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Chronically HIV-1-Infected Monocytic Cells Induce Apoptosis in Cocultured T Cells

Houchu Chen,* Y. K. Yip,† Italas George,* Max Tyorkin,* Erez Salik,* and Kirk Sperber2*

We have previously developed a human macrophage hybridoma model system to study the effect of HIV-1 infection on monocytic function. Upon coculture of one chronically (35 days postinfection) HIV-1-infected human macrophage hybridoma cell line, 43HIV, there was a dose-dependent decrease in the viability of cocultured Ag-stimulated T cells associated with an increase in DNA strand breaks. Enhanced apoptosis was determined by labeling with biotinylated UTP and propidium iodide, increased staining with annexin V, increased side light scatter and expression of CD95, and decreased forward light scatter and expression of Bcl-2. There was also increased DNA strand breaks as determined by propidium iodide staining in unstimulated T cells cocultured with 43HIV and in T cells stimulated with anti-CD3 mAb and PHA. Pretreatment with 5145, a human polyclonal anti-gp120 Ab that recognizes the CD4 binding region, as well as with an anti-Fas ligand mAb blocked apoptosis in CD4+ T cells but not in CD8+ T cells. A soluble factor with a Mr below 10,000 Da was defined that induced apoptosis in CD4+ and CD8+ T cells and B cells. SDS-PAGE analysis of the active fractions revealed a band of 6000 Da that, after electroelution, had proapoptotic activity. The pi of the activity was estimated to be between 6.5 and 7.0. In conclusion, chronically HIV-1-infected monocytic cells induce apoptosis in bystander-, Ag-, anti-CD3-, and mitogen-stimulated T cells by multiple factors, which may contribute to the depletion of lymphocytes induced by HIV-1. *The Journal of Immunology, 1998, 161: 4257–4267.

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Materials and Methods

Human macrophage hybridomas

Human macrophage hybridomas were obtained by fusing monocytes to mature into macrophages in Teflon
bag cultures) with a hypoxanthine-guanine phosphoribosyl transferase-deficient promonocytic line (U937) as previously described (30). We have uniformly infected and characterized one clone, 43, with HIV-1 (43HIV) (33).

**Monocyte isolation**

Mononuclear cells were separated from buffy coats obtained from normal healthy volunteers by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. The cells were washed three times with sterile PBS and resuspended in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS (Life Technologies, Grand Island, NY). Human macrophage hybridoma cell lines or with HIV-1 BaL-infected and uninfected monocytes (10 3 -10 5 ), and either uninfected monocytes and then assessed for the induction of apoptosis. In other experiments, T cells were incubated with varying concentrations of irradiated (cesium source) 43 cells, PHA (0.01–1 μg/ml) (Sigma, St. Louis, MO) or the anti-CD3 mAb 446 (1 μg/ml) (Leu 4, Becton Dickinson, Mountain View, CA) for 24, 48, and 72 h. The cells were then analyzed by flow cytometry to measure costaining of the mAbs with annexin V FITC-labeled annexin V, a phospholipid binding protein of the annexin family, was used to measure apoptosis with a commercially available kit (Coulter, Hialeah, FL). After incubating the HIV-1-infected human macrophage hybridomas with PHA, the cell samples were washed with ice-cold PBS followed by staining with FITC-phycocerythrin-labeled anti-CD3 mAb for 10 min in the dark. The cells were then analyzed by flow cytometry for DNA strand breaks and changes in cell size associated with apoptosis

**HIV-1 infection**

Monocytes or 43 cells were infected with HIV-1 from different clones (34, HIV-1ADA (35), HIV-1BaL (36), and HIV-1nat (37)) as previously described (31, 33). These reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD). Dilutions of HIV-1 containing supernatant standardized to contain reverse transcriptase activity of 80,000 cpm/ml were incubated for 90 min following by three washes with PBS.

**Ag-, mitogen-, and anti-CD3-induced apoptosis**

Clone 43 cells, 43HIV, and HIV-1-infected and uninfected monocytes were used as accessory cells in Ag-, mitogen-, and anti-CD3-induced T cell apoptosis. For these experiments, T cells were obtained from negative controls and were monocyte-depleted T cells (10 5 ) were cocultured with varying concentrations of irradiated (cesium source) 43 cells, PHA (0.01–1 μg/ml) (Sigma, St. Louis, MO) or the anti-CD3 mAb 446 (1 μg/ml) in 0.2 ml of CM at 37°C in a 5% CO 2 incubator for 24, 48, and 72 h. The cells were then analyzed by flow cytometry to measure costaining of the mAbs with annexin V.

