Interferon-α and -γ Differentially Reduce Rapid Immature T-Cell Death by Contact with HIV-1 Carrier Cell Clones In Vitro

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Abstract: The non-antigen specific rapid cytotoxic (CT) death of immature TdT⁺CD4⁺CD8⁺ T cells due to contact with HIV-1 carrier T-cell clones we have found recently is a novel phenomenon. The effects of interferons (IFN) on this CT reaction were studied in vitro. Treatment of the HIV-1 carrier clones, referred to as “effectors,” with IFN-α but not IFN-γ, or of the susceptible immature TdT⁺CD4⁺CD8⁺ T cells, referred to as “targets,” with IFN-γ but not IFN-α, for 24 hr prior to CT testing was found to reduce the CT reaction. Simultaneously, a down-regulated CD8 expression and an up-regulated antigen expression of both major histocompatibility antigen complex class I (MHC-I) and HIV-1 gp120/gp160 in the IFN-α treated effector (gp120⁺CD8⁺ HPB-ALL/HIV), and/or simultaneously up-regulated antigen expression of both CD8 and MHC-I in the IFN-γ treated target (CD4⁺CD8⁺ HPB-ALL) were found to be associated with reduced CT reaction. However, altered antigen expression in the IFN-γ treated effectors or IFN-α treated targets did not affect the ultimate degree of CT reaction. This study thus suggests a possible therapeutic efficacy of IFN by reducing the direct elimination of the T-cell precursors in HIV-1 infection.

Key words: T-cell death, Cytotoxic HIV-1 cells, IFN effects

Interferon (IFN) is known to inhibit the replication of HIV-1 in vitro and in vivo (11, 24, 25, 30). Recent findings (10, 33) indicate that viral activity and immune response are very high in HIV-1 infected individuals throughout the disease course, in that the CD4⁺ T cells seen in the blood at any given time actually represent a population with an estimated turnover rate of 1–2 × 10⁹ cells daily. In another report (17), it was shown that uninfected blood CD4⁺ T cells in HIV-1 infected patients were rapidly killed by autologous HIV-1 infected cells via the infected-cell-mediated killing mechanism (ICMK). The CD8⁺ T cells in the blood, on the other hand, are generally considered to be beneficial to HIV infected individuals because some of them have been shown to be immunologically specific cytotoxic lymphocytes against HIV-1 infected cells (8, 32), and because a sustained high level of blood CD8⁺ T cells is associated with a long-term nonprogressive disease status (2, 7, 21). In recent findings, however, the CD8⁺ T cells in bronchoalveolar lavages from AIDS patients were shown to harbor HIV-1 in four out of four cases examined (29), and in another study, blood CD8⁺ T cells were frequently shown to be infected with HIV-1 (12). We have recently observed a rapid cytotoxic (CT) phenomenon unique and exclusive to CD4⁺CD8⁺ double-positive (DP) immature T cells (“targets”), without requiring virus replication in them, by contact with HIV-1 infected T-cell clones (15). Most of the HIV-1 carrier T-cell clones (“effectors”) involved in this CT reaction happened to be CD8⁺ single-positive (SP) clones, although the CD8 expression on them was shown to be non-essential for the CT reaction (15). In view of the evi-
idence accumulated on multiple modes of CT potential by HIV-1 infected T cells, this study describes the effects of IFN on this novel CT reaction at the clonal level using leukemia cell lines and their HIV-1 carrier clones, which all have well defined stable clonal characteristics (13, 14).

Materials and Methods

Cell lines. The origins, establishment and characteristics of the parental leukemia cell lines and the CD8+ HIV-1 carrier cell clones used in this study are summarized in Table 1 (13, 14). For convenience, CD8+ HIV-1 carrier cell clones are referred to as “effectors” and the other cell populations to be combined with them for the CT assay are “targets.” The cell lines were maintained in RPMI-1640 medium (Nissui Pharmaceutical, Tokyo) supplemented with 5-10% heat-inactivated fetal bovine serum (FBS) (Gibco Oriental, Tokyo). Care was taken to maintain good cell viability (≥ 95%) and an exponential phase of growth by partial feeding daily. All cultures were monitored monthly for, and were consistently free from detectable microbiological contamination including Mycoplasma.

