Generation of endogenous tumour necrosis factor-α in MOLT-4 cells during the acute replication phase of human immunodeficiency virus type 1 determines the subsequent latent infection

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We have characterized the mechanism for human immunodeficiency virus type 1 (HIV-1) latent infection in a human T cell line MOLT-4 subclone no. 8 (MOLT-#8). The inocula used were HIV-1 recovered from MT-4 during the acute (NL-A) and persistent (NL-P) phases after HIV-1 infection. On infection of MOLT-#8 with NL-A, viral antigens first appeared in almost 100% of the cells whereafter the numbers of viable antigen-positive cells declined. In contrast, following infection with NL-P the expression of viral antigens was maintained in almost 100% of the cells. In fact, limiting dilution of NL-P-infected cells allowed us to isolate 43 subclones, all of which were positive for viral antigen expression in almost 100% of the cells (type I). In sharp contrast, only two of 41 subclones from NL-A-infected cells were of type I. Seven subclones were latently infected with HIV-1; latent HIV-1 in six subclones (type II), but not in one type III subclone, was activated by tumour necrosis factor (TNF)-α or phorbol 12-myristate 13-acetate. The remaining subclones were negative for the viral genome. Of particular note is the effect of endogenous TNF-α generated during the acute phase of virus replication which shifted the virus phenotype. Thus, the presence of TNF-α during the acute phase of virus replication seems to play a key role in the selective destruction of cells expressing higher levels of viral antigens and in subsequent establishment of latent infection in host T cells.

Introduction

An initial viraemia after primary infection with human immunodeficiency virus type 1 (HIV-1) is rapidly followed by virus clearance concomitant with specific cell-mediated immune responses (Koup et al., 1994; Panteleo et al., 1994). Thereafter, the persistence of low levels of virus in the blood for years prior to onset of disease is facilitated in part by the ability of the virus to establish persistent and latent infections in several tissues (Levy, 1993). Notwithstanding the latent form in most populations (Embretson et al., 1993a, b; Patterson et al., 1993), the clinical stage is significantly associated with all measures of virus load, including infectious virus, virus antigen levels and viral genome content (Michael et al., 1993). There is a high absolute number of productively and latently infected cells in lymphoid tissues throughout the early to late stages of infection (Embretson et al., 1993a, b; Panteleo et al., 1993). In fact, the dynamics of HIV-1 replication in vivo have been revealed by quantifying serial changes in the virus genotype and phenotype with respect to drug resistance in the plasma and peripheral blood mononuclear cells (Wei et al., 1995; Ho et al., 1995; Perelson et al., 1996). In addition to productive HIV-1 infection, HIV-1 activation in vivo from latency seems to be the major reason for increasing virus loads at later clinical stages. HIV-1 activation from latency is believed so far to be linked to immune activation, cytokine production (Poli & Fauci, 1992), or the presence of soluble forms of HIV-1 accessory gene products such as Vpr (Levy et al., 1994, 1995) and Nef (Fujinaga et al., 1995). Thus, elucidation of the mechanisms that determine the different HIV-1 life-cycles in host cells is likely to be important for an understanding of long-term pathogenicity leading to disease.

Our previous studies showed that infection of the CD4+ T cell line MT-4 with recombinant HIV-1 containing single or multiple accessory gene (vif, vpr, and vpu) mutations led to loss of cytopathogenicity and therefore to virus persistence (Nishino et al., 1991; Kishi et al., 1992, 1995). Nonsense
mutations in the \textit{vpr} gene and a misalignment deletion mutation from \textit{vif} to \textit{vpr} naturally arose in cells during acute infection and increased progressively with \textit{in vitro} serial passage of wild-type virus; this was concomitant with increased rates to establish virus persistence (Nishino et al., 1994; Nakaya et al., 1994, 1996).

In this report, we describe the establishment of virus latency in a human CD4\(^{+}\) T cell line MOLT-4 only by infection with HIV-1 recovered from acutely infected MT-4 cells and not with HIV-1 from persistently infected MT-4 cells. In addition, we also found that tumour necrosis factor (TNF-\(\alpha\)) had a significant effect on the various virus life-cycles in host cells. Thus, both viral and host factors seem to be involved in the multiple mechanisms of HIV-1 latency.