**Isolation of purified CD4 and CD8 populations**

Purified CD4 + and CD8 + populations were isolated using Ab-coated plates. Primary T cells were first separated by rosetting with neuraminidase-treated SRBC by previously established methods (38). Anti-mouse Ig (Accurate Antibodies, Westbury, NY) was diluted in PBS to a concentration of 100 μg/ml and coated onto a 100-mm plate (Nunc, Naperville, IL) at 4°C overnight followed by three PBS washes. T cells were incubated either with anti-CD4 or anti-CD8 mAb for 45 min at 4°C followed by three PBS washes and then allowed to settle onto the coated plates for 30 min at room temperature (39). The nonadherent cell population was removed, and the purity of the isolated population was assessed by flow cytometry as described below. Ninety percent of the isolated cells expressed either CD4 or CD8.

**B cell isolation**

PBMC were isolated from leucocyte concentrate packs obtained from normal blood donors at the Mount Sinai Blood Bank (New York, NY) as previously described (30). T and non-T cells were separated by SRBC rosetting, and non-T cells were depleted of monocytes by plastic adherence yielding B cell-enriched populations (38).

**Detection of DNA strand breaks and side and forward light scatter associated with apoptosis**

MHC-matched T cells were cocultured with HIV-1-infected and uninfected human macrophage hybridoma cell lines or with HIV-1infected and uninfected monocytes and then assessed for the induction of apoptosis. In other experiments, T cells were incubated with different concentrations of UV-irradiated supernatant (180 min in a 280-nm UV transilluminator) from HIV-1infected human monocytes or 43HIV, and then assessed for the induction of apoptosis. The cell-free supernatants were collected from chronically infected cells (4 wk after infection) and used fresh at varying concentrations. The cells were washed in sterile PBS three times and suspended in cacodylate buffer consisting of 0.2 M potassium, 25 mM Tris-HCl buffer (pH 6.6), 2.5 mM cobalt chloride (CoCl2), 0.25 μg/ml BSA, 100 μM terminal deoxynucleotidyl transferase, and 0.5 mM biotin for 30 min at 37°C. The cells were then fixed in 1% formaldehyde for 25 min at 4°C, washed in PBS, and re suspended in 100 μl of sodium citrate buffer consisting of 2.5 μg/ml fluorescein avidin, 0.1% Triton-X, and 5% (w/v) non-fat dry milk for 30 min at 25°C in the dark. The cells were then stained with propidium iodide (5 μg/ml propidium iodide and 0.1% RNase A) and analyzed by flow cytometry for DNA strand breaks and changes in cell size.

**Annexin V**

FITC-labeled annexin V, a phospholipid binding protein of the annexin family, was used to measure apoptosis with a commercially available kit (Coulter, Hialeah, FL). After incubating the HIV-1-infected human macrophage hybridomas with PHA, the cell samples were washed with ice-cold PBS followed by staining with FITC-phycocerythrin-labeled anti-CD3 mAb and analyzed by flow cytometry for 10 min in the dark. The cells were then analyzed by flow cytometry to measure the apoptosis assay or were precipitated with 10% TCA in preparation for SDS-PAGE analysis and silver staining.

**Fractionation of the monocytic supernatant**

Molecular sizing of the 43, 43HIV, monocyte, and HIV-1 infected monocyte supernatants was conducted on a Pharmacia Sepharcl SY-100 column using 0.5 mL elution fractions of cation exchange concentrate (Amicon, Danvers, MA; M cut-off, 500 Da) in 50 mM NaPO4 and 0.15 M NaCl, pH 7.0, as a buffer. One-milliliter fractions were collected from the column, and protein content was determined by absorbance at A280. The fractions were tested in the apoptosis assay or were precipitated with 10% TCA in preparation for SDS-PAGE analysis and silver staining.

**Western blot analysis**

Proapoptotic fractions from the Sepharcl SY-100 column were run on a 10% SDS-PAGE with 500 mA constant current for 4 h. The proteins were transferred onto a nitrocellulose membrane (Schleicher and Schuell, Keene, NH) in transfer buffer (20% methanol, 150 mM glycine, and 25 mM Tris, pH 8.3). After transfer, the nitrocellulose membrane was blocked with 5% milk in PBS, incubated for 2 h at room temperature with polyclonal anti-HIV serum (provided by Dr. A. Pinter) (41). After washing five times in washing buffer (0.05% Tween-20 in PBS), secondary Ab was added for 2 h (2 mg/ml; horseradish peroxidase-conjugated goat anti-human IgG, Cappel Laboratory, Durham, NC). After washing five times,
the membrane was developed by chemiluminescence (ECL, DuPont, Wilmington, DE) (32).

Electroelution of proapoptotic activity
In some experiments proapoptotic fractions were run on a 12% nondenaturing polyacrylamide gel, cut into 3-mm sections, and eluted for 30 min in 1 ml of buffer. The eluates were then used in the apoptosis assays described above (42).

