Markedly Disturbed Glutathione Redox Status in CD45RA+CD4+ Lymphocytes in Human Immunodeficiency Virus Type 1 Infection Is Associated With Selective Depletion of This Lymphocyte Subset

By Pål Aukrust, Asbjørn M. Svardal, Fredrik Müller, Bodil Lunden, Ingvild Nordøy, and Stig S. Froland

We investigated the percentage of CD45RA+ and CD45RO+ T cells in peripheral blood and the intracellular glutathione redox balance in these lymphocyte subsets in patients with human immunodeficiency virus type 1 (HIV-1) infection and healthy controls. In HIV-1-infected patients there was a preferential depletion of CD45RA+CD4+ cells, which was most pronounced in symptomatic patients. In CD4+ lymphocytes from HIV-1-infected patients the glutathione abnormalities were clearly most pronounced in the CD45RA+ subset with a marked increase in level of oxidized glutathione and decreased ratio of reduced to total glutathione as the major characteristics. These abnormalities were shown in CD45RA+ CD4+ lymphocytes from both symptomatic and asymptomatic patients, whereas similar abnormalities in CD45RO+CD4+ cells were found only in symptomatic patients. The glutathione abnormalities in CD45RA+CD4+ lymphocytes were significantly correlated with low numbers of total CD4+ lymphocytes, decreased proportion of CD45RA+CD4+ lymphocytes, and raised serum levels of tumor necrosis factor-α. In the CD8+ lymphocytes a decrease in both proportion and absolute numbers of CD45RA+ cells was found, with markedly increased level of oxidized glutathione and decreased ratio of reduced to total glutathione in this subset. These findings suggest that glutathione redox disturbances in CD45RA+ T cells may be of pathogenic importance for the preferential depletion of this subset considered to represent naive T cells, during HIV-1 infection.

© 1996 by The American Society of Hematology.

THE CONTINUOUS production of pro-oxidants during cell metabolism is normally balanced by their consumption by antioxidants.1 Oxidative stress results from imbalance between these processes resulting in accumulation of pro-oxidants with potential harmful consequences. Glutathione is a cysteine containing tripeptide that is found in eukaryotic cells as the dominant intracellular thiol, and plays a key role in protecting against oxidative stress.2 In addition, intact glutathione metabolism is required for a variety of immune functions including T lymphocyte proliferation, T- and B-lymphocyte differentiation, natural killer cell activation, and lymphocyte-mediated cytotoxicity.3,5

Oxidative stress and glutathione redox disturbances have been suggested to play an important role in the immunopathogenesis of human immunodeficiency virus type 1 (HIV-1) infection.6,7 We have recently shown markedly disturbed intracellular glutathione homeostasis in CD4+ lymphocytes from HIV-1-infected patients, particularly in patients with advanced clinical and immunological disease, with elevated levels of oxidized glutathione as the major feature.8 Peripheral blood (PB) T lymphocytes are divided into two functionally different subsets based on the reciprocal expression of CD45RO and CD45RA antigens.9,10 Responses to recall antigens are confined to T lymphocytes with the CD45RO phenotype.9,11 Moreover, as CD45RA+ cells lose CD45RA expression and concomitantly gain CD45RO expression after in vitro activation, it has been postulated that CD45RA and CD45RO expression define naive and memory cells, respectively.9,11,12 Recent studies have suggested that the decline in CD4+ lymphocyte counts during HIV-1 infection is primarily caused by a decrease in numbers of naive CD4+ lymphocytes.13,14 Because the naive and memory subsets have different functional activities (eg, activation requirements, proliferation, and cytokine secretion patterns),9,15,16 these changes may have important consequences. Furthermore, it is important to establish if the previously demonstrated glutathione abnormalities in CD4+ lymphocytes during HIV-1 infection6,7 merely reflect altered distribution of naive and memory CD4+ lymphocyte subsets. The mechanisms causing this preferential decline in naive T cells are unknown. However, as several lines of evidence suggest that disturbed intracellular glutathione redox balance may be of importance for the increased apoptosis in lymphocytes seen during HIV-1 infection,6,8 it is important to investigate if CD45RA+CD4+ lymphocytes differ from CD45RO+CD4+ lymphocytes with respect to intracellular glutathione redox balance in HIV-1–infected individuals. Therefore, in the present study we analyzed the percentage of CD45RA+ and CD45RO+ T cells in PB and the intracellular glutathione redox balance in these lymphocyte subsets in HIV-1–infected patients and healthy controls.

