Rapid Induction of Apoptosis by Cell-to-Cell Transmission of Human Immunodeficiency Virus Type 1

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The kinetics of human immunodeficiency virus type 1 (HIV-1)-induced cell death were investigated in cell-to-cell and cell-free models of virus transmission. Cocultivation of HIV-1 chronically infected H9 donor cells with uninfected H9 recipient cells resulted in rapid induction of programmed cell death. Within 8 h, apoptotic chromatin condensation was identified by histologic staining. In addition, many single cells with apoptotic nuclei were observed, indicating that stable cell fusion was not a requirement for apoptosis to occur. By 12 to 18 h of coculture, a DNA fragmentation ladder characteristic of apoptosis was detected by agarose gel electrophoresis. Quantitation of apoptosis by measurement of nuclear DNA content revealed that at least 20 to 30% of the nuclei were undergoing apoptosis by 24 h after cocultivation. The appearance of condensed nuclei and fragmented DNA occurred as HIV reverse transcription was completed, and it was not inhibited by zidovudine, suggesting that induction of apoptosis did not require new HIV replication. Soluble CD4 inhibited apoptosis, demonstrating that Env-CD4 interactions were required for apoptosis. In contrast to that in cell-to-cell transmission, apoptosis in cell-free HIV infections was markedly inefficient and was not observed until 70 to 90 h after infections were initiated. These findings indicate that HIV-1 induction of programmed destruction of the nucleus is initiated at the time of cell-cell cocultivation by a mechanism which requires CD4-Env interactions but not new HIV replication.

Human immunodeficiency virus type 1 (HIV-1) is transmitted to susceptible CD4+ cells by virion particles or infected cells. Although several critical steps in the HIV life cycle have been identified, the consequences of virus replication events for host cells are only poorly understood. HIV infection results in marked cytopathic effects in cultured T-lymphocytic cell lines, but the mechanisms of cell death remain unclear. Recently, several investigators (2, 3, 12, 25, 30, 32, 33, 43, 52) suggested that HIV infection initiates programmed cell death, a sequence of nuclear rearrangement and condensation events (7, 40, 57) which may culminate in apoptosis, with DNA digestion into megabase-sized (40, 41) or oligonucleosome-sized fragments (7). Laurent-Crawford and coworkers (25) have reported that HIV env gene products gp120 and gp41 were both required to induce apoptosis by engaging the HIV-1 receptor, CD4, suggesting that the cytopathic effect of HIV may be initiated upon virus entry. In contrast, several investigators have suggested that the cytopathic effects of HIV occur as a consequence of virus replication. Cloyd and Lynn (6) reported significant alterations in the lipid composition of host membranes after HIV infection, resulting in increased membrane permeability; others (4, 24, 30, 49) have reported toxic effects of env expression in env-inducible cell lines or in acute HIV infections. Klimkait and coworkers (23) noted increased cytopathic effect in cells infected with a vpu− HIV strain compared with that detected in cells infected with isogenic vpu+ virus. In virus expression studies, the asynchronous character of cell-free HIV infections did not permit direct comparison of the kinetics of virus replication and the induction of cell death.

We and others have recently characterized a cell-to-cell model for HIV transmission which provides a rapid and synchronous method for HIV transmission (29, 47). Coculture of HIV-1 chronically infected H9 cells with uninfected H9 cells results in new HIV-1 DNA synthesis of as many as 40 HIV-1 proviral copies per cell within 4 h of cocultivation and in release of progeny virions after 24 h of cocultivation. Kinetic analyses by Dimitrov et al. (8) revealed that cell-to-cell spread is 2 to 3 orders of magnitude more efficient than transmission of HIV by cell-free virions and therefore represents the predominant mode of HIV transmission after cell-free infection by HIV has taken place. In the present study, we investigated the synchronous cell-to-cell transmission model to identify the mechanism of cell death and to determine whether cell destruction was a product of HIV replication or was initiated upon virus entry. We obtained unambiguous evidence of apoptosis within 8 hours of coculture of uninfected H9 cells with H9 cells chronically infected with HIV. Programmed cell death occurred in the absence of new HIV replication and required Env-CD4 interactions. In contrast to the induction of apoptosis in the cell-to-cell transmission model, the induction of apoptosis following cell-free HIV infections was inefficient and markedly delayed. These results suggest that induction of apoptosis is principally mediated by Env-CD4 interactions between HIV-infected and uninfected cells.

