Thioredoxin and Protein Nitrotyrosine in Bone Marrow Supernatant From Patients With Human Immunodeficiency Virus Infection

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ABSTRACT

Background: **Balance and imbalance between oxidant stress and antioxidants contributes to the manifestations of human immunodeficiency virus (HIV) infection. Previously, we demonstrated a characteristic cytokine pattern in marrow supernatant from HIV patients who underwent diagnostic examinations.**

Methods: **In this report, we have determined the protein nitrotyrosine (pNT) concentration (an indicator of nitric oxide–superoxide interaction) as well as the concentration of the redox enzyme thioredoxin (Trx) in marrow supernatant from HIV patients, healthy controls, and other patients, and in serum from comparable subjects.**

Results: **pNT concentrations were similar in serum and marrow supernatant and did not differ between subject subsets. Trx concentrations in both marrow supernatant and serum were higher for HIV patients than for other subjects; serum Trx concentrations were significantly higher than marrow Trx concentrations for the non-HIV patients and controls. The ratios of pNT/Trx concentrations in serum were similar in all subsets tested. In marrow aspirate, however, these ratios differed widely and significantly, with the highest values observed in non-HIV patients and the lowest in HIV patients. Only for HIV patients were serum and marrow supernatant pNT/Trx ratios similar. In HIV patients, marrow Trx concentrations correlated with CD4 count, CD4/CD8 ratio, and marrow colony-forming unit E (CFU-E) concentration; marrow pNT concentration correlated with the concentration of tumor necrosis factor in marrow supernatant, and with platelet count. No correlations were observed in other subject subsets.**

Conclusions: **These findings suggest that there is a degree of local regulation of the redox state of the marrow microenvironment that varies with the patients' clinical statuses, and which is associated with effects on hematopoiesis. (J Investig Med 2002;50:10–18) Key Words: HIV • thioredoxin • peroxynitrite • oxidative stress • anemia**

INTRODUCTION

Cytopenias, such as leukopenia, thrombocytopenia, and particularly anemia, are common problems in patients with human immunodeficiency virus (HIV) infection.[1,2] The anemia found in HIV patients exhibits a number of significant parallels with the anemia of chronic disease. Both syndromes exhibit an impaired erythropoietin (EPO) response to anemia,[3] a therapeutic response to recombinant human (rh) EPO therapy,[4] impaired erythroid progenitor growth and differentiation,[5,6] and impaired mobilization of reticuloendothelial iron stores.[7–9]

It has been proposed that the anemia of chronic disease results from effects of cytokines that mediate immune or inflammatory responses, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and the interferons (IFNs).[10] Cytokines can exert their effects on hematopoietic progenitors through a number of mechanisms, one of which can be the induction of formation of reactive oxygen species (ROS).[11] ROS appear to play significant roles in a number of the pathologic manifestations of HIV infection.[12–21]

In biologic systems, the ROS superoxide reacts with nitric oxide (NO) to form peroxynitrite.[22] Peroxynitrite

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Supported by funds from the Medical Research Service, Department of Veterans Affairs and by grant HL-53703 from the National Institutes of Health.

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can then exert a variety of effects on cellular function.[19,23–25] Peroxynitrite causes irreversible nitration of protein tyrosine (pNT) residues,[22] which then serve as a persisting marker of its presence. Unlike other markers of NO production, such as nitrate–nitrite concentrations in tissue or biologic fluids, pNT content is not altered by recent diet.[26]

Thioredoxin (Trx) is a widely distributed redox enzyme that is induced in response to oxidative stress and which exerts cytoprotective effects against oxidant injury.[27,28] Trx concentrations can be measured in serum or plasma, and have been reported to be elevated in patients with HIV and other syndromes.[29–31] Trx appears to play a significant role in the response of monocytes (either primary cells[14] or cell lines[12]) to HIV infection.