MATERIALS AND METHODS

Patients and controls. Blood samples were obtained from 14 HIV-1–infected patients (11 homosexual men, 2 women with heterosexual transmission, and 1 hemophiliac). Patients were clinically classified according to the revised criteria from Centers for Disease Control and Prevention.14 Clinical and immunological characteristics of the study group are summarized (see Table 1). Patients with ongoing acute infection at the time of blood collection (2 months before or 1 week after), intravenous drug users, patients abusing alcohol, and patients with serum levels of alanine aminotransferase > 40 U/L or serum creatinine levels > 70 mmol/L were not included in the study. Six patients were receiving zidovudine (median dosage 600 mg/d, range 300 to 900 mg/d) and six patients were receiving Pneumocystis carinii prophylaxis at the time of the study. However, none of these patients had initiated therapy or changed dosage regime during the last 6 months.

Blood, Vol 88, No 7 (October 1) 1996: pp 2626-2633
GLUTATHIONE REDOX STATUS IN HIV INFECTION

Table 1. Clinical and Immunological Characteristics of the Study Group

<table>
<thead>
<tr>
<th>HIV-1–Infected Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>14</td>
</tr>
<tr>
<td>Age in yr, median</td>
<td>38-40</td>
</tr>
<tr>
<td>ranges</td>
<td></td>
</tr>
<tr>
<td>Males/females</td>
<td>12 (86%)/2 (14%)</td>
</tr>
<tr>
<td>CD4⁺ lymphocytes (×10⁹/L)</td>
<td>220 (150-310)</td>
</tr>
<tr>
<td>CD8⁺ lymphocytes (×10⁹/L)</td>
<td>680* (450-1000)</td>
</tr>
<tr>
<td>TNF-α (pg/mL) levels in serum</td>
<td>22 (20-53)</td>
</tr>
</tbody>
</table>

Six patients were classified as asymptomatic (CDG group A) and eight as symptomatic (six in CDC group B and two in CDC group C) HIV-1–infected patients. Data are given as medians and 25th to 75th percentiles if not otherwise stated.

* P < .01 compared with controls.
† P < .001 compared with controls.

Controls were 14 sex and age-matched healthy, volunteer, unpaid, HIV-1-seronegative blood donors (Table 1). The percentage of smokers was equal in the patient and the control group. Informed consent was obtained from all participants in the study.

Isolation of CD45RA⁺ and CD45RO⁺ lymphocyte subsets. PB mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque-Ficoll (Lymphoprep, Nycomed Pharma AS, Oslo, Norway) gradient centrifugation within 30 minutes after blood sampling. Mononuclear cells were washed twice in Hank’s balanced salt solution (HBSS; Gibco, Paisley, UK) and finally resuspended in phosphate-buffered saline (PBS) with 0.3% bovine serum albumin (BSA; Calbiochem, La Jolla, CA) at a concentration of 15 × 10⁹ PBMC/mL. Further positive selection of cell subsets by monodisperse immunomagnetic beads was done at 4°C as previously described.²³°²¹ Briefly, PBMC were mixed with beads coated with anti-CD4 antibodies (Dynabeads M-450 CD4; Dynal, Oslo, Norway) or anti-CD8 antibodies (Dynabeads M-450 CD8, Dynal) in a cell-to-bead ratio of 1:20 with anti-CD45RO (clone UCHL1, Pharmingen, San Diego, CA) coated magnetic beads. The mixture was incubated in a test tube on a rocking platform for 30 minutes and rosetting cells were isolated by application of a samarium cobalt magnet (Dynal) to the side of the test tube. After five consecutive washes in cold PBS/0.3% BSA, the isolated CD4⁺ lymphocytes (or CD8⁺ lymphocytes) were detached from beads by incubation with goat antimouse Fab antisera (DetachAbead, CD4/CD8, Dynal) for 1 hour in room temperature. The isolated cells were then divided in two portions, placed at 4°C, and mixed for 30 minutes in a cell-to-bead ratio of 1:20 with anti-CD45RA (clone L48, Becton Dickinson, San Jose, CA) and anti-CD45RO (clone UCHL1, Pharmingen, San Diego, CA) coated beads, respectively. The beads were precleaned as previously described.²¹ The positively selected cells were then washed five times in cold PBS/0.3% BSA, and the cell pellets were immediately placed in liquid nitrogen. The purity of the obtained cell populations was >96% as assessed by staining of cytosin preparations of positively selected cells by the alkaline phosphatase anti-alkaline phosphatase method²² using anti-CD4 (clone SK 3, Becton Dickinson), anti-CD8 (clone SK 1, Becton Dickinson), anti-CD45RA (clone L48, Becton Dickinson), and anti-CD45RO (clone UCHL1) monoclonal antibodies.

