropic for not only $CD4^+$ T cells but also macrophages [2,6,8,11,15,16]; thus, it was our objective to determine if the differences in disease susceptibility of the two species was related to increased susceptibility of these two target cells for SIV replication in the rhesus macaques. The data obtained demonstrate several interesting findings: a) SIV/SMM appears to replicate more efficiently in adherent cells, most of which are monocytes/macrophages of the sooty mangabey; b) the virus replicates better (as measured by RT activity) in monocytes from SIV seronegative mangabeys as compared with similar cells from seropositive mangabeys; c) while $CD8^+$ cells from uninfected rhesus macaques do not inhibit virus replication in either enriched populations of autologous $CD4^+$ cells or mono-

TABLE II. CD8$^+$ T cell mediated inhibition of SIV/SMM replication in vitro is MHC restricted[1]

Animal no.	SIV/SMM infected cultures	RT activity (mean cpm ± SD) in the presence of media (control)	autologous CD8$^+$	% of control[2]	Allogeneic CD8$^+$	% of control[2]
1	CD4$^+$ T cells	27,250 ± 877	14,250 ± 2,030	52.3	42,500 ± 4,009	155.9
	Monocytes	27,500 ± 2,410	1,975 ± 1,881	7.2	60,052 ± 4,993	218.4
2	CD4$^+$ T cells	40,000 ± 5,000	15,000 ± 1,904	37.5	72,500 ± 988	181.2
	Monocytes	28,250 ± 566	7,250 ± 423	25.6	40,000 ± 3,757	141.6

[1] Enriched populations of CD4$^+$ T cells or monocytes/macrophages (1×10^6/ml), with or without positively selected CD8$^+$ T cells, from two sooty mangabeys were cultured in vitro overnight with 1 × concentration of SIV/SMM. The cells were then rinsed and kept in media only. Cultures were performed in triplicate and supernatant fluid harvested on days 5, 10, 14, and 21 and assayed for RT activity. The data above represent mean RT activity on day 14 (peak activity) with a 1:1 ratio of CD8$^+$ cells to virus infected cells.

[2] Percent of control was calculated by dividing the mean cpm in the presence of CD8$^+$ cells by mean cpm in media control × 100.

cyte/macrophage cultures, $CD8^+$ cells from SIV infected sooty mangabeys and rhesus macaques markedly inhibit replication of the virus in autologous $CD4^+$ cells and enriched population of monocyte/macrophage culture. These data are compatible with the findings of Walker et al. [21] and Kannagi et al. [7]; d) the $CD8^+$ cells of the sooty mangabey, unlike those of the rhesus macaque, markedly suppress the replication of SIV in the monocyte/macrophage cultures; e) preliminary data indicate that this ability of $CD8^+$ cells to suppress SIV replication is MHC restricted.

Some of these findings (a,b) are quite contrary to our predictions. It was originally reasoned that the increased susceptibility of rhesus macaques to the development of disease was due to increased ability of the virus to replicate in the target cells of rhesus macaques. In contrast, our data show that, at least in vitro, once the $CD8^+$ cells are removed, the virus replicates more efficiently in the adherent monocytes/macrophages of sooty mangabeys than in similar populations from rhesus macaques. These differences were not secondary to endogenous dose of virus or to kinetics; nor were they due to major differences in the phenotypic frequency of either the effector or the target cells of the two species. Rather, these data imply a functional difference in the cell population of these two species.

It should be noted that no exogenous growth factors were added to culture media. We have found that the macrophage cultures in vitro are sustained quite well in 10% FBS conditioned media and, presumably, the plethora of factors which the macrophages release themselves upon activation by the plastic petri dishes. Thus, in this study, the complex effect of growth factors on the replication of SIV in the monocytes [9,18] was not addressed. (Further studies are underway.) It should also be emphasized that in these studies, the relative replication rate is being measured by RT activity only. Thus, replication incompetent viruses would not be assessed. An antigen capture assay may reveal differences in the measurement of total virus.

One might speculate that monocytes from seropositive mangebeys might contain defective or less virulent endogenous virus, which serves to attenuate the input virus. This hypothesis does not explain, however, why monocytes from seronegative rhesus do not replicate virus as well as do monocytes from seronegative mangabeys. Nonetheless, we plan to pass the input virus with monocytes from each species and then measure its ability to infect PBMC, monocytes, and even cell lines.

Why does the rhesus macaque die from SIV infection while the infected sooty mangabey remains clinically asymptomatic? One reason could be this unique property of the $CD8^+$ cells; in the mangabey, they are very potent,

having the ability (at least in vitro) to almost totally suppress the replication of this virus in the monocytes. The possibility that $CD8^+$ cells (or maybe a specific subset of $CD8^+$ cells) play an important role in protecting the sooty mangabeys is also supported by the following facts: i) there is a relatively high frequency of activated $CD8^+$ cells in the PBMC of mangabeys; ii) the frequency of $CD8^+$ cells diminishes dramatically in most SIV infected rhesus macaques prior to death (unpublished data).

Extrapolating from the data described here, one could speculate that the frequency of infected macrophages in the different tissues of the sooty mangabey will be much lower than in the rhesus macaque. In view of the important role attributed to the monocytes as reservoirs of HIV and SIV in the body, the importance of such findings to the understanding of the mechanisms leading to AIDS can clearly be seen.