In an earlier study, concentrations of TNF, soluble TNF receptors, IL-1, γ-IFN, and the chemokines macrophage inflammatory proteins (MIP); particularly, 1α, MIP-1β, and RANTES; in bone marrow supernatant from patients with HIV who underwent marrow examination for clinical indications were determined and compared to the values measured in marrow supernatant from other patients who had clinically indicated marrow examinations and from healthy volunteers. Each subject subset exhibited a characteristic marrow cytokine pattern. For HIV patients, concentrations of all cytokines or receptors measured correlated with the severity of anemia.[32]

In the present report, pNT and Trx concentrations were determined by immunoblot techniques in marrow supernatant from patients with and without HIV infection who underwent diagnostic marrow examination and from healthy volunteers, and these concentrations were compared to concentrations determined in serum specimens obtained from other, comparable patients who underwent marrow examination during the same time period. When serum and marrow values are compared, a characteristic pattern of pNT/Trx expression emerges for each subject subset.

METHODS

Specimen Collection and Processing

Marrow and serum specimens and data were collected under protocols approved by the Institutional Review Boards of the University of Cincinnati Medical Center and the Medical University of South Carolina. Following informed consent, 2–5 mL of bone marrow was aspirated from the posterior iliac crest of patients with and without HIV infection who underwent diagnostic bone marrow examination or healthy paid volunteers, and the marrow was collected in 5 mL of Iscove's modified Dulbecco's medium (IMDM; Sigma Chemical Co., St. Louis, Mo) that contained 10 U sodium heparin/mL. The cells were then centrifuged and the supernatant immediately collected and frozen in small aliquots at −80°C for later assays. Data on patients participating in this study were obtained from hospital and clinic records. Serum specimens and data were collected with informed consent from anemic patients who underwent diagnostic marrow examination as part of another study.[33] These specimens were also separated immediately from the cells and handled as described for marrow supernatant. The use of these specimens and this data for the current study was approved by the MUSC Institutional Review Board and by the Research & Development Committee of the Ralph H. Johnson VA Medical Center.

Protein Nitrotyrosine (pNT) Content Assay

The method used is a modification of the technique described by Oates et al.[34] Protein concentration in serum or marrow supernatants was determined using a commercially available kit (BioRad, Richmond, Calif). Five-microgram protein aliquots in 50 μL were then applied to a polyvinyl difluoride membrane under a constant low vacuum, and pNT was determined by an immunoblot technique. Results were quantified by comparison to a nitrotyrosine-containing protein standard (Upstate Biotechnology, Lake Placid, NY) from a single batch and expressed as equivalents of the nitrotyrosine content of 1 μg of the standard (μg equivalents). The result obtained, pNT μg equivalents/5 μg protein, was then normalized to the protein concentration. Anti-nitrotyrosine antibodies were purchased from Upstate Biotechnology, and represented using ECL Western blotting detection reagents (Amersham, Arlington Heights, Ill). Immunoblots were quantified using a Fotodyne Imaging Analysis System (Fotodyne, Inc., Hartland, Wisc). Marrow concentration values were corrected for dilution by IMDM and expressed as concentrations per volume of marrow aspirated.

Thioredoxin (Trx) Assay

Trx concentration was assayed using an immunoblot approach similar to that described for pNT, above. Fifty-microliter aliquots of serum or marrow supernatant (diluted 100- to 400-fold) were applied to a polyvinyl difluoride membrane under a constant low vacuum, and Trx concentration was determined. Results were quantified by comparison to *Escherichia coli* recombinant (r) Trx (Sigma), using standards between 1000 ng/mL and 7.8125 ng/mL. Antibodies generated against *E. coli* rTrx were used for detection, and represented and quantified as above. Also as above, marrow concentration values were corrected for dilution by IMDM and expressed as concentrations per volume of marrow aspirated.

TNFα Assays

Enzyme-linked immunosorbent assay (ELISA) kits for human TNFα were purchased from R&D Systems, Inc. (Minneapolis, Minn). ELISAs were performed using a BioTek (Winooski, Vt) EL312e microplate reader. A standard curve was determined with each experiment. Marrow cytokine values were corrected for dilution by IMDM and expressed as concentrations per volume of marrow aspirated. These values and some of their correlations, although not those discussed in this paper, have been reported previously.[32] Serum TNFα concentrations were determined specifically for this study.

