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Immune Restoration by Combination of a Cytostatic Drug (Hydroxyurea) and Anti-HIV Drugs (Didanosine and Indinavir)

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ABSTRACT

Cell activation is essential for HIV infection. $CD4^+$ T lymphocyte activation allows virus replication and $CD8^+$ T lymphocyte activation may contribute to pathogenesis. We combined hydroxyurea, a cytostatic drug that inhibits cell activation and proliferation, with two drugs that inhibit HIV (didanosine and indinavir), to block the "cell activation–virus production–pathogenesis" cycle. HIV was strongly suppressed in treated patients, and the average CD4 count increased to 224/mm^3. Compared with a matched group of patients who had declined antiretroviral treatment, treated patients had a significantly lower proportion of activated $CD8^+$ T lymphocytes and a significantly higher number of naive $CD8^+$ and $CD4^+$ T lymphocytes. The proliferative responses to allogeneic and influenza virus antigens were increased in treated patients, and a defect in CD3-ζ expression, the signaling chain of the T cell receptor complex, was reversed. The use of a cytostatic drug was not detrimental to the immune system; on the contrary, the combination of antiviral and cytostatic treatment improved all of the immune parameters tested.

INTRODUCTION

HIV RNA REVERSE TRANSCRIPTION starts both in quiescent and activated $CD4^+$ T lymphocytes after virus entry, but productive virus replication requires $CD4^+$ T lymphocyte activation/proliferation.[1–4] Some models predict that the number of cells supporting viral replication represents the limiting factor for HIV expansion.[5–7] Consistent with mathematical predictions, the increase in CD4 count during triple-drug induction therapy was a predictor of loss of viral suppression during single- and/or double-drug maintenance therapy.[8] $CD8^+$ T lymphocytes are also activated by HIV infection. The high rate of $CD8^+$ T lymphocyte turnover is evident by their telomeric shortening in the course of HIV infection.[9] Activation and proliferation of T lymphocytes represent the natural host response to HIV infection. $CD8^+$ T lymphocyte activation, however, might be more harmful than beneficial if (1) activated cytotoxic $CD8^+$ T lymphocytes (CTLs) contribute significantly to $CD4^+$ T lymphocyte depletion[10,11] or if (2) chronic proliferation exhausts HIV-specific or bystander $CD8^+$ T lymphocytes.[10,11]

Hydroxyurea inhibits the cellular enzyme ribonucleotide reductase, thus reducing the intracellular levels of deoxynucleotides. This blocks cell division during G1–S phase[12] and also inhibits HIV DNA synthesis.[13] In asymptomatic HIV-infected individuals hydroxyurea potentiates the antiviral effects both of didanosine,[14–19] and didanosine plus stavudine.[20] In these studies, however, although HIV production was controlled, only a modest increase in $CD4^+$ T lymphocytes was observed. This raised concerns about potential immunosuppressive effects of hydroxyurea-containing regimens.[18,20] The use of potentially immunosuppressive drugs during an infection leading to immunodeficiency might appear a paradox. However, since cell activation and proliferation are pivotal for HIV replication and may contribute to the immunopathology of HIV infection, early use of drugs that inhibit immune cell activation might limit the damage to the immune system.

MATERIALS AND METHODS

Patients

To be eligible in the study, each patient needed at least two independent consecutive assays scoring positive for the presence of HIV in the plasma. Patients received didanosine (200 mg twice a day) and indinavir (800 mg three times a day); pa-

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tients weighing <60 kg were also given hydroxyurea (300 mg three times a day) while patients weighing >60 kg were given hydroxyurea (400 mg three times a day). The principles outlined in the Declaration of Helsinki "Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects (1990)" have been followed. All patients signed an informed consent before starting (or not starting) treatment.

Surrogate markers

Plasma viremia was measured by a branch-DNA test (Chiron [Emeryville, CA], sensitivity of 500 equivalents/ml). $CD4^+$ and $CD8^+$ T lymphocyte counts (per mm^3) were performed following standard techniques.

Statistical analysis

To evaluate dynamic changes within groups a Wilcoxon matched pair test was used. To evaluate differences between two different groups of patients, the Mann–Whitney nonparametric *U* test was used, except for the analysis of plasma viremia, since no value could be attributed to <500. In this case a Fisher exact test analysis was performed, comparing the number of detectable and undetectable values in each group.