MATERIALS AND METHODS

Cells and virus. The Jurkat, H9, CEM-derived 12D7, and HIV chronically infected H9 (H9IIIIB) cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, glutamine, and antibiotics (maintenance medium [47]). HeLa cells were maintained in Dulbecco modified Eagle medium with supplements identical to those of the maintenance medium.

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HIV$_{imm}$ was obtained from cultures of H9IIIB cells (47); culture supernatants were pelleted at 1,000 rpm for 5 min to remove cells and debris, filtered, and then pelleted at 200,000 × g for 1 h. The pellets were resuspended in RPMI medium and used for infections or Western blotting (immunoblotting). The infectious titer of the concentrated HIV preparation was determined by endpoint dilution in H9 cells using reverse transcriptase activity in culture supernatants to demonstrate the presence of HIV. The titer was defined as the midpoint between the last dilution positive and the first dilution negative for reverse transcriptase activity. Reverse transcriptase was measured as previously described (50).

To obtain virus stocks of HIV molecular clones, HeLa cells were transfected with 15 μg each of the HIV-1 infectious molecular clones pNL4-3 (1), pNL4-3U35 (50), and pNL4-3 (10) by the calcium phosphate technique as described previously (50) except that the cells did not undergo glycerol shock but were washed and refed with maintenance medium. Culture medium was harvested at 48 h after transfection, clarified by centrifugation at 1,000 g for 5 min to remove released cells, and filtered through 0.45-μm-pore-size filters. H9 cells chronically expressing HIV were established by infecting 2.5 × 10⁶ cells with 15 μg of sheared, boiled salmon sperm DNA per ml [19] with a 32P-labeled probe obtained by random hexamer priming of a mixture of HindIII fragments of the infectious molecular clone pNL4-3 which span the HIV genome from positions 536 to 9606.

Induction of cell death by cocultivation. As previously described, cocultivation of uninfected H9 cells with H9 cells chronically infected with HIV-1 results in rapid transmission of

**RESULTS**

**Detection of HIV proteins.** HIV preparations and cell extracts were prepared, electrophoresed on 10% acrylamide gels, and electroblotted onto nitrocellulose membranes as previously described (50). HIV proteins were detected by Western blotting by sequential incubation of the membranes with a 1:1,000 dilution of plasma from two HIV-seropositive persons and a 1:1,000 dilution of ¹²⁵I-protein A (50). HIV proteins were visualized and quantitated with the Fuji phosphorimaging system.

**Induction of cell death by cocultivation.** As previously described, cocultivation of uninfected H9 cells with H9 cells chronically infected with HIV-1 results in rapid transmission of
HIV to uninfected cells; new HIV DNA synthesis occurs within 4 h, and new virions are produced after 24 to 48 h (47). Previous examinations of the cocultures revealed cell-cell fusions as rapidly as 15 min following cocultivation and the appearance of large balloon-like membrane protrusions within 24 h of coculture (47). To investigate whether the cytopathic effect observed after cell-to-cell HIV transmission included programmed cell death, we analyzed the appearance of nuclei in cocultivations by light microscopy. As shown in Fig. 1, thin sections of H9-H9IIIB cocultures stained with toluidine blue demonstrated the presence of nuclei with marked condensation and fragmentation, indicating that programmed cell death had been initiated; nuclear changes were evident as early as 2 h following cocultivation, when nuclei within fused cells began to condense (compare Fig. 1B, arrow, with Fig. 1A). The frequency of nuclei with distinct condensation and fragmentation increased after HIV cell-to-cell transmission was initiated (Fig. 1B to D, arrows); by 32 h following cocultivation, most cells exhibited apoptotic changes, although some of the fused cells still contained apparently normal-appearing nuclei (Fig. 1D). In whole mount specimens, nuclei from single cells with characteristic condensation (Fig. 2B, arrow) and condensation with extensive fragmentation (Fig. 2A) were observed. Cells undergoing apoptosis were easily distinguished from typical H9IIIB cells (Fig. 2C) or H9 cells (Fig. 2D), including cells in metaphase (Fig. 2C, arrow) or prophase (Fig. 2D, arrow). The presence of apoptosis in single cells suggested that in this cocultivation system, stable cell fusions were not required for programmed cell death to occur. The contribution of single cells to the total apoptotic population was minor and was most apparent soon after cocultivation; at 12 h after cocultivation, when relatively few cells were undergoing apoptosis, the number of hypercondensed nuclei in single cells was similar to the number of nuclei in syncytia undergoing apoptosis. By the peak of apoptosis, the majority of apoptotic bodies occurred in syncytia. These histologic studies permitted the earliest detection of apoptotic nuclei and demonstrated that a program of cell death was initiated rapidly after H9 and H9IIIB cells were cocultivated.