Reverse phase HPLC analysis
Reverse phase HPLC was performed on the proapoptotic fractions obtained from the Sephacryl S-100 column using a C4 (4.6 × 250 mm) column. Elution of bound proteins was developed using a linear gradient of 0.1% (v/v) trifluoroacetic acid in water and 70% (v/v) acetonitrile in 1% trifluoroacetic acid. A gradient volume of 60 ml was developed at a flow rate of 1 ml/min. Elution profiles were monitored at an absorbance of 215 nm. Solvent in the protein-containing fractions was removed by vacuum centrifugation in a Speed-Vac (Savant, Piscataway, NJ) (43).

Acetone precipitation
Acetone precipitation was conducted on crude supernatants from the 43 and 43HV cell lines. Acetone was chilled in an ice-salt bath to attain a temperature below 0°C. Proteins were fractionated from 43 and 43HV supernatants by precipitation in 50 and 95% (v/v) acetone sequentially. The precipitated proteins were collected by centrifugation, and the residual acetone in the precipitates was removed by vacuum centrifugation in a Speed-Vac (Savant).

Anion exchange chromatography
Anion exchange chromatography was performed with a Mono-Q HR 5/5 (5 × 50 mm) column on an FPLC system (Pharmacia). The elution gradient was developed using 20 mM Tris-HCl, pH 7.5 (buffer A), and NaCl in buffer A (buffer B) at a flow rate of 1 ml/min. Samples were prepared for anion exchange chromatography by exhaustive precipitation in the buffer A. The protein elution profile was monitored by absorbance at 280 nm (44).

Results
Chronically HIV-1-infected monocyte-depleted Ag-activated T cells
Our previous data demonstrated that chronically HIV-1-infected monocytes with other monocytotropic strains, including HIV-1BaL, induce apoptosis in bystander-, mitogen-, and anti-CD3-activated T cells (31). We investigated whether ongoing apoptosis by measuring DNA fragmentation and related parameters in stimulated MHC-matched T cells (10^5) were cocultured with different concentrations (10^3–10^5) of 43 and 43HV cells for varying periods (24, 48, and 72 h). There was a dose-dependent “left shoulder” in the DNA staining (hypodiploid cells; Fig. 1A) and a right shift in the UDP-biotin-stained DNA consistent with apoptosis. This diminished when the T cells were cocultured with decreasing concentrations of 43HV. There was no DNA fragmentation when the TT-stimulated T cells we cocultured with the uninfected 43 cells, and there was no DNA fragmentation in the 43HV cells (data not shown). Optimal results were obtained when the T cells were cocultured with 43HV for 48 h. We also assessed apoptosis by light scatter analysis and demonstrated a progressive increase in the side scatter and decreased forward scatter of TT-stimulated T cells cocultured with increasing concentrations of 43HV (data not shown). To confirm the induction of apoptosis, we stained the T cells with an anti-CD3 mAb and annexin V that has selective affinity for phosphatidylserine (45). Phosphatidylserine is exposed at the cell surface during early apoptosis and can be measured by annexin V staining in a variety of cell types (46). Consistent with the propidium iodide (PI), UDP-biotin, and light scatter analysis, the number of CD3^+ T cells staining with annexin V did increase when greater numbers of 43HV cells were added (Fig. 1B). No annexin V staining was observed in either the 43 or 43HV cells (data not shown). Increased surface expression of CD95 and reduced intracytoplasmic expression of Bcl-2 are also associated with apoptosis (6). To further assess the mechanism of induction of apoptosis in our system, we measured surface expression of CD95 and intracytoplasmic expression of Bcl-2 in the T cells cocultured with 43HV cells. Consonant with the DNA fragmentation experiments, the light scatter analysis, and the annexin V staining, there was reduced intracytoplasmic Bcl-2 staining (Fig. 1C). CD95 expression on the cocultured T cells was marginally increased. Multiple time points were determined, and reduced intracytoplasmic Bcl-2 expression was determined 16 h after coculture with 43HV.

