AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 15, Number 15, 1999, pp. 1333–1338
Mary Ann Liebert, Inc.

Hydroxyurea and Didanosine Long-Term Treatment Prevents HIV Breakthrough and Normalizes Immune Parameters

F. LORI,[1] E. ROSENBERG,[2] J. LIEBERMAN,[3] A. FOLI,[1] R. MASERATI,[4] E. SEMINARI,[4] F. ALBERICI,[5] B. WALKER,[2] and J. LISZIEWICZ[1]

ABSTRACT

Hydroxyurea and didanosine treatment suppressed HIV replication for more than 2 years, in the absence of viral breakthrough, in chronically infected patients. The profile of viral load reduction was unusual for a two-drug combination, since a continuous gradual decrease in viremia persisted despite residual viral replication. The increase in $CD4^+$ T cell counts was not robust. However, unlike those of patients treated by other therapies, $CD4^+$ T lymphocytes were functionally competent against HIV, mediating a vigorous HIV-specific helper T cell response in half of these patients. In addition, the percentages of naïve $CD4^+$ and $CD8^+$ T lymphocytes were not different from those in uninfected individuals. These results demonstrate that prolonged antiretroviral therapy with a simple, well-tolerated combination of two affordable drugs can lead to sustained control of HIV, normalization of immune parameters, and specific anti-HIV immune response.

INTRODUCTION

ERADICATION OF HIV appears an unrealistic goal because of the early establishment[1,2] and long persistence[2–4] of proviral latent reservoirs. Therefore, if any antiretroviral treatment is initiated, it must be a chronic treatment. Treatment of HIV infection is currently based on highly active antiretroviral therapies (HAARTs), which are combinations of drugs that decrease plasma viremia to undetectable levels. Since these drugs tend to generate resistance, maximal viral suppression is required to avoid the onset of drug-resistant mutants and to ensure long-term efficacy. Unfortunately, complicated dosing schedules and high cost make access to and compliance with HAART regimens difficult for most patients. Therefore, viral escape occurs in many cases, leaving individuals with multiple- and/or cross-resistant variants of HIV. Associated toxicity further limits the long-term use of these regimens.

Drugs that do not induce viral escape are an attractive alternative to HAART. Hydroxyurea inhibits HIV *in vitro*[5–7] and *in vivo*.[8–10] It inhibits a cellular protein, ribonucleotide reductase, and since cellular proteins are not prone to mutations, resistance to hydroxyurea is not expected.[11] We have shown that hydroxyurea reestablishes sensitivity to didanosine in HIV strains carrying genotype changes that confer resistance to didanosine.[9,12] Thus, combination of these two drugs might represent an alternative approach to long-term control of HIV infection.

MATERIALS AND METHODS

Patients

The 12 patients described in this study belonged to a previously described cohort of 57 asymptomatic patients ($CD4^+$ cell counts between 250 and 500 cells/μl) previously enrolled in a controlled clinical trial that showed that hydroxyurea potentiates the antiviral activity of didanosine.[9] The didanosine monotherapy arm (19 patients) was interrupted after 24 weeks,

[1]Research Institute for Genetic and Human Therapy (RIGHT) at Policlinico S. Matteo, 27100, Pavia, Italy and at Washington, D.C. 20007.
[2]Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129.
[3]Center for Blood Research, Boston, Massachusetts 02115.
[4]Clinica Malattie Infettive, Policlinico S. Matteo, 27100, Pavia, Italy.
[5]Divisione Malattie Infettive, 29100, Piacenza, Italy.

