Productive and Lytic Infection of Human CD4⁺ Type 1 Helper T Cells with Macrophage-Tropic Human Immunodeficiency Virus Type 1

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It is generally recognized that macrophage-tropic human immunodeficiency virus type 1 (HIV-1) is the predominant population during the acute and asymptomatic phases of HIV-1 infection. Here, we compared the proliferation and syncytium-inducing activities of different HIV-1 strains in primary CD4⁺ T cells expressing various helper T (Th)-type cytokine profiles. The macrophage-tropic HIV-1 strains HIV-1_{JR-CSF}, HIV-1_{NFN-SX}, and HIV-1_{SF162} could proliferate vigorously and generate syncytia in primary CD4⁺ T cells irrespective of their Th subtype, in contrast to the T-cell-line-tropic HIV-1 strains HIV-1_{NL4-3} and HIV-1_{IIIB}, which favored non-type 1 Th conditions. These results indicate that macrophage-tropic HIV-1 may be more invasive and virulent, since it kills more CD4⁺ Th1 cells than T-cell-line-tropic HIV-1 during the early stages of HIV-1 infection, when the Th1 immune response is dominant.

Human immunodeficiency virus type 1 (HIV-1) causes defects in T-helper (Th) immune function, which leads ultimately to AIDS, by infecting mainly CD4⁺ T cells and macrophages, both of which are essential for generating immune protection against pathogenic microorganisms in humans (7). HIV-1 isolates have been divided into two classes based upon their cell tropism: macrophage tropism or T-cell-line tropism (2, 3, 6). The macrophage-tropic HIV-1 class can proliferate efficiently in primary monocyte-derived macrophages as well as in primary CD4⁺ T cells but cannot proliferate in established CD4⁺ T-cell lines. In contrast, the T-cell-line-tropic HIV-1 class can proliferate in a wide range of T-cell lines as well as in primary CD4⁺ T cells but not in macrophages. Based upon the ability to generate syncytia in established CD4⁺ T-cell lines, T-cell-line-tropic and macrophage-tropic HIV-1 isolates have also been subdivided into a syncytium-inducing type and a non-syncytium-inducing type, respectively (20, 24). It is generally recognized that during the course of HIV-1 infection, infection is initiated by the macrophage-tropic HIV-1 strains, which persist as the predominant virus population in the acute phase as well as in the asymptomatic phase. As AIDS develops, the T-cell-line-tropic HIV-1 strains become dominant (24, 27). Thus, this shift from macrophage to T-cell-line tropism has been linked to depletion of CD4⁺ T cells and a progression to AIDS. Both groups of HIV-1 have been shown to cause killing of primary CD4⁺ T cells in vitro (26). However, the mechanisms of the shift and the shift's relationship to AIDS are not known.

It has been shown that human CD4⁺ Th cells are heterogeneous in their profiles of cytokine production, designated Th1, Th2, and Th0 (15, 21, 23). Th1 cells produce gamma interferon (IFN- γ), interleukin 2 (IL-2), and tumor necrosis factor beta (TNF- β) but not IL-4 or IL-5 and are principally involved in promoting cellular immunity. Th2 cells produce IL-4 and IL-5

but not IFN- γ , IL-2, or TNF- β and promote humoral immunity. Th0 cells produce both Th1- and Th2-type cytokines. The development of Th1- and Th2-like immune responses is promoted by IL-12 and IL-4, respectively (21). In HIV-1 infection, the Th1-like immune responses have been implicated in maintaining an asymptomatic status in infected individuals (9, 19). Based on comparative experiments using specimens from over 100 HIV-1 infected patients, it has been proposed that a switch from the Th1 to the Th2 cytokine profile occurs during the course of HIV-1 infection and that this is a critical step in the progression of HIV-1 infection (4, 5). Recently, however, two groups of investigators have argued that such a switch in HIV-1-infected individuals does not occur at the clonal CD4⁺ T-cell level; instead, they have suggested that a shift from Th1 to Th0 occurs during the course of infection, leading to AIDS (8, 13).