Analysis of phenotypic markers

Peripheral blood mononuclear cells (PBMCs) were suspended in 50 μl of Hanks' balanced salt solution (HBSS) and permeabilized using the Caltag Laboratories (Burlingame, CA) Fix and Perm kit according to the manufacturer's protocol. For CD3-ζ analysis, fixed cells were incubated for 15 min at room temperature with 2 μl of either CD3-ζ monoclonal antibody (MAb) 6B10.2 (Santa Cruz, Santa Cruz, CA) or MsIgG1 isotype-matched control antibody (Coulter, Hialeah, FL). After washing with 5 ml of HBSS, cells were stained with 2 μl of phycoerythrin (PE)-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin (Dako, Carpinteria, CA). Cells were then washed twice with 5 ml of HBSS buffer and resuspended in 50 μl of HBSS before adding 2 μl of CD4–Cy5 (PharMingen, San Diego, CA) and 2 μl of CD8–fluorescein isothiocyanate (FITC) (Immunotech, Miami, FL), 4 μl of CD20–FITC (Becton Dickinson, Mountain View, CA) and 2 μl of CD3–Cy5 (Immunotech), or IgG–FITC and –Cy5 conjugated controls. The samples were incubated for 15 min at 4°C, washed with 5 ml of HBSS, and fixed with 2% formaldehyde prior to analysis. For analysis of naive ($CD62L^+CD45RA^+$) cells, PBMCs ($2–10 \times 10^5$/tube), isolated by Ficoll-Hypaque density centrifugation from heparinized blood, were suspended in 100 μl of fluorescence-activated cell sorting (FACS) buffer (phosphate-buffered saline [PBS] with 2% fetal calf serum [FCS]) before adding 4 μl of CD45RA–FITC (MAb ALB11; Immunotech), 4 μl of a 1:10 dilution of CD62L–PE (MAb SCFI28T17G6; Coulter), and 4 μl of either CD8–Cy5 or CD4–Cy5 (MAb 13B8.2; Immunotech). After incubation for 15 min at room temperature, cells were washed and fixed in 2% formaldehyde in PBS. For analysis of activated ($CD38^+DR^+$) $CD8^+$ T lymphocytes, 4 μl of CD38–PE (MAb T16; Immunotech), 4 μl of HLA-DR–FITC (MAb 357, Immunotech) and 4 μl of CD8–Cy5 or 4 μl of IgG–FITC, –PE, and –Cy5 conjugated isotype-matched controls (Immunotech) were used. In all experiments, flow cytometry analysis was performed on a tightly gated lymphocyte population using FACScalibur (Becton Dickinson).

Proliferation in response to influenza virus and allogeneic antigens

Whole blood was drawn and collected in Vacutainer tubes containing preservative-free heparin (Becton Dickinson, Rutherford, NJ). Peripheral blood lymphocytes (PBLs) were separated in lymphocyte separation medium (Organon Teknika, Durham, NC) and washed in PBS, and the number of viable leukocytes was determined by trypan blue exclusion and a hemocytometer. All PBLs were tested as fresh samples. For proliferation assays, 3×10^5 PBLs were placed in flat-bottom wells of a microtiter culture plate (Costar, Cambridge, MA) in a final volume of 0.2 ml along with (1) no stimulation (medium background), (2) influenza A virus (A/Bangkok RX73 H3N2, 1:500 concentration) (flu); or (3) a pool of irradiated (50 Gy) allogeneic PBLs (1×10^5 cells/well) from two or more unrelated healthy control volunteers (allo). Three replicate cultures were performed for each stimulation. Pooled human plasma was added to each well (1:20 final concentration) 1 hr after sensitization of the PBLs. Cultures were pulsed with 1 μCi of [^{3}H]thymidine 6 days after antigenic stimulation and harvested 18 hr later. Proliferation indexes for flu and allo for each patient were obtained by averaging all triplicate results, then dividing the results obtained with flu and allo, respectively, and the medium background.

RESULTS

Eight patients were treated an average of 13 months after infection (Table 1) for an average of 5.0 months with the combination of hydroxyurea, didanosine, and indinavir. Before treatment, the average CD4 count was 481/mm^3. The CD4 count increased (average, +224/mm^3), as did the CD4/CD8 ratio (average before treatment, 0.7; after treatment, 1.0). Both the CD4 count and the CD4/CD8 ratio changes were significant (p = 0.007 and 0.012, respectively). Before treatment, the average viral load in the plasma was 142,715 copies/ml. Within the first 8 weeks of treatment, plasma viremia became undetectable in all treated patients, and remained undetectable throughout the observed period of treatment.

To evaluate the impact of our treatment on the immune system we compared the treated patients with a matched group of untreated HIV-infected individuals (Table 1). The mean age, estimated length of HIV infection, CD4 count, CD8 count, CD4/CD8 ratio, and plasma viremia (evaluated as number of undetectable samples per number of total samples by a Fisher exact test) were not statistically different between the two groups at the time samples were collected for the analysis of markers of immunologic function.