owing to the superior outcome of the combination therapy.[9] At week 40, no sign of rebound was observed in the 38 patients treated with hydroxyurea and didanosine.[9] Since 35 of 38 patients still had detectable plasma viremia (>500 copies/ml) and new drugs (such as protease inhibitors) capable of potent virus inhibition to undetectable levels had become available, 26 patients chose to initiate other therapies, and therefore were lost to follow-up. Twelve patients continued with the hydroxyurea (500 mg twice daily) and didanosine regimen (patients weighing <50 kg were given 100 mg twice daily; patients weighing 50–75 kg were given 200 mg twice daily; and patients weighing >75 kg were given 300 mg twice daily) for an average of 122 weeks. The availability of alternative treatments, and not a worse outcome, was the reason for changing treatment. In fact, at week 40, the group of patients who chose to continue taking the hydroxyurea plus didanosine double combination was not significantly different from the group of patients who changed to other therapies, with respect to viral load at week 40 ($p = 0.11$), decrease in viral load from baseline to week 40 ($p = 0.41$), $CD4^+$ cell counts at week 40 ($p = 0.14$), and change in $CD4^+$ cell counts from baseline to week 40 ($p = 0.38$).

Nine normal uninfected individuals and a control group of 12 untreated patients were selected to be matched to the group of 12 treated patients for the analysis of the immunological parameters.

Surrogate markers

Plasma viremia at baseline and at week 40 was determined by using a commercially available reverse transcriptase-polymerase chain reaction (RT-PCR) kit with a detection limit of 400 HIV RNA copies/ml of plasma (Roche Diagnostic Systems, Branchburg, NJ). At week 122, because plasma viremia was undetectable by standard PCR in 11 of 12 patients, viral load was measured by supersensitive PCR according to Bagnarelli *et al.*[13] (detection limit, 50 copies/ml). $CD4^+$ and $CD8^+$ cell counts were performed according to standard protocols.

Flow cytometry

For external staining, peripheral blood mononuclear cells (PBMCs; 2–10 × 10^5/tube), freshly isolated by Ficoll-Hypaque density centrifugation from heparinized blood, were suspended in 0.1 ml of fluorescence-activated cell sorting (FACS) buffer (phosphate-buffered saline [PBS] with 20% fetal calf serum [FCS]) before adding 4 ml of 1:5 dilution of CD28–fluorescein isothiocyanate (FITC) (monoclonal antibody [MAb] CD28.2; Immunotech, Westbrook, ME) and 4 ml of CD8–Cy5 (MAb B9.11; Immunotech); 4 ml of CD45RA–FITC (MAb ALB11; Immunotech), 4 ml of a 1:10 dilution of CD62L–phycoerythrin (PE) (MAb SCFI28T17G6; Coulter, Hialeah, FL), and 4 ml of either CD8–Cy5 or CD4–Cy5 (MAb 13B8.2; Immunotech); 4 ml of HLA-DR–FITC (MAb 357; Immunotech), 4 ml of CD38–PE (MAb T16; Immunotech), and 4 ml of CD8–Cy5; or 4 ml of IgG–FITC, –PE, and –Cy5 isotype-matched controls (Immunotech). After incubation for 15 min at room temperature, cells were washed and fixed in 2% formaldehyde in PBS. Flow cytometry analysis was performed on a tightly gated lymphocyte population, using a FACscalibur (Becton Dickinson, San Jose, CA). Gates were defined by requiring that fewer than 10% of the isotype control antibody-stained cells were positive.

Helper T cell analysis

Proliferation assays were performed by resuspending PBMCs in RPMI 1640 medium containing 10% human AB serum, HEPES buffer, L-glutamine, and penicillin–streptomycin. Cells (1×10^5 cells/well) were cultured in six replicate wells of 96-well U-bottom plates in the presence of HIV recombinant p24 protein at a final concentration of 5 mg/ml. Six days later, the cells were pulsed with [^{3}H]thymidine at 1.0 mCi/well, and uptake was measured 6 hr later with a scintillation counter (Topcount; Packard Instruments, Meriden, CT). The HIV p24 protein (Protein Science, Meriden, CT) used is a recombinant protein derived from the *gag* gene of HIV (NY-5 strain); it is produced in a baculovirus expression system and demonstrated 90 to 95% purity. A mixture of baculovirus proteins was used as a control antigen at a concentration of 1.5 mg/ml, which is equal to the baculovirus antigen concentration in the recombinant p24 protein. The stimulation index was calculated by taking the mean counts per minute (cpm) of incorporated [^{3}H]thymidine from cells stimulated with p24 and dividing this value by the mean counts per minute from cells stimulated with baculovirus control proteins.