To determine whether apoptotic changes affecting the nuclei of H9-H9IIIB cocultures progressed to include digestion of DNA, cocultivations were analyzed for the appearance of oligonucleosomal DNA fragments. Uninfected H9 cells and chronically infected H9IIIB cells were cultured at a ratio of one infected cell to four uninfected cells; cells were harvested throughout a 72-h period, and unintegrated DNA was prepared, electrophoresed on 1% agarose gels, and stained with ethidium bromide. As shown in Fig. 3, the presence of fragmented DNA in discrete oligonucleosome-sized bands typical of apoptosis (Fig. 3A) was detected by agarose gel electrophoresis after 12 h of mixing of cells and increased over 48 to 72 h. These findings indicated that programmed cell death initiated by the coculture included digestion of DNA into oligonucleosome-sized fragments. The onset of apoptosis shortly after the mixing of cells and the digestion of DNA into oligonucleosomal fragments coincided with early events in HIV
from the 1- and 2-LTR fragments (indicated by arrows) of 7,751 and 8,386 generated by 9607. The bands denoted by L represent fragments of 1,932, 2,203, and 5,094 bp with two-long terminal repeat (2-LTR) reverse transcript, and electrophoresed on restriction enzyme was prepared from cocultivations at the indicated times, digested with the restriction enzyme _Msc_ I, which cuts at positions 2203 and 4135 of the full-length two-long terminal repeat (2-LTR) reverse transcript, and electrophoresed on 0.6% agarose gels. DNA was transferred to Hybond-N+ membranes and hybridized with _HindIII_ fragments spanning the HIV genome from position 536 to position 9607. The bands denoted by L represent fragments of 1,932, 2,203, and 5,094 bp generated by _Msc_ I from linear HIV DNA, and those denoted by C are derived from the 1- and 2-LTR fragments (indicated by arrows) of 7,751 and 8,386 nucleotides, respectively, and are obtained from circular HIV DNA molecules.

Replication. As shown in Fig. 3B, Southern blot analysis of unintegrated DNA revealed that HIV reverse transcription was completed by 4 to 7 h following cocultivation; DNA fragments corresponding to one- and two-long terminal repeat circular forms of HIV DNA, indicative of DNA translocation to the nucleus, were also present, and they increased in amount during the initial 24 h of coculture. This comparison indicated that the early events of HIV replication, including translocation of HIV DNA, took place despite the initiation of apoptosis and nuclear destruction. It is not known whether unintegrated HIV DNA is a substrate for the endonucleases involved in apoptosis-associated DNA degradation; using HIV-specific probes, we have been unable to detect HIV sequences in the oligonucleosomal DNA fragments (data not shown). The delay between the first appearance of condensed nuclei (Fig. 1B) and the onset of oligonucleosomal DNA at 12 h (Fig. 3A) may be attributed to the time required for digestion sufficient to be detected by ethidium bromide staining of DNA in agarose gels.