Immunofluorescence analysis. Direct and indirect immunofluorescence analyses for cell surface and intracellular antigens were performed by flow cytometry (EPICS-PROFILE II; Coulter Electronics, Hialeah, Fla., U.S.A.) or fluorescent microscopy as described previously (13, 14). Antigens for terminal deoxynucleotidyl transferase (TdT) were detected using rabbit anti-calf TdT serum (P-L Biochemicals, Milwaukee, Wisc., U.S.A.) as the primary reagent and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit globulin (Cappel Laboratories, West Chester, Pa., U.S.A.) as the secondary reagent in methanol-fixed smear preparations. The panel of monoclonal antibodies used consisted of the following: NU-T3 (CD3), NU-TH/I (CD4), NUTS/C (CD8) and NU-la (HLA-DR, MHC class-II) (all from Nichirei, Tokyo); CH-11 (anti-Fas, CD95) (MBL, Nagoya, Japan); A3/10 (MHC-I) (provided by I. Trowbridge, The Salk Institute, La Jolla, Calif., U.S.A.); VAK-5 (anti-HIV p24 core protein) (provided by K. Sagawa, Kurume University, Fukuoka, Japan); 0.5β (anti-HIV-1 gp120) (provided by S. Matsushita, Kumamoto University, Kumamoto, Japan); b 12 (human monoclonal antibody against the CD4-binding site of HIV-1 gp120) (provided by D. Burton, Scripps Research Institute, La Jolla, Calif., U.S.A.); WT-31 (anti-TCR αβ heterodimer) (provided by W. Tax, St. Radboud Hospital, University of Nijmegen, The Netherlands); and pooled AIDS patients’ sera (anti-p24, gp41, and gp120/gp160) (provided by Y. Yamamura, AIDS Program, Ponce Medical School, Ponce, Puerto Rico). FITC-conjugated anti-mouse or -human immunoglobulin reagents (Cappel Laboratories) were used as secondary reagents. Like nuclear TdT, the cytoplasmic HIV-1 p24

Table 1. Characteristics of “effector” and “target” cell clones

<table>
<thead>
<tr>
<th>Clones</th>
<th>T-stage</th>
<th>Cellular markers CD3/CD4/CD8</th>
<th>TCR CD3/CD4</th>
<th>MHC-I/II</th>
<th>CD95 (%)</th>
<th>Sm-HIV (%)</th>
<th>p24/10⁶ cells</th>
<th>TCID₅₀/10⁶ cells (CAV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Effectors”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPB-ALL/HIV</td>
<td>II</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+/αβ</td>
<td>+/−</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>MOLT-4/HIV</td>
<td>III</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>−/−</td>
<td>+/−</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>MOLT-4/HIV</td>
<td>III</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>−/−</td>
<td>+/−</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>“Targets”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPB-ALL</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/αβ</td>
<td>+/−</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Steer 92</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/αβ</td>
<td>+/−</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Peer</td>
<td>IV</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+/γδ</td>
<td>+/−</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>MT-2</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+/αβ</td>
<td>+/−</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

4 Based on marker profiles (CD7, CD10, MHC class II and TdT antigens), five distinct stages of T-cell development had previously been defined, in order of maturation: stages I, II, III, IV and V (13, 14).

5 Direct or indirect immunofluorescence tests using monoclonal antibodies (except for nuclear TdT with polyclonal antibody), flow cytometric and visual fluorescent microscopic analyses were used. The data shown are mean values from multiple tests. + is ≥ 5% positive, − is <5% fluorescent cells.

6 Sm-HIV is the surface membrane expression of HIV-1 antigens (gp120 and gp160) as detected by either monoclonal antibodies or AIDS patients’ sera with high antibody titer using flow cytometry and fluorescent microscopy. The levels of persistent production of HIV-1 p24 antigen (measured by Abbott ELISA assay as ng/10⁶ cells) and of infectious HIV-1 particles (titrated by TCID₅₀ assay as TCID₅₀/CAV/10⁶ cells) within the cells of HIV-1 carrier clones are shown. The data presented are mean values from multiple tests.
antigen was detected in methanol-fixed cells by intracellular staining; all other antigens were detected on living-cell surface membranes.

