LAH 353

Intracellular Glutathione Levels in T Cell Subsets Decrease in HIV-Infected Individuals

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ABSTRACT

The authors have shown previously that intracellular glutathione (GSH) plays an important role in the regulation of human immunodeficiency virus (HIV) transcription and replication in vitro, through modulation of signal transduction by inflammatory cytokines. Moreover, intracellular GSH levels are known to regulate T-lymphocyte function. In multiparameter FACS studies presented here, we show that relative GSH levels in $CD4^+$ and $CD8^+$ T cells from HIV^+ individuals are significantly lower than in corresponding subsets from uninfected controls. These studies define the relative intracellular glutathione (GSH) levels in $CD4^+$ T cells, $CD8^+$ T cells, B cells, and monocytes from 134 HIV-infected individuals and 31 uninfected controls. The greatest decreases in intracellular GSH occur in subsets of T cells in individuals in the later stages of the HIV infection. In AIDS patients, GSH levels are 63% of normal in $CD4^+$ T cells ($p < 0.0001$) and are 62% of normal in $CD8^+$ T cells ($p < 0.0001$). Similarly, in AIDS-related complex (ARC) patients, GSH levels are 66% of normal in $CD4^+$ T cells ($p < 0.003$) and are 69% of normal in $CD8^+$ T cells ($p < 0.003$). These findings suggest that low intracellular GSH levels may be an important factor in HIV infection and in the resulting immunodeficiency.

INTRODUCTION

INFLAMMATORY CYTOKINES SUCH as tumor necrosis factor α (TNF-α), interleukin-1 (Il-1) and interleukin-6 (Il-6), stimulate human immunodeficiency virus (HIV) transcription and replication,[1-3] and therefore may play a central role in the progression of HIV infection to clinical acquired immunodeficiency syndrome (AIDS). Stimulation of cell lines with these cytokines results in the production of oxidants and consumption of intracellular glutathione (γ-glutamyl-cysteinyl-glycine, GSH).[4] Furthermore, Beauerle's group has shown recently that the oxidant hydrogen peroxide can directly stimulate HIV transcription in a Jurkat subclone.[5] These stimulations of HIV transcription (either by oxidants or cytokines) can be inhibited by N-acetylcysteine (NAC),[1,2,6,7] a cysteine analog that replenishes intracellular GSH. Moreover, cytokine-stimulated HIV transcription is enhanced by depleted intracellular GSH.[2]

These in vitro findings are of clinical importance because GSH levels are decreased in HIV^+ individuals. Previous studies, using bulk biochemical assays to measure GSH, have shown that HIV^+ individuals have lower GSH levels than uninfected controls in plasma,[8] lung epithelial lining fluid,[9] and extracts from peripheral blood mononuclear cells (PBMC).[8] Studies presented here, employing methods to study individual cells instead of bulk assays, confirm and substantially extend these findings. We use multiparameter Fluorescence Activated Cell

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Sorter (FACS) analyses to measure intracellular GSH levels in PBMC subsets (T cells, B cells, and monocytes) from 134 HIV$^+$ individuals whose clinical status ranges from asymptomatic to AIDS. We show (1) that the most dramatic decreases in intracellular GSH occur in the T-cell subsets (CD4$^+$ and CD8$^+$), but not in B cells and monocytes; (2) that these decreases are more severe in patients with AIDS and AIDS-related complex (ARC); and (3) that these decreases can mainly be accounted for by the selective loss of cells that have high intracellular GSH levels.

METHODS

Subjects

We recruited 134 HIV$^+$ patients, 31 HIV$^-$ healthy individuals without risk factors for HIV infection (normal controls), and seven HIV$^-$ homosexual males (at risk controls) in the San Francisco Bay area. The presence or absence of HIV infection was documented both by a commercially available ELISA kit (Abbott) specific for antibodies to the p24 antigen and confirmed by Western blot for antibodies against the p24 and gp160 antigens. The at-risk controls were in good health and their HIV$^-$ status was reconfirmed by ELISA and Western blot. Patients using thiol-enhancing drugs, such as N-acetylcysteine or glutathione-esters, were excluded from participation.