For negative selection of CD45RA⁺CD4⁺ lymphocytes PBMC were mixed with magnetic beads coated with anti-CD8 antibodies, anti-CD14 antibodies (Dynabeads M-450 CD14, Dynal), anti-CD19 antibodies (Dynabeads M-450 Pan-B, Dynal), anti-CD56 antibodies (clone ERIC-1, Southern Biotechnology, Birmingham, AL; bound to Dynabeads precoated with rat antimouse IgG1) and anti-CD45RO antibodies in a cell-to-bead ratio of 1:20 and placed on a rocking platform at 4°C for 60 minutes. After removing of rosetting cells by application of the magnet, nonrosetting cells were washed twice in cold PBS/0.3% BSA and immediately placed in liquid nitrogen. The purity of the negatively selected CD45RA⁺CD4⁺ cells was >91% as assessed by staining of cytosin preparations as previously described.

**Determination of intracellular glutathione levels.** After a median storage time in liquid nitrogen of 10 weeks (range 3 to 18 weeks) glutathione analysis was performed as previously described.²¹²³ Briefly, before thawing 0.3 mL ice-cold 5% sulfosalicylic acid (Merck, Darmstadt, Germany) containing 50 mmol/L dithioerythritol (Sigma, St Louis, MO) was added to the cell pellets to prevent in vitro oxidation of thiol groups.²² After thawing the precipitated protein and the immunomagnetic beads were immediately removed by centrifugation. The time from starting of thawing to removing of acid extract was less than 15 minutes. Storing of cells with immunomagnetic beads did not influence the intracellular glutathione levels (data not shown). Total free glutathione (reduced glutathione + glutathione disulfide + soluble glutathione mixed disulfide; for simplicity referred to as total glutathione level in the text) and reduced glutathione were determined in the acid extract according to a modification of a chromatographic procedure described previously.²³ The fraction of oxidized glutathione (glutathione disulfide + soluble glutathione mixed disulfide) was calculated by subtracting the amount of reduced from the total amount of glutathione. Measurement of intracellular glutathione levels was routinely performed on blinded samples in duplicates.

**Flow cytometry.** PBMC were examined by three-color immunophenotyping using peridinin chlorophyll protein (PerCP)-conjugated anti-CD4 (Leu-3a, Becton Dickinson) or anti-CD8 (Leu-2a, Becton Dickinson) in combination with phycoerythrin (PE)-conjugated anti-CD45RO (clone UCHL1, Pharmingen) and fluorescein isothiocyanate (FITC)-conjugated anti-CD45RA (clone L48, Becton Dickinson). Samples were fixed with 1% paraformaldehyde and analyzed using a FACScan flow cytometer (Becton Dickinson). All samples included staining with isotype matched control antibodies. Data were acquired with CellQuest software (Becton Dickinson), and list mode files were collected for 25,000 cells from each sample. Forward and side scatter were used to gate lymphocyte-sized cells, and the numbers of CD4⁺ and CD8⁺ lymphocytes in PB were determined by immunomagnetic quantification which has been shown to agree well with flow cytometry.²²²¹