The functionality of the T cells was analyzed by testing a phenotypic correlate of T cell function, the expression of CD3-ζ,[21] on $CD4^+$ as well as $CD8^+$ T lymphocytes. CD3-ζ expression was significantly greater for $CD4^+$ (p = 0.004) and $CD8^+$ (p = 0.002) T lymphocytes in the treated compared with the untreated group (Fig. 1A). The proliferative response of PBMCs to influenza virus (flu) and allogeneic (allo) antigens[22,23] was then compared in the treated and untreated patients (Fig. 1B). The average proliferation index in response to flu was twice as high in the group of treated patients compared with the untreated patients (8.3 ± 5.7 and 4.6 ± 3.8, respectively). The response to allogeneic target cells was also higher in the treated group (average,

Table 1. Characteristics of Treated and Untreated Patients

	HIV exposure/months		CD4			CD8			CD4/CD8			Plasma viremia	
Patient	Before treatment	Total exposure	Before treatment	Last value	Change	Before treatment	Last value	Change	Before treatment	Last value	Change	Before treatment	Last value
Treated patients													
1	1	7	570	747	177	940	704	−236	0.6	1.1	0.5	89,390	<500
2	2	4	349	775	426	227	491	264	1.5	1.6	0	756,100	<500
3	34	37	504	714	210	1,092	990	−102	0.5	0.7	0.3	1,243	<500
4	9	18	630	749	119	1,358	813	−545	0.5	0.9	0.5	30,820	<500
5	10	12	277	388	111	750	827	77	0.4	0.5	0.1	25,750	<500
6	10	18	480	1,072	592	970	972	2	0.5	1.1	0.6	45,290	<500
7	1	9	520	653	133	870	601	−269	0.6	1.1	0.5	192,000	<500
8	37	39	516	537	21	516	522	6	1	1	0	1,125	<500
Average:	13.0	18.0	481	704	224	840	740	−100	0.7	1.0	0.3	142,715	
SD:	14.4	13.3	115	198	190	348	192	248	0.4	0.3	0.2	255,586	
Median:	9.5	15.0	510	731	155	905	759	−50	0.5	1.0	0.4	38,055	
Untreated patients													
11	n.a.	5	n.a.	1,035	n.a.	n.a.	1,387	n.a.	n.a.	0.7	n.a.	n.a.	<500
12	n.a.	9	n.a.	461	n.a.	n.a.	885	n.a.	n.a.	0.5	n.a.	n.a.	42,000
13	n.a.	16	n.a.	650	n.a.	n.a.	1,123	n.a.	n.a.	0.6	n.a.	n.a.	<500
14	n.a.	17	n.a.	386	n.a.	n.a.	706	n.a.	n.a.	0.5	n.a.	n.a.	5,708
15	n.a.	8	n.a.	560	n.a.	n.a.	1,799	n.a.	n.a.	0.3	n.a.	n.a.	<500
16	n.a.	26	n.a.	815	n.a.	n.a.	664	n.a.	n.a.	1.2	n.a.	n.a.	<500
17	n.a.	35	n.a.	571	n.a.	n.a.	703	n.a.	n.a.	0.8	n.a.	n.a.	51,860
18	n.a.	43	n.a.	378	n.a.	n.a.	639	n.a.	n.a.	0.6	n.a.	n.a.	2,397
Average:		19.9		607			988			0.7			
SD:		13.7		225			420			0.3			
Median:		16.5		566			796			0.6			
p Value[a]		0.87		0.25			0.25			0.06			0.08

Abbreviation: n.a., Not available.

[a]*p* Values express lack of statistical differences between treated and untreated groups. For calculation of *p* values, see Materials and Methods.

30.8 ± 20.7) compared with the untreated group (average, 23.6 ± 24.3). Since CD3-ζ chain is essential for T cell receptor (TcR) signal transduction, a higher proportion of CD3-ζ-expressing cells might have increased the capacity of both $CD8^+$ and $CD4^+$ T lymphocytes to respond to antigens.

Naive T lymphocytes decrease during the course of HIV infection. Drugs that increase the number of naive T lymphocytes may create conditions favorable to *de novo* T cell development, a likely requirement for immune recovery. As shown in Fig. 2, treated patients had more naive ($CD45RA^+CD62L^+$) $CD4^+$ and $CD8^+$ T lymphocytes than the untreated group and the difference was statistically significant ($p = 0.02$ and $p = 0.01$, respectively). Interestingly, this was achieved quite early after treatment. After 5 months of treatment, 60.1 ± 6.5% $CD4^+$ T lymphocytes became $CD45RA^+$. In a previous report[24] recovery of $CD45RA^+$ cells (62 ± 25% of $CD4^+$ T lymphocytes) was significant only 12 months after starting other highly active antiretroviral therapies (HAARTs).

An elevated percentage of activated ($CD8^+CD38^+$) cells is a marker of poor prognosis of HIV infection[25,26] and $CD28^-$ cells are terminally differentiated effector cells that fail to respond to mitogen.[27] Treated patients had fewer activated $CD38^+DR^+$ $CD8^+$ T cells ($p = 0.004$) and more $CD28^-$ $CD8^+$ T cells than the untreated group (Fig. 3). Reversing the loss of CD28 antigen and the increase in activated cells is expected to have a beneficial impact on prognosis.[25] A decrease in activated $CD8^+$ T lymphocytes after other HAARTs[24] might be due to the decrease in HIV antigens. In our study, however, the two groups (treated and untreated patients) were not statistically different on the basis of their antigenemia (viral load), but the treated group had a significantly lower amount of activated $CD8^+$ T lymphocytes. Furthermore, the decrease in CD8 count did not correlate with the decrease of viremia after treatment ($r = -0.11$; $p = 0.79$). The cytostatic treatment may have therefore substantially contributed to the inhibition of $CD8^+$ T lymphocyte proliferation.