**Infectivity assay by TCID₅₀.** To characterize these HIV-1 carrier clones further, standardized levels of their persistent infectious virus production were obtained using 48-hr incubated cultures. The infectious virus titers were determined using samples of cell-free virus (FV) per ml and cell-associated virus (CAV) per 10⁶ cells prepared from standard 2-day-old cultures. The highly susceptible HTLV-1 transformed T-cell line MT-2 (9) was used in a TCID₅₀ infectivity assay according to a procedure described previously (13, 14). Serial 10-fold dilutions (0.2 ml) of virus sample were inoculated into triple wells (24-well plate: Iwaki Glass Ware, Tokyo) containing 0.8 ml MT-2 cells (approximately 5 × 10⁶ cells/well) in RPMI 1640 medium containing 5% FBS, 100 U/ml of penicillin and 50 μg/ml of streptomycin. The culture plates were then incubated at 37 C in a 5% CO₂ humidified incubator for 5 days. For the HIV-1 p24 antigen, HIV-1 infection was detected by immuno-fluorescence with VAK-5 antibody in triplicate wells at each 10-fold virus dilution, and the TCID₅₀ titer was calculated according to Reed and Muench (26).

**p24 ELISA assay.** The HIV-1 p24 antigen levels, being persistently produced in both FV and CAV samples in the HIV-1 carrier clones, were also measured using an Abbott ELISA kit (Abbott Monoantibody II, Abbott Diagnostics, North Chicago, Ill., U.S.A.).

**IFN treatment.** Natural human IFN-α and IFN-γ preparations (specific activities of 2 × 10⁶ IU/mg and 5.6 × 10⁶ IU/mg, respectively) were obtained from Fujisaki Institute of Hayashibara Biochemical Laboratories, Inc. (1, 6). As a preliminary study, the cell cultures were incubated at varying doses of IFN (10¹, 10², 10³ and 10⁴ IU/ml) for varying periods of time (24, 48, 72 and 96 hr) prior to the CT tests. The lower concentrations of IFN gave essentially the same results, but were often marginally significant. Prolonged treatment (periods) with IFN also gave similar results, but the lowered viability of IFN treated cells invariably compromised an accurate quantitative comparison of the cytotoxicity scores. With 10¹ IU/ml IFN treatment for 48 hr, for example, both HPB-ALL and HPB-ALL/HIV maintained good viability (97–99%). Both MOLT-4 and MOLT-4/HIV C3 maintained good viability (96–97%) with IFN-α, but with IFN-γ, viability was often unacceptable (84–89%) for the CT assay. Furthermore, a 10³/ml IFN-γ treatment for 48 hr showed a cell growth inhibition in the range of 15±5% relative to that of the untreated control culture.

Thus, the most satisfactory and reliable cytotoxic test protocol, a 24-hr treatment with a dose of 1,000 IU/ml IFN, was used throughout this study.

Similar to previous reports (24, 25, 30), IFN-α treatment caused a statistically significant inhibition in FV production, but not in CAV production, as determined by TCID₅₀ (percent relative inhibition of the control levels of untreated HIV-1 carriers; on average HPB-ALL/HIV was ≥60% and MOLT-4/HIV C3 was ≥30%). Using a p24 ELISA assay, however, there was no significant inhibition in any of the conditions examined. IFN-γ treatment had no significant anti-HIV-1 activity (data not shown).

**Propidium iodide (PI) flow cytometric CT test.** Because of the extreme radiation sensitivity of immature T-cell leukemia cells as reported previously (16), a flow cytometric CT assay using propidium iodide (PI) uptake as a measure of the percentage of dead cells (18) was used instead of the ³¹Cr release assay. Briefly, 0.2 ml each (approximately 5 × 10⁶ cells/ml) of CD8 SP HIV-1 carrier cells (effectors) and CD4CD8 DP or CD4 SP cells (targets) (effector/target ratio of 1/1) were combined, centrifuged for 2 min at 1,000 rpm and then incubated at 37 C for 3 hr. Then 50 μl of a 75-mm PI solution containing sodium citrate (34 mm) was added to each sample and the percentage of PI-stained dead cells (red fluorescence) was determined immediately by flow cytometry with a program for two-parameter analysis (red fluorescence and forward-light scatter). Immediately after flow cytometry, HIV-1 induced syncytium formation was determined visually using a trypan blue dye exclusion test. For the final CT score, the flow cytometric PI-CT points (0.0 to 20.0 points), which were a function of the percentage of PI-stained dead cells in the test sample divided by half the sum of the percentage of PI-stained effector alone and that of the target alone, were calculated as shown in the following formula:

\[
\text{CT score} = \frac{\% \text{PI-test sample}}{1/2 (\% \text{PI-effector alone} + \% \text{PI-target alone})}
\]

Syncytium formation in the test sample was, however, found to be independent of this rapid CT reaction and was not used in the CT scores. CT scores of ≥ 2.0 were arbitrarily considered cytotoxic for effector activity and susceptible for target sensitivity.