Statistical methods

Analysis of variance was performed by Scheffe test analysis. Groups were matched by using the Mann–Whitney nonparametric *U* test. In addition, to match the viral load values before interruption of treatment, comparison of detectable versus undetectable virus load values in each group was also performed by using the Fisher exact test.

RESULTS

Twelve patients were treated with hydroxyurea and didanosine for an average of 122 weeks (range, 102–154) (Table 1). When these patients began treatment, their average baseline plasma viremia was 51,795 (SD of 66,869) RNA copies/ml of plasma (Fig. 1). At week 40 the average plasma viremia was 1847 (SD of 2181) RNA copies/ml of plasma. At week 122 the average viremia further decreased to 186 (SD of 284) RNA copies/ml of plasma. Mean plasma viral RNA decreased by 1.14 $\log_{10}$ at week 40 and by 2.2 $\log_{10}$ at week 122. A continuous decrease in viremia despite residual viral replication is a novel feature of this two-drug combination. Maximal viral suppression was not required for long-term control of HIV by these drugs.

The lack of a robust increase in $CD4^+$ T lymphocytes is characteristic of hydroxyurea-containing combinations[9,14] and might be attributed to the cytostatic effects of this drug.[11] During the 122 weeks of follow-up of hydroxyurea and didanosine treatment, the average $CD4^+$ cell count increased by 30 cells/mm^3, from 376 ± 72 to 406 ± 118 cells/mm^3. Consistent with mathematical models[15] and with the results of the ACTG 343 study,[16] the lack of increase in $CD4^+$ T lymphocytes may partly explain the absence of HIV rebound observed in our study. In addition, since cell division is essential for viral repli-

TABLE 1. PATIENT CHARACTERISTICS

		Baseline				Last value			
Patient	*Weeks*[a]	*CD4*	*CD8*	*Ratio*	*PCR*[b]	*CD4*	*CD8*	*Ratio*	*ss-PCR*[c]
1	117	351	NA	NA	51,000	256	330	0.8	265
2	154	397	437	0.9	8,553	253	209	1.2	65
3	104	288	794	0.4	173,166	392	336	1.2	97
4	113	277	343	0.8	1,468	414	504	0.8	<50
5	121	385	NA	NA	21,854	562	783	0.7	<50
6	105	376	811	0.5	13,475	640	874	0.7	270
7	110	431	NA	NA	55,521	424	309	1.4	244
8	102	490	1,308	0.4	199,256	308	986	0.3	915
9	123	493	580	0.9	693	443	424	1.0	<50
10	123	308	736	0.4	66,245	314	551	0.6	60
11	141	320	743	0.4	29,708	491	685	0.7	119
12	149	399	1,033	0.4	602	375	711	0.5	<50
Average:	122	376	754	0.56	51,795	406	559	0.83	186[d]
SD:	18	72	294	0.23	66,869	118	248	0.31	284[d]

Abbreviation: NA, Not available.
[a]Weeks of treatment.
[b]PCR, Amplicor PCR; limit of detection, 400 copies/ml.
[c]ss-PCR, supersensitive PCR; limit of detection, 50 copies/ml.
[d]Calculated assuming that <50 = 49.

cation, the cytostatic effects of hydroxyurea could account for the impairment of HIV replication. However, since cytostatic effects were also exerted on $CD8^+$ T lymphocytes, the CD4/CD8 ratio significantly increased by 0.27, from 0.56 ± 0.23 to 0.83 ± 0.31 ($p = 0.039$) (Table 1).

