Lysis of Human Immunodeficiency Virus Type 1 Antigen-Expressing Cells by CD4 and CD8 T Cells Ex Vivo

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The aim of this study was to investigate the extent of lysis mediated by cytotoxic T lymphocytes (CTL) directed against human immunodeficiency virus (HIV) type 1 gag protein and envelope glycoprotein in peripheral blood mononuclear cells (PBMC) from HIV-1-infected subjects and to compare it with nonspecific envelope glycoprotein-directed cytotoxicity involving CD4 T cells. Most seropositive subjects exhibited antigen-specific cytotoxicity directed at one or both viral antigens in unstimulated or in vitro-stimulated PBMC (or both) mediated by CD8 T cells. In addition, all donors, including seronegative control persons, exhibited nonspecific calcium-independent cytotoxicity involving CD4 T cells and envelope glycoprotein-expressing cells. No calcium-dependent, antigen-specific CD4 T cell-mediated cytolysis was detected. In seropositive subjects, the vigor of nonspecific cytotoxicity was comparable to lysis by antigen-specific CD8 CTL and suggests that it may contribute to lysis of HIV-infected cells in vitro and in vivo.

Material and Methods

Patient population. Six HIV-1 seropositive patients from the Medical Policlinic and the Department of Internal Medicine (Würzburg University Medical School) were enrolled in this study. Their clinical characteristics and laboratory parameters at the time of the study were as follows: subject 9502: 31 years old, female, CDC group A1, CD4 cell count 625/mm³; subject 9503: 31 years old,
Results

HIV-1-directed cytotoxic response in unstimulated PBMC from seropositive subjects. PBMC were derived from asymptomatic HIV-seropositive subjects and separated into CD4 and CD8 T cell populations. These cells were then used as effector cells in cytotoxicity assays. PBMC from seronegative persons were used as control effector cells. Target cells were autologous B-LCL infected with recombinant vaccinia viruses expressing either the HIV-1 gag protein or the HIV-1 envelope glycoprotein. B-LCL infected with a vaccinia vector coding for an irrelevant protein served as a control. Expression of virus protein was monitored by flow cytometry and was comparable for all targets in each assay.

Figure 1 shows representative data from 3 seropositive persons and a seronegative control donor. HIV antigen-presenting autologous cells were lysed by unstimulated PBMC from all of these subjects. Similarly, CD4 T cells from all subjects lysed envelope glycoprotein–expressing cells. Envelope glycoprotein–directed lysis involving CD4 T cells was observed in both the absence and presence of EDTA, indicating nonspecific cytotoxicity related to cell-to-cell fusion. No antigen-specific calcium-dependent cytolytic response mediated by CD4 T cells was detected in the infected patients. In addition, CD8 T cell–mediated lysis of envelope glycoprotein and gag protein–expressing cells was observed in HIV-infected subjects 9503 and 9505. In contrast to the toxicity involving CD4 T cells, envelope glycoprotein–directed lysis exerted by CD8 T cells was calcium-dependent, indicating classical antigen-specific CTL activity.

The results obtained using unfractionated PBMC from the 3 seropositive subjects shown in figure 1 represent three different cytotoxicity response patterns. For instance, PBMC from subject 9503 exhibited both antigen-specific CD8 CTL activity and nonspecific envelope-directed lysis involving CD4 T cells. In contrast, PBMC from subject 9502 contained no antigen-specific cytotoxicity but a significant level of nonspecific cytotoxicity. In addition, unfractionated PBMC from subject 9505 exhibited antigen-specific cytotoxicity directed at gag and envelope targets but no CD4 T cell–mediated calcium-independent toxicity. However, after fractionation, CD4 cells from this individual exhibited calcium-independent envelope-directed lysis. The inability to demonstrate nonspecific envelope glycoprotein–directed lysis using PBMC from this subject reflects the low CD4 cell number in the peripheral blood of this particular donor.

HIV-1 gag- and envelope-directed cytotoxicity in stimulated T cells. Having demonstrated the presence of both antigen-specific CD8 T cell mediated cytotoxicity in unstimulated PBMC of HIV-infected subjects and nonspecific envelope-directed lysis involving CD4 T cells from both seropositive and seronegative persons, we examined in vitro–stimulated cells from these and several additional subjects. Seven PBMC samples from 6 HIV-1-seropositive subjects and 3 specimens from...
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3 seronegative control donors were tested. CD8 cells from infected but not control donors exerted gag- and envelope-specific cytotoxicity. In addition, similar to what has been observed using unstimulated cells, envelope glycoprotein-directed cytotoxicity was detectable in PBMC and CD4 cells from both seropositive and seronegative subjects. In contrast to envelope-specific lysis exerted by CD8 CTL, cytotoxicity involving CD4 cells was independent of extracellular calcium (figure 2). Moreover, lysis of envelope-expressing cells by CD4 cells was maintained when heterologous target cells were used, whereas CD8 CTL-mediated lysis was restricted to autologous cells (data not shown). No antigen-specific calcium-dependent cytolytic activity mediated by CD4 cells was detectable.