**Statistical analysis.** For most of the assay values, the Student’s t-test was used to determine significance in paired or unpaired samples.

**Results**

**Direct CT Reaction**

In a 3-hr PI flow cytometric CT assay, three HIV-1 carrier clones (effectors: HPB-ALL/HIV, MOLT-4/HIV
C1 and MOLT-4/HIV C3) were shown to exhibit rapid CT activity uniquely to the TdT⁺CD4⁺CD8⁺ targets (HPB-ALL and Steer 92), but not to the CD4⁺ targets (Peer and MT-2) as shown in Fig. 1 and Table 2. This rapid CT reaction was not found with inappropriate combinations such as cytotoxic effector and non-susceptible target or with two cytotoxic HIV-1 clones together (mean CT score 1.3±0.1 from 26 test). In our earlier publication (15), this rapid CT reaction was shown to be unidirectional indicating only target cell death (data not shown). None of the cellular characteristics in these individual HIV-1 carrier clones (virus production, CD95 Fas antigen expression or TCR type) correlated with the degrees of CT reaction (Tables 1 and 2). The CT reaction we present here appeared, however, to be limited to cells at an immature stage of T-cell maturation (TdT⁺CD4⁺CD8⁺ DP phenotype) and to HIV-1 infected cells, which happened to be those expressing CD8. Although two TdT-negative CD4 SP targets (Peer and MT-2), representing more advanced T-cell maturation stages, were not susceptible to this CT reaction, they invariably permitted a complete HIV-1 replication cycle in them leading to cytopathic effects in 36–48 hr.

Further characterization of this rapid CT reaction by blocking the relevant cell surface-antigen expression revealed that the expression of CD4, but not CD8 or MHC-I, in the targets and of HIV-1 gp120/gp160, but not CD8 or MHC-I, in the effectors is mutually obligatory.

Table 2. Immediate cytotoxicity of CD8⁺ HIV-1 carrier clones (effectors) to CD4⁺ CD8⁺ or CD4⁺ T-cell clones (targets)

<table>
<thead>
<tr>
<th>Targets</th>
<th>Cytotoxic scores of effectors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPB-ALL/HIV</td>
</tr>
<tr>
<td>HPB-ALL</td>
<td>6.0±0.3 (35)</td>
</tr>
<tr>
<td>Steer 92</td>
<td>5.0±0.3 (14)</td>
</tr>
<tr>
<td>Peer</td>
<td>1.3±0.2 (5)</td>
</tr>
<tr>
<td>MT-2</td>
<td>1.2±0.1 (5)</td>
</tr>
</tbody>
</table>

Cytotoxic scores were calculated as described in “Materials and Methods.”

Table 3. Effects of antigen blocking on immediate cytotoxicity

<table>
<thead>
<tr>
<th>Antigen block on HPB-ALL/HIV (effector) or HPB-ALL (target)</th>
<th>CT scores of effectors</th>
<th>Student’s t-test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPB-ALL/HIV + HPB-ALL (Control)</td>
<td>7.7±0.5</td>
<td></td>
</tr>
<tr>
<td>gp120-blocked HPB-ALL/HIV + HPB-ALL</td>
<td>3.4±0.3</td>
<td>0.003*</td>
</tr>
<tr>
<td>CD8-blocked HPB-ALL/HIV + HPB-ALL</td>
<td>8.0±1.4</td>
<td>0.840</td>
</tr>
<tr>
<td>MHC-I-blocked HPB-ALL/HIV + HPB-ALL</td>
<td>9.0±0.1</td>
<td>0.083</td>
</tr>
<tr>
<td>HPB-ALL/HIV + CD4-blocked HPB-ALL</td>
<td>3.3±1.3</td>
<td>0.037*</td>
</tr>
<tr>
<td>HPB-ALL/HIV + CD8-blocked HPB-ALL</td>
<td>8.4±0.4</td>
<td>0.389</td>
</tr>
<tr>
<td>HPB-ALL/HIV + MHC-I-blocked HPB-ALL</td>
<td>7.9±1.3</td>
<td>0.869</td>
</tr>
</tbody>
</table>

Antigen expression on either effector or target was individually blocked by incubating the cells with specific monoclonal antibodies as described in “Materials and Methods” for 1 hr prior to cytotoxicity testing (15).

