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Effects of Lymphocyte Isolation and Timing of Processing on Detection of CD127 Expression on T Cells in Human Immunodeficiency Virus-Infected Patients

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Decreases in the detection of CD127 expression on T cells of human immunodeficiency virus-infected patients by flow cytometry can occur by delayed processing or by peripheral blood mononuclear cell isolation and cryopreservation. These observations should be considered in the interpretation of functional studies and the planning of multicenter clinical trials.

Interleukin 7 (IL-7) is a nonredundant cytokine for T-lymphocyte development and function (8). IL-7 is essential for the survival and homeostatic proliferation of naïve cells (16, 18), as well as for the maturation and differentiation of memory $CD4^+$ and $CD8^+$ T cells (9–11). In CD4 lymphopenic states, such as human immunodeficiency virus (HIV) infection or chemotherapy, elevated levels of IL-7 in plasma have been observed, suggesting a potential compensatory mechanism (6, 14). These observations combined with animal data confirming the role of IL-7 in immune reconstitution (5) and the potential for peripheral T-cell expansion (7) have generated significant interest in therapeutic applications of IL-7 in immunocompromised states.

The IL-7 receptor is comprised of an α chain (CD127) and the γ_c chain (CD132, common cytokine chain) (2). CD127 is expressed mainly on B-cell precursors and mature T cells (8), and in animal models, its expression appears to be critical in $CD8^+$-T-cell memory development and maturation (9). Decreased expression of CD127 on $CD4^+$ and $CD8^+$ T cells in HIV-infected patients has been described, with increases seen after the introduction of antiretroviral therapy (12, 19).

In this study, we determined whether the timing of processing of peripheral blood or lymphocyte separation and cryopreservation procedures would have an effect on the detection of CD127 expression, as measured by flow cytometry, on $CD4^+$ or $CD8^+$ T cells of HIV-infected patients. The new Centers for Disease Control and Prevention guidelines for $CD4^+$-T-cell determinations with CD45 gating for HIV-infected persons state that peripheral blood can be held up to 72 h (13), a practice that can allow centralized laboratory testing in multicenter trials. Previous studies have also shown that density gradient separation and cryopreservation do not lead to loss of either $CD4^+$ or $CD8^+$ T cells (4), but measurable losses can be observed in specific T-cell subsets (3, 17).

In order to evaluate the effects of the time of processing and lymphocyte separation on the cytometric detection of CD127, a cohort of 53 HIV-seropositive patients with a median age of 47 years (range, 24 to 68 years) was studied after informed consent was obtained. The median CD4 count was 475 cells/μl (range, 16 to 1,258 cells/μl) and the median CD8 count was 788 cells/μl (range, 169 to 1,887 cells/μl). Twenty-four patients (45%) had viral loads of <50 copies/ml. The median viral load of the remaining patients was 1,728 copies/ml (range, 58 to 232,702 copies/ml). Four patients were not receiving antiretroviral therapy at the time of study.

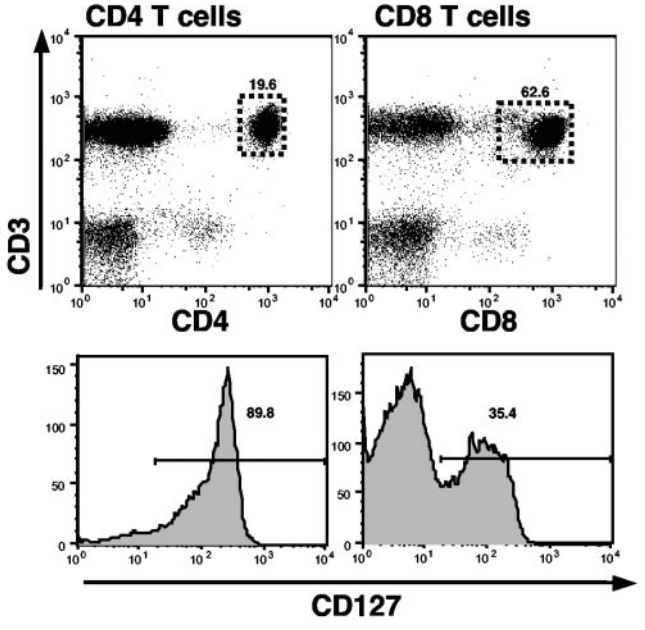

FIG. 1. Representative CD127 histograms from a study participant. Gating was done on $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ T cells.