ACKNOWLEDGEMENTS

The authors are deeply grateful for the excellent cooperation and help provided by Dr. Thomas Gordon of the Yerkes Regional Primate Research Center, Emory University, in the performance of these studies. In addition, we are deeply grateful to Dr. Daniel Anderson (YRPRC) for his critical review of the manuscript and to the following people for their technical assistance: Ms. Judy A. Scheppler of the Centers for Disease Control, Atlanta, GA; Ms. Ellen Lockwood of YRPRC; and Ms. Ann Mayne and Mr. Matthew M. Vuchetich of Emory University, School of Medicine. The editorial assistance of Ms. Cecile Long is also acknowledged. Supported by USPHS grants NIH-RO1-AI-27057-01 and NIH-DRR-00165.

REFERENCES

1. Collman R, Hassan NF, Walker R, Godfrey B, Cutilli J, Hastings JC, Friedman H, Douglas SD, Nathanson N: Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1): Monocyte-tropic and lymphocyte-tropic strains of HIV show distinctive patterns of replication in a panel of cell types. J Exp Med 170:1149–1163, 1989.
2. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK: Isolation of T cell tropic HTLV-III retrovirus from macaques. Science 228:1201–1204, 1985.
3. Folks T, Powell DM, Lightfoote MM, Benn S, Martin MA, Fauci AS: Induction of HTLV-III/LAV from a non virus-producing T cell line: Implications for latency. Science 231:600–602, 1986.
4. Folks TM, Justement J, Kinter A, Schnittman S, Orenstein J, Poli G, Fauci AS: Charac-

terization of a promonocyte clone chronically infected with HIV and inducible by 13-phorbol-12-myristate acetate. J Immunol 140:1117–1122, 1988.
5. Fultz PN, McClure HM, Anderson DC, Swenson RB, Anand R, Srinivasan A: Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cerocebus atys*). Proc Natl Acad Sci USA 83:5286–5290, 1986.
6. Gartner S, Markovitz P, Markovitz DM, Kaplan MH, Gallo RC, Popovic M: The role of mononculear phagocytes in HTLV-III/LAV infection. Science 233:215–217, 1986.
7. Kannagi M, Chalifoux LV, Lord CI, Letvin NL: Suppression of simian immunodeficiency virus replication *in vitro* by $CD8^+$ lymphocytes. J Immunol 140:2237–2242, 1988.
8. Klatzmann DF, Barre-Sinoussi DF, Nugeyre MT, Dauguet C, Vilmer E, Griscelli C, Brun-Vezinet F, Rouzious C, Gluckman JC, Chermann JC, Montagnier L: Selective tropism of lymphadenopathy associated virus (LAV) for helper inducer T lymphocytes. Science 225:59–63, 1984.
9. Koyangi Y, O'Brien WA, Zhao JQ, Golde DW, Gasson JC, Chen ISY: Cytokines alter production of HIV-1 from primary mononuclear phagocytes. Science 241:1673–1675, 1988.
10. Letvin NL, Daniel MD, Sehgal PK, Desrosiers RC, Hunt RD, Waldrom LM, Mackey JJ, Schmidt DK, Chalifoux LV, King NW: Induction of AIDS-like disease in macaque monkeys with T cell tropic retrovirus STLV-III. Science 230:71–73, 1985.
11. McDougal JS, Kennedy MS, Sligh JM, Cort SP, Mawle A, Nicholson JKA: Binding of HTLV-III/LAV to $T4^+$ T cells by a complex of the 110K viral protein and the T4 molecule. Science 231:382–385, 1986.
12. McClure HM, Anderson DC, Fultz PN, Ansari AA, Lockwood E, Brodie AR: Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. Vet Immunol Immunopath 21:13–24, 1989.
13. Meltzer MS, Gendelman HE: Effects of colony stimulating factors on the interaction of monocytes and the human immunodeficiency virus. Immunol Letters 19:193–1983, 1988.
14. Muller H, Falk S, Stutte HJ: Accessory cells as primary target of human immunodeficiency virus (HIV) infection. J Clin Pathol 39:1161–1167, 1986.
15. Nicholson JKA, Cross GD, Callaway CS, McDougal JS: In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). J Immunol 137:323–329, 1986.
16. Orenstein JM, Meltzer MS, Phipps T, Gendelman HE: Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1 treated human monocytes: An ultrastructural study. J Virol 62:2578–2586, 1988.
17. Pauza CD: HIV persistence in monocytes leads to pathogenesis and AIDS. Cell Immunol 112:414–424, 1988.
18. Pauza CD, Galindo J, Richman DD: Human immunodeficiency virus infection of monoblastoid cells: Cellular differentiation determines the pattern of virus replication. J Virol 62:3558–3564, 1988.
19. Spira TJ, Bozeman LH, Holman RC, Warfield DT, Phillips SK, Feorino PM: Micromethod for assaying reverse transcriptase of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. J Clin Microbiol 25:97–99, 1987.
20. Tsubota H, Lord CI, Watkin DI, Morimoto C, Letvin NL: A cytotoxic T lymphocyte inhibits acquired immunodeficiency syndrome virus replication in peripheral blood lymphocytes. J Exp Med 169:1421–1434, 1989.

21. Walker CM, Moody DJ, Stites DP, Levy JA: CD8$^+$ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 234:1563–1566, 1986.
22. Ward JM, O'Leary TJ, Baskin GB, Benveniste R, Harris CA, Nara PL, Rhodes RH: Immunohistochemical localization of human and simian immunodeficiency viral antigens in fixed tissue sections. Am J Path 127:199–205, 1987.

Dr. Aftab Ahmed-Ansari, Emory University School of Medicine, Winship Cancer Center, Room 5008, 1327 Clifton Road, N.E., Atlanta, GA 30322