Cytotoxic scores were calculated as described in “Materials and Methods.”

Mean±SE from 3 experiments.

* P values <0.05 from Student’s t-test are considered to be significant.
(for this CT reaction to occur). It was also revealed that the CT reaction required no MHC-I restriction, clearly indicating that this is a non-immunological reaction (Table 3).

**Effects of IFN-α or -γ Treatment on CT Reaction**

The cytotoxic HIV-1 effectors and susceptible TdT+ CD4+CD8+ targets were treated with IFN-α or -γ (1,000 IU/ml for 24 hr) prior to the CT test. Figure 2 illustrates representative results of the HPB-ALL/HIV and HPB-ALL combination with or without IFN treatment. Figure 3 summarizes the results of three experiments using this effector-target combination. While the treatment of effector HPB-ALL/HIV with IFN-α, and/or of target HPB-ALL with IFN-γ, reduced the CT reaction to a statistically significant level, treatment of the effectors with IFN-γ, or of the target with IFN-α, did not. For the less cytotoxic MOLT-4/HIV C1 and C3, IFN treatment under the same conditions resulted in insignificant to marginal protection of the cells from the CT reaction (data not shown). Treatment of non-susceptible TdT-negative CD4+ SP targets (Peer and MT-2) with IFN-α or -γ did not affect their susceptibility to the cytotoxic effectors regardless of IFN pretreatment (data not shown).

**Effects of IFN-α or -γ Treatment on Antigen Expression**

The particular sets of CD8, MHC-I, CD4 and/or HIV-1 gp120/gp160 antigens in effectors and targets, which appear to define the discrete stage of T-cell development so crucial to this unique CT reaction, were analyzed (Tables 1 and 2). The results of three such experiments...
are summarized in Table 4. The simultaneous occurrence of the reduced expression of CD8 and the increased expression of both MHC-I and HIV-1 gp120/gp160 on the IFN-γ treated effector HPB-ALL/HIV, or the increased expression of both CD8 and MHC-I on the IFN-γ treated target HPB-ALL, appeared to be associated with the reduced CT reaction. Figure 4 shows representative results of the changes in antigen expression in the IFN treated effectors and targets. Although it is intriguing that IFN-γ treatment also up-regulated HIV-1 gp120/gp160 expression in the effectors as with IFN-α treatment, it had no effect on the CT reaction (Table 4 and Fig. 3).

Discussion

The antiviral function of IFN has been extensively studied for a variety of viruses including HIV-1 (11, 23–25, 27, 30). In one of the studies, IFN-α, but not IFN-γ, significantly inhibited HIV-1 replication in vitro (30). The mechanism of the CT reaction of HIV-1 carrier clones, incidentally of CD8+ SP T cells, to the immature DP T cells we describe in this study apparently differs from the variety of IFN activities previously known. The HIV-1 carrier T-cell clone interacts with uniquely and crucially important cells in the immune system resulting in rapid immature T-cell death within 1 hr or less (15). As reported recently (17), the ICMK in which HIV-1 infected blood CD4 T cells were shown to directly kill uninfected CD4+ cells is different because the killing is not restricted to immature CD4+CD8+ T cells. This study is also different from those of immunologically specific cytotoxic lymphocytes (CTL) against HIV infected target cells (7, 8, 32). Despite the fact that the CT reaction described is clearly not an antigen-specific CT reaction, it is a cell-to-cell cytotoxicity of HIV-1

Table 4. Marker changes on effector (HPB-ALL/HIV) or target (HPB-ALL) after IFN treatment