All subjects gave informed consent. The HIV$^+$ group included 92 homosexual males; 2 bisexual males; 2 transsexual females; 2 heterosexual females (multiple sexual partners); 26 male and 9 female intravenous drug users; and 1 male infected by blood transfusion. No analyses were made concerning the dependences of our measurements on age, gender, or race, since this is a highly homogeneous cohort. In accordance with CDC classification, 31 (23%) subjects were asymptomatic, 32 (24%) were diagnosed with ARC, and 71 (53%) had a history of AIDS-defining diagnoses. Patients with current opportunistic infections were being treated with antimicrobials at the time of blood sampling.

Cell preparations and FACS measurements

Multiparameter FACS analyses were performed using fluorochrome-coupled monoclonal antibody staining reagents detecting CD8(Leu2a), CD4(Leu3a), CD3(Leu4), CD5(Leu1), CD20 (Leu16), CD14(LeuM3), CD7(Leu9), and CD16(Leu11c) (a gift from Becton Dickinson, San Jose, CA). PBMC from fresh blood samples (<4 h after drawing) were obtained by Ficoll-hypaque (Pharmacia, Alameda, CA) density centrifugation of 6–10 ml heparinized blood. Whole blood was diluted 1:1 in RPMI-1640, layered on Ficoll-hypaque and centrifuged for 20 min at 2400 rpm (1500 g). An additional 2 ml sample was used for a standard blood cell count and screening slide differential. Cells were washed and stained for intracellular GSH using a modification of a method employing monochlorobimane[10] (MCB, Molecular Probes, Portland, OR). Cells were stained first at room temperature for exactly 20 min with MCB in staining medium (deficient RPMI, 4% fetal calf serum (FCS), 10 mM HEPES pH 7.2) at a final concentration of 40 μM. The

reaction was stopped by the addition of excess (10 volumes) ice-cold staining medium and centrifugation through 0.5 ml FCS underlayered to remove unreacted MCB. Samples were then stained for cell surface phenotype in ice cold staining medium, fixed with paraformaldehyde (0.5% v/v final concentration) and were subsequently analyzed on a dual laser FACStarPlus (Becton Dickinson) with one laser providing 488 nm illumination and the other providing 361 nm ultraviolet (UV) illumination. A forward scatter threshold was set to exclude contaminating erythrocytes and cell debris. Data for 30,000 cells were collected for each sample, stored in list mode format, and analyzed later with FACS-DESK software (Stanford University) running on a VAX computer (Digital Equipment Corporation). One or two normal controls were included, as a reference for GSH levels, on each experiment day, in which 3 to 10 patient samples were studied.

Several studies have shown that the FACS-measured fluorescence is correlated linearly with intracellular GSH[10,11] (T. Kavanagh, personal communication). We have confirmed the linearity of the assay in PBMC, using high-performance liquid chromatography (HPLC) measurements of GSH on FACS-sorted subpopulations (data not shown). Similarly, high-GSH and low-GSH T cells (Fig. 2) were sorted and shown to have distinct levels of intracellular GSH by HPLC, as predicted by the FACS measurement. Controls also demonstrated that GSH levels in PBMC are stable for up to 24 h after drawing blood, and that the MCB fluorescence after staining is stable for several hours (during subsequent antibody staining and paraformaldehyde fixing of the cells).

Calculations and statistics

Absolute number of PBMC/μl of blood was determined from the screening differential as follows: (percentage of lymphocytes + percentage of monocytes) x total leukocytes. Absolute number of cells (per μl of blood) in each PBMC subset was determined by multiplying the absolute number of PBMC/μl by the percentage of cells in the subset, i.e., percentage of cells positive for a particular set of cell surface markers (as determined by FACS analysis). Subsets were defined as follows: CD4$^+$ T cells, Leu3a$^+$Leu4$^+$; CD8$^+$ T cells, Leu2a$^+$Leu4$^+$; B cells, Leu16$^+$; and monocytes, LeuM3$^+$Leu4$^-$. Additional forward and side scatter gates were used to distinguish lymphocytes and monocytes. Median GSH levels in each subset were calculated as the median of MCB fluorescence values for the cells in that subset; 90th and 10th percentile values were calculated as the MCB fluorescence level under which 90 and 10% of the cells reside. Statistical comparisons of the median GSH levels between each subject category (asymptomatic, ARC, AIDS, normal control) were computed with a single factor analysis of variance (unbalanced design) and Scheffe F-test. Statistically significant differences were determined for two group comparisons using the nonparametric Mann-Whitney U-test. The probabilities in Table 3 were calculated as the relative frequency of individuals in each category with a lower median GSH level than the lowest value observed in 30 of 31 healthy, uninfected controls (P1), or as the relative frequency of individuals with a CD4$^+$ T-cell count of less than 500/μl (P2).