Culture of Colony-Forming Unit E (CFU-E) in Plasma Clots

In some cases, marrow cells were resuspended in IMDM and enriched for light-density mononuclear cells (LDMN cells) by separation over Ficoll-Paque Plus 1.077 g/mL (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Marrow LDMN cells were cultured at concentrations of 1×10^5 cells/mL in 0.2 mL plasma clots with IMDM, 25% fetal calf serum (Hyclone Laboratories, Logan, Utah), 1% bovine serum albumin (Sigma), rhEPO 1 U/mL (Ortho Biotech, Raritan, NJ), penicillin, streptomycin, epsilon aminocaproic acid 1.5 mM, fibrinogen 1.3 mg/mL (Calbiochem-Novabiochem Co., La Jolla, Calif), and thrombin 0.2 U/mL (Parke-Davis Pharmaceuticals, Morris Plains, NJ). Cells were then cultured for 7 days at 37°C in 5% CO_2/95% air and then fixed and stained with benzidine–hematoxylin, as described by McLeod et al.[35] CFU-E were defined as giving rise to colonies of 8–49 hemoglobinized cells.[36] All cultures were performed in duplicate, representing 6–8 plasma clots per experiment, and results were expressed as CFU-E/10^5 LDMN cells.

Statistical Analysis

Statistical analysis was performed using an analytical software program. Groups of values were compared using the Mann-Whitney rank-sum test, and correlations between groups of values were evaluated using Pearson's product-moment correlation. Expected frequencies of events were compared using Fisher's exact test.[37]

RESULTS

Patient Demographics

The demographics of the patients who underwent diagnostic marrow examination and who were included in this study are shown in Table 1. The frequency of female patients was significantly greater in the non-HIV patient subsets compared to the HIV patient subsets ($P=0.02$) for both marrow and serum studies. Similarly, the distribution of ages of other anemic patients in the serum studies group is significantly more slanted towards older patients than in the HIV patient subset ($P<0.01$). However, no correlations between serum or marrow supernatant concentrations of pNT, Trx, or of the ratio of these concentrations and gender or age were observed in any subset. In addition, there are no significant demographic differences between HIV patients in the serum and marrow groups, or

Table 1. Characteristics of patient subsets who have undergone marrow examination.

	Marrow Supernatant Studies		Serum Studies	
	HIV	Non-HIV	HIV	Non-HIV
n	25	12	6	31
Median age, years	37	*	38	56†
Male/female‡	23/2	7/5	6/0	14/17
Race				
African-American	8	3	2	5
Caucasian	17	9	4	26
Indications for bone marrow examination:				
Abnormal blood count	15	9	3	16
Malignancy evaluation/staging	4	3	0	15
Fever evaluation	6	0	3	0

**Ages of all subjects in this group were not recorded. The median of the 7 recorded values was 38 years.*

†Age of non-HIV patients is significantly greater than that of HIV patients evaluated with serum studies ($P<0.01$).

‡The proportion of female patients was significantly greater in the non-HIV subset for both marrow supernatant and serum studies ($P=0.02$).

between non-HIV patients in the serum and marrow groups. Indications for bone marrow examination are also shown, and these are not significantly different when the anemic HIV patient and anemic non-HIV patient subsets in the marrow supernatant group are compared to the same patient subset in the serum group.

pNT and Trx Concentrations in Serum and Marrow Supernatant

Protein nitrotyrosine concentrations for bone marrow supernatant and serum are shown in Figures 1A and 2A,