To better understand HIV-1 pathogenesis, it is necessary to study the proliferation abilities of the two types of HIV-1 in CD4⁺ T cells expressing different Th-type cytokine profiles. To date, it has been reported that HIV-1 proliferates preferentially in Th2- and Th0-type cell clones but not in Th1-type cell clones (13, 25). However, these observations have been made with T-cell-line-tropic HIV-1 strains exclusively. Therefore, the proliferation potential of macrophage-tropic HIV-1 strains in CD4⁺ T cells of Th1 or non-Th1 type has not been determined. In this study, we have compared the proliferation activities of several macrophage- and T-cell-line-tropic HIV-1 strains in activated bulk and clonal CD4⁺ T cells of various Th subtypes. We report here that the macrophage-tropic HIV-1 strains can proliferate vigorously and generate syncytia in primary CD4⁺ T cells irrespective of their Th subtype, in contrast to the T-cell-line-tropic HIV-1 strains, which favor non-Th1 conditions.

MATERIALS AND METHODS

Reagents. RPMI 1640 medium supplemented with 12% fetal calf serum, 100 U of penicillin per ml, and 100 μ g of streptomycin per ml was used (hereinafter called RPMI medium). Recombinant IL-12 (rIL-12) and rIL-2 were kindly provided by the Genetics Institute, Inc. (Cambridge, Mass.), and Shionogi Pharma-

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ceutical Inc. (Osaka, Japan), respectively. rIL-4 was purchased from Biosource (Camarillo, Calif.), rIL-12, rIL-4, and rIL-2 were used at 100 U/ml. Anti-human CD3 monoclonal antibody (MAb) (OKT-3) was purchased from the American Type Culture Collection (Rockville, Md.).

Generation of polarized CD4⁺ bulk and clonal T cells. Peripheral blood lymphocytes (PBL) from normal donors were isolated by Ficoll-Hypaque gradient centrifugation. CD4⁺ T cells were purified from nonadherent PBL with immunomagnetic M-450 CD4 beads and M-450 CD4 DETACHaBEADS (DynaL Inc., Oslo, Norway) as indicated by the manufacturer. Purity of CD4⁺ T cells was more than 98% as analyzed by flow cytometry. These CD4⁺ T cells were cultured in 12-well culture plates (model no. 430345; Corning) coated with anti-CD3 MAb at a cell concentration of 10⁶ cells per ml in 100 U of IL-2-containing RPMI medium per ml in the presence of 100 U of IL-12 or IL-4 per ml. On days 3 and 6 of culture, these cells were split into halves in anti-CD3 MAb-coated wells and an equal volume of the fresh medium containing IL-2 and IL-12 or IL-4 was added to the cultures. Various CD4⁺ T-cell clones were generated from normal donors by stimulation with allogeneic (Raji) cells, 5 µg of anti-CD3 MAb per ml, or 10 µg of phytohemagglutinin P (PHA-P; Sigma) per ml by a limiting dilution (0.5 cell per well) by a method described by Manetti et al. (14). The Th types of bulk and clonal T cells were determined by assaying their culture supernatants for IFN-γ, IL-4, and IL-5 after stimulation of 5 × 10⁵ responder cells per ml with each stimulant for 3 days in IL-2-containing RPMI medium with enzyme-linked immunosorbent assay (ELISA) kits for IFN-γ and IL-4 (Biosource) and for IL-5 (R&D Systems, Inc., Minneapolis, Minn.).

Virus preparation. HIV-1_{NL4-3} (1), HIV-1_{JR-CSF} (12), and HIV-1_{NFN-SX} (16) were prepared by transfection of molecularly cloned viral DNA into COS cells. HIV-1_{SF162} (22) was prepared by infection of PHA-activated PBL, and HIV-1_{IIIB} was prepared from Molt-4 cells persistently infected with HIV-1_{IIIB} (18). Virus infectious titers (50% infective doses) of these culture supernatants were titrated on PHA-activated PBL.