Chronically HIV-1-infected human macrophage hybridomas induce apoptosis in bystander-, mitogen-, and anti-CD3-activated T cells
Bystander- and activation-induced apoptosis has been described in PBMC and lymph nodes of HIV-1-infected patients (8, 12). Since the 43HV cells induced apoptosis in the TT-stimulated T cells we next wanted to determine whether 43HV cells could also induce apoptosis in unstimulated bystander as well as T cells stimulated with mitogen and anti-CD3 mAbs. In these experiments, MHC-matched monocyte-depleted T cells were cocultured with 43 and 43HV cells and left unstimulated, while others were stimulated with phytohemagglutinin (PHA) and anti-CD3 mAb 446 (1 μg/ml). MHC-matched, uninfected T cells cocultured with uninfected 43 cells were also run as controls. Consistent with previously reported data, there was reduced production of gp120 in the 43HV cells induced apoptosis in the TT-stimulated T cells as well as T cells stimulated with mitogen and anti-CD3 mAbs. We next wanted to determine whether 43HV cells could also induce apoptosis in unstimulated bystander as well as T cells stimulated with mitogen and anti-CD3 mAbs. In these experiments, MHC-matched monocyte-depleted T cells were cocultured with 43 and 43HV cells and left unstimulated, while others were stimulated with PHA and anti-CD3 mAb 446 (1 μg/ml). MHC-matched, uninfected T cells cocultured with uninfected 43 cells were also run as controls. Consistent with previously reported data, there was reduced production of gp120 in the 43HV cells induced apoptosis in the TT-stimulated T cells as well as T cells stimulated with mitogen and anti-CD3 mAbs. We next attempted to block apoptosis in purified populations of CD4^+ T cells cocultured with 43HV cells by pretreating the 43HV cells with 5145, a human anti-gp120 Ab

The gp120 induces apoptosis in CD4^+ T cells cocultured with 43HV

Engagement of CD4 by gp120 resulting in aberrant T cell signaling has been implicated in the induction of apoptosis in CD4^+ T cells (14–16, 47). Since gp120 produced by virus may be playing a role in the 43HV cells, we first labeled the 43HV cell line with [35S]cysteine and [35S]methionine and immunoprecipitated gp120 to determine the amount produced by the 43HV cells compared with other HIV-1 proteins. The replication pattern of HIV-1 in macrophages is different from that in T cells. Consistent with previously reported data, there was reduced production of gp120 in the 43HV cells compared with other HIV-1 proteins, including p24 (Fig. 3A) (48). This is illustrated in the lane using the anti-HIV Ig, where only p24 is immunoprecipitated. We next attempted to block apoptosis in purified populations of CD4^+ and CD8^+ T cells by pretreating the 43HV cells with 5145, a human anti-gp120 Ab
that recognizes the CD4 binding site (49). Apoptosis was blocked with 5145 in the purified CD4\(^+\) T cell populations but not in the purified CD8\(^+\) T cell populations (Fig. 3B). The induction of apoptosis in the CD8\(^+\) T cells cocultured with 43\(_{HIV}\) was an unexpected finding in our system. However, in the lymph nodes of HIV-1-infected patients, apoptosis is not only restricted to CD4\(^+\) T cells but occurs in CD8\(^+\) T cells and in B cells as well (50, 51).

**FasL expression induces apoptosis in the cocultured T cells**

Fas-FasL interactions effectively induce apoptosis in T cells (52, 53). Up-regulation of FasL expression, which occurs in monocytes after HIV-1 infection and after cross-linking of CD4, has been proposed as a possible mechanism for bystander T cell death (28, 29). We performed surface immunofluorescence studies for FasL on the 43\(_{HIV}\) cells at different time points after HIV-1 infection to determine whether there was any potential role for FasL in the induction of apoptosis in our system. There was a modest increase in FasL expression 3 wk after infection (8 vs 22%) in the 43\(_{HIV}\) cells compared with the uninfected 43 cells (Fig. 4A). We next pretreated the 43\(_{HIV}\) cells with anti-FasL Ab, attempting to block apoptosis. The anti-FasL Ab blocked apoptosis in the purified CD4\(^+\) T cell population but not in the CD8\(^+\) T cells (Fig. 4B).
Accessory cell dysfunction may also lead to defective T cell activation, resulting in apoptosis rather than T cell proliferation. Abnormalities in expression of costimulatory molecules on accessory cells may impair T cell signaling (18, 54). To determine whether HIV-1 infection affects the expression of costimulatory molecules required in T cell activation, we performed surface immunofluorescence staining for LFA-1, LFA-3, CD80, and CD86 on 43 and 43 HIV cells at different time points. There was no difference in

![Image of Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** 43 HIV cells can induce apoptosis in bystander-, mitogen-, and anti-CD3-stimulated T cells. MHC-matched monocyte depleted T cells were cocultured with 43 and 43 HIV cells and either were left unstimulated or were stimulated with PHA (1 μg/ml) or the anti-CD3 mAb 446 (1 μg/ml). The DNA was stained with propidium iodide and analyzed by flow cytometry after 48 h. The dashed lines represent T cells cocultured with 43 HIV, while the solid lines are T cells alone. A0 is the subdiploid fraction. The results are representative of an experiment repeated three times.