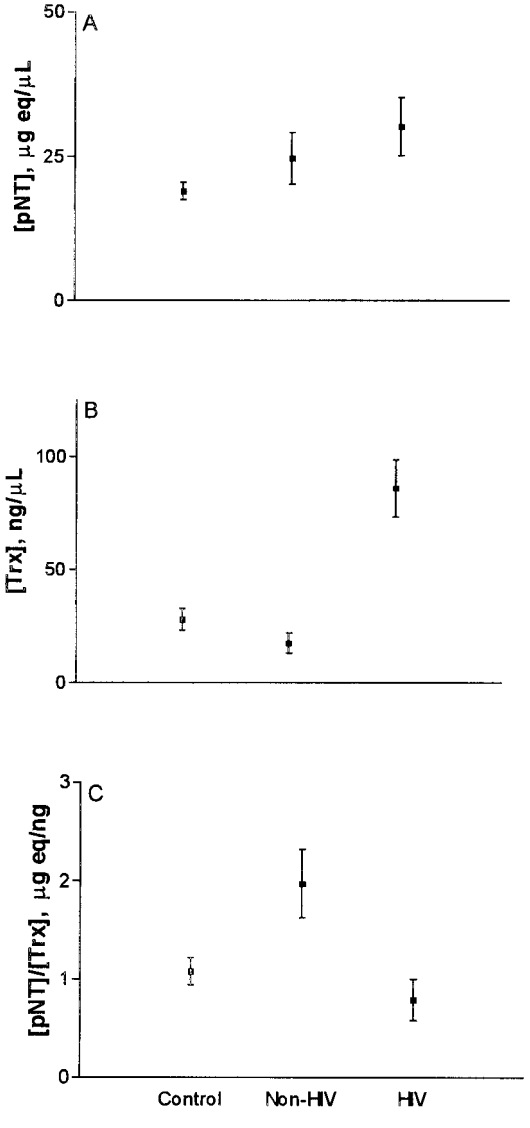

Figure 1. *Concentrations of protein nitrotyrosine (pNT) (A), thioredoxin (Trx) (B), and the ratios of these concentrations (C) measured in bone marrow supernatant from healthy controls, patients with HIV, and other patients. Results displayed as mean±SEM*

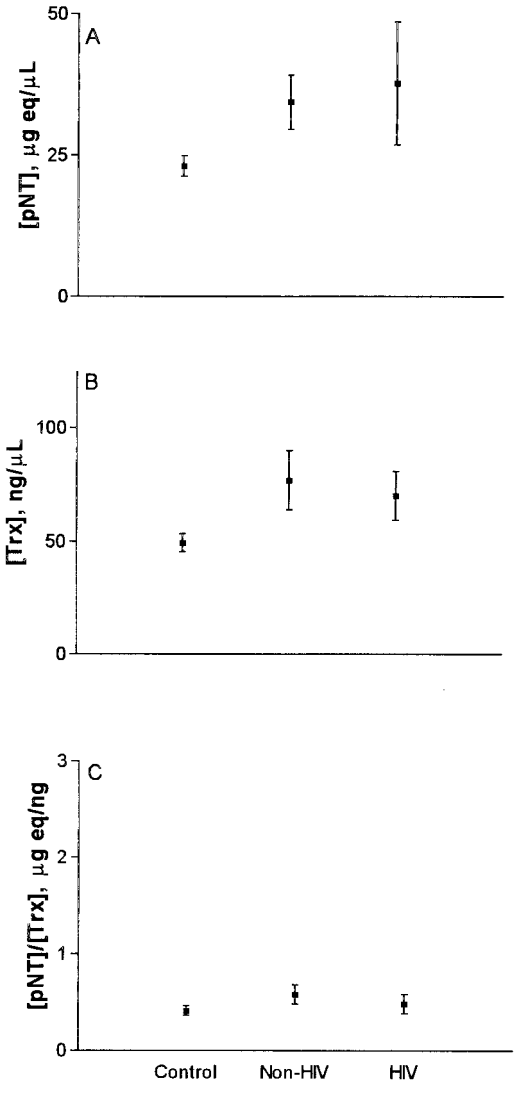

Figure 2. *Concentrations of protein nitrotyrosine (pNT) (A), thioredoxin (Trx) (B), and the ratios of these concentrations (C) measured in serum from healthy controls, patients with HIV, and other patients. Results displayed as mean±SEM.*

respectively. Concentrations were determined in marrow supernatant from 25 HIV patients, 12 other patients, and 35 healthy individuals, and in serum from 6 anemic patients with HIV, 31 other anemic patients, and 5 healthy individuals collected during the same time period. pNT concentrations in serum or in marrow supernatant did not differ between the subsets studied. Concentrations observed in serum were not significantly higher than those observed in marrow supernatant in the same subject subsets.