TABLE 1. GSH LEVELS AND NUMBER OF CELLS IN PBMC SUBSETS

Category	CD4+ T	CD8+ T	Monocytes	B
GSH levels[a]				
Random control (n = 31)	100 (93–100)	100 (98–104)	100 (93–105)	100 (95–103)
Risk group HIV-(n = 7)	102 (84–121)	95 (87–121)	95 (80–100)	97 (95–129)
Asymptomatic (n = 31)	72 (58–78)	74 (54–83)	84 (66–102)	88 (74–105)
ARC (n = 32)	66 (51–84)	69 (56–82)	76 (66–93)	82 (67–97)
AIDS (n = 71)	63 (49–76)	62 (51–82)	80 (65–98)	90 (77–110)
Number of cells per µL of whole blood				
Random control (n = 10)	1096 (428)	357 (127)	412 (168)	283 (143)
Risk group HIV-(n = 7)	745 (472)	409 (279)	497 (107)	526 (281)
Asymptomatic (n = 31)	516 (318)	727 (413)	361 (142)	181 (117)
ARC (n = 32)	283 (243)	678 (396)	338 (134)	213 (227)
AIDS (n = 71)	74 (120)	244 (252)	269 (246)	95 (118)

The median of GSH levels in random, normal control is set as 100 for each cell population. Patients' GSH levels are expressed as the relative level compared to the normals. Data are given as median (25th-75th percentile: interquartile range) for GSH levels and as mean (SD) for cell counts. In the random control group, cell counts are from 10 individuals; counts for other individuals in the group were not performed.

RESULTS

Analyses of GSH levels in PBMC subsets from HIV+ individuals reveal significant differences from normals. First, there are overall changes in GSH levels in all PBMC subsets (Table 1). Second, there is a selective loss from circulation of T lymphocytes with high GSH levels (Figs. 1 and 2). Together, these findings reveal a decrease in total intracellular GSH in PBMC from HIV+ individuals.

Data in Figure 1 (derived from FACS analyses such as those shown in Fig. 2) demonstrate that the relative levels of intracellular GSH in T-cell subsets in HIV+ individuals are decreased
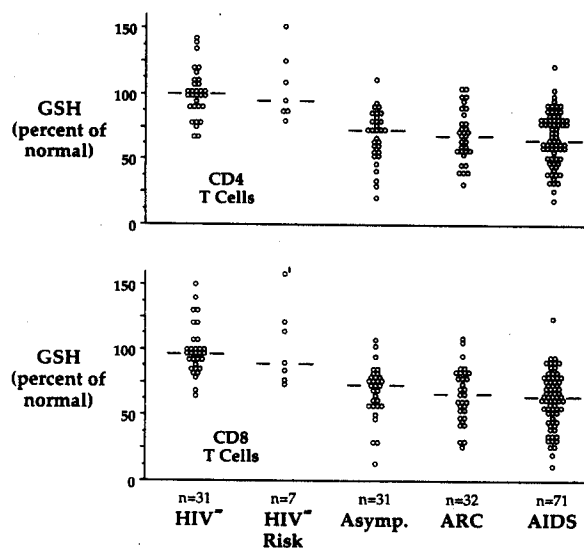

FIG. 1. Intracellular glutathione (GSH) levels in CD4+ and CD8+ T cells are lower in HIV+ than in HIV− individuals. Intracellular GSH is determined as the median of MCB fluorescence, with the average median of normal, healthy subjects (random control) normalized to 1.0 for each T cell subset. Each individual is indicated as a small circle; the median GSH level for each category is shown with a bar.

(single factor analysis of variance: CD4+ T cells, p < 0.0001; CD8+ T cells, p < 0.0001). Individuals in the two uninfected control groups (random and "at risk") have similar GSH levels. In contrast, GSH levels in most of the HIV+ individuals included within the study are below the median GSH level for normal, uninfected individuals, with the trend of lower GSH levels going from normal, asymptomatic, ARC to AIDS. Using the Mann-Whitney U-test, HIV+ patients had significantly lower relative intracellular GSH than the control groups in CD4+ T cells (asymptomatic: 72% of normal, p < 0.0023; ARC: 66% of normal, p < 0.003; AIDS: 63% of normal, p < 0.0001) and CD8+ T cells (asymptomatic: 74% of normal, p < 0.001; ARC: 69% of normal, p < 0.003, AIDS: 62% of normal, p < 0.0001). Since the distributions are not normal, parametric statistics cannot be used to compare these groups.