**Measurement of tumor necrosis factor-α (TNF-α) and lymphocyte subsets counts.** TNF-α concentrations in serum were quantified by enzyme immunoassay (Medgenix, Fleurus, Belgium) as previously described.²¹ The numbers of CD4⁺ and CD8⁺ lymphocytes in PB were determined by immunomagnetic quantification which has been shown to agree well with flow cytometry.²²²¹

**Statistical analysis.** For comparison of two groups of individuals, the two-tailed Mann-Whitney U test was used. When more than two groups were compared, the Kruskal-Wallis test was used. If a significant difference was found, Fisher’s least significant difference was computed on the ranks to determine the differences between each pair of group. Coefficients of correlation (r) were calculated by the Spearman Rank Test. The calculations were performed using the Statistica (StatSoft, Tulsa, OK) software package. Data are given as medians and 25th to 75th percentiles if not otherwise stated. P values are two-sided and considered significant when <.05.

**RESULTS**

Distribution of CD45RA⁺ and CD45RO⁺CD4⁺ lymphocyte subsets in HIV-1–infected patients and in healthy con-
trols. As shown in Table 2, flow cytometry analyses showed that HIV-1-infected patients were characterized by a significant decrease in proportion of CD45RA+CD4+ lymphocytes in PB compared with healthy controls. This decrease in proportion of CD45RA+CD4+ lymphocytes was most pronounced in advanced clinical disease (8.2% [7.0 to 14.2%] v 21.2% [16.4 to 29.8%], \( P < .01 \); symptomatic and asymptomatic HIV-1-infected patients, respectively). Furthermore, the decrease in proportion of CD45RA+CD4+ lymphocytes was significantly higher among symptomatic patients compared with healthy controls (data not shown). Similar to positively selected cells, no significant differences were found between CD45RA+CD4+ lymphocytes from asymptomatic HIV-1-infected patients and healthy controls in levels of either reduced or total glutathione (data not shown).

In 4 asymptomatic HIV-1-infected patients and 4 healthy controls the glutathione redox status was also analyzed in CD45RA+CD4+ lymphocytes isolated by negative selection. Also in negatively selected CD45RA+CD4+ lymphocytes we found that asymptomatic HIV-1-infected patients had significantly higher levels of oxidized glutathione and significantly decreased ratio of reduced to total glutathione than healthy controls (data not shown). Similar to positively selected cells, no significant differences were found between CD45RA+CD4+ lymphocytes from asymptomatic HIV-1-infected patients and healthy controls in levels of either reduced or total glutathione (data not shown).

As can be seen from Figs 1 and 3, among healthy controls intracellular levels of both total and reduced glutathione were markedly higher in CD45RA+ than in CD45RO+CD4+ lymphocytes (~120% \( P < .001 \) and ~100% \( P < .001 \) increase, reduced and total glutathione, respectively). Furthermore, although levels of oxidized glutathione were low in both CD45RO+ and CD45RA+CD4+ lymphocytes from healthy controls, the level of this glutathione species was ~100% higher \( ( P < .005 ) \) in the CD45RO+ than in the CD45RA+ subset (Figs 1 and 3). In contrast to healthy controls, we could not show any significant differences in these glutathione parameters between CD45RA+ and CD45RO+CD4+ lymphocytes from HIV-1-infected patients (Figs 1 and 3).