Mean serum Trx concentrations in both anemic HIV patients and other anemic patients were greater than were

those observed in healthy individuals (Figure 2B): 70.1±10.7 ng/μL (HIV patients; mean±SEM) or 76.8±13.1 ng/μL (other patients) vs. 49.4±3.9 ng/μL (healthy controls), although these differences do not attain statistical significance.

In contrast to the findings for pNT concentrations, Trx concentrations in bone marrow supernatant from HIV patients (85.9±12.7 ng/μL) were strikingly higher than those observed in other patients (17.5±4.4 ng/μL; $P<0.001$) or in healthy controls (28.0±4.8 ng/μL; $P<0.001$; Figure 1B). Trx concentrations observed in serum were significantly higher than those observed in marrow supernatant in non-HIV patients and in healthy subject subsets ($P<0.01$ for both). Serum and marrow supernatant Trx concentrations in HIV patients were not significantly different ($P=0.80$).

Because there is a statistically significant male predominance among HIV patients undergoing marrow examination, the possibility that the differences we observed may reflect an intrinsic gender difference in pNT and/or Trx concentration was evaluated. No statistically significant difference in marrow supernatant pNT or Trx concentrations, or in the ratio of these concentrations, was observed when results from male healthy volunteer controls (n=19) were compared to results from female volunteer controls (n=16).

pNT/Trx Ratio in Serum and Marrow Supernatant

In this study, serum pNT and Trx concentrations are similar to or higher than marrow supernatant concentrations observed in comparable individuals, so the possibility that the pNT and/or Trx that is detectable in marrow supernatant results from peripheral blood contamination cannot be ruled out by concentration determination alone. Because pNT content is a marker of superoxide activity,[22] and Trx is a redox protein that is induced by oxidants,[38] the ratio of the concentrations of these two proteins may provide a measure of net oxidative stress, and, if the ratios differ in the two compartments, it may provide evidence of local control of oxidation–redox activities.

The ratios of pNT concentration to Trx concentration in bone marrow supernatant and serum are shown in Figures 1C and 2C. Ratios observed in serum were very similar in all subject subsets, and all have a mean of approximately 0.50 (range 0.41–0.58; Figure 2C). In contrast, ratios differed broadly and significantly in marrow supernatant (Figure 1C). The highest mean ratio was observed in non-HIV patients (1.97±0.35; $P=0.01$ vs. either healthy controls or HIV patients). The lowest mean ratio occurred in HIV patients (0.79 ±0.21; $P=0.01$ vs. healthy controls). Healthy controls exhibited an intermediate mean ratio (1.08±0.14). The ratios observed in bone marrow aspirate supernatant were significantly higher than those observed in serum from comparable non-HIV patients and healthy controls ($P<0.001$ and $P=0.016$, respectively). Ratios observed in HIV patients did not differ significantly between serum and marrow supernatant ($P=0.73$).

Correlations Between Marrow Supernatant Trx or pNT Concentrations and Other Parameters in HIV Patients

Hematologic parameters (leukocyte count, platelet count, hemoglobin concentration, and mean corpuscular volume) and CD4 lymphocyte counts were available for 19 of the 25 HIV patients in whom marrow supernatant specimens were studied. CD8 lymphocyte counts were available for 16 of these 19 patients. Values are shown in Table 2. Marrow supernatant Trx concentration exhibited a significant correlation with CD4 count and with the CD4/CD8 ratio; band pNT concentration correlated with platelet count (Table 3). No other correlations with hematologic parameters were observed in HIV patients.

Blood counts were available on all 37 anemic patients in whom serum Trx and pNT concentrations had been determined. Whether the group was considered in its entirety or subdivided into HIV patients (n=6) versus other patients (n=31), no associations between hematologic results and serum Trx or pNT concentrations were found.

CFU-E colony assays were carried out with marrow cells from 23 healthy volunteers, 14 patients with HIV, and 6 other patients. The median number of CFU-E–derived colonies per 10^5 LDMN cells plated were similar

Table 2. Hematologic characteristics of HIV patients.