HIV-1 infection affects the expression of costimulatory molecules required in T cell activation, we performed surface immunofluorescence staining for LFA-1-α, LFA-3, CD80, and CD86 on 43 and 43 HIV cells at different time points. There was no difference in

| Table I. Induction of apoptosis by HIV-1BaL-infected monocytes |
|-------------------|----------------------|------------------------|----------------------|----------------------|
| Unstimulated Medium (%) | PHA T + M/T + M<sub>HV</sub> | 446 T + M/T + M<sub>HV</sub> | TT T + M/T + M<sub>HV</sub> |
| T cells | T + M/T + M<sub>HV</sub> | 4.2/10.9 | 5.0/13.5 |
| 6.2 | 8.6/12.3 | 16.6 | 16.7 |
| 8.7 | 5.0/7.4 | 12.7/24.3 | 4.2/4.6 |
| 5.6 | 4.2/6.8 | 12.0/13.5 | 5.4/10.9 |
| 4.5 | 5.8/15.9 | 11.3/10.9 | 5.5/6.8 |
| 5.6 | 6.4/25.5* | 17.0/49.1* | 14.2/28.0* |
| 4.5 | 5.8/15.9 | 17.5/18.2 | 20.2/21.7 |
| 6.9 | 5.6/22.9 | 14.9/22.8 | 14.8/24.6 |
| 4.5 | 5.6/11.9* | 11.2/24.5* | 7.7/14.8* |
| 2.0 | 1.7/5.9* | 6.2/16.2* | 2.8/12.4* |
| 4.2 | 8.2/13.8* | 6.9/19.8 | 7.5/18.3* |

HIV-1BaL-infected and uninfected monocytes from nine separate monocyte preparations were cocultured with autologous monocyte-depleted PBMC and either left unstimulated or stimulated with PHA, 446, and TT and cultured for 5 days. Apoptosis was assessed by propidium iodide staining. Bystander and activation-induced apoptosis (**) was observed in four experiments. The values represent the percentages of subdiploid fraction (A0).
the surface expression of LFA-1-α, LFA-3, CD80, or CD86 in the 43 HIV cells compared with the uninfected 43 cells at all time points tested (data not shown).

Identification of a soluble proapoptotic factor

Since we identified apoptosis in CD8+ T cells that was not mediated by FasL or gp120, we looked for the production of a soluble proapoptotic factor. Using UV-irradiated supernatant from 43 HIV cocultured with purified populations of CD4+ and CD8+ T cells, it was possible to demonstrate a dose-dependent increase in DNA fragmentation (as determined by propidium iodide staining) in both the CD4+ and CD8+ T cell populations (Fig. 5A). The supernatant-induced apoptosis was not blocked by the 5145 and anti-FasL Abs. The UV-treated supernatant also induced apoptosis in mitogen- and anti-CD3-stimulated T cells, and the proapoptotic activity was lost after treatment with pronase (data not shown). No DNA fragmentation was observed in the supernatant from the uninfected 43 cells (data not shown). Apoptosis of B cells has also been reported to occur in the lymph nodes of HIV-1-infected patients (50, 51). To determine whether the 43HIV supernatant had proapoptotic activity in these cells as well, we cocultured dilutions of UV-treated 43HIV supernatant with target B cell populations and assessed apoptosis by staining the DNA with propidium iodide. Similar to the data in the T cells, there was dose-dependent DNA fragmentation with increasing concentrations of UV-treated 43HIV supernatant (Fig. 5B). Consistent with the propidium iodide studies, there was also increased annexin V staining in the T cells cocultured with UV-treated 43HIV supernatant (data not shown).

We also sought to validate our findings in UV-treated supernatant from chronically HIVBaL-infected (14 days) peripheral blood monocytes. Similar to the supernatant data from the 43 HIV cells, there was a dose-dependent induction of DNA fragmentation in T cells cultured with different concentrations of UV-treated supernatants from four of the nine preparations of HIV-1BaL-infected monocytes (Table II).

Characterization of the proapoptotic activity

To more carefully characterize the proapoptotic activity in the 43HIV supernatant, we concentrated UV-treated supernatant from 43 HIV cocultured with purified populations of CD4+ and CD8+ T cells, and it was possible to demonstrate a dose-dependent increase in DNA fragmentation (as determined by propidium iodide staining) in both the CD4+ and CD8+ T cell populations (Fig. 5A). The supernatant-induced apoptosis was not blocked by the 5145 and anti-FasL Abs. The UV-treated supernatant also induced apoptosis in mitogen- and anti-CD3-stimulated T cells, and the proapoptotic activity was lost after treatment with pronase (data not shown). No DNA fragmentation was observed in the supernatant from the uninfected 43 cells (data not shown). Apoptosis of B cells has also been reported to occur in the lymph nodes of HIV-1-infected patients (50, 51). To determine whether the 43HIV supernatant had proapoptotic activity in these cells as well, we cocultured dilutions of UV-treated 43HIV supernatant with target B cell populations and assessed apoptosis by staining the DNA with propidium iodide. Similar to the data in the T cells, there was dose-dependent DNA fragmentation with increasing concentrations of UV-treated 43HIV supernatant (Fig. 5B). Consistent with the propidium iodide studies, there was also increased annexin V staining in the T cells cocultured with UV-treated 43HIV supernatant (data not shown).