\section*{Methods}

\subsection*{Cells and viruses.} Subclones MOLT-4 clone no. 8 (MOLT-\#8) (Kikukawa et al., 1986) and M10 (Yunoki et al., 1991) are derived from the human CD4\(^{+}\) T cell line MOLT-4 (Minowada et al., 1972) and a human T-lymphotropic virus 1-transformed human T cell line MT-4 (Miyoshi et al., 1982), respectively.

Wild-type HIV-1 (named NL) was initially obtained by transfection of SW480 cells with the infectious molecular clone pNL432 (Adachi et al., 1986), as described by Nishino et al. (1994). Since we previously found that persistently infected M10 cells were generated only by infection with highly passaged NL virus (Nishino et al., 1994), HIV-1 recovered from transfected SW480 cells was serially passed in M10 cells as described by Nishino et al. (1994). In this study, we compared two types of HIV-1, NL-A (HIV-1 in the conditioned medium from M10 cells acutely infected with NL virus at passage 20) and NL-P (HIV-1 from M10 cells persistently infected with NL virus similarly at passage 20) for any difference in the life-cycles in MOLT-\#8 after virus infection (Fig. 1).

Limiting dilution of these infected MOLT-\#8 cells was performed in 96-well, flat-bottomed microplates (Corning) for the establishment of persistently or latently HIV-1-infected subclones as described by Fujinaga et al. (1995). The cell lines and derived subclones were maintained in complete medium (RPMI 1640 supplemented with 10\% foetal bovine serum).

\subsection*{Chemicals and antibody.} TNF-\(\alpha\) and a neutralizing anti-TNF-\(\alpha\) antibody were purchased from Upstate Biotechnology. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma.

\subsection*{HIV-1 infection.} The MOLT-\#8 cells were infected with NL-A or NL-P at an m.o.i. of about 1. For pre-treatment with TNF-\(\alpha\), the cells were incubated for 1 h with TNF-\(\alpha\) (100 units/ml), washed with the medium and then infected with HIV-1. After infection for 3 h at 37 °C, the cells were washed and then cultured at 5 \times 10\(^5\) cells per ml in complete medium. For treatment with neutralizing anti-TNF-\(\alpha\) antibody, 1 \(\mu\)g/ml of the antibody was added after washing and virus adsorption. The cell number was adjusted to 5 \times 10\(^5\) cells per ml every 3 to 4 days in fresh complete medium, with or without the antibody (1 \(\mu\)g/ml). Virus replication kinetics were monitored by indirect immunofluorescence (IF), the reverse transcriptase (RT) assay or ELISA for viral Gag p24 antigen, as described below.

\subsection*{Indirect IF.} An indirect IF test was carried out using cell smears fixed with cold acetone. The cells were incubated with polyclonal antibodies to HIV-1 antigens (500-fold dilution of the serum from an HIV-1-seropositive subject; IF titre 1:4096) for 15 min at room temperature, then reacted with a 40-fold dilution of FITC-conjugated rabbit anti-human IgG (Dako) for 15 min at room temperature as described previously (Nishino et al., 1994).

\subsection*{Measurement of virus production.} RT activity was assayed as described previously (Kishi et al., 1992). Briefly, the medium was mixed with a reaction mixture containing poly(A)-oligo(dT) (Pharmacia-LKB Biotechnology) and [\(\alpha\)-\textsuperscript{32}P]dCTP (specific activity of 3000 Ci/mmol; NEN). After incubation for 3 h at 37 °C, each mixture was dotted on DEAE filter paper (DE81; Whatman) and the RT activity was quantified with an imaging analyser (BAS-1000; FUJI) using an ELISA for Gag p24 antigen as described below.

\subsection*{Measurement of TNF-\(\alpha\).} TNF-\(\alpha\) secreted into the conditioned medium of the cells was measured with an ELISA kit (Biosource) according to the manufacturer’s protocol.

![Fig. 1. Protocol for infecting MOLT-\#8 with HIV-1 NL-A and NL-P.](https://www.microbiologyresearch.org)
Detection of the viral genome in infected cells. Total cellular DNAs were extracted from subclones obtained from infected MOLT-8 cells as previously (Nishino et al., 1994). Virus sequences were amplified using a PCR kit (Perkin-Elmer Cetus) at the regions of the long terminal repeat (LTR), gag, env, vif, vpr and nef, as described previously (Kishi et al., 1995; Fujinaga et al., 1996).