Quantitation of HIV-1-induced programmed cell death. The relative extent of programmed cell death induced by cell-to-cell transmission of HIV was determined by using quantitative fluorescent staining of nuclear DNA to enumerate cells undergoing apoptosis. Three distinct populations of H9 or H9IIIB nuclei were discernible on the basis of DNA content: nuclei with the normal prereplication content of DNA (2N DNA) in G0/G1 phase (Fig. 4A); nuclei containing 4N DNA, i.e., nuclei in which DNA had been completely replicated (G2/M); and nuclei containing amounts of DNA between 2N and 4N (nuclei in S phase). As described by Nicoletti and coworkers (38), the presence of nuclei undergoing apoptosis is identified as a broad peak of nuclei with amounts of DNA less than 2N; to delineate this peak clearly, the fluorescence intensity was adjusted to log scale. Cocultivation of H9 and H9IIIB cells resulted in significant increases in numbers of apoptotic nuclei (Fig. 4A, lower panel), which were absent when the cells were mixed (Fig. 4A, upper panel). Time course studies (Fig. 4B) revealed that apoptotic nuclei were present after 18 to 24 h and increased in number over the 72-h cocultivation period; 24% of the nuclei assayed by this method were apoptotic at 24 h after coculture. The delay in the appearance of condensed nuclei detected by fluorescence-activated cell sorter (FACS) analysis compared with that detected by histologic staining may be attributed to the time necessary for fragmentation of the genome to sub-2N levels for the FACS-based detection.

DNA fragmentation induced by cocultivation could be inhibited by preincubating cells for 20 h with 10 μM ZnSO4, an agent known to prevent DNA fragmentation by a variety of inducers (Fig. 4C). Addition of ZnSO4 at the time of cocultivation did not prevent apoptosis (data not shown), suggesting that the rate of apoptosis induced by cocultivation was more rapid than the rate of inhibition by Zn2+. Preincubation of H9 and H9IIIB cells with other agents reported to inhibit apoptosis, such as N-acetyl cysteine (10 mM) and cyclosporin A (5 μg/ml), did not prevent apoptosis due to cell-to-cell virus transmission (Fig. 4C).

DNA content analysis was also performed to investigate the induction of apoptosis in the 12D7 clone of the CEM T-cell leukemia cell line. Because of the aneuploid nature of lymphoblastic cell lines (14), the DNA contents of H9IIIB and 12D7 cells are different. As a result, the DNA histograms of H9IIIB and 12D7 cells were not identical and the positions of the peaks representing the G0/G1 and G2/M phases for H9IIIB were distinct from those for 12D7 (Fig. 5; compare 12D7 with H9IIIB). Nevertheless, at 48 h following coculture, a broad hypodiploid peak similar to that obtained with H9-H9IIIB cocultivations was detectable; decreases in G0/G1 and G2/M peaks for both 12D7 and H9IIIB cell nuclei indicated that apoptosis had occurred for both 12D7 and H9IIIB nuclei (Fig. 5, 12D7 + H9IIIB). The presence of apoptosis was confirmed by identifying oligonucleosome-sized fragments upon analysis of unintegrated DNA from H9IIIB-12D7 cultures (data not shown). Similar DNA fragmentation was observed upon cocultivation of H9IIIB cells with Jurkat cells, and apoptosis was detected by using as donor cells H9 cells chronically infected with the HIV infectious molecular clones pNL4-3 and pNL4-3U35 (pjuiv) and an HIV molecular clone, pALA-5, with slow growth kinetics (data not shown). These results demonstrated that induction of apoptosis was not restricted to H9 as the recipient cell type or to HIV-1imm as the source of the virus.

Env-CD4 interactions, but not new HIV replication, were required for induction of apoptosis. To investigate whether new HIV DNA replication was necessary for apoptosis to occur in this system, parallel cultures were treated with 10 μM zidovudine (AZT). As shown in Fig. 6, analysis of cocultivations revealed no decreases in the relative amounts of apoptotic nuclei in cultures treated with AZT prior to coculture (Fig. 6A; compare +AZT with control). The apoptosis observed in the presence of AZT was not a toxic effect of the drug; as shown in Fig. 6A, treatment of H9 or H9IIIB cells with 10 μM AZT revealed no evidence of drug-induced apoptosis. The presence of oligonucleosome-sized DNA fragments in analyses of unintegrated DNA confirmed the presence of apoptosis in AZT-treated cultures (Fig. 6B). Analyses of unintegrated DNA obtained from H9-H9IIIB cocultures treated with 10 μM AZT revealed no HIV DNA, confirming previously
published observations (47) that AZT abolished new HIV DNA synthesis in this system (data not shown). These results indicate that synthesis of HIV DNA was not required for apoptosis.

