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Simian Immunodeficiency Virus Inhibits Bone Marrow Hematopoietic Progenitor Cell Growth

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The pathogenic mechanisms underlying the depressed hematopoietic functions seen in human immunodeficiency virus-infected individuals were explored in rhesus monkeys infected with the simian immunodeficiency virus of macaques (SIV_{mac}). Bone marrow hematopoietic progenitor cell colony formation, both granulocyte/macrophage (CFU-GM) and erythrocyte (BFU-E), was shown to be decreased in number in SIV_{mac}-infected rhesus monkeys. SIV_{mac} was readily isolated from bone marrow cells of infected monkeys and was shown to be harbored in macrophages rather than T lymphocytes. The in vitro infection of normal bone marrow cells by SIV_{mac} inhibited colony formation. A striking in vivo correlation between increased SIV_{mac} load in bone marrow cells and decreased hematopoietic progenitor cell colony growth was also shown. Finally, inhibition of SIV_{mac} replication in bone marrow macrophages resulted in increased progenitor cell colony growth from bone marrow cells. These results suggest that the infection of bone marrow macrophages by the acquired immunodeficiency syndrome (AIDS) virus may contribute to depressed bone marrow hematopoietic progenitor cell growth. Moreover, inhibition of AIDS virus replication in these macrophages might induce significant improvement in hematopoietic function.

Abnormalities in hematopoiesis with resultant peripheral blood cytopenias occur in the majority of patients with the acquired immunodeficiency syndrome (AIDS) (1, 26–29). The number of circulating monocytes is diminished in many of these individuals (29). Granulocytopenias have been reported in up to two-thirds of patients with AIDS or AIDS-related complex (29). An even higher incidence of anemia has been noted in a series of individuals with AIDS (27, 29). The pathogenic mechanisms which underlie these hematologic abnormalities remain poorly defined. The study of these mechanisms is complicated by coincident marrow suppression in patients with AIDS that occurs as a result of therapy for AIDS itself (21, 32) as well as therapy for common AIDS-related opportunistic infections (2). Studies of hematopoiesis in AIDS have also been hindered by the difficulties involved in obtaining frequent bone marrow samples from patients.

Lentiviruses related in their nucleotide sequences to human immunodeficiency virus (HIV) types 1 and 2 have recently been isolated from African and Asian nonhuman primates (8, 17). Some of these isolates, upon inoculation into macaque monkeys, induce a disease in those animals with striking similarities to human AIDS. Juvenile rhesus monkeys infected with the simian immunodeficiency virus of macaques (SIV_{mac}) develop a transient skin rash, subsequent lymphadenopathy syndrome, and immunologic abnormalities which include a reduction in the number of circulating CD4+ lymphocytes (5, 17, 22). These animals die with opportunistic infections, including disseminated adenovirus infections, disseminated *Mycobacterium avium intracellulare*, *Pneumocystis carinii* pneumonia, and intestinal cryptosporidiosis. Animals also die with lentiviral encephalitis, which is characterized by perivascular infiltrates of SIV_{mac}-infected macrophages and multinucleated giant cells. This SIV_{mac}-induced syndrome in the rhesus monkey provides an important animal model for studying the pathogenesis of AIDS (14, 30).

In the present studies, we have utilized the rhesus monkey SIV_{mac} system to explore the ramifications of lentivirus infection on hematopoiesis. We show that SIV_{mac} can be readily isolated from bone marrow macrophages of infected rhesus monkeys and that virus replication in these macrophages has a marked inhibitory effect on the development of granulocyte/macrophage and erythrocyte progenitor cell colonies.

MATERIALS AND METHODS

Animals and viruses. The rhesus monkeys (*Macaca mulatta*) used in these experiments included 10 uninfected monkeys, 8 monkeys experimentally infected with SIV_{mac} isolate 251 (7, 17) 1.5 to 3 years before initiating these studies, and 4 juvenile monkeys acutely infected with SIV_{mac} isolate 251.

Preparation of bone marrow cells. Heparinized bone marrow samples were aspirated from the posterior iliac crest of ketamine-anesthetized monkeys. Mononuclear cells were isolated by Ficoll (Pharmacia Fine Chemicals, Piscataway, N.J.)-diatrizoate density gradient centrifugation, washed, and suspended in Iscove modified Dulbecco minimum essential medium (IMDM; GIBCO, Grand Island, N.Y.) supplemented with 12.5% fetal bovine serum (FBS; Hyclone Laboratories, Logan, Utah)–12.5% horse serum (Flow Laboratories, Inc., McLean, Va.)–L-glutamine (1 mM)–penicillin (100 U/ml). For some experiments, the bone marrow mononuclear cells were depleted of T lymphocytes by the technique of complement-dependent lysis, using an anti-CD6 (3PT12B8; kindly provided by S. Schlossman, Dana-Farber Cancer Institute, Boston, Mass.) monoclonal antibody

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(MAb) in ascites form at a dilution of 1:200 and a 1:8 dilution of rabbit complement (Pel-Freeze, Brown Deer, Wis.).