**Table 2. Distribution of CD45RA+ and CD45RO+ Subsets Within the CD4+ and CD8+ Lymphocyte Subpopulations in Patients With HIV-1 Infection and Healthy Controls**

<table>
<thead>
<tr>
<th>CD4+ Lymphocytes</th>
<th>HIV-1-Sero-positive Patients</th>
<th>Controls</th>
<th>HIV-1-Sero-positive Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( 14 )</td>
<td>( 14 )</td>
<td>( 6 )</td>
<td>( 6 )</td>
</tr>
<tr>
<td>% CD45RA*</td>
<td>15.0* (7.7-24.2)</td>
<td>47.5 (36.1-53.8)</td>
<td>35.5 (26.6-59.8)</td>
<td>50.1 (41.8-70.3)</td>
</tr>
<tr>
<td>CD45RA* (x10(^6)L)</td>
<td>25* (10-110)</td>
<td>300 (265-330)</td>
<td>180 (135-200)</td>
<td>220 (190-240)</td>
</tr>
<tr>
<td>% CD45RO*</td>
<td>77.81 (60.9-87.0)</td>
<td>48.5 (45.3-59.0)</td>
<td>71.7 (60.7-80.0)</td>
<td>55.3 (33.5-62.9)</td>
</tr>
<tr>
<td>CD45RO* (x10(^6)L)</td>
<td>140* (120-220)</td>
<td>330 (300-450)</td>
<td>500 (400-580)</td>
<td>240 (180-320)</td>
</tr>
</tbody>
</table>

Distribution of lymphocyte subsets was analyzed by flow cytometry. Data are given as medians and 25th to 75th percentiles.

* \( P < .001 \) compared with controls.  
\( \dagger \) \( P < .01 \) compared with controls.  
\( \ddagger \) \( P < .05 \) compared with controls.

In intracellular glutathione levels in isolated CD45RA+ and CD45RO+CD4+ lymphocyte subsets. When analyzing the glutathione redox status in these CD4+ lymphocyte subpopulations, we could show that the most pronounced abnormalities in HIV-1-infected individuals were found in the CD45RA+ subset. This lymphocyte subpopulation was characterized by a significant decrease in levels of reduced glutathione (~40% decrease in symptomatic patients) and in particular, a marked increase in levels of oxidized glutathione (~1,300% increase in symptomatic patients) as well as a substantial decrease in ratio of reduced to total glutathione (~35% decrease in symptomatic patients) (Fig 1). Although these abnormalities were most pronounced among symptomatic HIV-1-infected patients, also asymptomatic patients had significant changes compared with healthy controls (Fig 1). As can be seen in Fig 2, these glutathione abnormalities in CD45RA+CD4+ lymphocytes were most pronounced in those HIV-1-infected patients with the lowest proportion of CD45RA+CD4+ lymphocytes in PB.

In isolated CD45RO+CD4+ lymphocytes from symptomatic HIV-1-infected patients we found significantly raised levels of oxidized glutathione (~100% increase) and significantly decreased ratio of reduced to total glutathione (~15% decrease) (Fig 3). However, these abnormalities were not as pronounced as those found in CD45RA+CD4+ lymphocytes (Figs 1 and 3). Furthermore, we could not show any significant abnormalities in levels of reduced glutathione in the CD45RO+CD4+ lymphocytes among HIV-1-infected patients (Fig 3). Finally, glutathione abnormalities in CD45RO+CD4+ lymphocytes were only demonstrated in symptomatic and not in asymptomatic HIV-1-infected patients (Fig 3).
GLUTATHIONE REDOX STATUS IN HIV INFECTION

Fig 1. Intracellular levels of reduced glutathione (A), total glutathione (B), ratio of reduced to total glutathione (C), and oxidized glutathione (D) in isolated CD45RA^CD4^ lymphocytes from 5 patients with asymptomatic HIV-1 infection, 8 patients with symptomatic HIV-1 infection, and 12 healthy controls. Bars represent median values.

Fig 2. Correlation between percentage of CD45RA^- cells of total CD4^ cells and intracellular levels of oxidized glutathione in CD45RA^-CD4^- lymphocytes in 13 HIV-1-infected patients. The levels of oxidized glutathione in CD45RA^-CD4^- lymphocytes were also significantly inversely correlated with the absolute numbers of this lymphocyte subset (r = -.73, P < .005). Also decreased ratio of reduced to total glutathione, but not decreased levels of reduced glutathione, in CD45RA^-CD4^- lymphocytes were significantly correlated with decreased proportion as well as decreased absolute numbers of CD45RA^-CD4^- lymphocytes (data not shown).
were significantly correlated with CD4+ lymphocyte counts or TNF-α levels (data not shown).

