The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 01 1999

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*J Immunol* (1999) 163 (7): 3914–3919.
https://doi.org/10.4049/jimmunol.163.7.3914

# Monocyte-Driven Activation-Induced Apoptotic Cell Death of Human T-Lymphotropic Virus Type I-Infected T Cells[1]

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**We attempted apoptotic cell death induction of T cells infected with human T lymphotropic virus type I (HTLV-I) which induces HTLV-I-associated myelopathy/tropical spastic paraparesis and adult T cell leukemia. T cells acutely infected and expressing HTLV-I*gag* Ags were killed by cross-linking their TCR with anti-CD3 mAb. Cells in apoptotic process were found by staining with annexin V. The apoptosis was not affected by costimulation through CD28 molecules and was resistant to ligation of Fas molecules. Whereas the virus-infected T cells expressed higher levels of HLA-DR, CD25, CD80, and CD86 Ags than apoptosis-resistant PHA-blasts, the T cell apoptosis was enhanced by addition of exogenous IL-2. Furthermore, in this apoptosis, monocytes played an important role because T cells infected in the absence of monocytes were resistant to the death signals. The apoptosis-sensitive T cells responded to TCR signaling more strongly by proliferating than those apoptosis-resistant cells. Monocytes weakly affected the expression levels of viral Ags on T cells. However, HTLV-I-infected monocytes primed T cells to die by subsequent TCR signaling. T cells primed with the monocytes, subsequently infected in the absence of monocytes, were killed by TCR signaling. These observations suggest that primed and infected T cells could be killed by activation-induced cell death. *The Journal of Immunology,* 1999, 163: 3914–3919.**

Human T-lymphotropic virus type I (HTLV-I)[3] is the causative agent of HTLV-I-associated myelopathy/tropical spastic paraparesis and adult T cell leukemia (ATL) (1, 2). The former is thought to be an autoimmune disease induced by activated autoreactive T cells (3), and the latter to be manifested by the expansion of leukemic T cells which are considered to be produced from HTLV-I-infected T cells by an unknown mechanism. The manifestation of these diseases is closely associated with an increase of virus-infected T cells. These diseases are manifested 40–60 years after infection with HTLV-I in the newborn period (2, 4). During the asymptomatic state, the number of virus-infected T cells is well controlled, and expansion of leukemic T cells is not allowed. In this process, HTLV-I Ag-specific CTL are believed to play a major role in controlling the expansion of these T cells. In addition to the cytolysis by CTL, however, induction of apoptosis of HTLV-I-infected T cells should be a desirable strategy to eliminate virus-infected T cells and to prevent a life-threatening disease such as ATL, if it could be induced under physiological conditions. After T cells are activated by responding to antigenic stimuli, they are generally killed by apoptotic mechanisms. Among the apoptotic mechanisms, activation-induced cell death (AICD) play a central role, especially in killing autoreactive T cells and in preventing hyperimmune responses (5–7). It has recently been reported that Jurkat cells artificially introduced with the HTLV-I*tax* gene undergo AICD by signaling through TCR (8).

The direction of the host immune response to exogenous Ags is largely determined by the quantity of Ags inoculated and the sort of APC that are enrolled in the immune responses. In the presence of a large amount of Ags and/or prolonged contact with Ags, T cells usually undergo anergic processes and enter an anergic or unresponsive state (9, 10). Therefore, the quick elimination of antigenic stimuli at the initial viral infection stage is extremely important for keeping T cell function normal. In this respect, the elimination of freshly HTLV-I-infected $CD4^+$ T cells by an apoptotic cell death induction should be a reasonable and effective tool for preventing the manifestation of HTLV-I-induced diseases. However, although HTLV-I-infected T cells are easily activated (11), there are very few consistent observations on the apoptosis of HTLV-I-infected T cells. Therefore, in this study we tried to induce AICD of acutely infected $CD4^+$ T cells and found that T cells infected with HTLV-I in the presence of monocytes for 1 wk could be killed by TCR signaling. In addition to the TCR signals, HTLV-I-infected monocytes played a major role in priming uninfected T cells to be feasibly killed by subsequent infection and a death signal.

## Materials and Methods

### *Cell preparation*

PBMCs were donated by uninfected healthy donors. The PBMCs were isolated from heparinized blood by using Ficoll-Paque Plus (Pharmacia, Uppsala, Sweden) and cryopreserved in liquid nitrogen until used as described previously (12). Plastic-adherent monocytes were obtained by culturing PBMCs for 60 min in the culture plate and by washing out nonadherent cells with prewarmed medium.