To determine whether Env-CD4 interactions were involved in induction of apoptosis, we investigated the ability of the inhibitor of HIV transmission soluble CD4 (sCD4) to prevent apoptosis in the cell-to-cell transmission model. Preincubation of donor and recipient cells with sCD4, which prevented CD4-Env interactions, cell-cell fusion, and HIV replication (47), also prevented induction of programmed cell death. As shown in Fig. 6A, no increases in numbers of apoptotic nuclei were detected at 48 h after mixing of H9 and H9IIIB cells in the presence of sCD4. These findings suggest that Env-CD4 interactions were necessary for induction of apoptosis. In control experiments, no increases in apoptosis were detected in cultures of H9IIIB cells treated with sCD4 (Fig. 6A), suggesting that CD4 alone cannot trigger this signal in H9IIIB cells.

**Marked delay in induction of apoptosis by cell-free HIV infections.** The rapid induction of apoptosis caused by cell-to-cell transmission of HIV was inhibited by sCD4, suggesting that Env-CD4 interactions triggered the apoptosis pathway. We investigated whether similar rapid induction of apoptosis occurred when cell-free HIV virions were used as a source of
Env protein. The kinetics of induction of apoptosis in cell-to-cell and cell-free HIV infections were compared; 2 × 10⁵ H9 cells were infected with cell-free HIV or chronically infected H9IIIB cells. As shown in Fig. 7A, induction of apoptosis in cell-free HIV infections was markedly delayed compared with that initiated by cell-cell transmission; with concentrated cell-free HIV corresponding to a multiplicity of infection of approximately 0.2, apoptotic nuclei were undetectable until 66 to 88 h after cell-free infections were initiated. As shown in the Western blot in Fig. 7B, the relative amount of inoculum HIV protein used in the cell-free infection was greater than that used in the cocultivation. The amount of cell-free virus (Fig. 7, lane 1:3) represents one-third of the virus inoculum used to infect H9 cells and is approximately equivalent to that present in 3.9 × 10⁵ to 7.8 × 10⁵ H9IIIB cells, which is 8- to 15-fold greater than the number of cells actually used in the parallel cocultivation experiment (5 × 10⁴ H9IIIB cells were used to infect 2 × 10⁵ H9 cells). Thus, despite the presence of 8- to 15-fold more virus protein in the cell-free inoculum, the peak of apoptosis was delayed by 70 h compared with that for the cocultures (Fig. 7A). These data demonstrate that cell-to-cell HIV transmission was much more efficient in inducing apoptosis than were cell-free virions, and they suggest that during in vitro infections with HIV, the apoptosis observed is due primarily to cell-to-cell HIV transmission.

Since apoptosis was not observed in cell-free infections until after one round of HIV replication, a possible explanation for the delay in the kinetics of apoptosis is that programmed cell death is exclusively associated with cell-to-cell transmission and that it was observed in cell-free infections only after the first round of HIV replication, when cell-to-cell HIV transmission takes place. To investigate whether HIV-induced apoptosis could occur in cells infected with virions in the absence of cell-to-cell HIV spread, we initiated infections of H9 cells and blocked cell-to-cell transmission of HIV by the addition of sCD4 to the medium at 2 h after infection. As shown in Fig. 7C, addition of sCD4 after the initial HIV infection did not prevent apoptosis following a cell-free HIV infection. Apoptotic nuclei were detectable at 130 h after infection in the presence or absence of sCD4, although the relative numbers of apoptotic nuclei in sCD4-treated cells were reduced. Light microscopic examination of May-Grunwald-Giemsa-stained cells confirmed the presence of single cells undergoing apoptosis (data not shown). These findings indicate that HIV induced apoptosis even when cell-to-cell transmission was blocked, suggesting that infection by HIV virions was a sufficient, albeit inefficient, inducer of apoptosis.