In vitro infection of bone marrow cell cultures with SIV_{mac}. The SIV_{mac} used in these experiments was a cell-free supernatant from a culture of isolate 251 maintained in lectin-activated normal human peripheral blood lymphocytes (PBLs) (17). Bone marrow cells were placed in culture medium at a concentration of 10^6/ml. After 7 days of culture, nonadherent cells were removed and the adherent cells were washed with medium two times. Adherent cells were then incubated for 2 h with a 1:20 dilution of SIV_{mac} containing culture supernatant. The medium was changed every 3 to 4 days, and harvested culture supernatant was saved.

Bone marrow cell cultures for colony assessment. To establish an adherent cell population, 2×10^7 nucleated bone marrow cells were placed into 25-cm^2 flasks containing 10 ml of culture medium. After 7 days of culture, nonadherent cells were removed. Adherent cells were then washed with medium and incubated for 2 h with a 1:20 dilution of SIV_{mac} containing culture supernatant. Freshly isolated bone marrow cells from the same monkeys were then added to these cultures in 10 ml of medium at a concentration of 10^6 cells per ml. At 3- to 4-day intervals, 5 ml of the medium was removed from each flask and replaced with fresh culture medium. Nonadherent cells were removed and assayed for colony formation.

Hematopoietic progenitor cell colony formation assays. A two-layer culture was established for the quantitation of granulocyte/macrophage progenitor (CFU/GM) colonies from bone marrow cells. The underlayer of 0.5% Noble agar contained 60 ng of recombinant human granulocyte/macrophage colony-stimulating factor (rGM-CSF) per ml (kindly provided by Genetics Institute, Inc., Cambridge, Mass.). The overlayer contained 10^5 bone marrow cells in IMDM supplemented with 12.5% FBS–12.5% horse serum in 0.3% Noble agar. It was layered onto the underlayer in Lux tissue culture dishes (35 by 10 mm) (Nunc, Inc., Naperville, Ill.), and the cultures were maintained at 37°C in a 5% CO_2 humidified atmosphere.

Erythrocyte progenitor (BFU-E) colonies were assessed in cultures of 10^5 bone marrow cells maintained in 0.9% (wt/vol) methylcellulose (Dow Chemical Co., Midland, Mich.) in IMDM supplemented with 30% FBS–0.9% deionized bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, Mo.)–5×10^{-5} M 2-mercaptoethanol–60 ng of rGM-CSF per ml–1 U of sheep erythropoietin (Step III, Connaught Laboratory, Willowdale, Ontario) per ml–5% phytohemagglutinin-stimulated rhesus monkey leukocyte conditioned medium.

CFU-GM and BFU-E colonies of greater than 50 cells were then counted under an inverted microscope 12 to 14 days after the cultures were established. In order to assess the effect of inhibiting SIV_{mac} replication on CFU-GM growth, anti-CD4 MAb (19Thy5D7; S. Schlossman) ascites or an MAb with an irrelevant specificity was added to the underlayer at the initiation of culture.

SIV_{mac} isolation from bone marrow cells of SIV_{mac}-infected monkeys. Bone marrow cells from SIV_{mac}-infected monkeys were placed in culture in two- or four-chamber Lab-Tek tissue culture slides (Nunc) at a cell concentration of 10^6/ml without the addition of lectin or cytokines. After 7 days of culture, nonadherent cells were removed and adherent cells were washed with medium. Medium was changed regularly, and supernatants were assayed for reverse transcriptase (RT) activity. In some experiments, anti-CD4 MAb ascites was continuously maintained in cultures at a final concentration of 1:100.

RT assay. RT activity in culture supernatants was measured as an indication of in vitro SIV_{mac} replication as previously described (15). Supernatants with an RT activity of five times background or greater were scored as positive for infection with SIV_{mac}.

Immunohistochemical analysis. Slides covered by adherent bone marrow cells from SIV_{mac}-infected rhesus monkeys were washed in phosphate-buffered saline and stored dessicated at −80°C until further processing. Before being stained, the sections were fixed in 2% paraformaldehyde (pH 7.2) for 10 min at 4°C. SIV_{mac} *gag* protein (p27) was detected in infected cells by using MAb R1C7 (kindly provided by M. Popovic and A. Minassian, National Cancer Institute, Bethesda, Md.) (19). This antibody, as well as MAbs which recognize CD2 (T11/3PT2H9; Coulter Immunology, Hialeah, Fla.), CD4 (NU-TH/I; kindly provided by M. M. Yokoyama and Y. Matsuo, Kurume University, Kurume, Japan), and a macrophage-specific antigen (EBM11; Dakopatts, Glostrup, Denmark), was used as previously described (23). Single antigen labeling was performed with the use of immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (Dakopatts) as previously described (6). Antigen was visualized by using a blue alkaline phosphatase substrate lot (Vector Laboratories, Burlingame, Calif.). The slides were counterstained with nuclear-fast red.

Electron microscopy. Adherent bone marrow cells from SIV_{mac}-infected monkeys were scraped from slides, fixed in half-strength Karnovsky fixative, washed in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and subsequently examined using a JEOL 100S transmission electron microscope.