Viral infection and p24 assay. CD4⁺ T cells (10⁶ cells), washed twice with RPMI medium, were resuspended in 1 ml of RPMI medium containing a 5 × 10³ 50% infective dose of HIV-1. After incubation at 37°C for 2 h, the cells were washed extensively and cultured in IL-2-containing RPMI medium at 5 × 10⁵ cells/ml in the 12-well plates. When these cells became confluent, they were split into halves and an equal volume of fresh medium was added. Virus proliferation was monitored by quantitating HIV-1 *gag* p24 produced in the culture supernatants with an ELISA kit (Coulter).

RESULTS AND DISCUSSION

Different proliferation potentials between HIV-1_{JR-CSF} and HIV-1_{NL4-3} in Th1-dominant bulk CD4⁺ T cells. IL-12 and IL-4 have been shown to be potent regulators of differentiation of naive CD4⁺ T cells into Th1 and Th2 cells, respectively (21). In order to generate Th1-like and control Th2-like bulk CD4⁺ T-cell cultures from normal donors, we purified CD4⁺ T cells from PBL and stimulated them with immobilized anti-CD3 MAb in the presence of IL-12 or IL-4. After three successive stimulations over 8 days, the IL-12-pretreated CD4⁺ T cells produced large amounts of IFN-γ (up to 200 ng/ml) and a small amount of IL-5 (less than 20 pg/ml). In contrast, the control IL-4-pretreated CD4⁺ T cells produced lower levels of IFN-γ (less than 8 ng/ml) but higher levels of IL-5 (more than 500 pg/ml). Although the IL-4 levels in the two cultures were low (less than 40 pg/ml), these distinct cytokine production patterns indicated that the IL-12-pretreated CD4⁺ T cells were Th1 dominant and that the control IL-4-pretreated CD4⁺ T cells were relatively Th2-like. Because IFN-γ has been suggested to diminish the production of IL-4 and inhibit proliferation of cells of the IL-4-producing phenotype (21), one of the reasons for low levels of IL-4 production in the Th2-like culture may have been the inhibitory effect of IFN-γ produced in the same culture from memory Th cells in the primary CD4⁺ T-cell populations. Unlike the cases for CD4⁺ T-cell clones, it was difficult to sharply discriminate the IL-4-pretreated CD4⁺ T cells as having the Th2 type. However, these cell populations can be used as controls for the Th1-dominant cell population, and we tentatively designate the phenotype of these IL-4-treated cells as Th2-like in this study.

These two distinct CD4⁺ T-cell populations were then infected with equal infectious units, with a multiplicity of infection (MOI) of 0.005, of two molecularly cloned representative

HIV-1 strains: macrophage-tropic HIV-1_{JR-CSF} and T-cell-line-tropic HIV-1_{NL4-3}. HIV-1 proliferation and cell growth were monitored for 2 weeks. Figure 1A shows that HIV-1_{JR-CSF} proliferated in both the Th1-dominant and the control Th2-like CD4⁺ T-cell cultures. This proliferation pattern was clearly different from that of HIV-1_{NL4-3}, of which proliferation in the Th1-dominant CD4⁺ T cells was about 100-fold less than that in the control Th2-like CD4⁺ T cells. The preference of T-cell-line-tropic HIV-1 for non-Th1 conditions has been observed previously (13, 25). However, this is the first evidence that macrophage-tropic HIV-1 can infect CD4⁺ T cells under both Th1-dominant and Th2-like conditions. To our surprise, the viable cell number of the Th1-dominant CD4⁺ T cells began to decrease 1 week after infection with HIV-1_{JR-CSF} but not with HIV-1_{NL4-3} (Fig. 1B), but the growth of the Th2-like CD4⁺ T cells was not significantly affected by infection with either virus type during the observation period. These observations suggest that macrophage-tropic HIV-1 is capable of inducing productive and lytic infection in Th1-dominant CD4⁺ T cells. It is interesting that there was no decrease in viability of Th2-like CD4⁺ T cells infected with either macrophage-tropic or T-cell-line-tropic HIV-1. One reason to explain the discrepancy may be differences in levels of production of other cytokines between these Th1-dominant and Th2-like CD4⁺ T-cell cultures. The Th1-dominant culture produced higher levels of TNF-α than the Th2-like culture (data not shown). Because TNF-α and IFN-γ have been shown to synergistically upregulate the Fas antigen and induce apoptosis of PBL (17), high levels of IFN-γ and TNF-α may be involved in the decrease in viable cells in the HIV-1-infected Th1-dominant CD4⁺ T-cell culture. Further studies are necessary to reveal the mechanisms.