Thus, the glutathione abnormalities in CD45RA+CD4+ lymphocytes in HIV-1 infection are not only associated with advanced clinical disease, but also significantly correlated with decreased numbers of CD4+ lymphocytes in PB and increased TNF-α activation reflecting advanced immunodeficiency.

**Intracellular glutathione levels in isolated CD45RA+ and CD45RO+CD8+ lymphocyte subsets.** In a recent study we could not show any significant abnormalities in glutathione metabolism in CD8+ lymphocytes from HIV-1–infected patients except for a slight increase in ratio of reduced to total glutathione.8 To elucidate if glutathione abnormalities in CD8+ lymphocytes during HIV-1 infection are masked by an altered distribution of naive and memory CD8+ lymphocyte subsets, we analyzed the proportion of CD45RA+ and CD45RO+CD8+ lymphocytes in PB and the intracellular glutathione ratios in these CD8+ lymphocyte subsets in 6 HIV-1–infected patients (4 asymptomatic and 2 symptomatic patients, CDC group B) and in 6 healthy controls.

As can be seen in Table 2, although the HIV-1–infected patients had significantly raised numbers of CD8+ lymphocytes in PB (Table 1), this rise was only found in the CD45RO+ subset. In fact, there was a decline rather than a rise, in both proportion and absolute numbers of CD45RA+CD8+ lymphocytes among HIV-1–infected patients, reflecting altered distribution of CD45RA+ and CD45RO+ subsets among CD8+ lymphocytes in HIV-1–infection.

When analyzing the glutathione redox status in isolated CD45RA+ and CD45RO+CD8+ subsets we found a significant decrease in ratio of reduced to total glutathione (20% decrease) and in particular, a marked increase in level of oxidized glutathione (400% increase) in the CD45RA+ subset in HIV-1–infected patients (Table 3). In fact, all HIV-1–seropositive patients had higher levels of oxidized glutathione in CD45RA+CD8+ lymphocytes than all controls. In contrast, no significant differences between HIV-1–infected patients and controls were found in the CD45RO+ subset (Table 3). Furthermore, as can be seen from Table 3, in healthy controls the CD45RO+ subset had significantly higher levels of oxidized glutathione than the CD45RA+ subset (200% increase, P < .05). In healthy controls the CD45RO+ subset also tended to have lower ratio of reduced to total glutathione than the CD45RA+ subset (Table 3).
GLUTATHIONE REDOX STATUS IN HIV INFECTION

Table 3. Intracellular Glutathione Levels in Isolated CD45RA+ and CD45ROCD8+ Lymphocytes in 6 HIV-1-Infected Patients and 6 Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>HIV-1-Infected Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45RA+CD8+ lymphocytes (nmol/10⁶ cells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>0.724 (0.679-1.187)</td>
<td>0.661 (0.600-0.672)</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>0.927 (0.684-1.160)</td>
<td>0.725 (0.647-0.751)</td>
</tr>
<tr>
<td>Oxidized glutathione</td>
<td>0.2031 (0.111-0.378)</td>
<td>0.053 (0.034-0.068)</td>
</tr>
<tr>
<td>Ratio of reduced to total glutathione</td>
<td>0.770* (0.758-0.845)</td>
<td>0.956 (0.889-0.972)</td>
</tr>
<tr>
<td>CD45ROCD8+ lymphocytes (nmol/10⁶/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>0.875 (0.785-1.091)</td>
<td>0.847 (0.423-1.109)</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>1.051 (0.624-1.291)</td>
<td>1.073 (0.705-1.189)</td>
</tr>
<tr>
<td>Oxidized glutathione</td>
<td>0.144 (0.051-0.201)</td>
<td>0.141 (0.081-0.221)</td>
</tr>
<tr>
<td>Ratio of reduced to total glutathione</td>
<td>0.871 (0.851-0.920)</td>
<td>0.852 (0.791-0.932)</td>
</tr>
</tbody>
</table>

Data are given as medians and 25th to 75th percentiles. * P < .01 versus controls.
† P < .005 versus controls.