Discussion

The HIV-specific cytolytic immune response mediated by CD8 CTL has been extensively examined in infected persons. In most studies, cytotoxic activity was assessed by chromium release assay using, as target cells, autologous or HLA-matched B cells, either infected with recombinant vaccinia viruses coding for HIV proteins or sensitized with synthetic viral peptides (reviewed in [1]). Lysis of target cells presenting the envelope glycoprotein was regarded as unique because it occurred in both an MHC-restricted and MHC-unrestricted fashion [2]. We recently demonstrated that rapid lysis of HIV-infected and envelope glycoprotein-expressing cells occurs in vitro in the presence of primary CD4 T cells. Although this cytolysis resembled antigen-specific cytotoxicity in the chromium release assay, it was MHC-unrestricted, independent of extracellular calcium, and appeared to be related to cell-to-cell fusion. It may therefore represent rapid disintegration of the cell fusion event [4].

On the basis of this observation, we evaluated the contribution of cytotoxic activity exerted by both mechanisms in PBMC
from infected persons. The data demonstrate that, in addition to antigen-specific CD8 CTL-mediated lysis of target cells, cytotoxicity involving envelope glycoprotein-expressing cells and CD4 T cells was readily detectable in the blood of HIV-infected subjects, using both unstimulated and in vitro-stimulated cells. Moreover, whereas antigen-specific CD8 cell-mediated lysis of envelope target cells was detected in only some HIV-infected subjects, nonspecific cytotoxicity was detectable in all donors. Similar results were obtained using either the complete recombinant vaccinia/HIV-1 envelope virus vPE16 or the truncated envelope construct vPE17, although lysis of vPE16-infected cells by CD4 cells was frequently less pronounced due to the reduced surface expression of the glycoprotein when this construct is used. The inability to detect nonspecific envelope-directed lysis in uninfected PBMC from seropositive donors with low CD4 T cell numbers and the fact that this type of toxicity is observed in CD4 T cells from these donors after separation indicate that a low CD4:CD8 T cell ratio in the blood may mask this type of cytotoxicity. No antigen-specific calcium-dependent cytolytic activity mediated by CD4 T lymphocytes was detected in the persons examined. This observation confirms reports by others (reviewed in [1]).

Previous reports suggested that non-MHC-restricted killing of envelope glycoprotein-expressing cells by PBMC was being mediated predominantly by NK and killer cells, which are armed with cytophilic antibodies [10-12]. Although cytolysis mediated by these killer cells was not specifically addressed in the present study, our results suggest that a significant portion of all envelope-directed cytolysis exerted by PBMC ex vivo could be attributed either to CD8 CTL or to nonspecific fusion-related lysis involving primary CD4 T cells.

Recognition and lysis by antigen-specific CTL require presentation of viral peptides in the context of MHC molecules. In contrast, fusion-related cytotoxicity needs expression of the native glycoprotein on the surface of infected cells. Therefore, since expression of the glycoproteins occurs at a later stage of viral replication, antigen-specific lysis mediated by CD8 CTL may to some extent precede cell-to-cell fusion toxicity in vivo. However, there is ample evidence indicating that target cells relevant for CD4 T cell fusion are present in the infected tissue.
For instance, the intracellular life cycle of HIV is relatively short and the interval between translation of early virus antigens and expression of the envelope glycoprotein may be only a few hours [13]. In addition, large numbers of virions are present in the serum of infected subjects and argue against efficient elimination of infected cells before viral synthesis has been completed [13, 14]. Moreover, fusion of viral and cellular membranes may render HIV-infected cells as targets for fusion-related cytolysis even before viral replication has taken place because envelope glycoproteins present in the HIV membrane become, though only for a limited time, part of the cell surface [15].

It is assumed, because the HIV-specific CTL response is exceptionally vigorous, that lysis of HIV-infected cells by CD8 CTL is relevant in the infected individual. In this study, the extent of cytotoxicity directed at HIV antigen-expressing cells exerted by CD8 CTL and CD4 T cells was in a similar range. Therefore, the similarity of the results obtained using an identical assay protocol for lysis involving CD8 CTL and CD4 T cells would suggest that nonspecific, CD4 T cell–involving cytotoxicity might contribute to lysis of infected cells in vivo.

In conclusion, both antigen-specific CD8 CTL–mediated cytotoxicity directed at multiple HIV antigens and fusion-related lysis of envelope glycoprotein–expressing cells involving CD4 T cells constitute the main components of cytotoxicity observed in PBMC from infected persons. Both factors may contribute to the destruction of virus-infected CD4 T cells in the infected individual.

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