Purified T cells were obtained as follows: Fresh or cryopreserved PBMCs were cultured for 60 min and nonadherent cells were obtained. They were further depleted of MHC class $II^+$ cells by using magnetic beads coated with mAb to MHC class II Ag (Dynabeads 450, Dynal, Oslo, Norway). Contamination of the purified population by HLA-DR-positive cells was <2%. The complete elimination of monocytes from the T cell population was accomplished by further treating the purified population with beads coated with anti-CD14 mAbs (Dynabeads 450). In this highly

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Received for publication May 27, 1999. Accepted for publication July 16, 1999.

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[1] This work was supported in part by a Grant-in-Aid for a Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.

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[3] Abbreviations used in this paper: HTLV-I, human T lymphotropic virus type I; ATL, adult T cell leukemia; AICD, activation-induced cell death; PI, propidium iodide; TRAIL, TNF-related apoptosis-inducing ligand; DC, dendritic cell.



0022-1767/99/$02.00

purified population, <0.5% of $CD14^+$ monocytes were included. Depletion of $CD8^+$ T cells from PBMCs was conducted by using beads coated with anti-CD8 mAb (Dynabeads 450).

### *Infection of T cells with HTLV-I*

The HTLV-I-producing cell line MT-2, a generous gift from Dr. I. Miyoshi, Kochi Medical School, was used as a viral source. T cells ($2.5 \times 10^5$/ml) from healthy donors were cocultured with mitomycin C (200 $\mu$g/ml)-treated MT-2 cells ($5 \times 10^4$/ml) in the presence or absence of uninfected monocytes. The mitomycin C treatment was done by incubating cells for 30 min at 37°C. Cocultivated T cells were maintained in the presence of 1 $\mu$g/ml PHA-P (Difco, Detroit, MI) for 7 days. At day 7 of culture, live and activated T cells were obtained by using 30 and 55% Percoll solutions (Pharmacia, Piscataway, NJ) and were maintained in the presence of 2 $\mu$g/ml PHA-P and 100 U/ml rIL-2 (TGP-3, Takeda Chemical Industries, Osaka, Japan). Monocytes were infected with HTLV-I by cocultivating them with mitomycin C-treated MT-2 cells for 5 days in the presence of 20% FCS. Priming of T cells by HTLV-I-infected monocytes was conducted by coculturing uninfected and purified T cells with virus-infected monocytes for 5 days. In some cases, the primed T cells were infected by using MT-2 after completely eliminating $CD14^+$ cells from the population. Infection of monocytes and T cells with HTLV-I was determined by cytometry analysis (FACScan, Becton Dickinson ImmunoCytometry Systems, San Jose, CA) for surface expression of HTLV-I*gag* and HTLV-I*env* proteins. HTLV-I*tax* protein was detected by FACScan by permeabilizing cells with 0.3% saponin solution (Sigma, St. Louis, MO).

### *Analysis of cell surface Ag*

The expression of cell surface Ags on the T cells was determined by using FACScan (Becton Dickinson). Live cells ($1 \times 10^4$) were gated and analyzed. To eliminate dead cells from analysis, propidium iodide (PI, Sigma) was used. We used FITC-conjugated mAb against CD25 (2A3), HLA-DR (L243, Becton Dickinson), and CD80 (BB1, PharMingen, San Diego, CA) and PE-labeled mAb to CD86 (IT2.2, PharMingen), and purified murine mAb to HTLV-I*gag* (GIN14), HTLV-I*env* (F10), and HTLV-I*tax* (LT4) (generously given to us by Dr. Y. Tanaka, Department of Hygiene, Kitazato University, Sagamihara, Japan) which were followed by FITC-labeled goat $F(ab')_2$ anti-mouse Igs (Tago Immunologicals, Camarillo, CA). The optimal concentrations of mAbs were determined in advance. T cells in an apoptotic process were determined by staining them with FITC-conjugated annexin V (Genzyme, Cambridge, MA) and PI. The T cells were preincubated for 20 min on ice with 2% BSA solution and were stained with 0.15 $\mu$g/ml annexin V conjugates.

### *Proliferation assay*

T cells infected with HTLV-I in the presence or absence of monocytes were stimulated with various concentrations of immobilized CD3 mAb. The infected T cells ($2.5 \times 10^4$/well) were plated in 96-well round-bottom tissue culture plates that were precoated with CD3 mAb. The cell proliferation during the last 16 h of the 48-h culture was quantified by incubating the cells with 1 $\mu$Ci/well [$^3$H]thymidine. The results are expressed as the mean value in cpm obtained from triplicate cultures.

