Destruction of primary CD4+ T cells by cell–cell interaction in human immunodeficiency virus type 1 infection in vitro

Hartmut Stocker, Carsten Scheller and Christian Jassoy

Infection of CD4+ T lymphocytes with human immunodeficiency virus (HIV) in vitro is accompanied by extensive cytopathicity. The mechanism of cell death is unclear, but may be related to expression of the viral envelope glycoprotein. Here, it is demonstrated that T cell destruction in primary T cells occurs upon contact of infected with uninfected lymphocytes. Cell death was due to the interaction of the envelope glycoprotein with CD4 and subsequent fusion of the cells. Agents that interfered with cell-to-cell fusion such as a monoclonal antibody to CD4 and the peptide T20 prevented T cell death and depletion. In contrast, single-cell lysis due to expression and intracellular processing of the envelope glycoprotein was insignificant. These results suggest that cell-to-cell fusion and concomitant rapid cell death promote the depletion of T cells in HIV-infected individuals.

Infection with human immunodeficiency virus (HIV) leads to a more or less steady decline of the number of T-helper cells in infected individuals. Both T cell destruction and a defect in the capacity for lymphocyte proliferation or replacement or both may underlie the loss of T cells in this disease (Pantaleo, 1999). Because of the highly cytopathic nature of HIV infection observed with both primary and transformed CD4+ T cells in vitro, the massive T-helper cell loss in infected individuals has been attributed mostly to virus-mediated processes. In addition, immune responses including HIV-specific cytotoxic T lymphocyte activity may contribute to some extent (Yang et al., 1996). The mechanism by which HIV replication causes death of T-helper cells in culture has not yet been determined. It was postulated that HIV infection of the cell represents a cytotoxic event that leads to the death of single cells (Cao et al., 1996; Leonard et al., 1988; Somasundaran & Robinson, 1987). Alternatively, infection of T cells with HIV in culture causes infected cells to fuse with neighbouring cells. This results in the development of syncytia that are not stable over an extended time-period (Lifson et al., 1986a, b; Sodroski et al., 1986). However, syncytia are not detectable in infected individuals except in the brain (Navia et al., 1986) and the tonsils (Frankel et al., 1996). Both syncytium formation and death in HIV-infected cultures have been attributed to the expression of the viral envelope glycoprotein. In particular, it was demonstrated that correct and efficient processing of gp160, CD4-binding ability and membrane fusion capacity are important for cell death in vitro (Cao et al., 1996). We and others have reported previously that co-culture of HIV-infected cells or cells expressing the envelope glycoprotein with CD4+ cells leads to rapid cell death (Heinkelein et al., 1995; Laurent-Crawford et al., 1993; Yoffe et al., 1987). The prerequisites for cell death were similar to those for cell-to-cell fusion, although the magnitude of cell destruction in this situation did not necessarily correlate with the extent of syncytium formation (Heinkelein et al., 1995). In addition, cell death was accompanied by signs of apoptosis (Corbeil & Richman, 1995; Laurent-Crawford et al., 1991, 1993, 1995; Nardelli et al., 1995; Ohnimus et al., 1997), indicating an active cell-death process that occurs beyond the cell-to-cell fusion event.

Previous studies of the mechanism of T-helper cell death were performed primarily with lymphoblast cell lines either infected with virus or virus vectors or transfected with the HIV envelope gene (Cao et al., 1996; Koga et al., 1990; Leonard et al., 1988; Lifson et al., 1986b; Sodroski et al., 1986; Somasundaran & Robinson, 1987). Alternatively, infected or HIV glycoprotein-expressing lymphoblasts were co-cultured with primary CD4+ T cells (Heinkelein et al., 1995, 1997; Ohnimus et al., 1997). Few studies have been carried out with primary cells (Nardelli et al., 1995). Since the pathology of HIV infection of primary CD4+ T lymphocytes may differ from that observed in T cell lines, analysis of such cells is crucial to the understanding of T cell death in HIV disease.

We examined whether the cytotoxicity mediated by the HIV envelope glycoprotein occurs in single cells or upon contact with CD4+ lymphocytes. To mimic the in vivo situation better, we used primary CD4+ T lymphocytes.