No relevant episodes of toxicity attributable to the drugs used in this study occurred during the average 122 weeks of therapy. This low level of toxicity is consistent with the scarcity of side effects registered in pilot as well as randomized controlled trials using hydroxyurea in combination with didanosine.[9,14,17,18] Long-term use of hydroxyurea has also proved to be safe in patients with oncologic and/or hematologic diseases.[11] Some of these patients have been treated with this drug for decades.[11]

A fundamental question was whether this therapy had beneficial effects on immune parameters and immune function. After an average 122 weeks of treatment, the 12 patients receiving hydroxyurea and didanosine were matched with a control

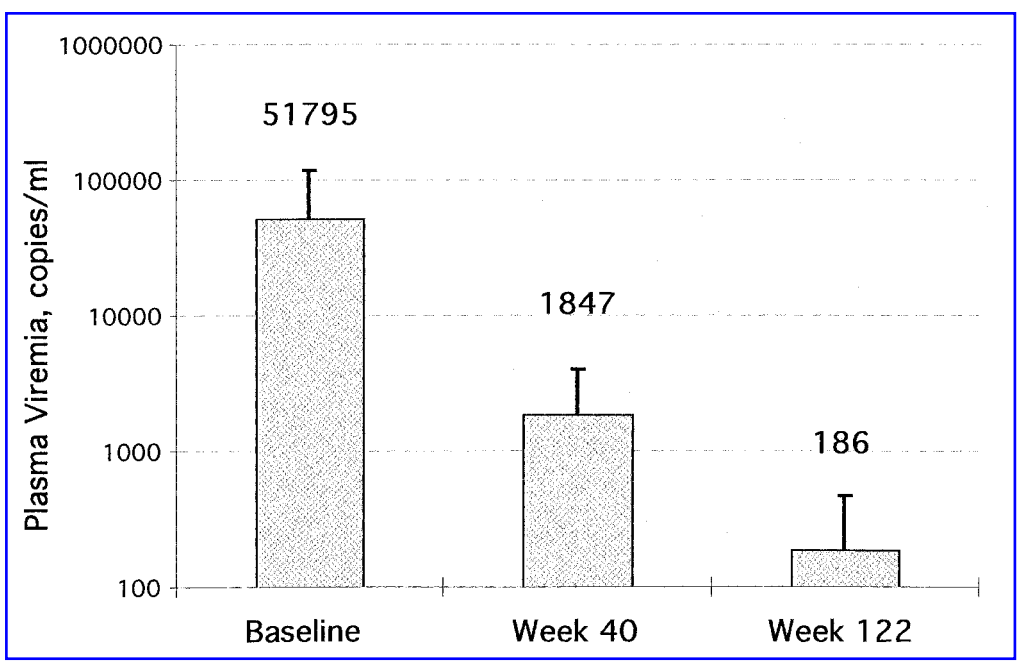

FIG. 1. Progressive decrease in plasma viremia in 12 patients treated with hydroxyurea and didanosine.

group of 12 chronically infected patients who declined to accept treatment. Treated and untreated patients were matched on the basis of the baseline $CD4^+$ cell count (average ± SD, 376 ± 72 and 401 ± 131, respectively, $p = 0.686$), and plasma viremia (51,795 ± 66,869 and 51,212 ± 83,308, respectively, $p = 0.525$).

It has been shown that the proportion of naïve T lymphocytes decreases during the course of untreated HIV infection.[19,20] Drugs that increase the number of naïve T lymphocytes may create conditions favorable to *de novo* T cell development, a likely requirement for immune recovery. *De novo* thymic or extrathymic T cell generation does not involve high levels of cell expansion. Therefore, resting thymocytes are not expected to be inhibited by cytostatic drugs. We found that, after 122 weeks of therapy with hydroxyurea and didanosine, the average percentages of naïve $CD8^+$ and $CD4^+$ T lymphocytes, measured by the expression of CD62L and CD45RA, were similar ($p = 0.25$ and $p = 0.99$, respectively) to the values of normal uninfected donors and significantly different ($p = 0.00053$ and $p = 0.001$, respectively) from those of HIV-infected untreated individuals (Fig. 2A).