RESULTS

SIV_{mac} replication and progenitor cell colony growth in bone marrow cells of SIV_{mac}-infected rhesus monkeys. As an initial approach to establishing the rhesus monkey SIV_{mac} system for studying the effects of AIDS virus infection on the bone marrow, bone marrow cells of four SIV_{mac}-infected monkeys were placed in culture without lectin stimulation, interleukin-2, or rGM-CSF. RT activities in the supernatants of these cultures were assessed as an indication of in vitro SIV_{mac} replication. As shown in Fig. 1, RT activity increased above baseline values by 4 weeks after initiation of the cultures and either persisted or continued to increase over the ensuing weeks. This RT activity did not reflect virus replication in contaminating peripheral blood T lymphocytes since treatment of these bone marrow cultures with an anti-CD6 antibody and complement did not diminish the in vitro generation of RT activity (see below). Thus, SIV_{mac} was present in bone marrow samples of these infected animals and replicated in the bone marrow cells in vitro.

To assess the functional ramifications of the SIV_{mac} infection of the bone marrow in monkeys, both CFU-GM and BFU-E progenitor colonies were assessed in the bone marrow cells. Bone marrow cells of five of eight SIV_{mac}-infected monkeys demonstrated significantly decreased numbers of CFU-GM colonies compared with bone marrow cells of healthy control animals (Table 1). A coincident decrease in the number of BFU-E colonies was also observed in the bone marrow cells of these animals. Therefore, as seen in bone marrow of the majority of humans infected with HIV type 1 (4, 16), bone marrow of many SIV_{mac}-infected mon-

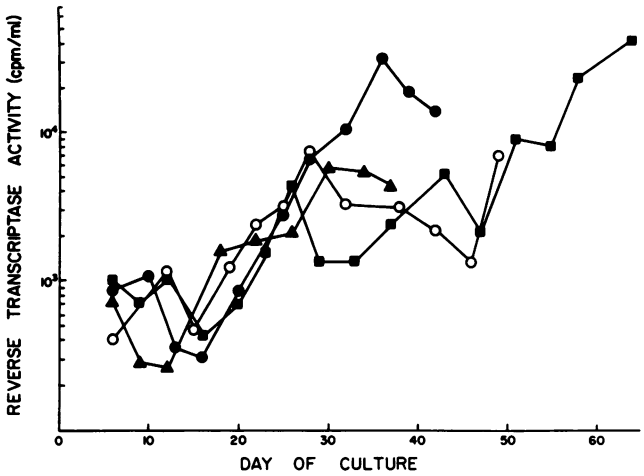

FIG. 1. SIV_{mac} replicated in bone marrow cells of SIV_{mac}-infected rhesus monkeys. Bone marrow cells of four SIV_{mac}-infected monkeys were placed in culture without lectin stimulation or cytokines. After 7 days of culture, nonadherent cells were removed. RT activity in the supernatants of these cultures was assessed as a measure of SIV_{mac} replication.

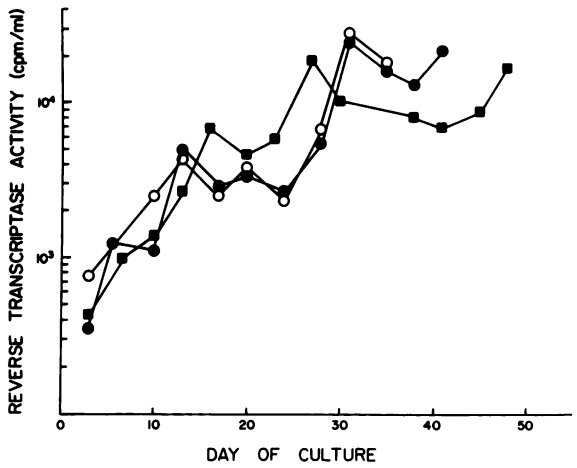

FIG. 2. SIV_{mac} replicated in bone marrow cells of normal rhesus monkeys after in vitro SIV_{mac} infection. Bone marrow cells of three normal rhesus monkeys were placed in culture. After 7 days of culture, adherent cells were infected with SIV_{mac}. Supernatants were harvested, and RT activity was measured.

keys also exhibits decreased CFU-GM and BFU-E colony growth in vitro.

SIV_{mac} replication and progenitor cell colony growth in bone marrow cells of normal rhesus monkeys after in vitro infection with SIV_{mac}. To determine whether these sequelae of the SIV_{mac} infection in the bone marrow represent a direct consequence of viral infection, SIV_{mac} replication and its ramifications were assessed in normal bone marrow cells after in vitro SIV_{mac} infection. As shown in Fig. 2, virus replication was detectable by approximately 2 weeks after in vitro infection of normal bone marrow cells with SIV_{mac}. An increase in RT activity in the culture supernatants occurred over the ensuing weeks. CFU-GM and BFU-E colonies were also quantitated in these SIV_{mac}-infected bone marrow cultures. Although normal bone marrow cells infected in vitro with SIV_{mac} maintained normal viability (data not shown), they generated decreased numbers of CFU-GM and BFU-E colonies (Table 2). These results suggest that SIV_{mac} infection may be involved in the decreased colony formation generated by bone marrow cells of SIV_{mac}-infected monkeys.

