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# A diffusible lymphokine produced by CD8$^+$ T lymphocytes suppresses HIV replication

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## SUMMARY

Peripheral blood CD8$^+$ T lymphocytes from human immunodeficiency virus (HIV)-infected individuals suppress replication of HIV in peripheral blood mononuclear cells (PBMC). This anti-viral activity appears to be mediated in part by a diffusible factor. Production of this lymphokine varies among infected individuals and may reflect the intrinsic ability of an individual's CD8$^+$ cells to control HIV infection. In some cases in which factor activity is not apparent, contact of the CD8$^+$ cells with infected CD4$^+$ cells can produce for suppression of virus replication. These observations could lead to approaches for enhancing anti-viral responses in HIV-infected individuals.


We have demonstrated that peripheral blood CD8$^+$ T lymphocytes from healthy, human immunodeficiency virus (HIV)-infected individuals prevent replication of HIV in cultured peripheral blood mononuclear cells (PBMC) (Walker *et al.*, 1986, 1989). Similar results have recently been reported with studies of the simian immunodeficiency virus (Kannagi *et al.*, 1988). Removal of CD8$^+$ cells from non-virus-producing PBMC cultures resulted in the rapid release of HIV (Walker *et al.*, 1986). This finding suggested that HIV-infected cells were not eliminated from the culture by cytotoxic cell activity; instead, virus production by these cells was suppressed. The data presented in this report provide evidence that this anti-viral activity of CD8$^+$ T cells is mediated at least in part by a lymphokine, and indicate that CD8$^+$ cells from different individuals vary in their ability to produce the anti-viral factor.

The PBMC from healthy, HIV-seropositive individuals were isolated on Ficoll–Hypaque gradients (Sigma, St Louis, MO), and T lymphocytes expressing the CD4 or CD8 surface antigens were enriched by the panning technique of Wysocki & Sato (1978). Briefly, plastic-adherent cells were first removed from the PBMC and then $20–30 \times 10^6$ of the remaining cells were incubated in 2 ml of phosphate-buffered saline (PBS) containing 10 $\mu$g/ml of monoclonal antibodies to either Leu 2a (CD8) or Leu 3a (CD4) (Becton-Dickinson, Mountain View, CA) for 20 min at room temperature. The cells were washed twice, resuspended in 4 ml of PBS containing 1% fetal bovine serum (FBS), and incubated for 2 hr at 4° on a plastic Petri dish coated with the F(ab)'$_2$ portion of goat antibody to mouse immunoglobulin G (Protos Lab., South San Francisco, CA). These capture plates had been treated with the goat anti-mouse antibody (10 $\mu$g/ml, 0·05 M Tris, pH 9·5) for 40 mins at 37°. To prevent non-specific attachment of the PBMC, plates were incubated with PBS containing 1% FBS prior to use. The non-adherent cells (i.e. the CD4- or CD8-depleted cell fraction) were removed from the plate by washing with cold PBS, and the CD4$^+$- or CD8$^+$-adherent cells were recovered with a forceful stream of PBS (Walker *et al.*, 1986). The effectiveness of the panning procedure was assessed by flow cytometry (Levy *et al.*, 1985). For the experiments described here, the enriched cell populations were greater than 95% pure.

Transwell tissue culture dishes (Costar, Van Nuys, CA) (Fig. 1) were used to determine if the CD8$^+$ cells produce a diffusible factor capable of preventing HIV replication in CD4$^+$ cells. Each 24·5-mm dish has a removable top chamber that is separated from the bottom chamber by a 0·45 micron filter. The culture medium in all experiments was RPMI-1640 supplemented with 10% FBS, 2 mM glutamine, 1% antibiotics (100 U/ml penicillin, 100 $\mu$g/ml streptomycin) and 10% human IL-2 (Electronucleonics, Silver Springs, MD). In most experiments, $5 \times 10^5$ CD4$^+$ T cells in 2 ml of culture medium were seeded into the top chamber; bottom chambers received $2 \times 10^6$ CD8$^+$ cells in 3 ml of culture medium. Phytohaemagglutinin (PHA) (Sigma Co., St Louis, MO) (3 $\mu$g/ml) was added at the initiation of the culture. After 3 days, cells in the top and bottom chambers were washed and re-fed with fresh IL-2-containing medium. Culture supernatants from the wells were assayed for Mg$^+$-dependent reverse transcriptase (RT) activity at 3-day intervals as described elsewhere (Hoffman, Banapour & Levy, 1985). Additional PBMC were not added to the cultures at any time. The presence of HIV antigens in cultured cells was measured by an indirect immunofluorescence assay using sera from HIV-infected individuals (Kaminsky *et al.*, 1985).