We currently are investigating the changes in GSH levels in other PBMC subsets (B cells, monocytes, NK cells). The decreased GSH levels in monocytes and B cells in the HIV+ categories (see Table 1) are not statistically significant (Scheffe *F*-test, nonparametric single analysis of variance). In fact, there is a greater disparity among GSH levels observed for these subsets. That is, many individuals have decreased GSH levels in their monocytes and/or B cells; however, some individuals show marked GSH increases in these subsets. We have not yet been able to determine whether the latter individuals fall into particular clinical categories (e.g., type of opportunistic infection).

We analyzed a number of our subjects a second or third time for their intracellular GSH levels. Samples from 23 individuals (7 asymptomatic individuals, 6 with ARC, and 10 with AIDS), who were clinically stable over time (e.g., no opportunistic infections), were investigated. Time between the samples varied from 7 to 104 days (mean 36 days). We did not observe any significant changes in the GSH status in the CD4+ and CD8+ T cells of these individuals (Wilcoxon signed rank test). Thus, the decreased GSH levels appear to be a constant characteristic of the T cells from these individuals and appear to be consistently lower than normal.

The decrease in GSH levels in HIV+ individuals traces to the selective loss of T cells that have high intracellular GSH levels.
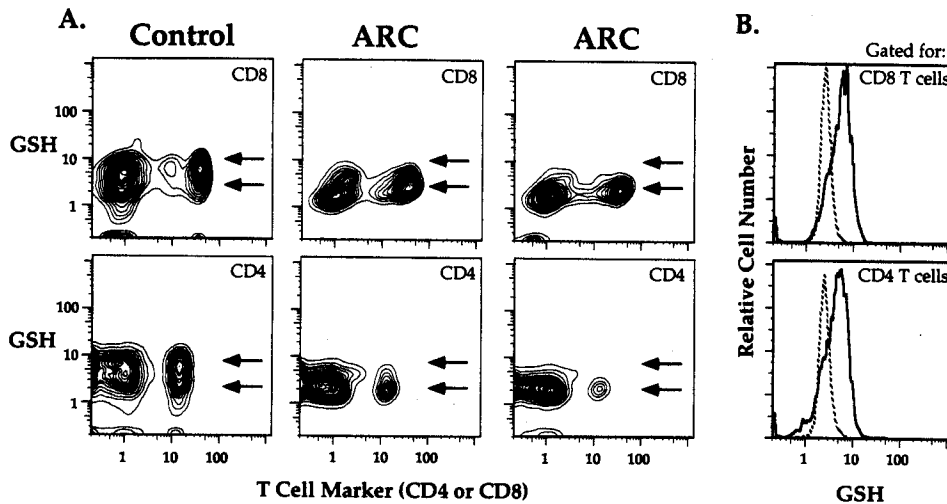
FIG. 2. FACS analysis of intracellular GSH in $CD4^+$ and $CD8^+$ T cells. (A) Dual parameter plot of MCB fluorescence (measuring GSH) on the ordinate versus CD4 or CD8 fluorescence on the abscissa. Left panel: HIV^- healthy control; middle and right panels: HIV^+ individuals classified as ARC. Note that the scale is logarithmic. Arrows indicate the approximate positions of the low- and high-GSH T cells (as found in the normal controls). The high-GSH containing Tcell classes are virtually missing in these individuals. While a significant loss of CD4 T cells was observed in these HIV^+ individuals, there was no decline in the number of CD8 T cells; however, the high-GSH T cells are missing in both subsets (cell counts: CD4: 806, 304, and 53/μl, respectively; CD8: 524, 974, and 393/μl). (B) Histograms of the GSH in the CD8 (top) or CD4 (bottom) cells from the HIV^- control (solid line) and the second HIV^+ individual (hatched line). Note that the low end of the distributions are at the same level of GSH for both individuals, indicating that the low-GSH T cells have not decreased in intracellular GSH content; rather, the representation of cells has shifted from predominantly high-GSH to exclusively low-GSH.