DISCUSSION

Despite the relatively small number of patients involved, we were able to document a robust HIV suppression and good indi-

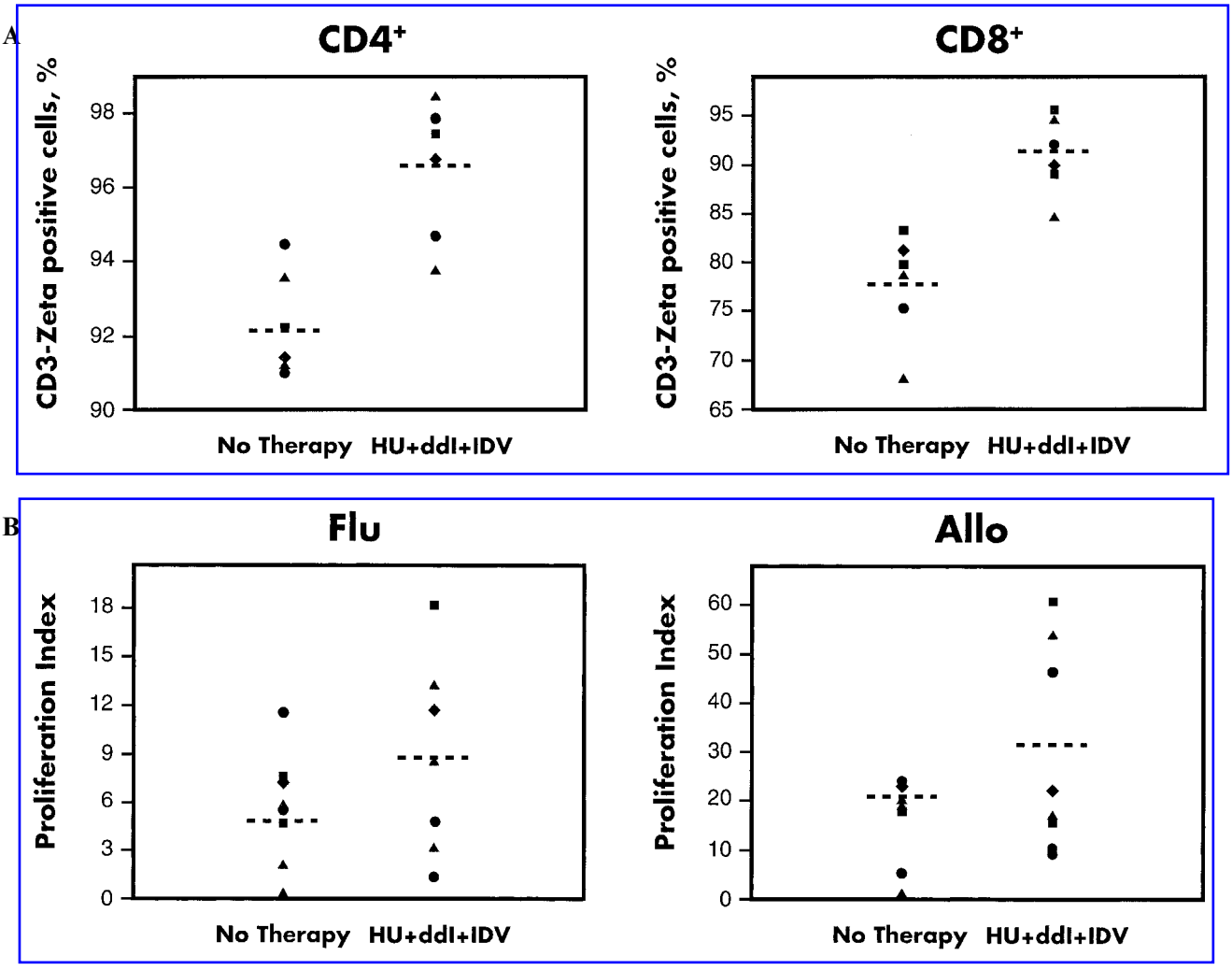

FIG. 1. **(A)** Percentage of CD3-ζ^+ cells in $CD4^+$ (left) and $CD8^+$ (right) T lymphocytes from samples of patients treated (HU + ddI + PI) or untreated (No Therapy). **(B)** Proliferation index in response to influenza virus antigens (flu, left side) and allogeneic antigens (allo, right side) from samples of patient treated (HU + ddI + PI) or untreated (No Therapy).