<table>
<thead>
<tr>
<th>Effector/Target</th>
<th>Percent change in MFI relative to control values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD (number of experiments)</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
</tr>
<tr>
<td>HPB-ALL/HIV (Effector)</td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>100</td>
</tr>
<tr>
<td>IFN-α treated</td>
<td>70±3 (3)</td>
</tr>
<tr>
<td>IFN-γ treated</td>
<td>72±9 (4)</td>
</tr>
<tr>
<td>HPB-ALL (Target)</td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>100</td>
</tr>
<tr>
<td>IFN-α treated</td>
<td>99±37 (3)</td>
</tr>
<tr>
<td>IFN-γ treated</td>
<td>122±22 (3)</td>
</tr>
</tbody>
</table>

¹ Interferon treatment at 1,000 IU/ml was done for a 24-hr period prior to the cytotoxic test as described in “Materials and Methods.”
² MFI is the mean fluorescence intensity as determined by flow cytometry.
³ Not detected.
⁴ Statistically these values are not significantly different from the control values (P>0.05).

Fig. 4. Changes in antigen expression after IFN treatment. FITC-labeled antigen expressions (CD8, MHC-I, HIV-1 gp120/gp160 and CD4) are shown in flow cytometric histograms. The numbers in each histogram are the mean fluorescence intensity (MFI); an MFI difference of >0.2 is considered significant. The effectors and targets with or without IFN treatment are the same cells used for the CT assays shown in Fig. 2.
carrier cells to specific immature T-cell targets representing TdT+CD4+CD8+ T-cell precursors, not to mature CD4+ T cells. The fact is that IFN-α treated CD8+ effector HIV-1 carrier cells and IFN-γ treated TdT+CD4+CD8+ target cells reduced the intensity of rapid cytotoxicity upon subsequent interaction with each other. We observed a more effective reduction of the CT reaction with IFN-α treated effectors than with the protective effects of IFN-γ treatment of the targets (Fig. 3). Neither the combination of those two nor longer periods of IFN treatment, however, increased the protective effects any further as judged by the method and criteria used in this study (data not shown). This limited protection from IFN treatment does not seem to be due to the antiviral activity of IFN because the CT reaction apparently does not require complete virus replication in the target cells (being killed). Actually, IFN treatment of the effectors resulted in up-regulated HIV-1 gp120/gp160 expression (on the cell surface) (4, Table 4), which is in accord with the reported effect of IFN-α demonstrating the accumulated HIV virions on the cell membrane (31).

For viral replication, the CT-nonsusceptible CD4+ Peer and MT-2 are actually highly susceptible to HIV-1 infection (13). Indeed, they both produce a high level of progeny infectious virus particles when the newly infected CD4+ T cells are cultured for longer periods of time (9, 13). While IFN treatment of the permissive cells is known to inhibit active virus replication in them, the rapid CT reaction by the IFN-α treated effectors, or by the IFN-γ treated targets, down-regulates the intensity of the ultimate CT reaction (Figs. 2 and 3). The up-regulation of HIV-1 gp120/gp160 expression in the IFN treated effectors is likely to establish an effective binding between the HIV-1 infected cells and the CD4 molecule on the target T cells. It is, however, not known why or how only the IFN-α treated, but not the IFN-γ treated HIV-1 gp120/gp160 reduces the intensity of the CT reaction as shown in Fig. 3. The circumstances of the lymphoid tissues during HIV-1 infection are indeed paradoxical in the side by side representation of both immune activation and suppression or dysregulation due to HIV-1 induced cytopathy (4, 10, 19, 33).

The CT activity occurs between the effector HIV-1 carrier cells often expressing CD8 and the TdT+CD4+CD8+ target cells which interestingly also express CD8. The cells being killed are the target, but not the HIV-1 carrier cells (15). These observations might bear significance in connection with a recent report (5) in which a profound apoptotic death of the lymph node cells of HIV-1 infected patients actually occurred in bystander cells, but not in virus-infected cells. Thus, this study provides further insight into the mechanism of CD4 T-cell depletion and into the roles of CD8+ T cells during HIV-1 pathogenesis. This study also provides a possible explanation for the devastating consequences of HIV-1 infection to thymocytes (28). Accordingly, this study indicates that IFN-α and IFN-γ can, at least partially, protect immature T cells in the thymus and/or in the primary active lymphopoietic tissues from rapid cytodestruction by contact with HIV infected cells (3–5, 19, 20, 28, 33).

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References