We have shown previously that approximately a third to half of the cells in the $CD4^+$ T cell and $CD8^+$ T cell subsets in healthy individuals have high levels of intracellular GSH.[12] GSH levels in the remaining cells in each subset have roughly three times less GSH (Fig. 2A, left panels). These differences in cell-associated GSH cannot be ascribed to cell size (as determined by light-scatter measurements; data not shown). Low GSH and high GSH T cells are present in the $CD4^+$ and $CD8^+$ T cell subsets in all healthy individuals (Fig. 2A, left panels). The relative frequencies of the two types of cells vary but both types are always detectable. However, the high-GSH T cells are missing in virtually all HIV^+ individuals (Fig. 2). This is true for both the $CD8^+$ and the $CD4^+$ T-cell subsets, and is independent of the loss of $CD4^+$ T cells (see below).

That the loss of high-GSH T cells in HIV-infected individuals is selective is illustrated in Figure 3. We calculated the intracellular GSH level at the 10th, 50th (median) and 90th percentiles in each individual for $CD4^+$ and $CD8^+$ T cells, and then averaged all individuals in each subject category. As the figure shows, the 90th percentile values are much lower in HIV-infected individuals whereas the 10th percentile values do not change significantly. Since the 90th percentile value is located within the high GSH class in uninfected individuals, the strong decline seen in this value for HIV-infected subjects points to a selective loss of high GSH cells. In contrast, the relative constancy of the 10th percentile values indicates that cells in the low GSH subset tend not to lose GSH.

Because of the large overlap between the high and low GSH T cells, it is impossible to set a threshold value which distinguishes them. Thus, in order to quantitate high-GSH T cells, we define a parameter related to the width of the distribution of GSH in T
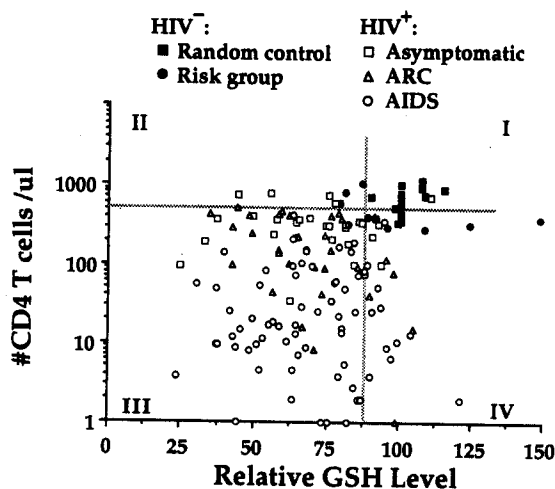
cells. This parameter is the ratio of GSH levels at 90th and 10th percentiles for each subset in an individual. This '90:10 ratio' is independent of the absolute GSH level, so that normalization to a control subject is not required (thus also removing a source of variation, that of the GSH level in the control subjects). A high 90:10 ratio indicates normal levels of the high-GSH T cells, whereas a low ratio indicates a deficit in these cells. For both $CD4^+$ and $CD8^+$ T cells, the 90:10 ratio is significantly different between normal controls and all HIV^+ groups (Table 2). Using this method, we also detect a significant difference between the asymptomatic and AIDS groups.

The relationship between the selective loss of the high-GSH T cells and the overall loss of T cells in HIV-infected individuals is not clear. Although asymptomatic individuals and ARC patients have lost relatively few $CD4^+$ T cells (see Table 1), they tend to have lost the majority of their high GSH cells (see Fig. 3). In patients with more advanced HIV infection, GSH levels tend to be lower, as do $CD4^+$ T cell numbers; however, these decreases are independent, in that the number of $CD4^+$ or $CD8^+$ T cells in a given individual is not predicted by the median GSH level in that subset (Fig. 4). The decrease in GSH in the subsets is not related directly to the loss of cells from the subsets; this is supported by the observation that the level of GSH in $CD4^+$ and $CD8^+$ T cells is highly correlated (r = 0.88) at all stages of HIV infection, whereas the $CD4^+$ T cells are lost much earlier in the disease.