Results

Comparison of the replication kinetics of NL-A and NL-P in MOLT-8

Infection of M10 cells with wild-type HIV-1 induces a drastic reduction in cell number by the killing of single cells, while in another human T cell line (MOLT-8) only some of the infected cells are killed by the induction of syncytia. We have previously shown that attenuated forms of HIV-1 used to establish persistent infection in M10 cells were generated by in vitro serial passage (Nishino et al., 1994). Among the viruses generated from M10 cells, HIV-1 NL-P was relatively homogeneous, whereas NL-A consisted of heterogeneous populations (Nishino et al., 1994; Nakaya et al., 1994, 1996). Therefore, we focused on differences in the virus life-cycles in MOLT-8 after acute infection with NL-A and NL-P.

The long-term cultures revealed a clear difference in the replication kinetics of the two viruses (Fig. 2). Markedly faster replication kinetics were observed with NL-A infection than with NL-P infection. In addition, the viral antigen-positive cell number reached nearly 100% then declined to about 10% in NL-A-infected cells, whereas the expression of viral antigens in almost 100% of the NL-P-infected cells was maintained for at least several months. Cytopathogenicity against the host cells was observed only with NL-A infection, although it was only transient during the acute phase of infection in which nearly 100% of the cells expressed the viral antigens. Cytopathogenicity of NL-A against MOLT-8 was similar to that observed against M10 cells. Interestingly, the decline in the percentage of viral antigen-positive cells was observed only in NL-A-infected MOLT-8 (Fig. 2). Such a decline was not observed in NL-A- or NL-P-infected M10 cells (Nishino et al., 1994).

Effect of TNF-α

In order to clarify the mechanism for the different kinetics of viral antigen expression in NL-A-infected MOLT-8 and M10 cells, we measured the amounts of TNF-α in the conditioned media of infected cells in different phases after virus infection. It is well-known that TNF-α induces HIV-1 expression through activation of the transcription factor nuclear factor (NF)-κB, which binds to the HIV-1 LTR sequence, thus increasing virus transcription (Duh et al., 1989; Osborn et al., 1989; Rosenberg & Fauci, 1990) as well as cytopathogenicity (Matsuyama et al., 1989) in HIV-1-infected cells.

A significant amount of TNF-α was secreted into conditioned media of infected M10 cells, compared with those of infected MOLT-8 cells (Fig. 3). The M10 and MOLT-8 cells were similarly infected with NL-A at an m.o.i. of 1, as in Fig. 2. The MOLT-8 cells were used at 20 and 60 days post-infection in the acute and persistent phases of virus infection, respectively, according to the results in Fig. 2. On the other hand, the M10 cells were used at 4 and 30 days post-infection [acute and persistent phases of virus infection, respectively, according to the kinetics for the appearance of viral antigens (Nishino et al., 1994)]. TNF-α production levels from M10 were 45 pg/ml in mock infection, 360 pg/ml in the acute phase, 250 pg/ml in the persistent phase and 2000 pg/ml during PMA treatment; those from MOLT-8 were 10 pg/ml in mock infection, 35 pg/ml in the acute phase, 10 pg/ml in the persistent phase and 10 pg/ml during PMA treatment. Thus, higher levels of TNF-α were generated during both phases in M10 cells compared with those produced in MOLT-8 lines.

In addition, the amount of TNF-α produced during the acute phase in MOLT-8 cells was slightly higher than that observed following PMA treatment (Fig. 3). This result indicates that TNF-α may contribute towards higher viral antigen expression and cytopathogenicity in NL-A-infected MOLT-8 cells during the acute phase than in the persistent phase (Fig. 2). In fact, TNF-α treatment for 4 days enhanced virus production from persistently infected MOLT-8 (Fig. 4) to a level similar to that from persistently infected M10 cells (not shown). Since the conditioned medium of NL-P-infected M10 cells contained markedly higher levels of TNF-α (Fig. 3), it was plausible that the TNF-α in the conditioned medium used as virus inocula might have significant effects on HIV-1 replication or virus life-cycles in MOLT-8 host cells.