DISCUSSION

The present study clearly shows that the glutathione redox disturbances in CD4+ lymphocytes seen in HIV-1 infection are most pronounced in CD45RA+CD4+ lymphocytes as distinct from memory CD4+ lymphocytes. Furthermore, the significant correlation between the glutathione abnormalities in this naive CD4+ lymphocyte subpopulation and the decrease in absolute numbers as well as proportion of circulating CD45RA+CD4+ lymphocytes might suggest that dysregulated intracellular glutathione homeostasis may be implicated in the preferential depletion of naive CD4+ lymphocytes in HIV-1-infected patients. Finally, although we in a recent study could not find any glutathione abnormalities in isolated CD8+ lymphocytes from HIV-1-infected patients, the present study shows that purified CD45RA+CD8+ lymphocytes from these patients are characterized by important glutathione abnormalities.

Although it has been claimed that the use of other surface markers in addition to CD45RA and CD45RO are necessary to precisely delineate naive and memory T cells, and that some memory cells can phenotypically masquerade as naive cells, it appears established that the T-cell memory resides within the CD45RO+ subset and that the naive T cells reside within the CD45RA+ subset.

The results of the present study indicate in accordance with other recent reports, that naive CD4+ as well as naive CD8+ lymphocytes are preferentially lost as total CD4+ lymphocyte counts decline during HIV-1 infection. However, discrepant results have been reported possibly reflecting methodological differences and differences in the degree of immunodeficiency among patient groups studied. In fact, it appears as found in the present study, that the preferential loss of CD45RA+ T cells is most prominent in advanced clinical and immunological disease. Nevertheless, a loss of naive T lymphocytes in HIV-1 infection may have important immunological consequences including inability to mount responses to novel antigens, eg, opportunistic pathogens as well as the continuously mutating HIV-1 itself. Furthermore, as naive and memory T lymphocytes appear to differ in their potential for cytokine synthesis, altered distribution of naive and memory T lymphocytes may be involved in the dysregulated cytokine production observed in HIV-1-infected individuals. Interestingly, it has been reported that naive CD8+ lymphocytes are potent sources of macrophage inflammatory protein 1α and RANTES, and these chemokines have very recently been shown to suppress HIV replication in vitro, possibly reflecting the "soluble anti-HIV factor(s)" derived from CD8+ lymphocytes.

We have previously shown increased levels of oxidized glutathione and decreased ratio of reduced to total glutathione as the major intracellular glutathione abnormalities in CD4+ lymphocytes during HIV-1 infection. The present study clearly shows that these previous findings do not merely reflect altered distribution of naive and memory subsets in CD4+ lymphocytes from HIV-1-infected individuals. On the contrary, although glutathione abnormalities were present in both CD45RO+ and CD45RA+CD4+ lymphocytes, they were clearly most pronounced in the CD45RA+CD4+ lymphocyte subpopulation of which a decreased proportion was found in HIV-1-infected patients. Furthermore, although abnormalities in CD45RO+CD4+ lymphocytes were only shown in advanced clinical disease, significant abnormalities were found in CD45RA+CD4+ lymphocytes from both asymptomatic and symptomatic HIV-1-infected patients.

In contrast, our previous report of isolated CD8+ lymphocytes from HIV-1-seropositive patients showing a normal glutathione redox status except for a slight increase in ratio of reduced to total glutathione, seems partly to reflect altered distribution of naive and memory CD8+ lymphocytes. In fact, although there was an increase in the CD45RO+ subpopulation comprising normal glutathione redox status, there was a decrease in proportion of CD45RA+CD8+ lymphocytes with raised levels of oxidized glutathione and decreased ratio of reduced to total glutathione in HIV-1-infected patients. These findings underscore the importance of examining isolated naive and memory T cells in addition to CD4+ and CD8+ lymphocytes when studying T cells in HIV-1 infection.