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shown). Again to validate our findings in the 43 HIV cell line, we pooled and concentrated the supernatants from the HIV-1 BaL-infected primary monocytes with Amicon ultrafiltration (Mr cut-off, 500 Da) and passed it over the Sephacryl S-100 column sizing column. Proapoptotic activity was present in those fractions corresponding to a Mr, 10,000 Da (data not shown). No proapoptotic activity was present in the concentrated supernatant from the uninfected monocytes. We further characterized fractions 5 and 6 from the 43HIV supernatant by reverse phase HPLC analysis. We compared HPLC elution profiles of fractions 5 and 6 and demonstrated that unique fractions that had proapoptotic activity were present in the 43HIV supernatant but not in the 43 supernatant. We then attempted to isolate proapoptotic activity by precipitation with acetone. Pro-apoptotic activity could not be precipitated with acetone at a concentration <80% saturation, which is a characteristic that is observed with smaller peptides. Results from peptide binding to anion exchange matrixes at different pH values indicated that the pI of the proapoptotic activity is estimated to be between 6.5 and 7.0.

**Discussion**

Although apoptosis is an important factor in the T cell depletion that occurs during the course of HIV-1 infection, it is unclear how this process occurs. Cross-linking of CD4 by gp120, involvement of cytokines, superantigen activity encoded by HIV-1 proteins, and aberrant accessory cell function have all been proposed as potential mechanisms to account for the induction of apoptosis (6). Recent evidence, however, underscores the importance of accessory cells, including dendritic cells and monocytes, as effector cell populations in inducing lymphocyte apoptosis in AIDS (22–29, 57, 58). In chimpanzees, in which HIV-1 is unable to infect monocytes, the persistent infection in T cells occurs without the development of T cell apoptosis (24–27). In the hu-PBL-SCID mouse
inducing apoptosis (31). Ag-, anti-CD3-, and mitogen-stimulated T cells as well as unstimulated bystander T cells underwent apoptosis when exposed to the chronically HIV-1-infected mononcytic cells (Figs. 1 and 4B). The induction of apoptosis by the primary HIV-1-infected monocytes was more variable than that observed with the chronically HIV-1-infected monocytes, which may relate to the different rates of infection with HIV-1 or the level of chronicity of the infection. In both systems, costimulatory mechanisms by which HIV-1-infected monocytes induced apoptosis included expression of gp120 and FasL and production of a proapoptotic factor. Interestingly, anti-FasL Abs did not abrogate the increased surface expression of the costimulatory molecules CD95L and Fas on 43 HIV cells after infection. Defective interactions between costimulatory molecules on APCs, especially the B7 family of proteins, and T cells (CD28/CTLA-4) have been implicated in the induction of apoptosis (60). Anti-CD28 Abs may provide a rescue signal to block apoptosis in T cells of HIV-1-infected patients (8), while stimulation with anti-CTLA-4 Ab promotes apoptosis (54).

All these mechanisms may play a role in the induction of apoptosis in T cells in HIV-1-infected patients, although perhaps to varying degrees. The replication pattern of HIV-1 in monocytes is different from that in T cells (48). In monocytes the virus produces lesser amounts of gp120 and accumulates in intracytoplasmic vacuoles with relatively little shedding (61). The amount of gp120 exposed on the cell surface needed to engage CD4 and induce apoptosis is reduced compared with that in T cells (Fig. 3A). Furthermore, in our system gp120 would only account for apoptosis in the CD4+ T cells, not in CD8+ T cells (Fig. 4B). Increased surface expression of FasL on 43 HIV cells induced apoptosis in the cocultured bystander T cells (Fig. 4A). However, in line with the published results of others, FasL-mediated apoptosis occurred in CD4+ T cells but not in CD8+ T cells (62) (Fig. 4B). In our system, apoptosis of CD4+ T cells could be explained by gp120 and FasL expression but not in CD8+ T cells and B cells. Even though the anti-gp120 and anti-FasL Abs blocked apoptosis in CD4+ but not in CD8+ T cells, the proapoptotic factor induced apoptosis in both CD4+ and CD8+ T cells (Fig. 5A). In these experiments the supernatant is from cells continuously growing in culture, so that the concentration of the proapoptotic factor is higher than that in the blocking studies. The interaction between gp120 and FasL, and the proapoptotic factor is uncertain. There may also be differences in signaling pathways. The anti-gp120 and anti-FasL Abs may suppress the production or release of the proapoptotic factor from 43 HIV cells.