In a first set of experiments, peripheral blood mononuclear cells were prepared by density-gradient centrifugation from...
The results depicted in Fig. 1(a) demonstrate that a significant fraction of uninfected CD4+ T lymphocytes was lysed upon co-culture with HIV-infected cells. The fraction of cells destroyed increased with the duration of culture. However, since co-culture with infected cells may lead to infection of the uninfected cells, single cell lysis due to cytopathicity of virus replication and cell death due to cell–cell fusion could not be differentiated in this assay.

To address this issue, experiments were performed with recombinant vaccinia viruses that express either an intracellularly truncated envelope glycoprotein (vPE17, vac/env) (Heinkelein et al., 1995) or polymerase (vCF21, vac/pol) of the HIV-1 strain IIIB/LAI. Expression of the HIV glycoprotein in this and the other experiments was determined by flow cytometry by using the FITC-labelled anti-gp120 MAb 902 (Chesebro & Wehrly, 1988). PHA-activated CD4+ T cells were infected with the vaccinia virus encoding either the HIV glycoprotein or the polymerase gene (vac/pol; filled bar) or the polymerase gene (vac/env; shaded bar) or the polymerase gene (vac/pol; filled bar) or the polymerase gene (vac/env; shaded bar) were co-cultured for 6 h with autologous uninfected cells at a ratio of 1:3.

In order to dissect cytolysis upon cell-to-cell contact from cytopathicity due solely to HIV glycoprotein expression, PHA-activated CD4+ T cells were labelled with Na251CrO4 for 60 min, washed twice with medium and split into three fractions. Cells were infected for 1 h either with the envelope vaccinia virus construct or with wild-type vaccinia virus (strain Copenhagen) at an m.o.i. of 15 p.f.u. per cell. The third culture was not infected. Cells (2 × 106) were placed in duplicate in 96-well plates and the anti-CD4 MAb SIM.2 (60 µg/ml) (McCallus et al., 1992), which inhibits gp120–CD4 binding, cell-to-cell fusion and cytolysis (Heinkelein et al., 1995), or the peptide T20 (2 µg/ml), which inhibits post-binding fusion events, were added to some of the cultures. T lymphocytes were incubated for an additional 12 h at 37°C. Supernatants were harvested and counted in a gamma counter. Percentage lysis was determined from the formula 100 × (release from vaccinia virus-infected culture—spontaneous release)/(maximum release—spontaneous release). Maximum release was determined by lysis of targets in 1·5% Triton X-100.

The blood of uninfected individuals and depleted of CD8+ cells by using antibody-covered immunomagnetic beads (Dynal). T cell preparations contained less than 5% CD8+ T cells. Cells were stimulated with PHA (2 µg/ml) and cultured in RPMI-1640 medium supplemented with 10% FCS, HEPES, antibiotics and 100 U/ml interleukin-2 (Proleukin, Eurocetus) for 3–5 days. Activated CD4+ T cells were infected with HIV-1 strain IIIB/LAI at an m.o.i. of 0·01 TCID50 per cell. More than 70% of the cells were HIV Gag-positive after 3–4 days of culture. Uninfected CD4+ T cells (3 × 106) were labelled with 100 µCi Na251CrO4 for 60 min and added to the same number of autologous HIV-infected cells in 96-well round-bottomed microtitre plates. Cells were incubated for 5·5–15·5 h, after which time supernatants were harvested and counted in a gamma counter. Percentage lysis was determined from the formula 100 × (experimental release—spontaneous release)/
did not account for the cytotoxicity described. Analogous experiments with HIV could not be performed, because agents that block CD4-gp120 contact or cell-to-cell fusion inhibit cytotoxicity similarly due to prevention of virus infection.

Having demonstrated that CD4+ T cell destruction occurs upon contact of infected with uninfected lymphocytes, cell loss was examined additionally by quantitative flow cytometric analysis. T cells were infected with vPE17 at an m.o.i. of 20 p.f.u. per cell. Cells were split into fractions of 1.5 × 10^6 cells and placed in 24-well plates and the anti-CD4 MAb was added to some of the cultures. Cells were incubated for 10 h at 37 °C. After 8, 9 and 10 h incubation, the fraction of glycoprotein-expressing cells was determined and flow cytometric analysis of the number of CD4+ T cells was performed as described previously (Heinkelein et al., 1997). Briefly, cells were transferred from the plate to flow cytometry tubes and incubated in the presence of fluorescent dye-conjugated antibodies against CD4 (FITC; Dako) and CD3 (PE-Cy5; Dako). Cells were fixed in formaldehyde, washed in PBS containing 0.1% BSA and 0.02% sodium azide and resuspended in 300 µl staining solution containing 3 × 10^4 FITC-conjugated microbeads. For adjustment of quantification, reference beads were located in the forward/sideways scatter position and, in addition, in the fluorescence 1/fluorescence 2 position. The flow cytometer was programmed to stop the collection of data after one-sixth of the beads had appeared in the reference gate. Absolute numbers of cells in the sample were obtained by multiplying the number of cells counted by six.