Characteristic	Median (range)
Bone marrow supernatant donors (n=19)	
Hemoglobin, g/dL	9.3 (6.2–15.0)*
Mean corpuscular volume, fL	91.0 (70.6–104.2)
Leukocytes, $10^3/\mu L$	3.1 (1.0–6.8)
Platelets, $10^3/\mu L$	130 (16–431)
CD4, /μL	20 (0–377)
CD8, /μL (n=16)	403 (86–1228)
CD4/CD8 ratio	0.03 (0–1.07)
Serum donors (n=6)	
Hemoglobin, g/dL	9.4 (7.3–11.3)
Mean corpuscular volume, fL	88.0 (76.0–97.3)
Leukocytes, $10^3/\mu L$	2.4 (1.1–4.2)
Platelets, $10^3/\mu L$	114 (10–358)

**Only one of these patients had a normal hemoglobin concentration.*

Table 3. Correlations of bone marrow supernatant concentrations of thioredoxin or protein nitrotyrosine with other parameters in patients with HIV.

Concentration	Correlation Coefficient	*P*
Thioredoxin concentration		
Peripheral CD4 lymphocyte count (n=19)	0.497	0.03
CD4/CD8 ratio (n=16)	0.525	0.025
CFU-E per 10^5 LDMN marrow cells (n=14)	0.789	0.001
Protein nitrotyrosine concentration		
Platelet count (n=19)	0.683	0.001
TNFα concentration (n=19)	0.657	0.002

in all three subsets, although slightly lower for HIV patients: 306 colonies/10^5 LDMN cells for HIV patients, compared with 418 colonies/10^5 LDMN cells for healthy donors and 384 colonies/10^5 LDMN cells for other patients. These differences were not statistically significant. In HIV patients, higher concentrations of Trx in marrow supernatant correlated with greater numbers of CFU-E colonies (P=0.001; Table 3 and Figure 3); this was not observed in the other subject subsets.

TNFα concentrations in bone marrow supernatants in which pNT and Trx concentrations had been measured were available for 19 HIV patients, 7 healthy donors, and 4 other patients. Only one of the latter group had detectable TNFα in marrow supernatant. In contrast, 16/19 HIV patients and 6/7 healthy subjects had detectable TNFα in marrow supernatant. Although values in supernatant from HIV patients tended to be higher than those observed in healthy donors (median 11.4 pg/mL vs 6.0 pg/mL), there was sufficient overlap to make these differences insignificant (P=0.20).[32] pNT concentrations in marrow supernatant from HIV patients were correlated with TNFα concentrations in marrow supernatant (Table 3). No similar correlations were observed in the other subject subsets.

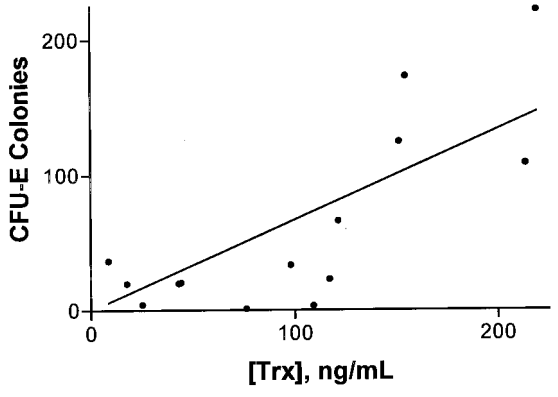

Figure 3. *Association between colony-forming unit–E (CFU-E) colonies per 10^5 light-density mononuclear cells marrow cells and thioredoxin (Trx) concentrations in marrow supernatant of HIV patients. The regression line for these variables is also shown.*

TNFα concentrations in serum were determined in serum specimens from all 37 anemic patients and 5 healthy controls. Two of the 6 HIV patients, 1 of 31 other patients, and none of the controls had detectable serum TNFα concentrations. The two HIV patients with detectable TNFα concentrations also had the two highest serum pNT concentrations in that patient subset. The significant differences in the frequency with which TNFα is detectable in marrow supernatant compared with serum (P=0.032, 0.015, and 0.002 for HIV patients, controls, and other patients, respectively) are consistent with the observation in our earlier report that marrow TNF levels are higher than serum levels in the same patients.[32]

No correlations with the pNT/Trx ratio or any of the parameters described here were observed in either serum or marrow. Marrow supernatant concentrations of pNT, Trx, and the pNT/Trx ratio did not differ between HIV patients who underwent marrow examination as part of an evaluation of a fever and those who underwent marrow examination for other indications ($P \geq 0.78$ for all parameters).