### *Apoptotic cell death induction*

HTLV-I-infected T cells or PHA-blasts were stimulated with 2 $\mu$g/ml CD3 mAb. The CD3 mAb was precoated in 96-well round-bottom tissue culture plates, and an excess of mAb was washed out with PBS before use. T cells ($2.5 \times 10^4$) were plated and harvested 16 h after stimulation. The apoptosis of T cells was determined by using FACScan. In some cases, T cells were stimulated with CD3 mAb in the presence of mAbs antagonistic to Fas (ZB4, Medical and Biological Lab., Nagoya, Japan) and/or Fas ligand (NOK-2, Sumitomo Electric, Yokohama, Japan).

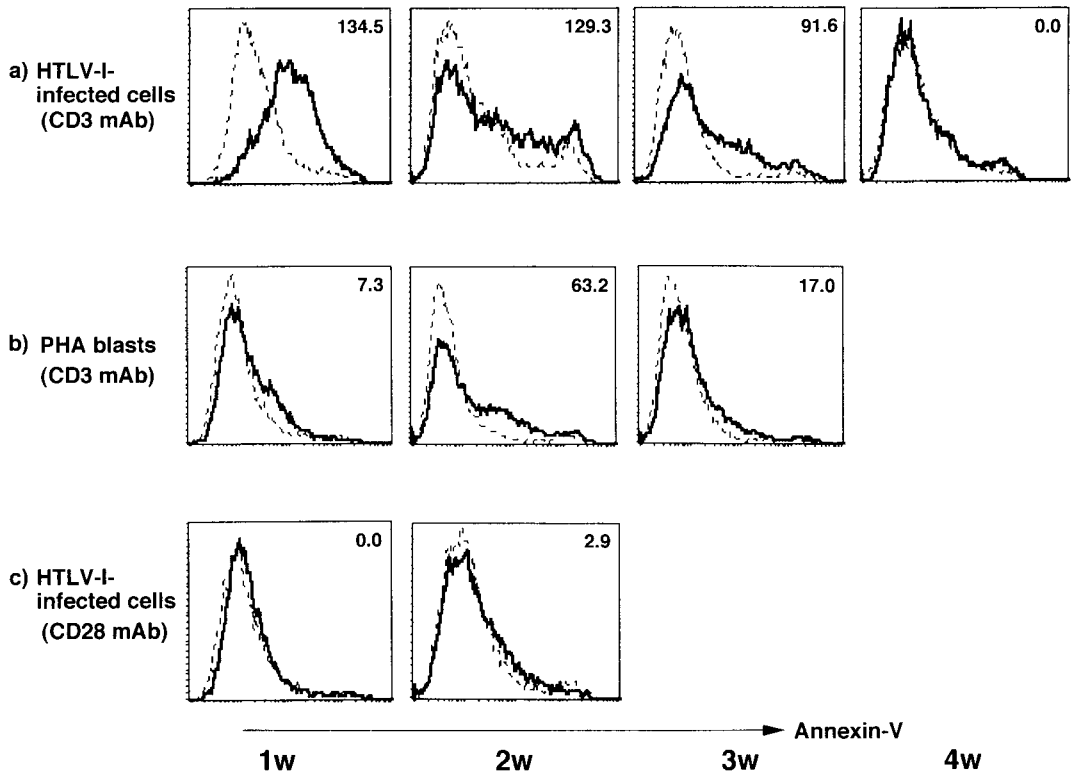


**FIGURE 1.** Apoptotic cell death induction in HTLV-I-infected T cells. PBMCs were donated by an uninfected donor and infected with HTLV-I. T cells ($5 \times 10^5$) were cocultured with $1.0 \times 10^5$ MT-2 cells in the presence of 1 $\mu$g/ml PHA. The infected T cells were maintained for 4 wk by using PHA and IL-2 (100 U/ml). PHA-blasts were produced by stimulating T cells with PHA. The T cells were stained with 150 ng/ml of annexin V after restimulation with CD3 mAb or CD28 mAb. – – – –, annexin V staining of cells prestimulated with the mAb; ——, annexin V staining of cells poststimulated with the mAb. The number represents the difference in mean fluorescence intensity between – – – – and ——. A representative of more than three independent experiments is shown. *a*, HTLV-I-infected cells stimulated with CD3 mAb; *b*, PHA-blasts stimulated with CD3 mAb; *c*, HTLV-I-infected cells stimulated with CD28 mAb.