Fig. 2 shows that, in the absence of the anti-CD4 MAb, the number of CD4+ T cells decreased rapidly. The fraction of cells expressing the envelope glycoprotein was similar in the absence and presence of the anti-CD4 MAb, reflecting the fact that the antibody did not interfere with vaccinia virus infection and indicating that the T cell loss was not due to destruction of the cells by vaccinia virus infection. The anti-CD4 MAb prevented T-helper cell loss. The degree of T cell loss in the absence of inhibiting antibody was correlated negatively with the level of HIV glycoprotein expression. In this and related experiments with primary cells, T cell destruction and depletion were only marginally affected by an inhibitor of the apoptosis-mediating caspase enzymes (data not shown).

Syncytium formation and rapid cell death may represent alternative outcomes of the cell-to-cell contact in HIV infection. Whereas syncytium formation is observed readily in vitro, cytosis may be the preferential outcome of cell-to-cell contact in vivo. Our data compare favourably with the cytotoxicity results presented previously by Nardelli et al. (1995). In addition, they conform with most of the findings of Cao et al. (1996), who demonstrated that gp160 processing, CD4 binding and membrane fusion were required for cell death in HIV-infected Jurkat T lymphoblasts. However, in the latter study, the addition of soluble CD4 at a concentration that inhibited syncytium formation did not prevent cell death when cells were cultured for several days, leading the authors to conclude that cell death occurred by single-cell lysis rather than by cell-to-cell fusion. In contrast to what would be expected from this report, no lysis of single primary T cells due to HIV glycoprotein expression from recombinant vaccinia virus was detectable in our system. This discrepancy may be due to the cell system tested and, in particular, to the time-period examined. Cao et al. (1996) studied cell death and the ability of CD4 to inhibit cytotoxicity over a period of up to 12 days. In contrast, we analysed cytotoxicity in the first hours of cell-to-cell contact. This difference is important, because we demonstrated that recombinant CD4 inhibits both syncytium formation and cytosis readily when primary CD4+ T lymphocytes were cultured for 6–15 h together with HIV-infected Jurkat cells or glycoprotein-expressing B lymphoblasts (Heinkelein et al., 1995; Ohnimus et al., 1997).

Since HIV glycoprotein expression on the cell surface is part of the virus particle synthesis process in infected cells, our data suggest that the mechanism of T cell destruction observed in vitro operates in vivo. The T cell death described involves...
both infected and uninfected cells. The question of whether cell
loss in HIV infection is due solely to the death of infected cells
or involves both infected and uninfected cells is important. In
either model system, T cell loss correlates with the number of
infected cells. This compares favourably with the situation
observed in infected individuals, where the virus RNA load
correlates with the pace of disease progression (Mellors et al.,
1996). However, in contrast to the situation with single-cell
killing, cytotoxicity involving uninfected bystander cells points
to a scenario in which T-helper cell death exceeds the
number of infected cells significantly at any given time-point.
Moreover, if cell death in vivo is mediated by cell-to-cell
contact, prevention of virus replication and inhibition of the
interaction of infected with uninfected cells may act syner-
gistically in terms of prevention of T-helper cell depletion.

The peptide T20 inhibits HIV infection and syncytium
formation by preventing the gp41 molecule from folding
adequately after binding of gp120 to its receptor molecules
and insertion of the gp41 fusion peptide into the cell membrane
(Wild et al., 1994). It was observed that treatment of HIV-
infected individuals with this agent led to potent suppression
of HIV replication in vivo (Kilby et al., 1998). The results of
our study suggest an additional beneficial effect of this and similar
agents on T-helper cell numbers in infected individuals,
through interference with the lethal consequences of the
contact of HIV-infected with uninfected cells.