As shown in Table 1, when CD4$^+$ T lymphocytes from three

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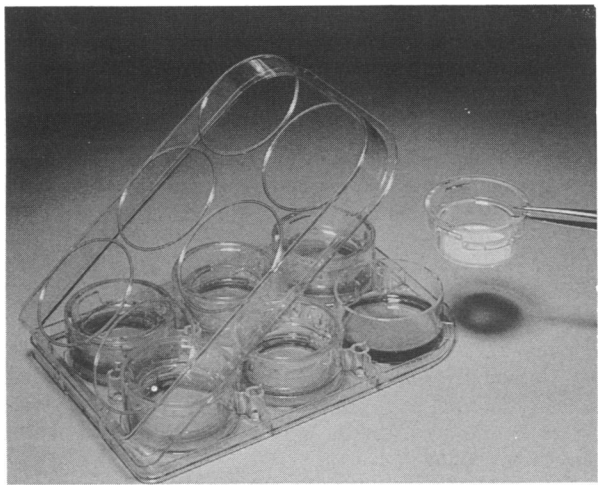

**Figure 1.** Transwell tissue culture plate. Each 24·5-mm dish has a removable insert that divides it into two compartments separated by a 0·45 micron filter (0·4 × magnification).

**Table 1.** Inhibition of HIV replication by CD8+ T cells is mediated by a diffusible factor

| Experiment | CD4+ cells (top well) | CD8+ cells (bottom well) | RT activity in culture supernatants† |
|---|---|---|---|
| 1 | + | − | 1669·1 |
| | + | + | 85·8 |
| | − | + | 2·8 |
| 2 | + | − | 1320·3 |
| | + | + | 22·2 |
| | − | + | 0·9 |
| 3 | + | − | 857·3 |
| | + | + | 0·9 |
| | − | + | 2·3 |

* PBMC from three different HIV-seropositive individuals were separated into CD4+ and CD8+ subsets by the panning technique (Wysocki & Sato, 1978). CD4+ cells ($5 \times 10^5$) and CD8+ cells ($2 \times 10^6$) were seeded into the top and bottom wells, respectively, of the transwell tissue culture dish.

† Values represent reverse transcriptase (RT) activity (c.p.m. × $10^{-3}$) per ml of culture supernatant on Day 6 after the initiation of culture.

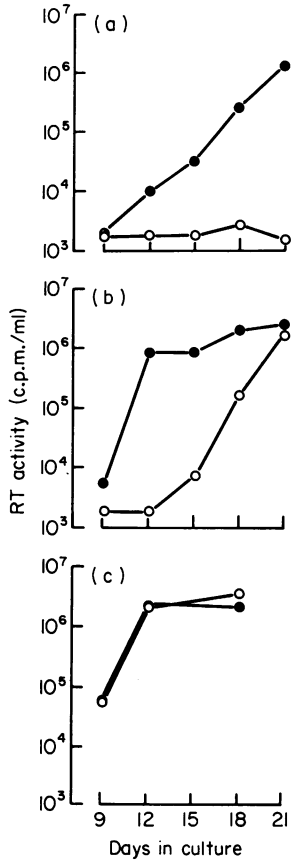


**Figure 2.** Production of CD8+ cell-derived lymphokine varies among infected individuals. CD4+ and CD8+ T lymphocytes were enriched from the PBMC of eight healthy seropositive individuals and cultured in the transwell dishes. CD4+ T cells ($5 \times 10^5$) were cultured in the top chamber with (○) or without (●) the addition of $2 \times 10^6$ autologous CD8+ T cells to the bottom chamber. Each panel shows data from a representative experiment.

healthy subjects were cultured in the top chamber in the absence of CD8+ cells, high levels of RT activity were detected in culture supernatants. This virus production was accompanied by the appearance of multinucleated giant cells and degenerative ballooning of cells that stained for HIV antigens. As expected, the supernatant from cultures containing only the non-HIV-susceptible CD8+ cells showed no replicating virus. When the bottom well with CD8+ cells was placed together with the top well containing infected autologous CD4+ cells, the appearance of both RT activity, HIV antigen production, and cytopathic changes in the cells was prevented or sharply reduced. This observation demonstrated that suppression of virus replication by CD8+ cells can be mediated by a diffusible factor. At the time of HIV suppression, the number of viable CD4+ T cells in the top chamber was approximately equal to the number of CD4+ cells in chambers cultured alone. These findings suggest that the CD8+ cell-derived factor acted by directly interfering with virus replication, rather than by reducing the proliferation of the target cells for HIV growth.