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TABLE 1. Percentage of $CD4^+$ and $CD8^+$ T cells expressing CD127 in peripheral blood at 4, 24, 48, and 72 h

T cells	% in PB at[a]:				Difference from value at <4 h[b]	r[c]
	<4 h	24 h	48 h	72 h		
$CD4^+$	83.4 (50–100)	79.4 (40–100)	79.1 (40–100)	75.8 (25–100)	24 h: −2.2 (−17.8 to +4.2)	0.90
					48 h: −3.0 (−15.4 to +4.2)	0.95
					72 h: −6.5 (−25.0 to +4.2)	0.92
$CD8^+$	45.5 (13.8–76.1)	38.7 (19.5–71.7)	40.6 (19–71.7)	39.0 (17.7–66.7)	24 h: −2.9 (−11.9 to +9.2)	0.96
					48 h: −1.8 (−11.9 to +7.7)	0.96
					72 h: −3.3 (−19.2 to +10.4)	0.90

[a] PB, peripheral blood. Values are medians, with ranges in parentheses ($n = 40$).
[b] Paired comparisons with freshly isolated peripheral blood at <4 h by the Wilcoxon signed-rank test. All P values were <0.001.
[c] Spearman correlation coefficient (correlation between values from each individual at <4 h versus 24, 48 or 72 h). P values for all correlations were <0.001.

Immunophenotyping of peripheral blood drawn in EDTA was done at 4, 24, 48, and 72 h after drawing of blood from 40 participants, according to the manufacturer's instructions, by using a modification of the Centers for Disease Control and Prevention guidelines in a Clinical Laboratory Improvement Act (CLIA)-certified laboratory (3). Cells were lysed after staining with Optilyse C (Beckman Coulter, Hialeah, Fla.), washed twice, and resuspended in 500 μl of phosphate-buffered saline (Cambrex, Walkersville, Md.). Samples were analyzed immediately on an Epics XL flow cytometer (Beckman Coulter) (1). The antibodies used were CD45-fluorescein isothiocyanate (clone 2D1), CD14-phycoerythrin (PE) (clone MΦ Pq), immunoglobulin G1 PE-Cy5 (clone G18-145), CD3 FITC (clone SK7), CD4 PE-Cy5 (clone RPA-T4), and CD8 PE-Cy5 (clone RPA-T8) from BD/Pharmingen (San Jose, Calif.) and CD127 PE (clone R34.34) (15) from Beckman-Coulter. Gating on $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$ T cells was used to determine the expression of CD127 (Fig. 1).

In addition, peripheral blood was collected in a heparinized syringe from a total of 28 participants, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque separation (1). A fraction of the PBMC were processed immediately after separation for immunophenotyping, and the remaining PBMC were cryopreserved (1). After 1 week, the cryopreserved PBMC were thawed in a 37°C water bath (1), washed once in complete medium, counted, and assessed for viability with propidium iodide prior to immunophenotyping

Median values and ranges are reported. All paired comparisons (within individuals) were done with the Wilcoxon signed-rank test, and Spearman correlation coefficients were used to describe associations between variables.

The percentage of $CD4^+$ and $CD8^+$ T cells expressing CD127 at 24 h after the blood draw was significantly lower than that in peripheral blood processed within 4 h (Table 1). In the $CD4^+$-T-cell subset, a further decrease was seen at 72 h, and decreases at 72 h were significantly different from those at 4, 24 and 48 h ($P < 0.001$ for all comparisons). Although the decreased CD127 expression in the $CD8^+$-T-cell subset was observed at 24 h, no further decreases were noted (Table 1). Results were not affected by the type of anticoagulant used (EDTA versus heparin; data not shown). Similar results were obtained with a cohort of 11 HIV-seronegative volunteers, with 4.5% decreases in CD127 expression on both $CD4^+$ ($P = 0.002$) and $CD8^+$ ($P = 0.008$) T cells at 72 h.