Macrophages, not T lymphocytes, harbor replicating SIV_{mac} in bone marrow cultures. We then sought to determine which bone marrow-derived cell harbors replicating SIV_{mac} under these in vitro conditions. Bone marrow cells were isolated from two SIV_{mac}-infected rhesus monkeys and placed in culture without interleukin-2 or GM-CSF. These cells were treated with complement alone or with an anti-CD6 MAb and complement to eliminate any contaminating PBLs or bone marrow T lymphocytes. The anti-CD6 and complement-treated cells consisted of <3.5% CD2+ or CD6+ cells. At 1 month after the initiation of these cultures, RT activity was measurable in the supernatants of both the complement alone and the anti-CD6 and complement-treated bone marrow cell cultures (Fig. 3). There was no quantitative difference in the RT activity measured in the supernatants of these cultures. Thus, there was no evidence that virus replication in these bone marrow cultures represented replication in bone marrow T lymphocytes. Similarly, as

TABLE 1. SIV_{mac} replication and number of colony-forming cells from bone marrow of rhesus monkeys chronically infected with SIV_{mac}

Rhesus monkey	SIV_{mac} isolation[a]	No. of colonies[b]	
		CFU-GM/ 5×10^4 cells	BFU-E/ 2×10^5 cells
Normal healthy (n = 10)		222 ± 16	76 ± 4
Chronically infected with SIV_{mac}			
Mm355-78	++	24	20
Mm156-85	++	42	8
Mm202-84	+	46	38
Mm167-84	+	48	16
Mm159-86	++	114	ND[c]
Mm318-70	−	216	90
Mm445-82	−	213	ND
Mm434-82	−	308	64

[a] SIV_{mac} isolations were performed on adherent bone marrow cell populations, assessing RT activity in the supernatants of bone marrow cultures. Results are shown as follows: −, <1,000 cpm/ml; +, 1,000 to 5,000 cpm/ml; ++, >5,000 cpm/ml.

[b] Number of colonies shown is the mean of duplicate cultures.

[c] ND, Not done.

TABLE 2. Decreased number of colony-forming cells from bone marrow of normal rhesus monkeys after SIV_{mac} infection in vitro

Bone marrow cells	No. of colonies[a]			
	CFU-GM/5×10^4 cells		BFU-E/2×10^5 cells	
	Day 7	Day 14	Day 7	Day 14
Uninfected (n = 4)	137 ± 13	155 ± 14	35 ± 6	48 ± 8
SIV_{mac} infected (n = 4)	49 ± 16[b]	62 ± 6	10 ± 2	2 ± 2

[a] Cultures of bone marrow cells from normal rhesus monkeys were infected in vitro with SIV_{mac}. At 7 and 14 days after infection, CFU-GM and BFU-E were assessed in these cells. Results were expressed as mean ± standard error.

[b] $P < 0.001$ compared with uninfected groups (paired, two-tailed Student's t test).

TABLE 3. Effect of T-lymphocyte depletion of bone marrow cells on the number of colony-forming cells after SIV_{mac} infection in vitro

Bone marrow cells		No. of colonies[a]	
Treatment	Infection	CFU-GM/ 5×10^4 cells	BFU-E/ 2×10^5 cells
Untreated	Uninfected	129	56
	SIV_{mac} infected[b]	68	0
T lymphocyte depleted[c]	Uninfected	147	56
	SIV_{mac} infected	67	5

[a] Results are expressed as mean of duplicate cultures counted on day 14 of culture.
[b] Virus isolated from untreated and T-lymphocyte-depleted bone marrow cultures had peak RT activities of 4,672 and 5,648 cpm/ml, respectively.
[c] Bone marrow cells from a normal rhesus monkey were depleted of T lymphocytes by the technique of complement-dependent lysis, using an anti-CD6 MAb.

shown in Table 3, SIV_{mac} replicated with equal efficiency in untreated and CD6+ lymphocyte-depleted normal bone marrow cell cultures. Moreover, colony formation in the T-lymphocyte-depleted SIV_{mac}-infected bone marrow cells was decreased to the same degree as that seen in the unfractionated SIV_{mac}-infected bone marrow cells. These studies suggested that virus in these bone marrow cell cultures was not replicating in T lymphocytes.

Immunohistochemical studies were then performed on cultured bone marrow cells of SIV_{mac}-infected monkeys to ascertain the cell population which harbors replicating virus. No staining for SIV_{mac} antigen expression was detected on bone marrow cells after 7 days in culture. Such staining was detected on cells from some animals by 14 days and on cells from all animals by 21 days after initiation of culture. By 40 days in culture, less than 5% of the cells in these cultures expressed the T-lymphocyte-specific molecule CD2 (Fig. 4A). Greater than 90% of the large, round nonstromal cells expressed the EBM11 surface molecule, indicating that they were of macrophage lineage (Fig. 4B). At least 60% of these macrophages expressed the CD4 surface molecule, although CD4 was only weakly detected on many of the macrophages (not shown). Greater than 80% of the macrophages demonstrated SIV_{mac} core antigen cytoplasmic and membrane staining (Fig. 4C). Electron microscopic studies confirmed the presence of budding lentivirus particles in macrophages