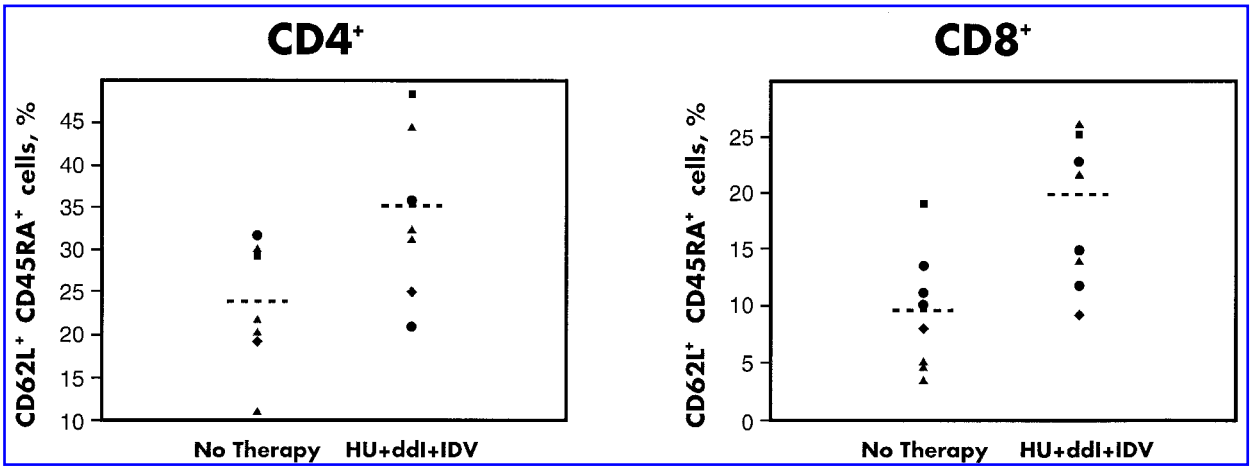

FIG. 2. Percentage of naive ($CD62L^+CD45RA^+$) cells in $CD4^+$ (left) and $CD8^+$ (right) T lymphocytes from samples of patients treated (HU + ddI + PI) or untreated (No Therapy).

cations of a quite rapid immune normalization. Furthermore, the only examples of complete viral suppression without rebound after drug withdrawal have been reported in patients treated with hydroxyurea-containing antiviral regmens.[28,29] We cannot conclusively explain the reasons for the apparent peculiarity of these results. One possibility is that the hydroxyurea-containing combinations have potent antiretroviral effects, thus leading to a marked virus suppression and consequent immune recovery. Another explanation might be the presence of a cytostatic agent in the drug combination. Cytostatic effects on $CD4^+$ T lymphocytes might be advantageous, since resting cells do not support HIV replication.[1–4] If immunostimulation (mainly represented by $CD8^+$ T lymphocyte proliferation) and consequent T cell-mediated immunopathology play a major role in the immunopathogenesis of HIV infection,[10,11] a cytostatic treatment is likely to be beneficial.

The use of a cytostatic and potentially immunosuppressive drug during the course of an infection causing immunodeficiency has raised theoretical concerns.[18,20] In the present study, however, no signs of immunosuppression were detected. All the immune system parameters tested here showed improvement, and a clear increase in $CD4^+$ T lymphocytes was documented. Other immunomodulatory agents suppressing T cell activation, such as cyclosporin A, have also been tested. This drug influenced the kinetics of primary SIV infection in some monkeys[30]; however, studies in humans did not show a clear anti-HIV activity.[31,32] The present study, therefore, represents the first demonstration that a cytostatic drug, if used in combination with potent antiretroviral drugs, can improve immune functions. Future randomized studies comparing drug combinations without hydroxyurea with drug combinations containing hydroxyurea will define the precise role of this drug in the reconstitution of the immune system.

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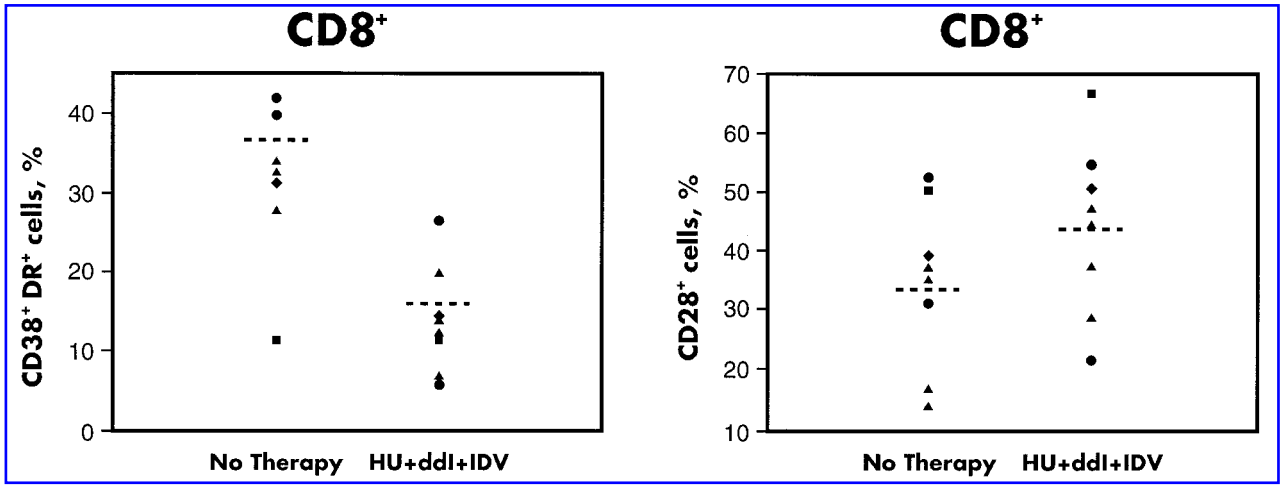