A small but statistically significant decrease in the detected percentage of $CD4^+$ T cells expressing CD127 was observed in freshly isolated and cryopreserved PBMC compared to peripheral blood (Table 2). The percentage of $CD4^+$ T cells expressing CD127 in peripheral blood was strongly correlated with that in PBMC and in cryopreserved PBMC, suggesting a homogeneous effect on all studied donors (Table 1). A larger and statistically significant decrease was observed in the detected percentage of $CD8^+$ T cells expressing CD127 in freshly isolated or cryopreserved PBMC compared to peripheral blood (Table 2). Significant correlations were noted between the percentage of $CD8^+$ T cells expressing CD127 in peripheral blood and that in freshly isolated or cryopreserved PBMC. No statistically significant differences in CD127 expression were noted between freshly isolated and cryopreserved PBMC in either the $CD4^+$- or $CD8^+$-T-cell subset. Similar findings were obtained in the HIV-seronegative cohort, with decreases in CD127 expression on both cryopreserved $CD4^+$ (9%, $P = 0.001$) and $CD8^+$ (4%, $P = 0.001$) T cells.

These data suggest that PBMC separation can result in decreases in the percentage of T cells expressing CD127 in HIV-infected patients. These decreases seem to occur predomi-

TABLE 2. Percentage of T cells expressing CD127 in peripheral blood, PBMC, and cryopreserved PBMC, and absolute differences between PBMC or cryopreserved PBMC and PB[a]

T cells	% in:			PB vs. PBMC			PB vs. cryopreserved PBMC		
	PB	PBMC	Cryopreserved PBMC	Difference	P value[b]	r[c]	Difference	P value[b]	r[c]
$CD4^+$	86.5 (67.3–97)	81.7 (62.5–93)	81.3 (67–94.9)	−4.5 (−15.3 to +3.6)	<0.001	0.84	−3.3 (−17 to +5.9)	0.002	0.68
$CD8^+$	48.5 (11–77)	30.4 (5–64)	36.1 (10–66)	−9.8 (−30 to +1.7)	<0.001	0.87	−9.6 (−31.2 to +6.4)	0.0001	0.80

[a] PB, peripheral blood. Values are medians, with ranges in parentheses ($n = 28$).
[b] Paired comparisons were done by the Wilcoxon signed-rank test between PBMC or cryopreserved PBMC and PB at <4 h.
[c] Spearman correlation coefficient (correlation between values from each individual at <4 h in PB versus PBMC or cryopreserved PBMC). P values for all correlations were <0.001.

nantly during PBMC separation and affect disproportionally the $CD8^+$-T-cell subset. This should be taken into consideration, since many functional T-cell assays are performed on separated and/or cryopreserved PBMC. Despite the observed decreases, the expression of CD127 on PBMC correlated strongly with that obtained from staining of freshly collected peripheral blood, suggesting an overall proportional cross-donor effect. Finally, although optimal results were obtained with immediate processing after a blood draw, the observed decreases would be acceptable for most laboratories at both 24 and 48 h, allowing the potential for off-site flow cytometry in multicenter clinical trials.