DISCUSSION

Redox changes appear to play a significant role in the pathophysiology of HIV infection. Production of H_2O_2 by monocytes from patients with HIV correlates with viral load,[14] and in vitro exposure of human T-lymphocytes to H_2O_2 at micromolar concentrations induces expression and replication of HIV-1. This effect is mediated by NF-κB.[21] The antioxidant N-acetyl-cysteine blocks this effect by preventing NF-κB activation by H_2O_2; it also blocks NF-κB activation by TNFα, IL-1, and a variety of other agents, which suggests that it is oxidation that represents the final common pathway for the conversion of IF-κB to its active form.[21] In addition, HIV infection can itself alter the cellular redox state. HIV Tat protein, which enhances activation of NF-κB by TNF as well as TNF cytoxicity,[16] appears to do so by suppressing expression of manganese superoxide dismutase.[16,39] Tat mutants that are unable to suppress manganese superoxide dismutase do not potentiate TNF effects.[16] ROS also appear to be partially responsible for some of the toxic effects of zidovudine.[18]

In addition to the above findings, peroxynitrite has been implicated in the pathologic manifestations of HIV infection as well. HIV Tat–induced apoptosis of hippocampal neurons in culture appears to be peroxynitrite-dependent.[17]

Staining for tissue nitrotyrosine is more commonly found and, when found, is more intense in brain sections from demented patients with HIV than in brain sections from HIV patients without dementia.[19] Peroxynitrite is also implicated in zidovudine cardiac toxicity.[18]

Associations between Trx and apoptosis have been reported in HIV-infected monocytes.[12,14] In the early stages of HIV infection, there appears to be an enhancement of cellular oxidative stress, which is associated with a parallel decline in Trx content and Bcl-2 expression and results in apoptosis.[12] As persistent infection becomes established, both Bcl-2 and Trx expression recover; cellular Trx protein concentrations eventually exceed those of uninfected controls.[12] These findings are observed in infected monocytic cell lines as well as in primary monocytes obtained from infected patients at different stages of their disease.[12,14]

It could be anticipated that Trx concentrations in marrow supernatant from HIV patients and some of the other patients would be higher than those found in healthy controls. Elevated Trx concentrations have been reported in the plasma of patients with HIV[29] and also in the plasma of patients who underwent open heart surgery,[30] as well as in the serum of patients with hepatocellular carcinoma.[31] Serum Trx concentrations as measured by the technique described here were significantly higher than those that other groups have reported for either serum or plasma using different assay techniques and reagents.[29–31,40] For this reason, it would not be valid to compare specific concentration values reported here to those reported by other investigators; however, as in a previous report of plasma Trx concentrations in a much larger population,[29] the majority of HIV patients in this study (4/6) have serum Trx concentrations higher than the highest serum value observed in a healthy subject.

Protein nitrotyrosine concentrations in either marrow supernatant or serum do not differ among subject subsets (Figures 1A, 2A). This suggests that the degree of NO–superoxide interaction is similar in each group, but it does not necessarily imply that the degree of NO activity is similar. Nitric oxide effects can occur in pathways that do not involve superoxide,[41] and the cellular effects of NO and ROS may differ.[42,43]

The major observation that arises from the determination of pNT and Trx concentrations in the specimens studied was the divergence of pNT/Trx ratios in marrow supernatant between the three subject subsets, whereas ratios observed in serum samples from comparable subjects were essentially identical. Although initially conceived as a rough approximation of net oxidative stress, the pNT/Trx ratio more correctly represents the relative balance between a specific pro-oxidant pathway (NO–superoxide–peroxynitrite) and a specific antioxidant system (Trx). The findings described in the present study suggest that there is a unique pattern of oxidant/antioxidant balance in patients with HIV; the divergence between marrow and serum ratios in non-HIV patients and healthy individuals suggests that there are mechanisms operating in bone marrow that allow maintenance of a particular local oxidative environment. The determining component of this local, syndrome-specific regulation appears to be Trx. In patients with HIV, for any given degree of peroxynitrite generation in marrow supernatant, there is a greater Trx response than is observed in healthy controls or in other patients. In contrast, the Trx response that is detectable in serum is similar for all levels of pNT.