Finally, within our patient sample, the probability of having less than 500 $CD4^+$ T cells (a commonly used threshold) is essentially equivalent to the probability of having a median GSH level in $CD4^+$ T cells that is below the normal range (determined as the lowest value observed in 30 of 31 noninfected controls;
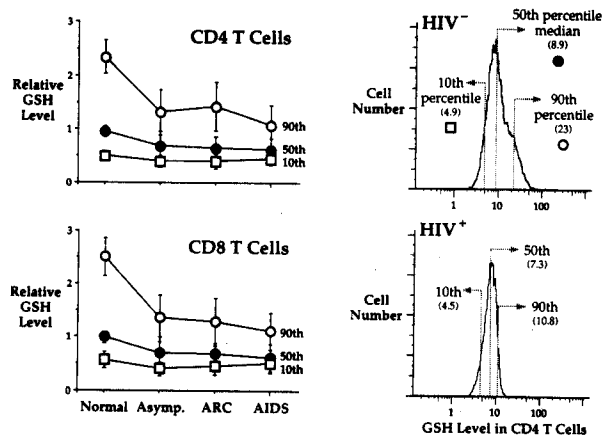
FIG. 3. High GSH subset of CD4$^+$ and CD8$^+$ T cells is progressively lost during HIV infection. Right: explanation of the 10th, 50th, 90th percentiles of GSH levels. The histograms show the MCB fluorescence (which measures intracellular GSH) for CD4$^+$ T cells from an uninfected, normal control (top) and an HIV$^+$ individual (bottom). Note the loss of the high-GSH T cells in the HIV$^+$ individual, as evidenced by the loss of the shoulder in the GSH distribution. The 10th percentile value indicates the GSH level under which 10% of the cells reside. Similarly, the 50th (median) and 90th percentiles indicate the GSH level under which 50% and 90% of the cells reside. The values in the histograms represent the measured fluorescence prior to normalization and averaging. Left: the average 10th, 50th, 90th pecentiles for CD4$^+$ and CD8$^+$ T cells in each subject group. The median GSH level (50th percentile) for HIV-controls is set as 1.0; all other values (10th and 90th percentiles, as well as medians for the other groups) are normalized to this value. Bars represent ± 1 standard deviation. There is a much greater drop in the 90th percentile (which, in normal subjects, is primarily in the high-GSH T cells) as compared to the 10th percentile (which is primarily in the low-GSH T cells); see also Table 2.

these boundaries are illustrated in Fig. 4); and, the probability of being low for both of these characteristics is essentially equal to the product of the probabilities for each (see Table 3). This demonstrates that these two parameters (CD4 count, GSH level)

TABLE 2. LESS HETEROGENEOUS DISTRIBUTION OF GSH IN T CELLS FROM HIV$^+$ INDIVIDUALS

Category	90:10 ratio CD8	90:10 ratio CD4
HIV$^-$ controls	4.9 ± 0.6	4.7 ± 0.8
asymptomatic	3.0 ± 1.1	3.0 ± 1.0
ARC	2.6 ± 0.8	2.9 ± 0.8
AIDS	2.4 ± 0.8	2.4 ± 0.8

The '90:10 ratio' is the ratio of the 90th and 10th percentiles of GSH levels in CD4$^+$ and CD8$^+$ T cells (see Fig. 3). Data are given as mean ± SD for each subject category. The differences in 90:10 ratio for both CD4$^+$ and CD8$^+$ T cells are significant (Scheffe F-test, 95% confidence level) between uninfected controls and all other categories, and between asymptomatic individuals and AIDS patients.

FIG. 4. The number of CD4$^+$ T cells and median GSH level in CD4$^+$ T cells are independent. The number of CD4$^+$ T cells versus relative GSH level are shown for each subject in the different categories. Normal individuals for which cell counts were not available are not included in this figure. The lines indicate the boundaries of 500 CD4$^+$ T cells and the lowest relative GSH level observed in 30 of 31 normal controls. The linear correlation between the two plotted parameters is not significant (r = 0.29). See also Table 3.

vary independently. As shown in Table 3, this independence applies to all patient categories, as well as to the HIV-infected group as a whole.

DISCUSSION

Previous studies have demonstrated GSH deficiencies in plasma, in lung epithelial lining fluid,[9] and in bulk extracts of PBMC[8] from HIV-infected individuals. Our findings are consistent with these decreases; however, the more detailed analysis that we present demonstrates that some PBMC subsets are substantially more affected than others. Specifically, we demonstrate significant decreases in CD4$^+$ and CD8$^+$ T-cell GSH levels in HIV-infected individuals and show further that these decreases are largely explained by the selective depletion of cells with high intracellular GSH levels from these subsets.

The mechanism(s) underlying this selective depletion are not clear at present. In fact, we do not know whether the high GSH cells represent distinct subsets of CD4$^+$ and CD8$^+$ T cells that are depleted in HIV-infected individuals, or whether cells with high intracellular GSH levels merely lose some of their GSH and become indistinguishable from their low GSH counterparts. Nevertheless, the loss of the high GSH cells, which is detectable even in asymptomatic patients with nearly normal numbers of CD4$^+$ T cells, suggests that this loss represents a significant aspect of the disease.