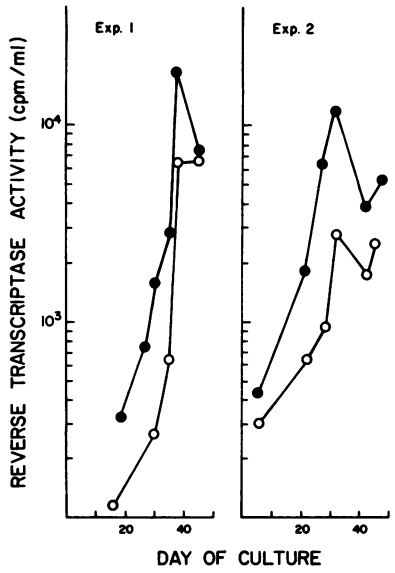

FIG. 3. SIV_{mac} replicated with equal efficiency in bone marrow cells of SIV_{mac}-infected rhesus monkeys treated with complement alone (○) and with anti-CD6+ complement (●).

(Fig. 5). Syncytia of SIV_{mac}-infected macrophages were not seen. These studies, in concert with the T-lymphocyte-depletion experiments, indicated that SIV_{mac} replication in bone marrow cells reflects virus replication in macrophages.

Progenitor cell colony growth is correlated with SIV_{mac} load in bone marrow of rhesus monkeys. The rhesus monkey SIV_{mac} system was then utilized to assess the infection of bone marrow macrophages and its sequelae early in the clinical course of an AIDS virus infection. Four normal rhesus monkeys were inoculated with SIV_{mac}, and their bone marrows were assessed on a regular schedule for virus isolation and colony formation. As shown in Table 4, virus was readily isolated in large quantities from the bone marrow of monkeys Mm129-86 and Mm244-86 as early as 2 weeks after virus inoculation and on every subsequent isolation attempt. Both CFU-GM and BFU-E colonies were decreased in number in studies of bone marrow cells done after virus inoculation of these two monkeys. In contrast, SIV_{mac} was isolated only sporadically and, when isolated, in smaller quantities from monkeys Mm161-86 and Mm179-86. Mm161-

TABLE 4. Virus isolations and numbers of colony-forming cells from bone marrow of rhesus monkeys after inoculation with SIV_{mac}

Week after inoculation with SIV_{mac}	Mm129-86			Mm161-86			Mm179-86			Mm244-86		
	SIV_{mac} isolation[a]	No. of colonies[b]		SIV_{mac} isolation	No. of colonies		SIV_{mac} isolation	No. of colonies		SIV_{mac} isolation	No. of colonies	
		CFU-GM	BFU-E		CFU-GM	BFU-E		CFU-GM	BFU-E		CFU-GM	BFU-E
Preinoculation		224	82		167	66		341	100		201	86
2	++	167	102	−	165	110	−	321	84	++	144	62
4	++	238	118	−	266	ND	−	286	ND	++	214	ND[c]
14	++	184	66	+	266	60	+	344	102	ND	62	12
18	+	180	92	−	190	98	+	310	54	+	126	20
22	++	161	6	+	247	50	+	315	72	+	167	24
27	++	102	ND	Monkey died			ND	ND	ND	+	104	ND
31	++	108	24				+	240	ND	++	86	20

[a] SIV_{mac} isolations were performed on adherent bone marrow cell populations with RT activity assessed on culture supernatants. Results are shown as follows: −, <1,000 cpm/ml; +, 1,000 to 5,000 cpm/ml; ++, >5,000 cpm/ml.
[b] CFU-GM and BFU-E colony counts shown are the means of duplicate cultures per 5×10^4 and 2×10^5 cultured bone marrow cells, respectively.
[c] ND, Not done.