FIG. 3. Percentage of activated ($CD38^+DR^+$, left side) or $CD28^+$ (right side) $CD8^+$ T lymphocytes from samples of patients treated (HU + ddI + PI) or untreated (No Therapy).

REFERENCES

1. Zagury D, Bernard J, Leonard R, Cheynier R, Feldman M, Sarin PS, and Gallo RC: Long-term cultures of HTLV-III-infected T cells: A model of cytopathology of T-cell depletion in AIDS. Science 1986;231:850–853.
2. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, and Chen IS: HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. Cell 1990;61: 213–222.
3. Stevenson M, Stanwick TL, Dempsey MP, and Lamonica CA: HIV-1 replication is controlled at the level of T cell activation and proviral integration. EMBO 1990;9:1551–1560.
4. Lori F, di Marzo Veronese F, de Vico AL, Lusso P, Reitz MS Jr, and Gallo RC: Viral DNA carried by human immunodeficiency virus type 1 virions. J Virol 1992;66:5067–5074.
5. Phillips AN: Reduction of HIV concentration during acute infection: Independence from a specific immune response. Science 1996;271:497–499.
6. McLean AR, Emery VC, Webster A, and Griffiths PD: Population dynamics of HIV within an individual after treatment with zidovudine. AIDS 1991;5:485–489.
7. De Boer RJ, Boucher CA, and Perelson AS: Target cell availability and the successful suppression of HIV by hydroxyurea and didanosine. AIDS 1998;12:1567–1570.
8. Havlir DV, Marschner IC, Hirsch MS, Collier AC, Tebas P, Bassett RL, Ioannidis JPA, Holohan MK, Leavitt R, Boone G, and Richman DD: Maintenance antiretroviral therapies in HIV-infected subjects with undetectable plasma HIV RNA after triple-drug therapy. N Engl J Med 1998;339:1261–1268.
9. Wolthers KC, Bea G, Wisman A, Otto SA, de Roda Husman AM, Schaft N, de Wolf F, Goudsmit J, Coutinho RA, van der Zee AG, Meyaard L, and Miedema F: T cell telomere length in HIV-1 infection: No evidence for increased $CD4^+$ T cell turnover. Science 1996;274:1543–1547.
10. Zinkernagel RM and Hengartner H: T-cell-mediated immunopathology versus direct cytolysis by virus: Implications for HIV and AIDS. Immunol Today 1994;15:262–268.
11. Zinkernagel RM: Immunology taught by viruses. Science 1996; 271:173–178.
12. Donehower RC: An overview of the clinical experience with hydroxyurea. Semin. Oncol. 1992;19:11–19.
13. Gao WY, Cara A, Gallo RC, and Lori F: Low levels of deoxynucleotides in peripheral blood lymphocytes: A strategy to inhibit human immunodeficiency virus type 1 replication. Proc Natl Acad Sci USA 1993;90:8925–8928.
14. Lori F, Malykh A, Cara A, Sun D, Weinstein JN, Lisziewicz J, and Gallo RC: Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. Science 1994;266:801–805.
15. Biron F, Lucht F, Peyramond D, Fresard A, Vallet T, Nugier F, Grange J, Malley S, Hamedi-Sangasari F, and Vila J: Anti-HIV activity of the combination of didanosine and hydroxyurea in HIV-1-infected individuals. J Acquir Immune Defic Syndr Hum Retroviral 1995;10:36–40.
16. Vila J, Biron F, Nugier F, Vallet T, and Peyramond D: 1-year follow-up of the use of hydroxycarbamide and didanosine in HIV infection. Lancet 1996;348:203–204.
17. Jessen H, Foli A, Lisziewicz J, and Lori F: Long-term suppression of HIV-1 by hydroxyurea and didanosine. JAMA 1997;277: 1437–1438.
18. Lori F, Malykh AG, Foli A, Maserati R, De AA, Minoli L, Padrini D, Degli AA, Barchi E, Jessen H, Wainberg MA, Gallo RC, and Lisziewicz J: Combination of a drug targeting the cell with a drug targeting the virus controls human immunodeficiency virus type 1 resistance. AIDS Res Hum Retroviruses 1997;13: 1403–1409.
19. Montaner JS, Zala C, Conway B, Raboud J, Patenaude P, Rae S, O'Shaughnessy MV, and Schechter MT: A pilot study of hydroxyurea among patients with advanced human immunodeficiency virus (HIV) disease receiving chronic didanosine therapy: Canadian HIV trials network protocol 080. J Infect Dis 1997;175: 801–806.
20. Rutschmann OT, Opravil M, Iten A, Malinverni R, Vernazza PL, Bucher HC, Bernasconi E, Sudre P, Leduc D, Yerly S, Perrin LH, and Hirschel B: A placebo-controlled trial of didanosine plus stavudine, with and without hydroxyurea, for HIV infection. The Swiss HIV Cohort Study. AIDS 1998;12:F71–F77.
21. Trimble LA and Lieberman J: Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3 zeta, the signaling chain of the T-cell receptor complex. Blood 1998;91:585–594.
22. Clerici M, Stocks NI, Zajac RA, Boswell RN, Lucey DR, Via CS, and Shearer GM: Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. J Clin Invest 1989;84:1892–1899.
23. Shearer GM and Clerici M: Protective immunity against HIV infection. Has nature done the experiment for us? Immunol Today 1996;17:21–24.
24. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, Katlama C, Debre P, and Leibowitch J: Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112–116.
25. Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K, and Detels R: Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: Results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr 1993; 6:904–912.
26. Mocroft A, Bofill M, Lipman M, Medina E, Borthwick N, Timms A, Batista L, Winter M, Sabin CA, Johnson M, Lee CA, Phillips A, and Janossy G: CD8+,CD38+ lymphocyte percent: A useful immunological marker for monitoring HIV-1-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14:158–162.
27. Borthwick NJ, *et al.*: Lymphocyte activation in HIV-1 infection. II. Functional defects of CD28− T cells. AIDS 1994;8:431–441.
28. Lisziewicz J, Jessen H, Finzi D, Siliciano RE, and Lori F: HIV-1 suppression by early treatment with hydroxyurea, didanosine, and a protease inhibitor. Lancet 1998;352:199–200.
29. Vila J, Nugier F, Bargues G, Vallet T, Peyramond D, Hamedi-Sangsari F, and Seigneurin JM: Absence of viral rebound after treatment of HIV-infected patients with didanosine and hydroxycarbamide. Lancet 1997;350:635–636.
30. Martin LN, Murphey-Corb M, Mack P, Baskin GB, Pantaleo G, Vaccarezza M, Fox CH, and Fauci AS: Cyclosporin A modulation of early virologic and immunologic events during primary simian immunodeficiency virus infection in rhesus monkeys. J Infect Dis 1997;176:374–383.
31. Andrieu JM, Even P, Venet A, Tourani JM, Stern M, Lowenstein W, Audroin C, Eme D, Masson D, Sors H, *et al.*: Effects of cyclosporin on T-cell subsets in human immunodeficiency virus disease. Clin Immunol Immunopathol 1988;47:181–198.
32. Phillips A, Wainberg MA, Coates R, Klein M, Rachlis A, Read S, Shepherd F, Vellend H, Walmsley S, Halloran P, *et al.*: Cyclosporine-induced deterioration in patients with AIDS. Canadian Med Assoc J 1989;140:1456–1460.