An elevated percentage of activated $CD8^+$ T lymphocytes expressing CD38 and HLA-DR indicates a poor prognosis in HIV infection.[21,22] Lymphocytes lacking the CD28 surface marker are terminally differentiated effector cells that fail to proliferate in response to mitogen.[23] Reversing the loss of CD28 antigen and increasing numbers of activated cells are expected to have a beneficial impact on prognosis.[21] The percentage of activated $CD8^+CD38^+DR^+$ T lymphocytes in patients treated with hydroxyurea plus didanosine was higher than in normal individuals ($p = 0.008$), but lower than in untreated individuals ($p = 0.0046$), whereas the percentage of $CD8^+CD28^+$ cells was lower than in normal individuals ($p = 0.042$) and higher ($p = 0.0015$) than in untreated patients (Fig. 2B), consistent

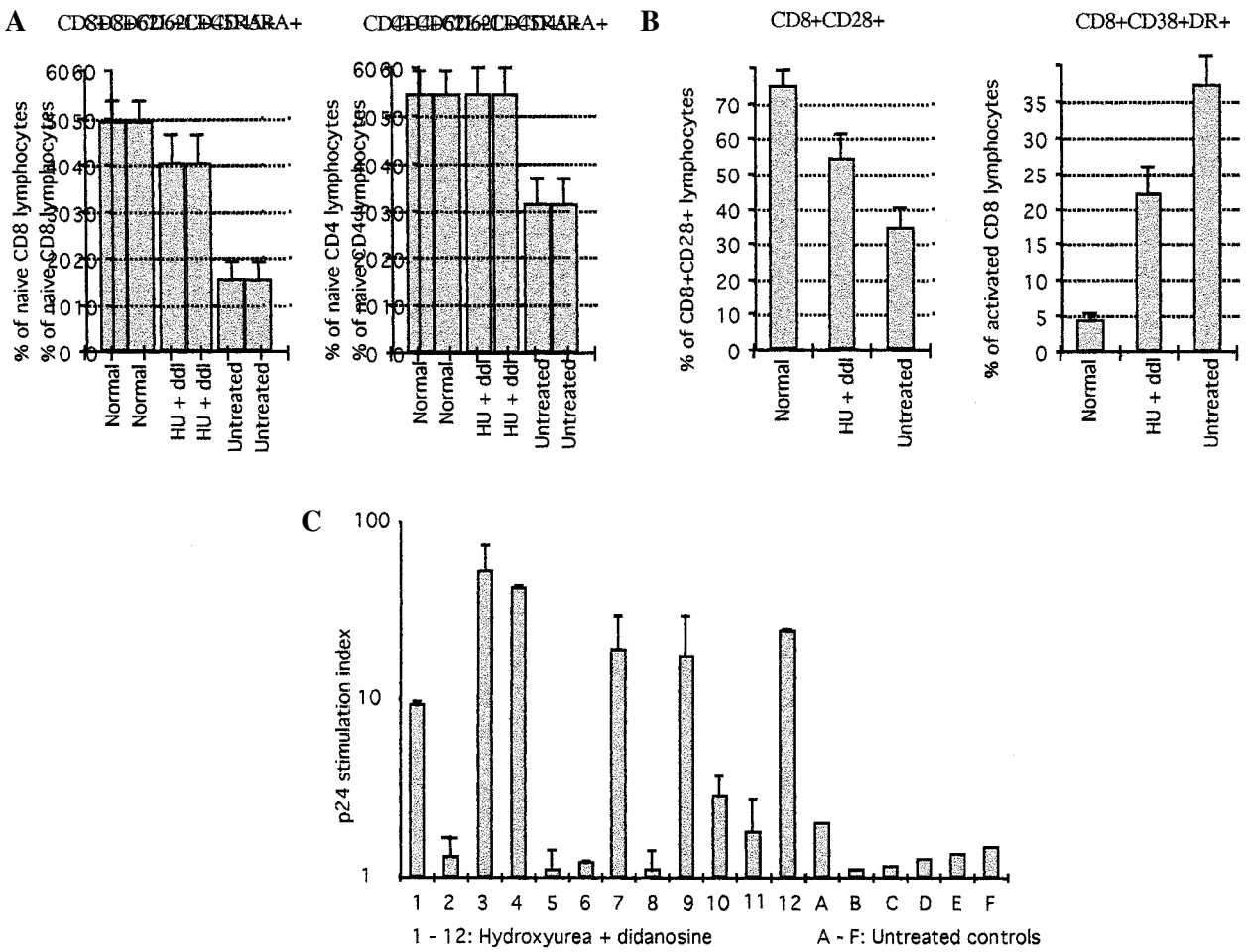

FIG. 2. Immunophenotypic and functional markers of T lymphocytes of chronically HIV-infected patients after an average 122 weeks of treatment with hydroxyurea and didanosine, compared with normal uninfected individuals and a matched control group of untreated patients. **(A)** Percentage of naïve $CD8^+$ T lymphocytes ($CD8^+CD62L^+CD45RA^+$, left) and naïve $CD4^+$ T lymphocytes ($CD4^+CD62L^+CD45RA^+$, right). **(B)** Percentage of $CD8^+CD28^+$ T lymphocytes (left) and activated $CD8^+$ T lymphocytes ($CD8^+CD38^+DR^+$, right). Bars represent standard deviations. Normal, Nine uninfected individuals; HU + ddI, 12 HIV-infected patients treated with hydroxyurea and didanosine for an average of 122 weeks; untreated, 12 HIV-infected asymptomatic patients (matched control group) not treated with antiretroviral drugs. **(C)** Columns 1–12: HIV-specific helper T cell proliferative responses (p24 stimulation index) in the 12 hydroxyurea + didanosine-treate d patients. Columns A–F: Untreated controls, six patients from the untreated matched control group.

with strongly reduced but still residual viral replication. Although increases in naïve T lymphocytes and decreases in activated $CD8^+$ T lymphocytes have been described after HAART,[24] we found similar results while using only two drugs.

HIV-specific helper T cell responses are associated with control of viremia.[25] HIV-specific $CD4^+$ T lymphocyte-proliferative responses are typically absent in persons with progressive infection.[25,26] Unfortunately, these responses have not been restored by HAART during the chronic phase of infection.