Consistent with this hypothesis, pre-treatment of uninfected MOLT-8 cells with TNF-α followed by infection with NL-A showed earlier kinetics of virus replication (Fig. 5 a). However, the cytopathogenicity induced by infection during the acute phase and the subsequent decline in the percentage of viral antigen-positive cells were not significantly affected by this treatment (Fig. 5 a, b). Interestingly, generation of endogenous TNF-α from host cells just after the acute phase of virus replication was observed only in the absence of pre-stimulation with TNF-α, but not in pre-stimulated host cells (Fig. 5 c). The high levels of TNF-α observed before virus replication seem to be due to remaining exogenously added TNF-α. Although the amount of TNF-α from MOLT-8 cells in the acute phase was much lower than that in the cultures of M10 cells (Fig. 3), such endogenously generated TNF-α might play a significant role in amplifying the virus production rate which could lead to selective killing of host cells expressing higher levels of HIV-1 antigens. Therefore, it is likely that pre-treatment of MOLT-8 with TNF-α would allow the survival of the host cells expressing higher levels of HIV-1 antigens.

Next, the effects of a neutralizing anti-TNF-α antibody during HIV-1 infection were examined (Fig. 5 d). In the presence of the neutralizing anti-TNF-α antibody, the decline in the percentage of viral antigen-positive cells, as observed in
Fig. 2. Kinetics of replication and cytopathic properties of NL-A and NL-P. MOLT-8 cells were infected with NL-A (a) and NL-P (b) at an m.o.i of 1 for 1 h. After washing, the cells were seeded at 5 x 10^5 cells per ml in complete medium. Viable cells were counted every 3 or 4 days after infection by trypan blue dye exclusion. Similarly, percentages of HIV-1 antigen-positive cells in the viable cell population were determined by the indirect IF test with polyclonal anti-HIV-1 antibodies.

Fig. 3. Production of TNF-α. The M10 and MOLT-8 cells were similarly infected with NL-A at an m.o.i. of 1, as in Fig. 2. The M10 cells at 4 and 30 days post-infection and MOLT-8 cells at 20 and 60 days post-infection were used as acute and persistent phases of virus infection, respectively. The levels of TNF-α in the conditioned media were quantified by an ELISA kit. Mock-infected M10 and MOLT-8 cells were used as negative controls. Levels of TNF-α in the conditioned media of the M10 and MOLT-8 cells stimulated with PMA (25 ng/ml) for 3 days were similarly quantified and used as positive controls.

Fig. 4. Effects of TNF-α on virus production from MOLT-8 cells. MOLT-8 cells persistently infected with HIV-1 were stimulated with (+) or without (-) 100 units/ml of TNF-α for 4 days. The amount of Gag p24 was quantified by ELISA.

Fig. 5. Effects of neutralizing anti-TNF-α antibody on HIV-1 replication in NL-A-infected MOLT-8 cells. The MOLT-8 cells persistently infected with HIV-1 were treated with neutralizing anti-TNF-α antibody (Fig. 5d,e) or mouse IgG (not shown). The levels of TNF-α in the conditioned media of the M10 and MOLT-8 cells stimulated with PMA (25 ng/ml) for 3 days were similarly quantified and used as positive controls.

Isolation of latently infected subclones only from NL-A-infected MOLT-8 cells

To characterize the mechanism of the different kinetics of viral antigen expression in MOLT-8 cells after acute infection
with NL-A and NL-P, subclones were obtained by limiting dilution of the infected cells just after the viral antigen-positive cells reached almost 100% (see Figs 2 and 5) as shown in Fig. 1. The subclones obtained were examined for viral antigen expression by the IF test using polyclonal anti-HIV-1 antibodies. The characteristics of these subclones are summarized in Table 1. The subclones were classified into three types depending on the levels of viral antigen expression: type I, which continuously expressed HIV-1 antigens in nearly 100% of the cells and continuously produced high levels of virus
Table 1. Characterization of subclones isolated from HIV-1-infected MOLT-#8

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Treatment</th>
<th>Number of subclones</th>
<th>Number of subclones with no provirus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Type I</td>
</tr>
<tr>
<td>NL-P</td>
<td>None</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>NL-A</td>
<td>None</td>
<td>41</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Anti-TNF-α</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

Fig. 6. IF of individual subclones from MOLT-#8 infected with HIV-1. Representative subclones isolated, MOLT-20-2 (a and b), -5-5 (c and d), -5-17 (e and f), -5-16 (g and h), -5-13 (i and j), -5-8 (k and l) and -1-8 (m and n), were unstimulated (a, c, e, g, i, k and m) or stimulated with 100 units/ml of TNF-α (b, d, f, h, j, l and n) for 3 days. The cells were stained with polyclonal anti-HIV-1 antibodies.

particles in the conditioned media, indicating that HIV-1 in all subclones belonging to this type remained active with productive replication; type II, which expressed HIV-1 antigens at very low levels; and type III, which did not express HIV-1 antigens.