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1. J. M. Benito, M. López, S. Lozano, C. Ballesteros, J. González-Lahoz, V. Soriano. 2007. Hydroxyurea exerts an anti-proliferative effect on T cells but has no direct impact on cellular activation. *Clinical & Experimental Immunology* **149**:1, 171-177. [CrossRef]
2. José Miguel Benito , Mariola López , Celia Ballesteros , Sara Lozano , Laura Capa , Pablo Barreiro , José Sempere , Juan Gonzalez-Lahoz , Vincent Soriano . 2006. Immunological and Virological Effects of Structured Treatment Interruptions following Exposure to Hydroxyurea Plus DidanosineImmunological and Virological Effects of Structured Treatment Interruptions following Exposure to Hydroxyurea Plus Didanosine. *AIDS Research and Human Retroviruses* **22**:8, 734-743. [Abstract] [PDF] [PDF Plus]
3. Mark T. Bloch, Don E. Smith, Dick Quan, John M. Kaldor, John J. Zaunders, Kathy Petoumenos, Kathy Irvine, Matthew Law, Pat Grey, Robert J. Finlayson, Robert McFarlane, Anthony D. Kelleher, Andrew Carr, David A. Cooper. 2006. The Role of Hydroxyurea in Enhancing the Virologic Control Achieved Through Structured Treatment Interruption in Primary HIV Infection. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **42**:2, 192-202. [CrossRef]
4. Franco Lori, Laurene M Kelly, Andrea Foli, Julianna Lisziewicz. 2004. Safety of hydroxyurea in the treatment of HIV infection. *Expert Opinion on Drug Safety* **3**:4, 279-288. [CrossRef]
5. Mariola L??pez, Jos?? M Benito, Sara Lozano, Pablo Barreiro, Pilar Mart??nez, Juan Gonz??lez-Lahoz, Vincent Soriano. 2004. Enhanced HIV-specific immune responses in chronically HIV-infected patients receiving didanosine plus hydroxyurea. *AIDS* **18**:9, 1251-1261. [CrossRef]
6. R□gis T. Costello, Hac□ne Zerazhi, Aude Charbonnier, Jean-Marc Schiano de Colella, Claude Alzieu, Isabelle Poizot-Martin, Rolande Cohen, Val□rie-Jeanne Bardou, Luc Xerri, Daniel Olive, Meyer Nezri, G□rard Lepeu, Jean-Albert Gastaut. 2004. Intensive sequential chemotherapy with hematopoietic growth factor support for non-Hodgkin lymphoma in patients infected with the human immunodeficiency virus. *Cancer* **100**:4, 667-676. [CrossRef]
7. Julianna Lisziewicz, Andrea Foli, Mark Wainberg, Franco Lori. 2003. Hydroxyurea in the Treatment of HIV Infection. *Drug Safety* **26**:9, 605-624. [CrossRef]
8. Marina Núñez , Rafael Rodríguez-Rosado , Vincent Soriano . 2001. Intensification of Antiretroviral TherapyIntensification of Antiretroviral Therapy. *AIDS Research and Human Retroviruses* **17**:6, 499-506. [Citation] [PDF] [PDF Plus]
9. Fran??ois Biron, B??n??dicte Ponceau, Damien Bouhour, Andr?? Boibieux, Bernard Verrier, Dominique Peyramond. 2000. Long-Term Safety and Antiretroviral Activity of Hydroxyurea and Didanosine in HIV-Infected Patients. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **25**:4, 329-336. [CrossRef]
10. Fran??ois Biron, B??n??dicte Ponceau, Damien Bouhour, Andr?? Boibieux, Bernard Verrier, Dominique Peyramond. 2000. Long-Term Safety and Antiretroviral Activity of Hydroxyurea and Didanosine in HIV-Infected Patients. *Journal of Acquired Immune Deficiency Syndromes* 329-336. [CrossRef]
11. MARK W. KLINE, NANCY R. CALLES, CARA SIMON, HEIDI SCHWARZWALD. 2000. Pilot study of hydroxyurea in human immunodeficiency virus-infected children receiving didanosine and/or stavudine. *The Pediatric Infectious Disease Journal* **19**:11, 1083-1086. [CrossRef]
12. Marina Núñez , Vincent Soriano . 2000. Salvage Therapy for HIV Infection: When and HowSalvage Therapy for HIV Infection: When and How. *AIDS Patient Care and STDs* **14**:9, 465-476. [Citation] [PDF] [PDF Plus]
13. Olivier T Rutschmann, Pietro L Vernazza, Heiner C Bucher, Milos Opravil, Bruno Ledergerber, Amalio Telenti, Raffaele Malinverni, Enos Bernasconi, Catherine Fagard, Dominique Leduc, Luc Perrin, Bernard Hirschel. 2000. Long-term hydroxyurea in combination with didanosine and stavudine for the treatment of HIV-1 infection. *AIDS* **14**:14, 2145-2151. [CrossRef]