[24,27,28] In contrast, chronically infected patients treated with hydroxyurea and didanosine had a vigorous $CD4^+$ T lymphocyte-proliferative response to HIV p24 (Fig. 2C). It is possible that this response was elicited because of the incomplete block of HIV replication in our patients and the consequent constant exposure of HIV antigen to the immune system, whereas current HAART regimens may lower viremia to levels so low that the immune system is "off guard" to HIV, "leaving the patient naïve to the virus, like an uninfected individual, rather than one prepared to control HIV infection."[29] Six of 12 of these patients had a stimulation index above 9, whereas none of the asymptomatic, chronically infected drug-naïve patients had a p24 stimulation index above 3.5 (Fig. 2C). The presence of an HIV-specific immune response in chronically infected patients had been described only in long-term nonprogressors,[25,26] who control HIV in the absence of therapy.

DISCUSSION

These findings indicate that long-term treatment with hydroxyurea and didanosine has novel characteristics: progressive reduction of viremia, absence of viral breakthrough in the presence of detectable virus, and induction of an HIV-specific immune response.

The progressive reduction of viremia in the presence of detectable active viral replication is unusual for a two-drug combination and might be explained by the peculiar characteristics of the drugs. Hydroxyurea inhibits the cellular enzyme ribonucleotide reductase. Cellular proteins are not prone to mutations. No resistance of ribonucleotide reductase to hydroxyurea has been observed in 40 years of clinical use.[11] Hydroxyurea-based treatments might therefore be associated with a better resistance profile. A confirmation of this hypothesis came from the assessment of the emergence of didanosine resistance in patients receiving didanosine monotherapy or the hydroxyurea–didanosine combination[12] during the RIGHT 411 study.[9] Although the two-drug combination was more effective in reducing plasma viremia than didanosine monotherapy, resistance to didanosine at week 24 was more pronounced in the combination therapy arm. This paradox was explained by assessing the sensitivity of these mutants to didanosine in the presence of hydroxyurea. Although genotypically resistant, the mutants retained a phenotypic sensitivity to didanosine in the presence of hydroxyurea. The finding that phenotypic sensitivity of the didanosine-resistant mutants is retained in the presence of hydroxyurea appears to be explained by the favoring of incorporation of didanosine triphosphate by resistant reverse transcriptase in the setting of the low levels of dATP resulting from hydroxyurea treatment. The data have been confirmed and extended to the combination of hydroxyurea and PMEA or PMPA.[30] These results might have clinical applications in both maintaining antiviral response to initial treatment for prolonged periods (as described here) and in reestablishing response once resistance has emerged.