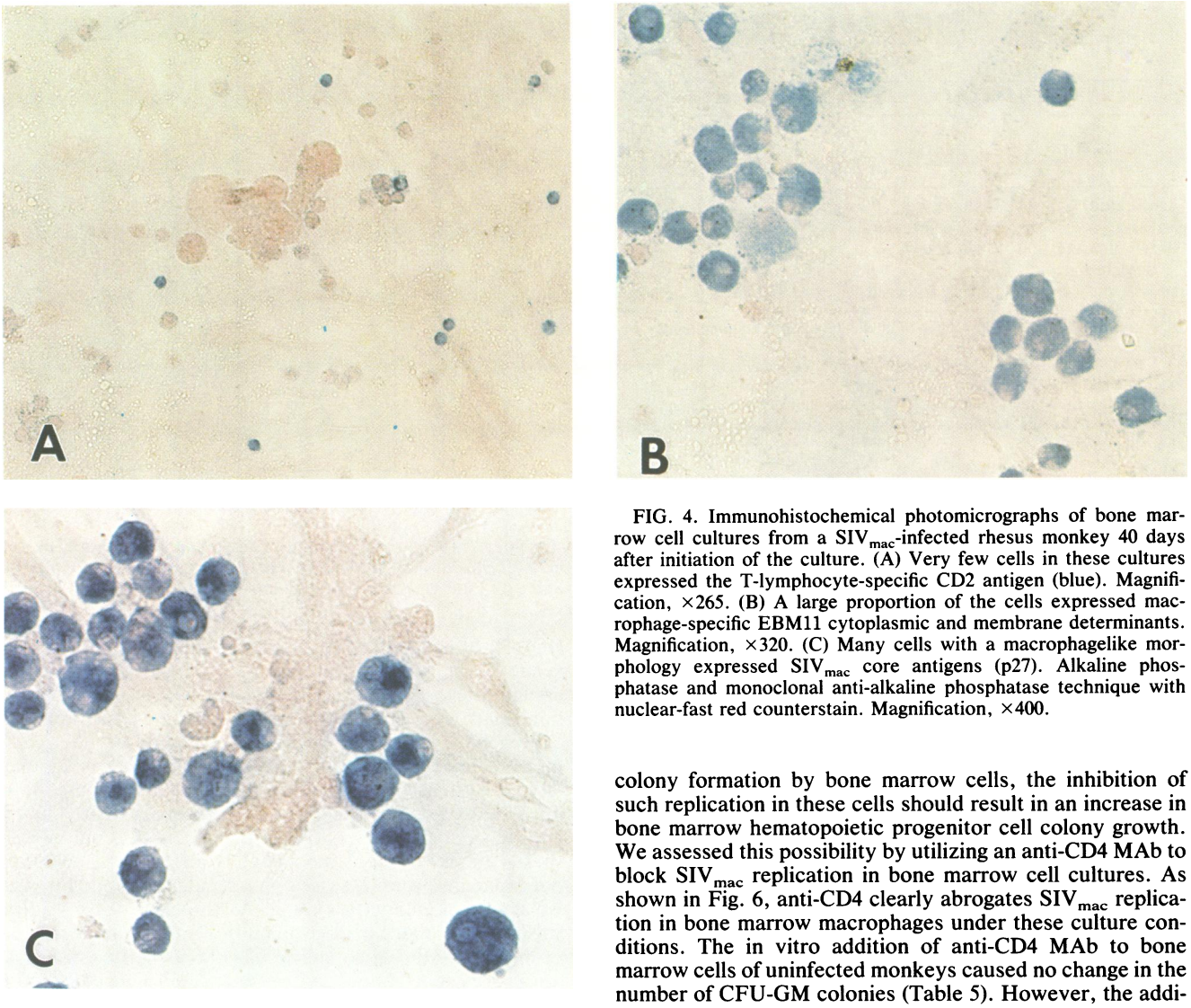

FIG. 4. Immunohistochemical photomicrographs of bone marrow cell cultures from a SIV_{mac}-infected rhesus monkey 40 days after initiation of the culture. (A) Very few cells in these cultures expressed the T-lymphocyte-specific CD2 antigen (blue). Magnification, ×265. (B) A large proportion of the cells expressed macrophage-specific EBM11 cytoplasmic and membrane determinants. Magnification, ×320. (C) Many cells with a macrophagelike morphology expressed SIV_{mac} core antigens (p27). Alkaline phosphatase and monoclonal anti-alkaline phosphatase technique with nuclear-fast red counterstain. Magnification, ×400.

86 died 22 weeks after virus inoculation, never showing a significant decrease in the number of bone marrow CFU-GM or BFU-E colonies. Bone marrow of Mm179-86 also showed no significant decrease in the numbers of progenitor cell colonies formed. These studies suggest a possible correlation between virus load in bone marrow cell populations and a decrease in the generation of hematopoietic progenitor cell colonies.

A possible correlation between SIV_{mac} load in bone marrow cells and hematopoietic progenitor cell colony growth was also explored in a study of eight monkeys chronically infected with SIV_{mac}. SIV_{mac} was readily isolated from bone marrow cells of five of these eight animals (Table 1). Bone marrow from the same five monkeys exhibited decreased progenitor cell colony growth. Conversely, bone marrow cells from the three animals which did not yield virus on in vitro cultivation also demonstrated normal progenitor cell colony growth. These findings further strengthen the correlation between bone marrow virus load and progenitor cell colony growth.

Inhibition of SIV_{mac} replication augments CFU-GM colonies in bone marrow cells. If SIV_{mac} replication in bone marrow macrophages were responsible for the inhibition of colony formation by bone marrow cells, the inhibition of such replication in these cells should result in an increase in bone marrow hematopoietic progenitor cell colony growth. We assessed this possibility by utilizing an anti-CD4 MAb to block SIV_{mac} replication in bone marrow cell cultures. As shown in Fig. 6, anti-CD4 clearly abrogates SIV_{mac} replication in bone marrow macrophages under these culture conditions. The in vitro addition of anti-CD4 MAb to bone marrow cells of uninfected monkeys caused no change in the number of CFU-GM colonies (Table 5). However, the addition of anti-CD4 antibody to similar cultures of bone marrow cells of SIV_{mac}-infected monkeys resulted in a marked increase in CFU-GM colonies (Table 5). Similarly, in analogous experiments, an anti-CD4 but not a control MAb augmented CFU-GM in vitro in bone marrow cells of SIV_{mac}-infected monkeys (data not shown). These studies demonstrated that SIV_{mac} replication in bone marrow cells causes an inhibition of CFU-GM colonies in those cells.