In conclusion, the results of this study demonstrate rapid
cytolysis and cell loss upon contact and fusion of primary HIV-
infected and HIV glycoprotein-expressing T cells with un-
infected cells. The data support the view that T-helper cell
destruction in HIV infection in vitro and in vivo is mediated at
least partially by a lethal cell-to-cell contact. Cell–cell fusion
and cytolysis may represent different aspects of a related
intercellular process. Since destruction involves both infected
and uninfected cells, cell death is extensive. This may
contribute to the large depletion of T-helper cells in HIV-
infected individuals.

The authors thank Ingeborg Euler-König for technical assistance
and Martin Heinekein for helpful discussion. The T20 peptide was kindly
provided by T. J. Matthews, Duke University Medical Center, and
Trimeris Inc., Durham, NC, USA. The anti-CD4 MAb SIM.2 was
obtained from J. Hildreth, the anti-gp120 MAb from B. Chesebro and the
HIV isolate from R. Gallo through the AIDS Research and Reference
Reagent Program, Division of AIDS, NIAID, NIH. The vaccinia viruses
vPE17 and vCF21 were kindly provided by B. Moss, Bethesda, MD,
USA. This work was supported by DFG grant Ja 662/4-1 and EU
contract BMH4-CT97-2055 (to C.J.).

References

determinants of acute single-cell lysis by human immunodeficiency virus

quantitative focal assay for human immunodeficiency virus infectivity.

subsequent interaction of CD4–gp120 at the cellular membrane is
required for HIV-induced apoptosis of CD4+ T cells. Journal of General
Virology 76, 681–690.

Frankel, S. S., Wenig, B. M., Burke, A. P., Mannan, P., Thompson,
(1996). Replication of HIV-1 in dendritic cell-derived syncytia at the

immunodeficiency virus type 1-infected and uninfected CD4+ T
lymphocytes is highly cytoytic for both cells. Journal of Virology 69,
6925–6931.

Rapid and selective depletion of CD4+ T lymphocytes and preferential
loss of memory cells on interaction of mononuclear cells with HIV-1
glycoprotein-expressing cells. Journal of Acquired Immune Deficiency
 Syndromes and Human Retrovirology 16, 74–82.

Lee, J. Y., Aldredge, L., Hunter, E., Lambert, D., Bolognesi, D.,
Matthews, T., Johnson, M. R., Nowak, M. A., Shaw, G. M. & Saag, M. S.
(1998). Potent suppression of HIV-1 replication in humans by T-20, a
peptide inhibitor of gp41-mediated virus entry. Nature Medicine 4,
1302–1307.

(1990). Cytotoxic effect determined by the amount of CD4 molecules
in human cell lines expressing envelope glycoprotein of HIV. Journal of
Immunology 144, 94–102.

Laurent-Crawford, A. G., Krust, B., Muller, S., Rivière, Y., Rey-Cuillé,
The cytopathic effect of HIV is associated with apoptosis. Virology 185,
829–839.

Laurent-Crawford, A. G., Krust, B., Rivière, Y., Desgranges, C., Muller,
expression of HIV envelope glycoproteins triggers apoptosis in CD4
cells. AIDS Research and Human Retroviruses 9, 761–773.

Laurent-Crawford, A. G., Coccia, E., Krust, B. & Hovanessian, A. G.
(1995). Membrane-expressed HIV envelope glycoprotein heterodimer is
a powerful inducer of cell death in uninfected CD4+ target cells.
Research in Virology 146, 5–17.

in T cells is linked to the last stages of virus infection. Proceedings of the
National Academy of Sciences, USA 85, 3570–3574.

Lifson, J. D., Feinberg, M. B., Reyes, G. R., Rabin, L., Banapour, B.,
Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K. S. & Engleman,
E. G. (1986a). Induction of CD4-dependent cell fusion by the HTLV-

Lifson, J. D., Reyes, G. R., McGrath, M. S., Stein, B. S. & Engleman,

McCallius, D. E., Ugen, K. E., Sato, A. I., Williams, W. V. & Weiner,
system producing a bioactive CD4 molecule. Viral Immunology 5,
163–172.


**Ohnimus, H., Heinkelein, M. & Jassoy, C. (1997).** Apoptotic cell death upon contact of CD4+ T lymphocytes with HIV glycoprotein-expressing cells is mediated by caspases but bypasses CD95 (Fas/Apo-1) and TNF receptor 1. *Journal of Immunology* **159**, 5246–5252.


Received 28 December 1999; Accepted 12 April 2000