The precise mechanisms leading to CD4+ lymphocyte depletion in general and in particular, to the preferential depletion of CD45RA+ T cells in HIV-1 infection are still unknown. A loss of thymopoietic capacity has been sug-
gested as an important factor.5,13,41 Our present findings of marked glutathione redox abnormalities in CD45RA+CD4+ lymphocytes significantly correlated with decreased numbers of CD45RA+CD4+ lymphocytes in PB and increased TNF-α activity may also represent important, pathogenic factors in this preferential depletion of naive T lymphocytes during HIV-1 infection. Several lines of evidence suggest that disturbed intracellular glutathione redox homeostasis might be involved in the increased apoptosis of T lymphocytes during HIV-1 infection, particularly when associated with increased TNF-α activity.5,18,42,45 However, caution should be used when drawing conclusions from correlation analyses, and other mechanisms leading to depletion of naive T cells may also be involved in HIV-1-infected individuals. For example, recent in vitro studies have suggested that Fas antigen stimulation is of importance for the increased apoptosis of T lymphocytes during HIV-1 infection.43 Although this mechanism may also possibly be influenced by intracellular glutathione redox status, the significance of intracellular redox status on Fas-mediated apoptosis has recently been questioned.45

The present study also shows that in healthy controls the CD45RO+CD4+ lymphocytes have markedly lower levels of both total and reduced glutathione and also increased levels of oxidized glutathione, than the CD45RA+CD4+ lymphocytes as we have previously suggested.21 These findings, demonstrating that the intracellular glutathione levels in healthy individuals seem to be markedly different in CD45RA+, compared with CD45RO+CD4+ lymphocytes, extend our knowledge of potentially important differences between human CD45RO+ and CD45RA+ lymphocytes. Furthermore, the altered glutathione redox status in CD45RO+, compared with CD45RA+CD4+ lymphocytes, together with the recent demonstration of decreased Bcl-2 expression in these memory cells,15 might be of importance for the increased susceptibility to apoptosis normally found in human memory T lymphocytes.13

A preferential loss of CD45RA+CD4+ lymphocytes in PB may not be restricted to HIV-1 infection. For example, we and others have shown this phenomenon in patients with common variable immunodeficiency (CVI).21,46 If in fact, there are several immunological similarities between HIV-1-infected patients and subgroups of CVI patients including decreased numbers of CD4+ lymphocytes and increased numbers of CD8+ lymphocytes in PB and persistent immune activation in vivo.21,46-49 Interestingly, we have recently found that CD45RA+CD4+ lymphocytes from CVI patients are also characterized by marked glutathione abnormalities, and these glutathione redox disturbances were correlated with increased TNF-α activity.5 Thus, the mechanisms leading to altered glutathione homeostasis in naive T cells in HIV-1-infected individuals may not be unique for this infection, but may possibly reflect a state of persistent immune activation, particularly in the TNF system, also occurring in other clinical disorders.

In conclusion, the markedly disturbed glutathione homeostasis in naive T cells in HIV-1-infected patients may have profound consequences contributing to both the progression of immunodeficiency and clinical manifestations in these patients. The results of the present study represent further justification for attempts at therapeutical intervention with drugs with effect on the disturbed glutathione metabolism in HIV-1-infected patients.

ACKNOWLEDGMENT

We thank Audun Haylandskjær and Lisbeth Wikeby for excellent technical assistance.

REFERENCES

17. Roederer M, Staal FJT, Osada H, Herzenberg LA, Herzenberg LA: CD4 and CD8 T cells with high intracellular glutathione levels are selectively lost as the HIV infection progresses. Int Immunol 3:933, 1991
GLUTATHIONE REDOX STATUS IN HIV INFECTION


42. Mayer M, Noble M: N-acetyl-L-cysteine is a pluripotent protector against cell death and enhancer of trophic factor-mediated cell survival. Proc Natl Acad Sci USA 91:7496, 1994


Markedly disturbed glutathione redox status in CD45RA+CD4+ lymphocytes in human immunodeficiency virus type 1 infection is associated with selective depletion of this lymphocyte subset

P Aukrust, AM Svardal, F Muller, B Lunden, I Nordoy and SS Froland