It is unlikely that the suppression was caused by CD8+ cells passing through the 0·45 micron membrane. First, the CD8+ cells were placed in the bottom chamber, and thus had no opportunity to come in contact with the membrane supporting the CD4+ cells. Second, at the end of the experiment FACS analyses of the cells in the top chamber showed they were >90% CD4 positive. Finally, replication of HIV commenced when the cells in the top chamber were removed from the influence of CD8+ cells in the bottom chamber.

The transwell culture dishes were subsequently used to determine if there was variation in the ability of CD8+ cells from eight randomly selected healthy HIV-seropositive subjects to produce the anti-viral factor. As shown in Fig. 2, three different patterns of responsiveness were observed. For two of the subjects, high levels of RT activity could only be detected in

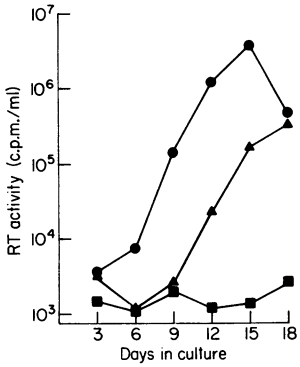


**Figure 3.** Cell contact is required for efficient suppression of HIV growth by CD8+ T cells. $5 \times 10^5$ CD4+ T cells were cultured alone (●) or with $2 \times 10^6$ CD8+ T cells (■). In a third approach, the two cell types were separated in the transwell culture plate (▲). A representative experiment is shown.

cultures containing CD4+ T cells alone; the addition of CD8+ cells to the bottom chamber completely suppressed virus replication (Fig. 2a). For three additional subjects, the presence of CD8+ cells in the bottom chamber delayed but did not prevent virus replication (Fig. 2b). Finally, three asymptomatic individuals were identified whose CD8+ cells did not affect the kinetic of virus replication by CD4+ cells in the top chamber (Fig. 2c).

It was not clear whether the absence of CD8+ cell control via factor production reflected a complete defect in anti-viral response; therefore, suppression of HIV replication was evaluated by both the chamber and co-culture procedures in five infected individuals who previously showed low level factor production. The results from a representative experiment are shown in Fig. 3. The CD8+ cells delayed virus replication when they were separated from CD4+ cells in the transwell culture dishes, but HIV production was completely abrogated when cell:cell contact was permitted. This finding suggests the production and/or delivery of the anti-viral factor to the target cells is more efficient when cells are in close contact. Alternatively, the anti-viral activity observed with cell:cell contact could be mediated by a different mechanism.

Results similar to these findings with HIV have been obtained in studies of T-cell immunity to Epstein–Barr virus (EBV). Transformation of B lymphocytes by EBV can be prevented by adding immune T lymphocytes to the culture (Thorley-Lawson, 1981; Moss, Rickinson & Pope, 1978). This suppression of transformation has two phases: an early phase occurring after infection but before transformation (< 24 hr), and a late phase observed 6 days post-infection (Thorley-Lawson, 1981). The early phase of transformation is prevented by leucocyte (alpha) interferon produced when the T cells encounter the infected cell, while the later phase can only be inhibited by a process that requires direct contact between the T and B cells (Thorley-Lawson, 1981; Rickinson, Yao & Wallace, 1985).

In our system, the identification of the CD8+ cell-derived anti-viral factor awaits the development of a rapid assay for its detection. Addition of large amounts of interferon-gamma ($5 \times 10^5$ units) to the CD4+ cell cultures every 3 days did not suppress replication of the virus, suggesting that this lymphokine is not responsible. Nevertheless, suppression of virus growth could result from production of other cytokines such as interferon-alpha (Yamamoto *et al.*, 1986), lymphotoxin, tumour necrosis factor (Meston *et al.*, 1986; Wong *et al.*, 1988) or a combination of lymphokines (Wong et al., 1988). Finally, whether the effect of cell to cell contact reflects a different anti-viral mechanism requires future evaluation. These results provide additional information on the anti-HIV activity of CD8+ cells and suggest future directions at possible strategies for antiviral therapy.

## ACKNOWLEDGMENTS

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