The induction of an HIV-specific immune response in the hydroxyurea–didanosine-treated patients might have also contributed to the long-term control of HIV. We have described[31] how early treatment with hydroxyurea, didanosine, and a protease inhibitor was associated with a vigorous anti-HIV-specific immune response capable of controlling HIV after treatment discontinuation.

The combination of hydroxyurea and didanosine is simple to take, inexpensive, well tolerated, and accessible to the majority of people living with HIV.

ACKNOWLEDGMENTS

We are grateful to Michael Stevens and BMS-Immunology for their support. We thank Carmine Tinelli for statistical analysis, and Franco Maggiolo, Angelo Pan, Francesco Castelli, Daniela Padrini, Pierluigi Viale, Giuditta Comolli, and Sergio Lo Caputo for data collection. We also thank Toni Battle for editorial assistance.

REFERENCES

1. Chun TW, Engel D, Berrey MM, Shea T, Corey L, and Fauci AS: Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci USA 1998;95:8869–8873.
2. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, and Siliciano RF: Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295–1300.
3. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, and Fauci AS: Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci USA 1997;94:13193–13197.
4. Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, and Richman DD: Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278:1291–1295.
5. 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.
6. 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.
7. Malley SD, Grange JM, Hamedi-Sangsari F, and Vila JR: Synergistic anti-human immunodeficiency virus type 1 effect of hydroxamate compounds with 2′,3′-dideoxyinosine in infected resting human lymphocytes. Proc Natl Acad Sci USA 1994;91:11017–11021.
8. Biron F, Lucht F, Peyramond D, Fresard A, Vallet T, Nugier F, Grange J, Malley S, Hamedi-Sangsari 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 Retrovirol 1995;10:36–40.
9. 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.
10. Lisziewicz J, Jessen H, Finzi D, Siliciano RF, and Lori F: HIV-1 suppression by early treatment with hydroxyurea, didanosine, and a protease inhibitor. Lancet 1998;352:199–200.
11. Donehower RC: An overview of the clinical experience with hydroxyurea. Semin Oncol 1992;19:11–19.
12. De Antoni A, Foli A, Lisziewicz J, and Lori F: Mutations in the *pol* gene of human immunodeficiency virus type 1 in infected patients receiving didanosine and hydroxyurea combination therapy. J Infect Dis 1997;176:899–903.
13. Bagnarelli P, Valenza A, Menzo S, Manzin A, Scalise G, Varaldo PE, and Clementi M: Dynamics of molecular parameters of human immunodeficiency virus type 1 activity in vivo. J Virol 1994; 68:2495–2502.
14. 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.
15. 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.
16. 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.
17. Biron F, Lucht F, Peyramond D, Fresard A, Vallet T, Nugier F, Grange J, Malley S, Hamedi SF, and Vila J: Pilot clinical trial of the combination of hydroxyurea and didanosine. Antiviral Res 1996;29:111–113.
18. 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.
19. Rabin RL, Roederer M, Maldonado Y, Petru A, and Herzenberg LA: Altered representation of naive and memory CD8 T cell subsets in HIV-infected children. J Clin Invest 1995;95:2054–2060.
20. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA, and Herzenberg LA: CD8 naive T cell counts decrease progressively in HIV-infected adults. J Clin Invest 1995;95:2061–2066.
21. 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.
22. 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.
23. Borthwick NJ, *et al.*: Lymphocyte activation in HIV-1 infection. II. Functional defects of CD28− T cells. AIDS 1994;8:431–441.
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. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, and Walker BD: Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 1997;278:1447–1450.
26. Schwartz D, Sharma U, Busch M, Weinhold K, Matthews T, Lieberman J, Birx D, Farzedagen H, Margolick J, Quinn T, *et al.*: Absence of recoverable infectious virus and unique immune responses in an asymptomatic HIV+ long-term survivor. AIDS Res Hum Retroviruses 1994;10:1703–1711.
27. Plana M, Garcia F, Gallart T, Miro JM, and Gatell JM: Lack of T-cell proliferative response to HIV-1 antigens after 1 year of highly active antiretroviral treatment in early HIV-1 disease. Immunology Study Group of Spanish EARTH-1 Study. Lancet 1998;352:1194–1195.
28. Pitcher CJ, Quittner C, Peterson DM, Connors M, Koup RA, Maino VC, and Picker LJ: HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nature Med 1999;5:518–525.
29. Levy JA: Caution: Should we be treating HIV infection early? Lancet 1998;352:982–983.
30. Palmer S, Shafer R, and Merigan TC: Both nucleoside and nucleotide analogues are potentiated by hydroxyurea against drug-susceptible and drug-resistant HIV isolates. In: *2nd International Workshop on HIV Drug Resistance and Treatment Strategies 1998 Lake Maggiore, Italy, Abstract No. 3.*
31. Lisziewicz J, Rosenberg E, Lieberman J, Jessen H, Lopalco L, Siliciano R, Walker B, and Lori F: Control of HIV despite the discontinuation of antiretroviral therapy. N Engl J Med 1999;340: 1683–1684.