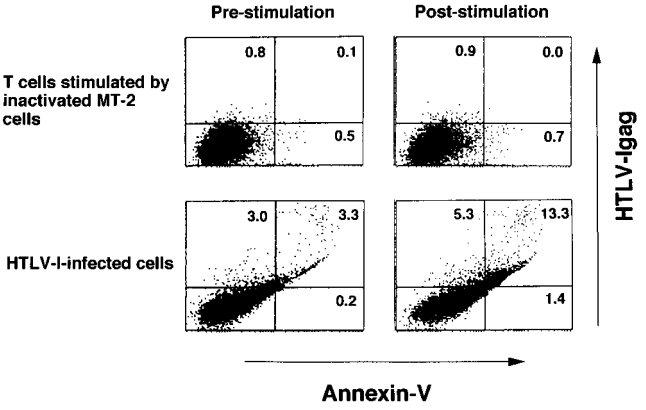


**FIGURE 2.** Double staining of T cells with HTLV-I*gag* mAb and annexin V. Uninfected T cells were stimulated with MT-2 cells or heat (56°C, 30 min)-inactivated MT-2 cells in the presence of PHA. One week after stimulation, live T cells were stained with HTLV-I*gag* mAb (GIN-14) and annexin V. The number represents percent positive cells. A representative of three independent experiments is shown.

## Results

### *Apoptotic cell death induction of HTLV-I-infected T cells*

$CD4^+$ T cells were obtained from normal uninfected donors and infected with HTLV-I. One week after infection, live viral infected T cells were purified and tested for their ability to undergo AICD after cross-linking with CD3 mAb (Fig. 1). These restimulated T cells were positively stained with annexin V (Fig. 1*a*). T cells were infected by using various amounts of MT-2 cells, and they consistently showed apoptosis (not shown). The apoptosis level decreased in a time-dependent manner after infection, and the cells maintained for 4 wk showed no apoptosis (Fig. 1*a*). Several donors were examined and most viral infected T cells changed to be resistant to CD3 mAb stimulation 2–4 wk after infection (not shown). However, massive apoptotic cell death was not induced in T cells stimulated by PHA and IL-2 and restimulated by CD3 mAb (Fig. 1*b*). No apparent changes were induced on HTLV-I-infected T cells by CD28 mAb stimulation (Fig. 1*c*). Furthermore, the effect of CD28 mAb costimulation to CD3 stimulation in the T cell apoptosis was not observed (not shown). To confirm that the HTLV-I-infected T cells were killed by CD3 mAb stimulation, we conducted double staining analysis by using mAb to HTLV-I*gag* Ags and annexin V (Fig. 2). Although 3.5% of T cells among the whole population showed apoptosis before restimulation, most of the apoptotic cells (94.3%) were positively stained with HTLV-I*gag* Ags. By restimulation with CD3 mAb, both HTLV-I-infected cells and apoptotic cells increased, and again, most of the apoptotic cells (90.5%) expressed HTLV-Igag Ags. In contrast to HTLV-I-infected T cells, T cells stimulated with heat-inactivated MT-2 cells were minimally stained with HTLV-I*gag* mAb and showed no apparent apoptotic cell death. Therefore, HTLV-I-infected T cells were considered to be killed mainly by CD3 mAb stimulation.

### *Contribution of Fas ligand and $CD8^+$ T cells to apoptosis of HTLV-I-infected T cells*

To clarify the roles of Fas and Fas ligand molecules and $CD8^+$ T cells in the apoptosis, we induced the virus-infected T cell death in the presence of mAbs antagonistic to Fas, Fas ligand, and both (Table I). The concentrations of the antagonistic mAbs were enough to block apoptotic death of Jurkat cells induced by mAb agonistic to Fas Ag. However, no apparent reduction of apoptosis by those mAbs was observed. Furthermore, T cells infected in the absence of $CD8^+$ T cells showed a level of apoptosis similar to those infected in the presence of $CD8^+$ T cells.

### *The possibility of activation-induced cell death in HTLV-I-infected T cells*

The minor contribution of Fas and Fas ligand to the apoptosis prompted us to check the possibility of Fas signaling-independent AICD of HTLV-I-infected T cells by CD3 mAb stimulation. Therefore, we compared the expression level of various activation-related molecules. When we compared the expression of these molecules on virus-infected T cells with that on uninfected PHA-blasts, the former expressed significantly higher levels of HLA-DR (126 vs 95 in the mean fluorescence intensity), CD86 (94 vs 19), and CD25 (192 vs 39) Ags (Fig. 3*A*). Furthermore, because IL-2 is known to activate T cells, we checked the effect of exogenous IL-2 on the apoptosis. Addition of exogenous IL-2, especially at 100 U/ml, significantly enhanced the ability of the virus-infected T cells to undergo apoptosis to a subsequent cross-linking of the CD3 mAb (Fig. 3*B*).