An additional new finding of this study was that HIV-1_{JR-CSF} but not HIV-1_{NL4-3} generated syncytia in both types of T-cell cultures as the viral load increased. The numbers of syncytia were about 50 to 100 and 10 to 20 per 2 × 10⁶ Th1-dominant and Th2-like cells, respectively (data not shown). Together, these results indicate that macrophage-tropic HIV-1_{JR-CSF} can proliferate even in Th1-dominant CD4⁺ T cells and that proliferation of the T-cell-line-tropic HIV-1_{NL4-3} is limited in Th1-dominant CD4⁺ T cells.

Macrophage-tropic HIV-1 strains can proliferate in Th1 cells. Experiments were repeated with CD4⁺ T cells from two independent donors and three additional HIV-1 strains: macrophage-tropic HIV-1_{NFN-SX} and HIV-1_{SF162} and T-cell-line-tropic HIV-1_{IIIB}. Representative data on virus proliferation (Fig. 2) showed that all three macrophage-tropic HIV-1 strains could proliferate in both Th1-dominant and Th2-like bulk CD4⁺ T cells. However, proliferation of the two T-cell-line-tropic HIV-1 strains was limited in the Th1-dominant CD4⁺ T cells compared to that in Th2-like CD4⁺ T cells. As with the results shown in Fig. 1B, a decrease in the viable cell number in these cultures was observed in only the Th1-dominant CD4⁺ T cells infected with all of the macrophage-tropic HIV-1 strains over the first 2 weeks of infection (not shown). Again, in these experiments, we also observed that all the macrophage-tropic HIV-1 strains, but none of the T-cell-line-tropic HIV-1 strains, generated a number of syncytia in these bulk CD4⁺ T cells. Interestingly, both the sizes and numbers of syncytia generated by HIV-1_{NFN-SX} and HIV-1_{SF162} were larger than those generated by HIV-1_{JR-CSF}, as confirmed later with CD4⁺ Th1 clones.

Macrophage-tropic HIV-1 strains can proliferate in CD4⁺ Th1 clones. In order to further confirm the proliferation potential of macrophage-tropic HIV-1 in the Th1-dominant environment, we established a panel of CD4⁺ T-cell clones from

FIG. 1. Proliferation of macrophage-tropic HIV-1 in both Th1-dominant and Th2-like human CD4⁺ T cells. Human CD4⁺ bulk T cells were driven from nonadherent CD4⁺ T cells into Th1-dominant or control Th2-like stages by activation with immobilized anti-CD3 MAb three times (days 0, 3, and 6) in 100 U of rIL-2 per ml and 100 U of rIL-12 or rIL-4 per ml, respectively. On day 8 of cultivation, these cells were infected with either macrophage-tropic HIV-1_{JR-CSF} or T-cell-line-tropic HIV-1_{NL4-3} at an MOI of 0.005, and virus production and cell growth were monitored for 2 weeks. (A) Total HIV-1 p24 produced in culture supernatants; (B) total viable cell numbers in each culture. Values represent means of triplicate determinations.

three normal donors by stimulation of peripheral CD4⁺ T cells with allogeneic Raji cells, anti-CD3 MAb, or PHA. Their cytokine production patterns and permissiveness for HIV-1 are summarized in Table 1. Macrophage-tropic HIV-1 proliferated in most CD4⁺ T-cell clones in which T-cell-line-tropic HIV-1 proliferation was greatly suppressed.