All 43 subclones isolated from NL-P-infected MOLT-#8 were classified as type I. In contrast, only two of the 41 subclones from NL-A-infected MOLT-#8 were classified as type I, six subclones (MOLT-20-2, -5-5, -5-17, -5-16, -5-13 and -5-8) were classified as type II (i.e. the population of cells expressing HIV-1 antigens was less than 5%) (Fig. 6a, c, e, g, i, k), while one subclone (MOLT-1-8) was of type III (Fig. 6m). PCR analyses using primer sets corresponding to the regions of LTR, gag, env, vif, vpr and nef revealed that the other 32 subclones carried no HIV-1 genes (not shown), indicating that these subclones might be derived from cells which were resistant to HIV-1 infection. On the other hand, following pre-stimulation of host cells with TNF-α, the ratio of subclones belonging to type I significantly increased, compared with the subclones obtained from unstimulated cells (Table 1). This observation was surprising, since the decline in the percentage of viral antigen-expressing cells was also observed in TNF-α-treated MOLT-#8 cells (Fig. 5a). One of two type II subclones derived from the TNF-α-treated, NL-A-infected cells
of Gag p24 in the conditioned media of cells that were enhanced virus production from uncloned infected MOLT-CII subclones, the amounts were increased from C with TNF-α compounds. Again, the type III MOLT-1-8 subclone was not a subclone belonging to type I, which was isolated from NL-P-infected cells. All type II subclones, but not in one type III subclone. The IF results for one type III and six type II subclones obtained in NL-A-infected MOLT-#8 cells. As expected from virus replication kinetics shown in Fig. 5(d, e, f), all of the 13 and 12 subclones isolated from NL-A- and NL-P-infected cells, respectively, which were cultured in the presence of neutralizing anti-TNF-α, were found to belong to type I (Table 1). Thus, HIV-1 phenotypes in the subclones obtained were almost consistent with those of parental cells before cell clonning, as shown in Figs 2 and 5.

TNF-α or PMA-mediated activation of HIV-1 from latency

Seven representative subclones which were latently infected were examined for activation of HIV-1 from latency by several chemical compounds, including TNF-α, to confirm the enhanced virus production from uncloned infected MOLT-#8 cells (Fig. 4). TNF-α (100 units/ml) or PMA (25 ng/ml) effectively induced viral antigen expression in nearly 100% of all type II subclones, but not in one type III subclone. The IF results for one type III and six type II subclones obtained in NL-A-infected MOLT-#8 cells before and after treatment with TNF-α for 3 days are shown in Fig. 6. Fig. 7 shows the amount of Gag p24 in the conditioned media of cells that were unstimulated or stimulated with either TNF-α or PMA. In type II subclones, the amounts were increased from ~2-fold (MOLT-20-2) to ~8-fold (MOLT-5-5) as a result of stimulation with TNF-α or PMA. Similarly, even a MOLT-P-8 subclone belonging to type I, which was isolated from NL-P-infected MOLT-#8 cells, was further activated by such compounds. Again, the type III MOLT-1-8 subclone was not sensitive enough for activation of latent HIV-1 by treatment with TNF-α or PMA. Thus, most of the isolated subclones with the latent form of HIV-1 became competent for viral antigen expression and virus production by stimulation with TNF-α or PMA.

Discussion

Several MOLT-#8-derived subclones were isolated as in vitro models to characterize the mechanisms of latent HIV-1 infection and their activation from latency (Table 1). The subclones obtained were classified into at least three types in terms of HIV-1 antigen expression rate: type I, which continuously expressed HIV-1 antigens in nearly 100% of the host cells; type II, which continuously expressed viral antigens in only a low population (less than 5%) but with the potential to increase the antigen expression rate to nearly 100% on stimulation by TNF-α or PMA; and type III, which showed no viral antigen expression even after stimulation by TNF-α or PMA (Figs 6 and 7).