### *The role of HTLV-I-infected monocytes in virus-infected T cell apoptosis*

To clarify the role of APCs in the activation of HTLV-I-infected cells, we removed monocytes from PBMCs, infected them with HTLV-I, and induced apoptosis (Fig. 4). As observed before (Fig. 1), T cells infected with HTLV-I 1 wk before in the presence of monocytes were sensitive to the apoptosis signal, whereas those infected 2 wk before were less sensitive to the signaling. However, surprisingly, in contrast to these T cells, T cells infected 1 or 2 wk before in the absence of monocytes showed little apoptosis by CD3 mAb stimulation. This difference might come from a difference in sensitivity to CD3 mAb stimuli. T cells infected in the presence or absence of monocytes were stimulated by various concentrations

Table I. *Role of Fas Ag and $CD8^+$ T cells in induction of apoptosis of HTLV-I-infected T cell apoptosis*[a]

| Expt. | Cells Infected with HTLV-I | Stimulation | Antagonistic mAb to | % Cell Death | |
|---|---|---|---|---|---|
| | | | | Annexin $V^+$ $PI^-$ | Annexin $V^+$ $PI^+$ |
| 1 | Whole PBMCs | CD3 mAb | None | 22.5 | 2.4 |
| | Whole PBMCs | CD3 mAb | Fas | 20.4 | 2.0 |
| | Whole PBMCs | CD3 mAb | FasL[b] | 21.9 | 2.0 |
| | Whole PBMCs | CD3 mAb | Fas + FasL | 20.6 | 1.8 |
| 2 | Whole PBMCs | CD3 mAb | None | 32.3 | 4.0 |
| | $CD8^-$ PBMCs | CD3 mAb | None | 32.7 | 5.2 |

[a] Unseparated or CD8-depleted population of PBMCs were infected with HTLV-I for 1 wk and were stimulated with CD3 mAb (2 $\mu$g/ml) in the presence of the indicated antagonistic mAbs. The mAb to Fas was used at 5 $\mu$g/ml, and that to FasL was used at 2.5 $\mu$g/ml.

[b] FasL, Fas ligand.

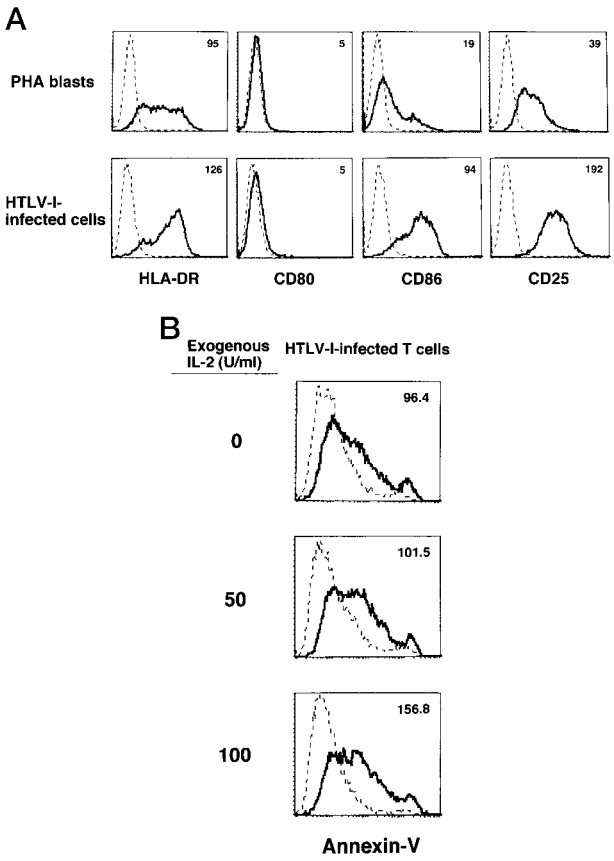


**FIGURE 3.** *A,* Expression of various molecules on PHA-blasts and HTLV-I-infected T cells. T cells from a healthy donor were infected with HTLV-I by using MT-2 cells or were stimulated with PHA for 1 week. The T cells were stained with mAbs to the indicated Ags. – – – –, control mAb; ——, indicated mAb. A representative of three independent experiments is shown. The number represents the mean fluorescence intensity. *B,* Influence of an exogenous rIL-2 on apoptotic cell death induction of HTLV-I-infected T cells. T cells were infected with HTLV-I by using MT-2 cells for 1 wk and were stained with annexin V after restimulation with CD3 mAb in the presence of various concentrations of exogenous rIL-2. – – – –, annexin V staining of cells before CD3 mAb stimulation; ——, annexin V staining of cells after the stimulation. The number represents the difference in mean fluorescence intensity between – – – – and ——. A representative of three independent experiments is shown.