14. Elisabetta Ravot, Giuseppe Tambussi, Heiko Jessen, Carmine Tinelli, Adriano Lazzarin, Julianna Lisziewicz, Franco Lori. 2000. Effects of hydroxyurea on T cell count changes during primary HIV infection. *AIDS* **14**:5, 619-622. [CrossRef]

15. A. Fraternale, A. Casabianca, A. Tonelli, G. Vallanti, L. Chiarantini, G. Brandi, A. G. Celeste, M. Magnani. 2000. Inhibition of Murine AIDS by Alternate Administration of Azidothymidine and Fludarabine Monophosphate. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **23**:3, 209-220. [CrossRef]

16. A. Fraternale, A. Casabianca, A. Tonelli, G. Vallanti, L. Chiarantini, G. Brandi, A. G. Celeste, M. Magnani. 2000. Inhibition of Murine AIDS by Alternate Administration of Azidothymidine and Fludarabine Monophosphate. *Journal of Acquired Immune Deficiency Syndromes* **23**:3, 209-220. [CrossRef]

17. Christopher N. Mayhew, Jonathan D. Phillips, Richard N. Greenberg, Nicholas J. Birch, Howard L. Elford, Vincent S. Gallicchio. 1999. In Vivo and In Vitro Comparison of the Short‐Term Hematopoietic Toxicity Between Hydroxyurea and Trimidox or Didox, Novel Ribonucleotide Reductase Inhibitors with Potential Anti‐HIV‐1 Activity. *Stem Cells* **17**:6, 345-356. [CrossRef]