Virally infected lymphocytes produce Trx at a high and constitutive level,[44] and in that context, the association between CD4 lymphocyte count and higher Trx levels seems predictable. On the other hand, Elbim and colleagues reported that monocytes from asymptomatic HIV patients with CD4 counts $>500/\mu$L have lower Trx content than healthy controls, and elevated Trx levels are only observed in patients with AIDS, who have lower CD4 counts.[14] All but one of the patients with HIV from whom marrow supernatant was collected in this series meet the 1993-case definition for AIDS;[45] it may be that increased numbers of CD4 lymphocytes predict higher Trx concentrations among this subset of individuals with elevated baseline Trx concentrations.

The association between increased CFU-E colony formation and marrow supernatant Trx concentration was only noted in HIV patients, which probably reflects the fact that only these subjects had significantly elevated Trx concentrations. Apoptosis is a major regulator of erythropoiesis; one pathway to apoptosis in CFU-E involves members of the Bcl-2 family.[46] In patients with HIV, higher Trx cellular concentrations are associated with higher Bcl-2 concentrations.[12,14] The viability of chronic lymphocytic leukemia clones in long-term cultures is enhanced in the presence of Trx; chronic lymphocytic leukemia cells in short-term cultures that contain μg/mL concentrations of Trx maintain expression of Bcl-2 longer than do cells not exposed to Trx.[47] Mechanisms by which Trx inhibits apoptosis that are not dependent on Bcl-2 have also been described.[48] Protection of CFU-E colony formation by antioxidants such as Trx may explain the apparent paradox of preserved hematopoietic colony formation in patients with either early[49] or more advanced[50,51] HIV infection. It is noteworthy that a recent report suggests that Trx is a growth factor that enhances hematopoietic colony formation in vitro.[52]

Associations between TNF and NO production have been reported in a variety of circumstances.[53–55] The as-

sociation between pNT and TNFα concentrations may have been observed only in patients with HIV because those were the subjects in whom elevated TNF concentrations were routinely detectable. It is unclear why elevated pNT levels in marrow supernatant from patients with HIV correlate with higher platelet counts, while this is not the case with their serum pNT levels.

In both the marrow and supernatant subsets, a significantly greater frequency for females was observed in the patient controls, yet the differences observed cannot be attributed solely to this fact, because no correlation with sex was observed in any of the variables tested. Similarly, no correlation was observed between the age of subjects and the variables evaluated. We believe the results described reflect the effects of HIV infection and underlying diseases that are associated with it. Also, the characteristics of the patients with HIV and the other anemic patients in the serum group were very similar to those observed in the same subsets of the marrow supernatant group, which indicates that the differences we observed in pNT/Trx ratios between serum and marrow cannot be explained on the basis of selection.

In summary, the ratios of peroxynitrite production (as measured by pNT concentration) to Trx production, which were measured in serum, fall into a narrow range and do not differ significantly between patients with HIV, other patients, and healthy controls. In contrast, the ratios observed in marrow supernatant from similar patients differ markedly. In HIV patients, as opposed to the other subjects studied, the balance between pNT and Trx observed in serum is preserved in the marrow supernatant. This suggests that the redox environment of the bone marrow is regulated by local factors that differ depending upon clinical status, and that this local redox environment may contribute to the regulation of hematopoiesis.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Brenda Paulson, MT(ASCP), and Kimberley Walker, MT(ASCP), in collecting specimens; of the staff of the Infectious Diseases/AIDS Clinical Trials Center of the University of Cincinnati in identifying candidates and collecting clinical data; and of Melissa North, RN, in the early laboratory phases of this project.

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