DISCUSSION

Accruing evidence has implicated the macrophage as an important reservoir for HIV and SIV_{mac} in the infected host (6, 11–13, 18, 20, 24, 25). Techniques have been developed for the cultivation of these lentiviruses in a variety of macrophage populations, including peripheral blood monocytes and alveolar macrophages. We now demonstrate an approach for maintaining replicating AIDS viruses in bone marrow macrophages for greater than 2 months in the absence of lectins or cytokines. This simple method provides a powerful means of isolating virus from a readily sampled macrophage source from individuals infected with the AIDS virus. This in vitro technique also facilitates the study not only of the effects of virus infection on the macrophage but

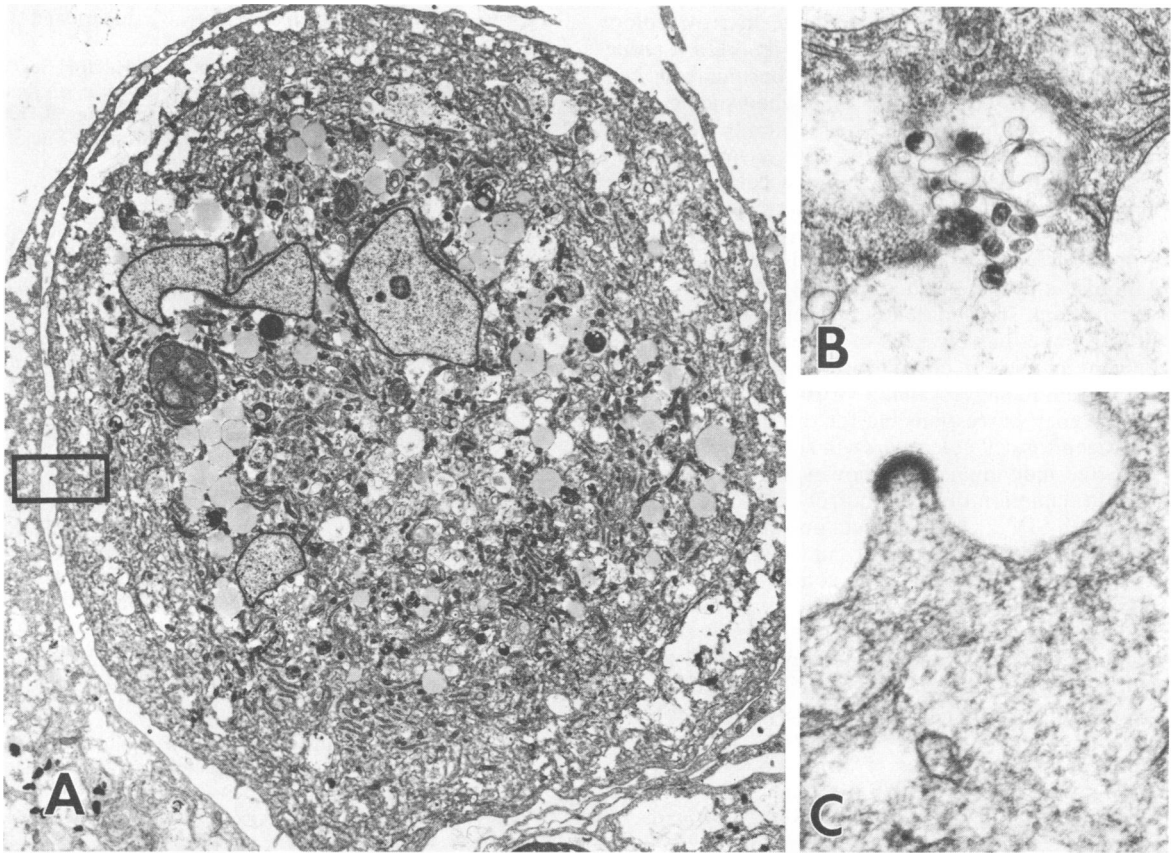

FIG. 5. Transmission electron photomicrographs of bone marrow cells from a SIV_{mac}-infected rhesus monkey after 43 days in culture. (A) A bone marrow macrophage contains cytoplasmic lipid droplets, small phagocytic vacuoles, electron-dense granules, and swollen rough endoplasmic reticulum. A solitary SIV budding particle is present (enclosed; enlarged in panel C). (B) In other bone marrow macrophages, mature SIV particles are found within cytoplasmic vacuoles. Uranyl acetate and lead citrate. Magnifications: panel A, ×3,600; panel B, ×25,000; panel C, ×75,000.