The decrease in the high GSH T cells in both the CD4$^+$ and CD8$^+$ subsets is not likely to be due to infection of these cells, since HIV only infects CD4$^+$ cells and only a small fraction of these are infected. Thus we suspect that HIV infection or related opportunistic infections induces the production of cytokines that

TABLE 3. PROBABILITIES FOR LOW GSH LEVEL AND LOW CD4⁺ T-CELL NUMBER

	All HIV⁺ (n = 134)	Asymp (n = 31)	ARC (n = 32)	AIDS (n = 71)
P1 (low for intracellular GSH)	0.88	0.88	0.91	0.86
P2 (low for CD4⁺ number)	0.85	0.64	0.72	0.98
P3 (low for both)	0.73	0.56	0.63	0.85
P1 × P2 (expected)	0.74	0.56	0.65	0.85

Low GSH level is defined as having a GSH level lower than the lowest GSH level we observed in 30 of 31 uninfected controls (88% of the average level for all normal controls). Low CD4⁺ T cell number is defined as having a CD4⁺ T cell count of less than 500/μl. The 'expected' row shows the calculated product of the probabilities for the above parameters. There is essentially no difference between the expected (P1 × P2) and the observed (P3) probability for having both low GSH levels and low CD4⁺ T cell counts, indicating that these parameters are independent.

either selectively deplete the GSH from the high GSH cells or selectively deplete these cells from circulation.

HIV-infected individuals, particularly those at the later stages of disease, have been reported to have elevated serum levels of tumor necrosis factor alpha (TNF-α) and other inflammatory cytokines.[13-17] These cytokines, which stimulate HIV expression in a variety of cell types via a GSH/oxidants-regulated pathway[1,2] are known to induce the production of reactive oxidants in cells.[18-20] GSH, which reduces (and thereby detoxifies) intracellular oxidants, is consumed by this process. However, to explain the selective loss of the high GSH cells, such cells would have to be selectively sensitive to stimulation by one or more of the cytokines. There is no evidence arguing either for or against this hypothesis at present.

Whatever the mechanism of the depletion of high GSH T cells following HIV infection, the decreased frequency of these cells could have severe consequences. The critical need for adequate levels of GSH in lymphocyte function is well established: GSH is important for mixed lymphocyte reactions,[21] T-cell proliferation,[21] T- and B-cell differentiation,[22] cytotoxic T-cell activity,[22] NK activity,[23] and cell protection against oxidants.[24] In view of the requirement of adequate GSH levels for proper lymphocyte function, it is possible that a decrease in the levels of this metabolite may contribute to the immunodeficiency seen in the later stages of HIV infection.

The alterations in GSH content of T cells during HIV infection that we report may have implications regarding future therapy for HIV disease. We have suggested that GSH levels could potentially be restored by oral administration of N-acetylcysteine (NAC),[1] a well-known, nontoxic drug used clinically to replenish GSH in acetaminophen overdose.[25-27] OTC (L-2-oxothiazolidine-4-carboxylic acid),[28] GSH-esters,[29,30] and GSH itself,[31] also could be used to restore GSH levels. Clinical trials, underway with NAC and starting with OTC soon, will answer whether thiol-replacement therapy (with these drugs) is effective in treatment of AIDS.

In addition to implications for therapy, the decreased GSH levels in T-cell subsets of HIV-infected individuals may provide a valuable marker for prognosis and staging of HIV infection and/or for monitoring experimental therapies. Although therapeutic effectiveness does not necessarily mean restoration of GSH levels, the significant differences that we have demonstrated between the infected and noninfected groups clearly provide a potential basis for distinguishing effective from ineffective therapies. The question of whether GSH levels can be used to monitor disease progression in individuals, in contrast, is still an open question. The largely cross-sectional study we report here was not designed to address this point. (The small longitudinal study that we completed only tested the stability of the GSH levels in selected patients whose clinical condition and therapy profile did not change during the period of observation.) Thus a full longitudinal study is still in order.

In any event, there is good reason to believe that the decreased intracellular GSH levels we have described here have profound physiological consequences and play a role in the progression of HIV infection and the development of the immunodeficiency.

ACKNOWLEDGMENTS

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