The proapoptotic factor produced by 43 HIV induced apoptosis in CD4+ and CD8+ T cells as well as B cells (Fig. 5, A and B). We...
could also identify proapoptotic activity in the supernatants from four preparations of HIV-1-BaL-infected primary monocytes 2 wk after infection (Table II). When we fractionated the pooled supernatant from 43HV cells and primary HIV-1-BaL-infected monocytes, we could identify proapoptotic activity in fractions corresponding to $M_r < 10,000$ Da (Fig. 6). In other studies describing the effect of monocytes on the induction of apoptosis in cocultured T cells, no soluble factor was identified (29, 57–59). In these studies monocytes were treated with PMA, macrophage CSF, or anti-CD4 mAb, but not infected with HIV-1. Our preliminary analysis suggests that this factor is a peptide (since its activity is lost after pronase treatment) that does not appear to be HIV-1 derived and can induce apoptosis not only in bystander T cells but also in mitogen-, antigen-, and anti-CD3-activated T cells. Since the low $M_r$ peptide induced apoptosis in a variety of cell types, the production of this factor by HIV-1-infected monocytes may contribute to the generalized state of apoptosis for CD4$^+$, CD8$^+$ T cells and B cells that has been described in HIV-1-infected patients (7, 12, 13). In lymph nodes, apoptosis is related to a general state of immune activation but not viral load or stage of disease (52). It is uncertain what role this peptide has in the induction of apoptosis in HIV-1-infected individuals, and studies are presently underway to define this factor biochemically and to measure levels in HIV-1-infected individuals and PBMC at different stages of disease.

There is precedence for the concept of macrophage-derived proapoptotic factors. Macrophages have been reported to produce proapoptotic cytokines as well as apoptosis promoting low $M_r$ molecules, such as reactive oxygen molecules, PGs, and nitric oxide (6). After HIV-1 infection, there is increased production of proinflammatory cytokines, including IL-6, IL-8, and TNF-$\alpha$ (63). In HIV-1-infected individuals, this cytokine imbalance may contribute to apoptosis. Both TNF-$\alpha$ as well as IFN-$\gamma$ promote apoptosis (64–70). Recent evidence also suggests that two other predominantly macrophage-derived cytokines, IL-10 and IL-12, are involved in the regulation of apoptosis. IL-10 promotes apoptosis, while IL-12 prevents it (70). We have demonstrated that HIV-1 infection of the human macrophage hybridomas induces IL-6, IL-8, and IL-10 production along with loss of IL-1 and IL-12 production (31, 32). There may be synergy in the induction of apoptosis between the cytokines induced by HIV-1 infection and the low $M_r$ proapoptotic factor.

The transduction of a proapoptotic signal at least through Fas involves the activity of proteases, including IL-1$\beta$-converting enzyme (71, 72). It has also been possible to block apoptosis in vitro in murine systems (73) and in PBMC of HIV-1-infected individuals by treatment with protease inhibitors (11). Similarly, IL-2 has been reported to inhibit apoptosis in PBMC from HIV-1-infected patients by increasing intracytoplasmic levels of Bcl-2 (74). Although T cells cocultured with the 43HV cells had decreased Bcl-2 corresponding to a $M_r < 10,000$ Da. The dashed lines represent unstimulated T cells cocultured with 43HV supernatant, and the solid lines are T cells alone. $A_o$ is the subdiploid fraction. These results are representative of an experiment repeated three times. C. Fractions 5 and 6 were electrophoresed on a 10% polyacrylamide gel followed by silver staining. These results are representative of an experiment repeated three times. D. Proapoptotic activity was eluted from a nondenaturing SDS-PAGE gel of fractions 5 and 6. Proapoptotic activity, as determined by PI staining in unstimulated target T cells, was detected in 3-mm gel slices corresponding to different $M_r$ (29, 15, and 6 kDa). The dashed lines represent T cells cocultured with the electroeluted gel slices, while the solid lines represent T cells alone. $A_o$ is the subdiploid fraction. These results are representative of an experiment repeated three times.
expression (Fig. 1C) and some increase in FasL expression, there was no change in either CD95 or Bel-2 expression in T cells cocultured with the low M<sub>f</sub> factor. It remains to be determined whether apoptosis induced by this peptide uses pathways that can be blocked with IL-1β-converting enzyme protease inhibitors and IL-2.