Address reprint requests to:
Franco Lori
RIGHT
Medical-Dental Bldg. SW307
3900 Reservoir Rd. NW
Washington, D.C. 20007

This article has been cited by:

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. 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]
3. Julianna Lisziewicz, Jeffrey Trocio, Lucia Whitman, Georg Varga, Jianqing Xu, Nyasha Bakare, Patrick Erbacher, Cecil Fox, Ruth Woodward, Phil Markham, Suresh Arya, Jean-Paul Behr, Franco Lori. 2005. DermaVir: A Novel Topical Vaccine for HIV/AIDS. *Journal of Investigative Dermatology* **124**:1, 160-169. [CrossRef]
4. Julianna Lisziewicz, Andrea Foli, Mark Wainberg, Franco Lori. 2003. Hydroxyurea in the Treatment of HIV Infection. *Drug Safety* **26**:9, 605-624. [CrossRef]
5. D Johnson. 2002. Therapeutic management of HIV. *Oral Diseases* **8**:s2, 17-20. [CrossRef]
6. Elissa Schwartz, Avidan Neumann, Avelino Teixeira, Leslie Bruggeman, Jay Rappaport, Alan Perelson, Paul Klotman. 2002. *Aids* **16**:3, 341-345. [CrossRef]
7. Ying Zhang , Miriam Huber , Benedikt Weissbrich , Gerald Voss , Peter Langmann , Hartwig Klinker , Christian Jassoy . 2001. Characterization of HIV-Specific Proliferative T Cell Responses in HIV-Infected PersonsCharacterization of HIV-Specific Proliferative T Cell Responses in HIV-Infected Persons. *AIDS Research and Human Retroviruses* **17**:7, 623-629. [Abstract] [PDF] [PDF Plus]
8. Jason J. Coull, Diana Turner, Thomas Melby, Michael R. Betts, Randall Lanier, David M. Margolis. 2001. A Pilot Study of the Use of Mycophenolate Mofetil as a Component of Therapy for Multidrug-Resistant HIV-1 Infection. *JAIDS Journal of Acquired Immune Deficiency Syndromes* **26**:5, 423-434. [CrossRef]
9. Jason J. Coull, Diana Turner, Thomas Melby, Michael R. Betts, Randall Lanier, David M. Margolis. 2001. A Pilot Study of the Use of Mycophenolate Mofetil as a Component of Therapy for Multidrug-Resistant HIV-1 Infection. *Journal of Acquired Immune Deficiency Syndromes* **26**:5, 423-434. [CrossRef]
10. Veronica Miller. 2001. Structured treatment interruptions in antiretroviral management of HIV-1. *Current Opinion in Infectious Diseases* **14**:1, 29-37. [CrossRef]
11. 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]
12. 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]