of CD3 mAb (Fig. 5). The proliferative responses of T cells infected with HTLV-I in the presence of monocyte to CD3 mAb stimuli were significantly higher than that of T cells infected in the absence of monocytes. Therefore, the former viral infected T cells seemed more sensitive to CD3 mAb stimuli than the latter ones. This observation might reflect that viral infection level of T cells is influenced by monocytes. However, there was no apparent correlation between the number of monocytes and the infection level of T cells (Fig. 6). T cells ($5 \times 10^5$) were infected in the presence of various numbers ($0–1 \times 10^5$) of monocytes. The expression level of HTLV-I*gag* Ags on T cells was not influenced by the presence of monocytes. Then, to clarify the action of monocytes on T cells, we checked another possibility that HTLV-I-infected monocytes worked to prime T cells to die by subsequent viral infection and CD3 mAb stimuli (Fig. 7). Plastic-adherent monocytes were infected in vitro and cocultured with highly purified $CD4^+$ T cells for 7 days. Only T cells primed with virus-infected monocytes were weakly stained with annexin V after CD3 mAb stimulation. Furthermore, when T cells primed by the infected

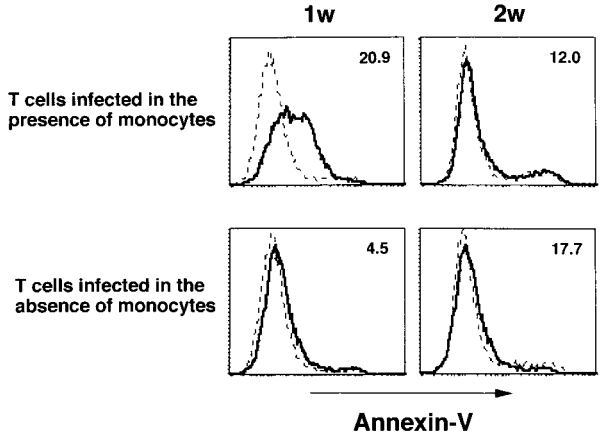


**FIGURE 4.** Influence of monocytes on HTLV-I-infected T cell apoptosis. T cells from a healthy donor were infected with HTLV-I in the presence or absence of monocytes. One and two weeks (w) after infection, T cells were purified, stimulated with CD3 mAb, and stained with annexin V. – – – –, annexin V staining of cells before CD3 mAb stimulation; ——, annexin V staining of cells after the stimulation. The number represents the difference in mean fluorescence intensity between – – – – and ——. A representative of three independent experiments is shown.

monocytes were infected with HTLV-I after complete depletion of $CD14^+$ monocytes and were restimulated with CD3 mAb, massive apoptosis was induced solely in T cells primed with virus-infected monocytes. No apparent apoptosis was observed in T cells primed with uninfected monocytes and infected with HTLV-I.

## Discussion

In this study, we induced apoptotic cell death of acutely HTLV-I-infected $CD4^+$ T cells by TCR signaling in combination with virus-infected monocytes. In general, Fas/Fas ligand signaling (13, 14), TNF/TNFR binding (15, 16), CTLA-4 cross-linking (17, 18), AICD (5–7), and cell lysis by CTL and NK cells are considered