In conclusion, the human macrophage hybridoma cell lines induce apoptosis by multiple mechanisms, including gp120, FasL expression (Fig. 1C).


Letter of Retraction

We wish to retract the manuscript titled “Chronically HIV-1-Infected Monocytic Cells Induce Apoptosis in Cocultured T Cells” by Houchu Chen, Y. K. Yip, Italas George, Max Tyorkin, Erez Salik, and Kirk Sperber. *The Journal of Immunology*, 1998, 161: 4257–4267. The manuscript contains errors in the presentation of data in some of the figures.

Fig. 3B demonstrating the apoptotic effect of gp120 on CD4 and CD8 cells, Fig. 4B depicting the apoptotic effect of Fas-FasL interactions in CD4 and CD8 T cells cocultured with 43HIV cells, and Fig. 6B showing the apoptotic activity of fractionated supernatant from the 43HIV cell line are inaccurate. We published the corrected figures as errata in the December 15, 2005 issue of *The JI*. However, given the errors made in these figures, we wish to retract the manuscript.

We deeply regret these errors and the need to take this action.

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In Figure 1, panel C was omitted. The corrected figure is shown below. The error has been corrected in the online version, which now differs from the print version as originally published.

In the author line, the sequence of the first two authors is reversed. The corrected author line is shown below.

Krishnamurthy Malathi, Xiaogui Li, Olga Krizanova, Karol Ondrias, Kirk Sperber, Vitaly Ablamunits, and Thottala Jayaraman


The fourth author’s name, Cindy Banh, was omitted. The correct list of authors and affiliations is shown below.

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In Materials and Methods, in the first sentence under the heading Intranasal administration of recombinant adeno-virus-containing HO-1 cDNA, the source for adenoviral HO-1 cDNA was incorrectly attributed. The source is stated in the corrected sentence below.

Mice were anesthetized with methoxyflurane, and then 5 × 10⁸ PFU of adenoviral HO-1 (Ad-HO-1) (a gift from K. Kolls, University of Pittsburgh Medical Center, Pittsburgh, PA, and J. Alam, Alton Ochsner Medical Foundation, New Orleans, LA) (29) or adenoviral β-galactosidase (Ad-LacZ) (BD Biosciences) were administered intranasally to each mouse in a volume of 50 μl as described previously (12).

The authors also wish to add the reference shown below.


In Figure 1, a sentence regarding the solid and broken lines was omitted from the legend. The corrected legend is shown below.

**FIGURE 1.** Specificity of the CM4 mAb. A, YB2 or RNK cells transfected with Ly49 constructs were stained with medium or first layer Abs followed by AF488 goat anti-mouse Ig. Solid lines: staining by CM4. Left broken line: medium control. Right broken line: staining by positive control Abs Ly49A = A1, Ly49B = 1A1, Ly49C = 4D12, Ly49D = 4E5, Ly49E = 4D12, Ly49F = HBF, Ly49G = 4G11, Ly49H = 3D10, Ly49I = YBI. B, Cross-competition between Abs. YB2 cells transfected with Ly49E (YB2-E) and RNK cells transfected with Ly49F (RNK-F) were incubated with medium or saturating quantities of the unlabeled Ly49 Abs shown on the y-axis. After 20 min, AF488-labeled CM4, 4D12, or HBF Ab was added, and incubation was continued for an additional 20 min. Median fluorescence values were determined by flow cytometry, and the percentage inhibition caused by pretreatment with each unlabeled Ab is plotted on the y-axis. The likelihood that the inhibition observed was due to chance variation was determined by Student’s *t* test (*, *p* < 0.05, **, *p* < 0.01, ***, *p* < 0.001). The experiments shown are representative of three similar experiments of each type that were performed.

In Figure 9A, the gel image labeled Ly49A is inverted. The corrected figure is shown below.
Figure 10, demonstrating intracellular trafficking of HLA-DR after the introduction of HIV proteins, is incorrect. The corrected figure is shown below.


In Materials and Methods, in the first sentence under the heading RSV infection, the designation of the virus type should be human RSV A strain, not A2 strain.


In Materials and Methods, in the first sentence under the heading Virus and infection, the designation of the virus type should be human RSV A strain, not A2 strain.

Figure 3B, demonstrating the apoptotic effect of gp120 on CD4 and CD8 cells; Figure 4B, depicting the apoptotic effect of Fas-FasL interactions in CD4 and CD8 T cells cocultured with 43HV cells; and Figure 6B, showing the apoptotic activity of fractionated supernatant from the 43HV cell line, are inaccurate. The corrected figures are shown below.

In Figure 5, demonstrating the inability of HIV-1-infected 43 cells to present antigen to HLA-DR2 and DR4 T cells, panels A and B are the same. The corrected figure is shown below.