Table 1 also shows that there was a difference in abilities to infect CD4⁺ T-cell clones among the macrophage-tropic HIV-1 strains (HIV-1_{NFN-SX} proliferated more aggressively than HIV-1_{JR-CSF}). Several explanations are possible. First, although HIV-1_{NFN-SX} and HIV-1_{JR-CSF} have the same amino acid sequence in the V3 region which determines cell tropism (22), differences in the envelope structures, other than those of the V3 regions, of the two HIV-1 strains may affect their infectivity (10). Second, some clones may produce inhibitory factors for HIV-1 and their inhibitory effects may be dependent on host cell Th type and HIV-1 strain. Since we confirmed that exogenously added IFN- γ and IL-4 exhibited only a partial inhibition of HIV-1_{JR-CSF} proliferation in the bulk Th1-dominant cell culture (data not shown), we assume that these cytokines may not be the major factors for the inhibition in

even clonal Th cultures. It remains to be determined what kinds of factors, other than IFN- γ and IL-4, were produced in these various CD4⁺ Th clones.

As mentioned above, it should be noted that all the macrophage-tropic HIV-1 strains tested generated obvious syncytia in the bulk and clonal CD4⁺ T cells (Fig. 3) but that neither HIV-1_{NL4-3} nor HIV-1_{IIB} possessed this capability even in Th2-like cultures. These results were consistently reproducible. The size and the number of syncytia varied depending upon the type of the Th culture and strain of macrophage-tropic HIV-1. Because these macrophage-tropic HIV-1 strains do not induce syncytia in the MT-2 cell line (11), an indicator cell line for syncytium induction, these HIV-1 strains have been classified as non-syncytium-inducing HIV-1. Our new findings therefore suggest a new classification of HIV-1 strains based on their syncytium-inducing ability in purified CD4⁺ Th1-dominant cell cultures or Th1 clones.

In conclusion, our study revealed that macrophage-tropic HIV-1 strains can proliferate in various types of CD4⁺ T cells expressing a Th1 phenotype, induce syncytia, and kill infected Th1 cells. This phenotype of the macrophage-tropic HIV-1

FIG. 2. Proliferation of macrophage-tropic HIV-1 in both Th1-dominant and Th2-like human CD4⁺ T cells from different donors. Bulk Th1-dominant and control CD4⁺ T cells obtained from two different donors and stimulated as stated in the legend to Fig. 1 were infected with two T-cell-line-tropic HIV-1 strains, HIV-1_{NL4-3} and HIV-1_{IIB}, and three macrophage-tropic HIV-1 strains, HIV-1_{JR-CSF}, HIV-1_{NFN-SX}, and HIV-1_{SF162} at an MOI of 0.005. Twelve days after infection, total p24 produced in the supernatants was quantitated.

strains is quite different from that of the T-cell-line-tropic HIV-1 strains, which favored non-Th1 conditions to proliferate (13, 25) and generated no syncytia under our experimental conditions. The broader cell tropism of the macrophage-tropic HIV-1 strains may be of great advantage for their proliferation

in vivo, as opposed to the limited cell tropism and proliferation abilities of T-cell-line-tropic HIV-1 strains. Therefore, macrophage-tropic HIV-1 may be more aggressive in spreading throughout the body and in killing CD4⁺ Th1 cells than T-cell-line-tropic HIV-1 during the early stages of HIV-1 infection,