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REFERENCES

1. **Baseler, M., R. A. Stevens, V. Natarajan, J. C. Gea-Banacloche, and J. A. Metcalf.** 2002. Immunologic evaluation of patients with human immunodeficiency virus infection, p. 758–771. *In* N. R. Rose, R. G. Hamilton, and B. Detrick (ed.), Manual of clinical laboratory immunology, 6th ed. ASM Press, Washington, D.C.
2. **Candeias, S., K. Muegge, and S. K. Durum.** 1997. IL-7 receptor and VDJ recombination: trophic versus mechanistic actions. Immunity **6:**501–508.
3. **Centers for Disease Control and Prevention.** 1997. 1997 revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV). MMWR Recomm. Rep. **46:**1–29.
4. **De Paoli, P., M. Reitano, S. Battistin, C. Castiglia, and G. Santini.** 1984. Enumeration of human lymphocyte subsets by monoclonal antibodies and flow cytometry: a comparative study using whole blood or mononuclear cells separated by density gradient centrifugation. J. Immunol. Methods **72:**349–353.
5. **Fry, T. J., B. L. Christensen, K. L. Komschlies, R. E. Gress, and C. L. Mackall.** 2001. Interleukin-7 restores immunity in athymic T-cell-depleted hosts. Blood **97:**1525–1533.
6. **Fry, T. J., E. Connick, J. Falloon, M. M. Lederman, D. J. Liewehr, J. Spritzler, S. M. Steinberg, L. V. Wood, R. Yarchoan, J. Zuckerman, A. Landay, and C. L. Mackall.** 2001. A potential role for interleukin-7 in T-cell homeostasis. Blood **97:**2983–2990.
7. **Fry, T. J., M. Moniuszko, S. Creekmore, S. J. Donohue, D. C. Douek, S. Giardina, T. T. Hecht, B. J. Hill, K. Komschlies, J. Tomaszewski, G. Franchini, and C. L. Mackall.** 2003. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. Blood **101:**2294–2299.
8. **Hofmeister, R., A. R. Khaled, N. Benbernou, E. Rajnavolgyi, K. Muegge, and S. K. Durum.** 1999. Interleukin-7: physiological roles and mechanisms of action. Cytokine Growth Factor Rev. **10:**41–60.
9. **Kaech, S. M., J. T. Tan, E. J. Wherry, B. T. Konieczny, C. D. Surh, and R. Ahmed.** 2003. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat. Immunol. **4:**1191–1198.
10. **Kondrack, R. M., J. Harbertson, J. T. Tan, M. E. McBreen, C. D. Surh, and L. M. Bradley.** 2003. Interleukin 7 regulates the survival and generation of memory CD4 cells. J. Exp. Med. **198:**1797–1806.
11. **Li, J., G. Huston, and S. L. Swain.** 2003. IL-7 promotes the transition of CD4 effectors to persistent memory cells. J. Exp. Med. **198:**1807–1815.
12. **MacPherson, P. A., C. Fex, J. Sanchez-Dardon, N. Hawley-Foss, and J. B. Angel.** 2001. Interleukin-7 receptor expression on CD8(+) T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. J. Acquir. Immune Defic. Syndr. **28:**454–457.
13. **Mandy, F. F., J. K. Nicholson, and J. S. McDougal.** 2003. Guidelines for performing single-platform absolute CD4+ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. MMWR Recomm. Rep. **52:**1–13.
14. **Napolitano, L. A., R. M. Grant, S. G. Deeks, D. Schmidt, S. C. De Rosa, L. A. Herzenberg, B. G. Herndier, J. Andersson, and J. M. McCune.** 2001. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Nat. Med. **7:**73–79.
15. **Pandrau-Garcia, D., B. de Saint-Vis, S. Saeland, N. Renard, S. Ho, I. Moreau, J. Banchereau, and J. P. Galizzi.** 1994. Growth inhibitory and agonistic signals of interleukin-7 (IL-7) can be mediated through the CDw127 IL-7 receptor. Blood **83:**3613–3619.
16. **Rathmell, J. C., E. A. Farkash, W. Gao, and C. B. Thompson.** 2001. IL-7 enhances the survival and maintains the size of naive T cells. J. Immunol. **167:**6869–6876.
17. **Reimann, K. A., M. Chernoff, C. L. Wilkening, C. E. Nickerson, A. L. Landay, et al.** 2000. Preservation of lymphocyte immunophenotype and proliferative responses in cryopreserved peripheral blood mononuclear cells from human immunodeficiency virus type 1-infected donors: implications for multicenter clinical trials. Clin. Diagn. Lab. Immunol. **7:**352–359.
18. **Tan, J. T., E. Dudl, E. LeRoy, R. Murray, J. Sprent, K. I. Weinberg, and C. D. Surh.** 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc. Natl. Acad. Sci. USA **98:**8732–8737.
19. **Vingerhoets, J., E. Bisalinkumi, G. Penne, R. Colebunders, E. Bosmans, L. Kestens, and G. Vanham.** 1998. Altered receptor expression and decreased sensitivity of T-cells to the stimulatory cytokines IL-2, IL-7 and IL-12 in HIV infection. Immunol. Lett. **61:**53–61.