DISCUSSION

The process of programmed cell death is critical in executing structural changes in development and metamorphosis and in balancing cell populations in multicellular organisms (7). Apoptosis has also been identified as a pathologic response to virus infections by members of the retrovirus, orthomyxovirus, iridovirus, togavirus, picornavirus, parvovirus, chicken anemia virus, herpesvirus, and adenovirus families (5, 11, 17, 22, 27, 35–37, 39, 42, 53, 56). Apoptosis has been reported to occur in HIV-infected cells, but the relationship between HIV replication and the induction of cell death is unclear. We investigated the mode and tempo of cell death produced by synchronous cell-to-cell HIV transmission, for which the kinetics of HIV replication are well defined (8, 47). We found that apoptosis occurred rapidly after coculture of chronically infected and uninfected cells, and a variety of techniques provided complementary data regarding the process of programmed cell death. Microscopic studies detected chromatin condensation in single nuclei, were extremely sensitive in identifying the first nuclei undergoing apoptosis (2 to 8 h), and demonstrated that apoptosis occurred in single cells and in cell fusions; FACS analyses permitted analyses of entire populations and provided approximate quantitations of the extent of apoptosis. Analyses of unintegrated DNA by gel electrophoresis demonstrated that apoptosis proceeds to degradation of DNA into oligonucleo-

![Figure 5](https://jvi.asm.org) Induction of apoptosis in 12D7 cells. 12D7 cells and H9IIIB cells were cocultivated at a ratio of 1:4 for 48 h, and apoptosis was detected by FACS DNA content analysis. Data are presented in linear scale to demonstrate differences in positions of G₀/G₁ and G₂/M peaks for the two cell lines. The log scale demonstrates the appearance of apoptotic nuclei (arrow) after cocultivation.

![Figure 6](https://jvi.asm.org) Effects of sCD4 and AZT on induction of apoptosis. (A) H9 and H9IIIB cells were preincubated in 10 μg of sCD4 per mL, 10 μM AZT, or media for 1 h prior to cocultivation. Cells were harvested at 48 h after cocultivation, nuclear DNA content was quantitated by FACS analysis, and the percentages of total nuclei present in the apoptotic fraction are presented. (B) H9 and H9IIIB cells were treated in the presence or absence of 10 μM AZT as described for panel A; unintegrated DNA prepared from each of the cultures was electrophoresed in 1% agarose gels and stained with ethidium bromide. M is the 1-kb marker described in the legend to Fig. 3.
somal DNA fragments. Although Env-CD4 interactions were required for apoptosis to occur and were blocked by sCD4, no new HIV replication was necessary. These findings support those of Laurent-Crawford and coworkers, who identified Env-mediated induction of apoptosis in cells containing vaccinia virus-expressed Env and CD4 (26), and are consistent with a model in which HIV Env protein cross-links CD4 on the cell surface and triggers apoptosis upon HIV transmission. These findings do not rule out other mechanisms of HIV-induced cell death which rely on virus replication, but they do suggest that these additional mechanisms may take place in cells already committed to cell death.

An unexpected finding of these studies was the marked inefficiency of the induction of apoptosis in cell-free infections compared with the induction in cell-to-cell HIV infections. Differences could not be explained by the amount of HIV protein in virus preparations compared with that in infected cells used to transmit the infection, and they were unlikely to be due to cell-cell fusion alone, since many single cells were observed undergoing apoptosis within 24 to 48 h in cell-cell, but not cell-free, cultures. It is possible that the concentration of Env protein at the apposition of donor and recipient cell membranes is much greater than any ever achieved by points of contact between HIV virions and recipient cell membranes or that it is much more effective in CD4 cross-linking, thereby generating a more potent apoptosis signal. In this regard, the virion inoculum may have downregulated the CD4 receptor and reduced the ability of these cells to become infected, perhaps explaining the reduced amount of apoptosis observed in virion infections in the presence of sCD4 (Fig. 7C). Alternatively, interaction of other cell surface proteins not present on virions may enhance the induction of apoptosis. Nevertheless, the greater efficiency of apoptosis induction by cell-to-cell transmission suggests that during the course of in vitro HIV infections, observed apoptosis is likely to be the product of cell-to-cell HIV transmission.