TABLE 1. Macrophage-tropic HIV-1 proliferation in CD4⁺ Th1 clones^a

Donor	Clone	Stimulation	Phenotype	Production (ng/ml) of:		Clone type	p24 (ng/ml) produced by infection with:		
				IFN- γ	IL-4		HIV-1 _{NL4-3}	HIV-1 _{JR-CSF}	HIV-1 _{NFN-SX}
1	Ra-1	Raji cells	CD4 ⁺	1.3	0.05	1	<0.1	60.0	90.0
	Ra-2	Raji cells	CD4 ⁺	1.6	0.12	1	<0.1	0.9	12.0
	Ra-3	Raji cells	CD4 ⁺	2.0	8.8	0	<0.1	0.4	22.0
	Ra-4	Raji cells	CD4 ⁺	3.8	4.5	0	<0.1	10.0	40.0
	Ra-5	Raji cells	CD4 ⁺	2.1	2.0	0	<0.1	0.7	55.0
	Ra-6	Raji cells	CD4 ⁺	2.8	3.5	0	0.15	5.0	90.0
	Ra-7	Raji cells	CD4 ⁺	3.0	0.04	1	<0.1	40.0	150.0
	Ok-7	Anti-CD3 MAb	CD4 ⁺	3.2	0.1	1	<0.1	<0.1	<0.1
	Ok-8	Anti-CD3 MAb	CD4 ⁺	5.0	0.23	1	<0.1	55.0	120.0
	Ok-14	Anti-CD3 MAb	CD4 ⁺	5.5	0.78	1 or 0	<0.1	12.0	40.0
	Ok-10	Anti-CD3 MAb	CD4 ⁺ CD8 ⁺	5.5	0.11	1	<0.1	<0.1	<0.1
	Ok-18	Anti-CD3 MAb	CD4 ⁺ CD8 ⁺	50.0	3.5	1 or 0	<0.1	0.2	18.0
	Ph-111	PHA	CD4 ⁺	8.0	25.0	0	<0.1	42.0	150.0
	Ph-114	PHA	CD4 ⁺	3.8	0.01	1	0.6	20.0	80.0
	Ph-115	PHA	CD4 ⁺	5.0	10.0	0	1.0	5.0	140.0
	Ph-116	PHA	CD4 ⁺	3.1	3.6	0	<0.1	0.1	140.0
	Ph-218	PHA	CD4 ⁺	80.0	0.12	1	0.3	0.2	130.0
	Ph-113	PHA	CD8 ⁺	300.0	0.19	1	<0.1	<0.1	<0.1
	Bulk CD4 ⁺	Anti-CD3 MAb plus IL-12	CD4 ⁺	187.0	0.04	Bulk Th1	0.3	60.0	80.0
	Bulk CD4 ⁺	Anti-CD3 MAb plus IL-4	CD4 ⁺	4.7	0.03	Bulk Th2	26.0	43.0	200.0
2	Ok-301	Anti-CD3 MAb	CD4 ⁺	8.0	0.20	1	<0.1	62.0	125.0
	Ok-302	Anti-CD3 MAb	CD4 ⁺	7.5	0.78	1	<0.1	22.0	40.0
3	Ok-401	Anti-CD3 MAb	CD4 ⁺	19.2	<0.1	1	0.18	4.5	20.1
	Ok-402	Anti-CD3 MAb	CD4 ⁺	<0.1	9.0	2	1.2	2.5	11.0

^a Various CD4⁺ and CD8⁺ T-cell clones were generated from three normal donors by stimulation with allogeneic cells (Raji cells), anti-CD3 MAb, or T-cell lectin (PHA) by a limiting dilution (0.5 cell per well). Their subtypes were determined by assaying their culture supernatants after stimulation of 5×10^5 responder cells per ml with respective stimulants for 3 days for IFN- γ and IL-4. These T-cell clones and control bulk Th1-dominant and Th2-like CD4⁺ T cells were infected with each HIV-1 strain at an MOI of 0.005. Ten days after infection, p24 produced in the culture supernatants was quantitated by ELISA.

FIG. 3. Generation of syncytia in clonal CD4⁺ Th1 cells by infection with macrophage-tropic HIV-1. The clonal CD4⁺ Th1 cells (Ra-1 in Table 1) were infected with T-cell-line-tropic HIV-1_{NL4-3} and the macrophage-tropic strains HIV-1_{JR-CSF}, HIV-1_{NFN-SX}, and HIV-1_{SF162} at an MOI of 0.005 and then cultured for 10 days. Syncytia were examined with an inverted microscope at $\times 100$ magnification. Similar syncytium formation was also observed in bulk CD4⁺ T cells infected with the three macrophage-tropic HIV-1 strains (data not shown).

when the Th1 immune response is dominant (4, 5). It is possible that the unique proliferation potential of macrophage-tropic HIV-1 can well explain the peculiar observations that primary viremia is mainly caused by macrophage-tropic, but not T-cell-line-tropic, HIV-1 in infected individuals.

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