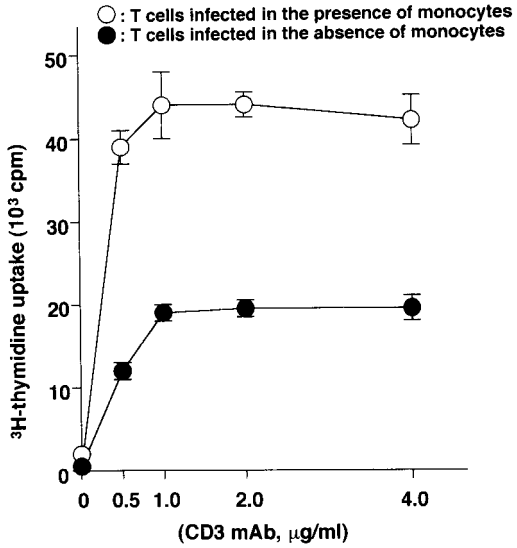


**FIGURE 5.** Proliferative responses of HTLV-I-infected T cells to CD3 mAb stimuli. T cells were infected with HTLV-I in the presence or absence of monocytes for 1 wk. T cells ($1.25 \times 10^5$/ml) were cultured for 48 h in wells precoated with various concentrations of CD3 mAb. Assay was done in triplicate and the mean SD is shown. A representative of three independent experiments is shown.

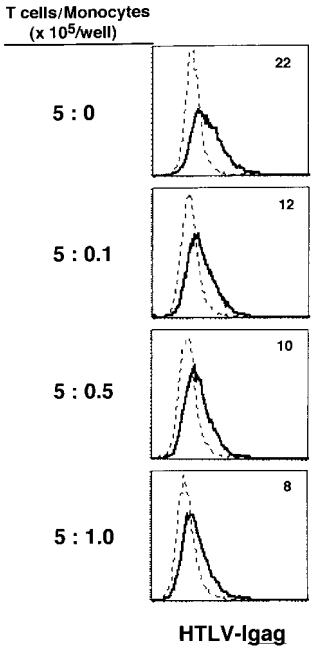


**FIGURE 6.** Expression of HTLV-I*gag* Ags on HTLV-I-infected T cells. T cells were infected with HTLV-I for 1 wk in the presence of various amounts of uninfected autologous monocytes. The T cells were stained with mAb to HTLV-I*gag* Ags. – – – –, control mAb; ——, HTLV-I*gag* mAb. The number represents the difference in mean fluorescence intensity between – – – – and ——.

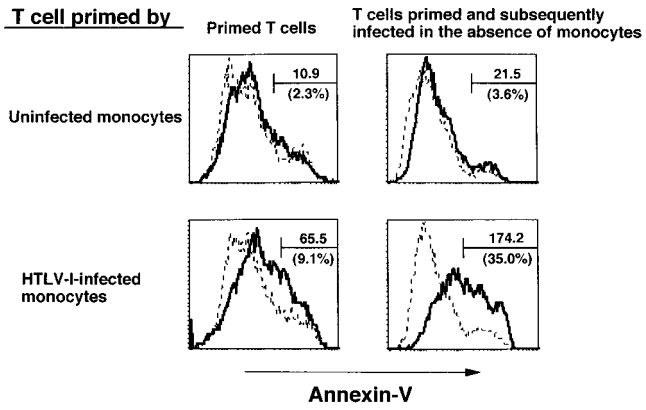


**FIGURE 7.** Priming effect of HTLV-I-infected monocytes on HTLV-I-infected T cell apoptosis. Monocytes were infected with HTLV-I by cocultivating them with MT-2 cells in the presence of 20% FCS. The virus-infected or uninfected monocytes ($5 \times 10^5$/ml) were treated with mitomycin C and cocultured with autologous uninfected T cells ($1 \times 10^6$/ml) for 7 days. The cocultivated T cells were stimulated with CD3 mAb or were infected with HTLV-I after complete elimination of CD14$^+$ monocytes and subsequently stimulated with CD3 mAb. – – – –, annexin V staining of cells before CD3 mAb stimulation; ——, annexin V staining of cells after the stimulation. The number represents the difference in mean fluorescence intensity between – – – – and ——. The number in parentheses represents the percent positive cell number subtracted from background value. A representative of three independent experiments is shown.

representative mechanisms for apoptotic cell death. Among them, Fas-independent AICD seemed the most appropriate mechanism for the apoptosis of HTLV-I-infected cells. The virus-infected T cells were more strongly activated than PHA-blasts and were more sensitive to CD3 mAb-mediated proliferative stimuli than apoptosis-resistant T cells. Furthermore, the apoptosis was enhanced by an addition of exogenous IL-2. IL-2 seems essential for induction of Fas-independent AICD as well as Fas-dependent AICD (19–21). The role of IL-2 is considered to be to enter cells at the S stage of the cell cycle in which the cells are most sensitive to apoptotic signals (22). However, the AICD was not induced in cells infected for more than 4 wk. The exact reason for this resistance is unknown, but they were least sensitive to CD3 mAb in proliferative responses (not shown). The reduced sensitivity of chronically infected T cells to CD3 mAb signaling might be an important mechanism for their resistance.