Studies reported here and elsewhere demonstrate a central role for Env-CD4 interactions in initiating apoptosis, but the pathway of programmed cell death subsequent to the Env-CD4 interaction has not been elucidated. Multiple independent pathways culminating in apoptosis have been identified (7, 45, 51), and it is unclear whether HIV-induced cell death occurs by a known triggering mechanism or by a pathway yet to be defined. Whether HIV proteins other than Env participate in, or modulate, the apoptotic process is unknown; recent reports suggest that expression of the HIV and human T-cell leukemia virus type 1 transactivator proteins Tat and Tax, respectively, may protect (59), accelerate (55), or induce apoptosis (28, 58).
In our cocultivation experiments, some cell fusions showed marked degenerative changes in nuclear appearance within 2 to 8 h of coculture, while others contained nuclei that appeared normal even after 32 h of cocultivation (Fig. 1). In addition, HIV infections of H9 cells characteristically yield survivor cells chronically expressing infectious virus without apoptosis, demonstrating that some infected cells can escape cell death. We are presently investigating the expression of viral and cellular genes involved in apoptosis in the rapidly apoptotic and the nondegenerating nuclei to characterize the pathway of apoptosis occurring after HIV infection.

One consequence of rapid induction of apoptosis is that the expression of HIV RNA and protein occurs in cells undergoing marked changes in nuclear organization. It is not clear how nuclear destruction affects the functions of HIV proteins such as Tat, Rev, and Vpr. Tat and Rev have been associated with intact nucleoli, which are destroyed early in the apoptotic process. It will be of interest to determine whether the transactivating functions of Tat and the splicing and RNA transport activities attributed to Rev (20) still occur in nuclei undergoing programmed cell death.

CD4+ T-cell depletion remains a key unexplained characteristic of acquired immunodeficiency, which predisposes the patient to lethal infectious and neoplastic complications of AIDS and for which no therapy is effective. Programmed cell death has been postulated as a potential mechanism for CD4+ T-cell loss in HIV-infected persons (15, 33, 43, 52) because lymphocytes from infected persons undergo accelerated apoptosis after cultivation in vitro. It is of interest that no accelerated apoptosis was detected in lymphocytes cultured from HIV-infected chimpanzees, animals which become viremic but show no CD4 T-cell decline and no disease progression after HIV infection (16, 48). No correlation between susceptibility to apoptosis in vitro and CD4 decline or AIDS disease progression has been observed (34), and apoptosis has been observed in peripheral blood mononuclear cells cultured from patients with chronic illnesses other than HIV infection, including leukemia and systemic lupus erythematosus (9, 21, 46), suggesting that accelerated programmed cell death ex vivo may represent a nonspecific defect in peripheral blood mononuclear cell longevity or a result of imperfect cultivation conditions and not a specific pathologic consequence of HIV infection. Recently, apoptotic cells have been identified in lymph nodes of HIV-infected children and simian immunodeficiency virus-infected primates, primarily among bystander cells and not virus-infected cells (13). Our finding that infected cells efficiently and rapidly induce apoptosis following cell-to-cell transmission of HIV suggests apoptosis may contribute to progressive lymphocyte depletion within organs such as lymph nodes, in which relatively few infected cells are present (44) but extensive cell-cell interactions are possible. In addition, direct T-cell killing has been inferred in explaining CD4 cell decline in HIV infection and the recovery of CD4 cells after treatment with HIV protease inhibitors (18, 54). In this regard, we have detected rapid induction of apoptosis after cocultivation of peripheral blood mononuclear cells from uninfected donors with chronically infected H9 cells (unpublished data), demonstrating that HIV-associated apoptosis is efficiently induced in lymphocytes as well as in T-cell lines. By continuing to characterize the multiple mechanisms that result in the elimination of CD4+ cells, including apoptosis, we may identify novel approaches to prevent lymphocyte depletion and prolong the disease-free period following the initial exposure to HIV.

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