All type II or III subclones were derived from MOLT-#8 persistently infected with NL-A but not with NL-P (Table 1). An additional two subclones belonging to type II were also obtained from MOLT-#8 cells pre-treated with TNF-α followed by infection with NL-A, although most of the subclones from these cells were of type I (Table 1). All subclones from NL-P-infected MOLT-#8 cells were of type I (Table 1). The phenotypes of HIV-1 expression in the subclones obtained were consistent with those of parental cells infected with NL-A or NL-P (Fig. 2).

The TNF-α included in inocula of both NL-A and NL-P preparations from M10 cells (Fig. 3) had a significant effect on subsequent virus replication in MOLT-#8 cells. In fact, exogenous addition of TNF-α resulted in a significant increase in virus production in persistently infected MOLT-#8 cells (Fig. 4). Pre-treatment with TNF-α induced a slight increase in replication kinetics (Fig. 5a), although this treatment had no apparent effects on the decline in the percentage of viral antigen-positive cells (Fig. 5a) or cytopathogenicity (Fig. 5b). However, it is noteworthy that endogenous TNF-α was generated only in untreated cells and not in pre-treated cells (Fig. 5c). Pre-treatment with TNF-α allowed isolation of 10 type I subclones but only two of type II (Table 1). In addition, culture of NL-A-infected MOLT-#8 cells in the presence of neutralizing anti-TNF-α allowed the isolation of only type I and not type II or III subclones (Table 1). Cultures grown in the presence of the antibody showed no decrease in the numbers of viral antigen-positive cells (Fig. 5d). These results are consistent with a previous report that TNF-α could induce selective killing of host cells producing higher levels of HIV-1 (Matsuyama et al., 1989). Thus, TNF-α, especially that generated just after acute virus replication, seems to play a critical role in determining the subsequent establishment of HIV-1 latency in host cells. The reason why such endogenous TNF-α was not generated during acute NL-A infection in TNF-α-pre-treated MOLT-#8 is not clear at present. Although exogenously added TNF-α amplified the overall level of virus production from MOLT-#8 cells (Fig. 4), the generation of endogenous TNF-α during the acute phase of virus replication was not observed in MOLT-#8 cells pre-treated with TNF-α.

![Graph](image-url)
followed by NL-A infection (Fig. 5c). Thus, it is likely that TNF-α, especially during the acute replication phase, might be critical for the selective killing of infected cells which would become virus-producing type I subclones and for the establishment of latent infection.

In this study, we observed a change in the phenotype of NL-A virus in the presence of neutralizing anti-TNF-α, i.e. NL-A virus in infected cells in the presence of the neutralizing anti-TNF-α antibody had a similar phenotype to that of NL-P virus in terms of cytopathogenicity (Fig. 5). Pre-treatment of the host cells with TNF-α increased the isolation rate of type I subclones (Table 1). This change could be explained by the failure of TNF-α secretion in pre-treated cells (Fig. 5c), although the exact role of endogenous TNF-α secreted into the culture medium of MOLT-#8 during the acute virus replication phase has to be clarified. Herbein et al. (1996) showed that pre-treatment of tissue culture-differentiated macrophages, but not T cells, with TNF-α inhibited entry of HIV-1. In contrast, our results indicated that only short exposure (1 h) of cells to TNF-α enhanced the virus replication rate (Fig. 5a) which affected the virus life-cycle (Table 1). Thus, TNF-α might exhibit various effects on virus replication and such effects seem to be mainly dependent on the virus replication phase when TNF-α is present. It is well-known that cytokines activate latent HIV-1 in cell lines or peripheral blood mononuclear cells from HIV-1 carriers (Poli & Fauci, 1992). However, cytokines could also exhibit significant effects only at specific stages of the HIV-1 life-cycle in the host.

In conclusion, we have established differentially HIV-1-infected cell models (types I to III) in MOLT-#8. This heterogeneity seemed to be due to both host and viral factors. We have shown here that the presence or absence of TNF-α during acute virus replication determined the subsequent virus phenotypes.

We thank Dr I. M. Jones of NERC Institute of Virology, Oxford, UK, for helpful discussion. This work was supported in part by the Research Fellowship of the Japan Society for the Promotion of Science by a Young Scientist (K.F.), a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture, a Grant-in-Aid for AIDS Research from the Ministry of Health and Welfare, and a Special Grant-in-Aid for the promotion of Education and Science in Hokkaido University provided by the Ministry of Education, Science, Sports and Culture of Japan.

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Received 29 May 1997; Accepted 26 September 1997