The contribution of CD8$^+$ T cells to the virus-infected T cell lysis seems less likely because cell death was induced in the absence of CD8$^+$ T cells. However, we could not rule out the contribution of CD4$^+$ CTL. There are some uninfected CD4$^+$ T cells remaining in the T cell population. It is reported that CD4$^+$ CTL kill their target in a TNF-related apoptosis-inducing ligand (TRAIL)-mediated fashion (23) and that some CD4$^+$ T cells undergo AICD by signals through the TRAIL (24). Therefore, we checked the possibility by using an inhibitory mAb to TRAIL, RIK-2 (25). However, the AICD of HTLV-I-infected T cells was not suppressed by RIK-2 (not shown).

Thus far two mechanisms are reported concerning the apoptosis of HTLV-I-infected T cells. One of them is the cell death by Fas-Fas ligand signaling. Debatin et al. (26) reported that the virus-infected T cells were sensitive to Fas signaling. However, in our study, both acutely and chronically infected T cells were resistant to a mAb agonistic to Fas in spite of expressing a high level of the Ag (not shown), and the apoptosis of acutely infected T cells was not blocked by a mAb antagonistic to Fas ligand (Table I). These observations are in line with many other reports in which HTLV-I-infected T cells are reported to be Fas insensitive for unknown reasons (27, 28). Quite recently, however, mutation of the *Fas* gene was found in HTLV-I-infected ATL cells (29, 30). These observations might be able to explain the resistance of virus-infected T cells to Fas signal. The second mechanism is HTLV-I*tax*-mediated apoptosis. The fibroblasts and osteoblasts expressing genetically introduced HTLV-I*tax* molecules have easily undergone apoptosis (31, 32) and, in some cases, the Tax-mediated apoptosis was observed in thymocytes (33). Quite recently, it was reported that Jurkat cells transfected with HTLV-I*tax* showed AICD by TCR signaling (8). However, in our study, there were no marked differences in the Tax-expressing level between AICD-sensitive and AICD-resistant virus-infected T cells as determined by FACS (not shown). Therefore, a contribution of HTLV-I*tax* to our AICD seems less likely.

In addition to TCR signaling, HTLV-I-infected monocytes played a major role in the T cell apoptosis. Our data clearly indicate that, on one hand, monocyte depletion prevents sensitization of T cells for subsequent AICD and that, on the other hand, uninfected T cells primed with virus-infected monocytes showed weak AICD on TCR signaling, and their apoptosis was clearly enhanced by subsequent HTLV-I infection in the absence of the monocytes. In the former case, limited cell death was observed. This might be due to poor viral transmission efficiency from monocytes to T cells. Depending on the experimental conditions, monocytes are suggested to transfer virus to T cells (34). However, in our case, apparent transmission was not observed (not shown). Monocytes activated by PHA or superantigens induce apoptosis of target cells by secreting a soluble form of Fas ligand (35), although in another study soluble Fas ligands made target cells resistant to apoptosis (36). In addition, HIV-1-infected monocytes directly killed uninfected CD4$^+$ T cells by signaling through gp120 (37). In our study, however, the direct killing of HTLV-I-infected T cells by virus-infected monocytes was not observed (not shown),

and the monocytes primed T cells solely by up-regulating their sensitivity to apoptosis signals. To our knowledge, this is the first report indicating that HTLV-I-infected monocytes were able to prime T cells to be killed by subsequent viral infection.

There are still 20 million people infected with HTLV-I (38), and they have a possibility of manifesting HTLV-I-associated myelopathy/tropical spastic paraparesis or ATL. Elimination of virus-infected T cells is a quite important therapeutic strategy. In our system, virus-infected monocytes and TCR signaling are solely required for the T cell killing. Monocytes are sensitive to HTLV-I-infection both in vitro and in vivo (39),[4] and TCR signaling can be provided by virus-infected dendritic cells (DCs). DCs are also sensitive to HTLV-I infection (40), and the infected DCs can strikingly stimulate $CD4^+$ T cells in a MHC-dependent manner (40). Therefore, both HTLV-I-infected monocytes and TCR signaling will be easily prepared in vitro that should be useful for the development of a new immunological treatment.

## Acknowledgments

We thank Ms. N. Makino for preparation of the manuscript, Dr. M. L. Robbins for reviewing the manuscript, and Drs. N. Kayagaki and H. Yagita (Juntendo University, Tokyo, Japan) for donating RIK-2.

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