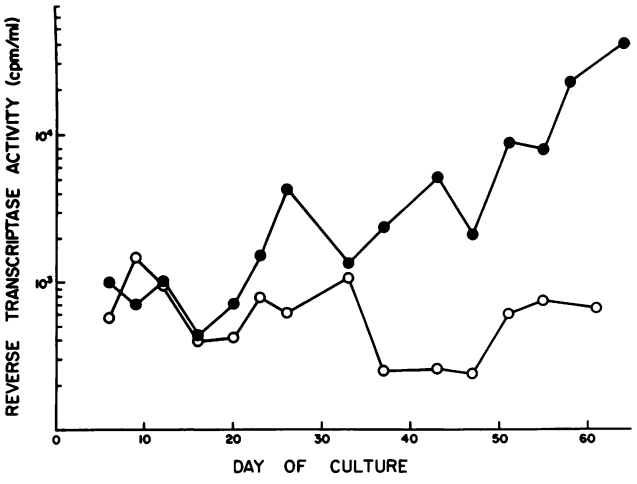

FIG. 6. Anti-CD4 MAb blocked SIV_{mac} replication in bone marrow cells of a SIV_{mac}-infected rhesus monkey. Anti-CD4 MAb ascites at a final concentration of 1:100 was added to a bone marrow culture established from a SIV_{mac}-infected rhesus monkey. Bone marrow cell cultures were maintained in the absence of MAb (●) or in the presence of anti-CD4 MAb (○).

of the effects of infected macrophages on cells of other lineages and the therapeutic efficacy of antiviral agents against infected macrophages.

A number of studies have demonstrated that hematopoietic progenitor cell colony growth in cells from bone marrow of individuals infected with HIV is decreased, perhaps explaining the cytopenias which occur in the setting of AIDS

TABLE 5. Increase in CFU-GM in bone marrow cells from SIV_{mac}-infected monkeys after in vitro addition of anti-CD4 antibody

Disease status and monkey no.	CFU-GM/5 × 10^4 cells[a] after MAb		
	Control	Anti-CD4 at 1:100 titer	Anti-CD4 at 1:1,000 titer
SIV_{mac} infected			
Mm129-86	108 (1.0)[b]	153 (1.42)	126 (1.17)
Mm244-86	86 (1.0)	146 (1.70)	94 (1.09)
Mm156-85	99 (1.0)	179 (1.81)	105 (1.06)
Uninfected			
Mm120-84	225 (1.0)	228 (1.01)	231 (1.03)
Mm122-84	172 (1.0)	170 (0.99)	173 (1.01)
Mm220-79	234 (1.0)	247 (1.06)	227 (0.97)

[a] Number of colonies shown is the mean of duplicate cultures.

[b] The number in parentheses is the ratio of the CFU-GM number generated in the presence of anti-CD4 MAb divided by the CFU-GM number generated in the absence of an MAb.

(4, 16). The etiology of this reduced bone marrow colony growth has been unclear. Donahue et al. reported a reduction in colony formation in cultures of bone marrow cells from individuals infected with HIV only when such cultures were maintained in the presence of serum from patients containing anti-gp120 antibody (9). These investigators, however, rigorously depleted bone marrow cells of adherent macrophages before initiating colony assays. In the present studies, progenitor cell colony formation was decreased in washed, unfractionated bone marrow cells of many SIV_{mac}-infected monkeys in the absence of monkey sera.

Carlo-Stella et al. showed that depletion of T lymphocytes from cultured bone marrow cells of patients with AIDS led to a significant increase in colony formation from those cells (4). This led them to suggest that a virus-induced T-lymphocyte response may be responsible for the decreased hematopoietic progenitor cell colony growth seen in bone marrow of HIV-infected individuals. We, however, have found that T-lymphocyte depletion of bone marrow cells before infection in vitro with SIV_{mac} had no effect on subsequent colony formation. Moreover, analysis of an SIV_{mac}-specific T-lymphocyte response in the same cohort of four rhesus monkeys studied prospectively in these experiments provides no evidence for a correlation between the presence of virus-specific T cells and hematopoietic progenitor cell colony growth. While an SIV_{mac} *gag*-specific cytolytic T lymphocyte was readily demonstrated in PBLs of Mm179-86 and Mm244-86 (data not shown), bone marrow colonies were decreased in number in studies of Mm244-85 but remained normal in number in Mm179-86. Although Mm129-86 and Mm161-86 never developed measurable SIV_{mac} *gag*-specific T-cell responses (data not shown), bone marrow colony numbers decreased in studies of Mm129-86 but remained normal in Mm161-86.

The results of the current studies are certainly consistent with the possibility that the bone marrow stem cell or both early myelocyte and erythrocyte progenitor cells might be infected with SIV_{mac}, resulting in depressed hematopoietic progenitor cell colony formation (10). However, the present studies suggest that SIV_{mac} infection of the progenitor cells could not alone be responsible for inducing a depression in bone marrow cell colony formation. The introduction of an anti-CD4 MAb into the bone marrow cells of SIV_{mac}-infected monkeys augments colony formation, presumably through an inhibition of SIV_{mac} replication in a CD4+ cell population. Since CD4 is expressed on bone marrow macrophages but not on the stem cell or committed progenitor cells, virus replication in bone marrow macrophages must play a part in the depression of bone marrow colony growth.

The suppression of colony growth may be mediated by an already characterized factor such as tumor necrosis factor (3) or a factor such as that described by Leiderman et al. (16). By whatever mechanism, the exquisite sensitivity of this inhibition to the addition of anti-CD4 MAb suggests that the effect is rapidly reversible. This finding suggests that antiviral agents which decrease lentivirus load in bone marrow cells may induce a rapid improvement in hematopoietic function in individuals with AIDS. Such a therapeutic response was seen in recombinant soluble CD4-treated SIV_{mac}-infected rhesus monkeys (31).

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