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**Impaired base excision repair and accumulation of oxidative base lesions in CD4<sup>+</sup> T cells of HIV-infected patients**

**Running head: Aukrust et al. Repair of oxidative DNA damage in HIV infection**

Pål Aukrust<sup>1,2</sup>, Luisa Luna<sup>4,5</sup>, Thor Ueland<sup>2,3</sup>, Rune F. Johansen<sup>4,5</sup>, Fredrik Müller<sup>2</sup>, Stig S. Frøland<sup>1,2</sup>, Erling C. Seeberg<sup>4,5</sup>, Magnar Bjørås<sup>4,5</sup>

<sup>1</sup>Section of Clinical Immunology and Infectious Diseases, <sup>2</sup>Research Institute for Internal Medicine, <sup>3</sup>Section of Endocrinology, Medical Department, <sup>4</sup>Department of Molecular Biology, Institute of Medical Microbiology, <sup>5</sup>Centre for Molecular Biology and Neuroscience, University of Oslo, The National Hospital, N-0027, Oslo, Norway.

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**Corresponding authors:**

Pål Aukrust, Section of Clinical Immunology and Infectious Diseases, Medical Department, Rikshospitalet, N-0027 Oslo, Norway.

Phone: 47-23070000, Fax: 47-23073630, e-mail: [pal.aukrust@rikshospitlet.no](mailto:pal.aukrust@rikshospitlet.no).

Magnar Bjørås, Department of Molecular Biology, Institute of Medical Microbiology, University of Oslo, The National Hospital, N-0027, Oslo, Norway.

Phone: 47-23074060, Fax: 47-23074061, e-mail: [magnar.bjoras@medisin.uio.no](mailto:magnar.bjoras@medisin.uio.no)

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## **Abstract**

Several studies have reported enhanced oxidative stress in HIV infection. An important pathophysiological consequence of increased oxidative stress is endogenous DNA damage, and the base excision repair pathway is the most important mechanism to withstand such deleterious effects. To investigate the role of base excision repair in HIV infection we examined 8-oxoguanine (8-oxoG) levels, as a marker of oxidative DNA damage, and DNA glycosylase activities in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from HIV-infected patients and controls. These results showed that HIV-infected patients, particularly those with advanced disease, were characterized by increased accumulation of 8oxoG in CD4<sup>+</sup> T cells accompanied by a marked decline in DNA glycosylase activity for repair of oxidative base lesions in these cells. In contrast, CD8<sup>+</sup> T cells from HIV-infected patients, with 8-oxoG levels similar to those in healthy controls, showed enhanced capacity to repair oxidative DNA damage. Finally, highly active antiretroviral therapy induced increased glycosylase activity in CD4<sup>+</sup> T cells accompanied by normalization of 8-oxoG levels. This imbalance between accumulation of oxidative DNA damage and the capacity to repair such lesions in CD4<sup>+</sup> T cells may represent a previous unrecognized mechanism involved in the numerical and functional impairment of CD4<sup>+</sup> T cells in HIV infection.

## **Introduction**

Highly reactive oxygen species (ROS) are formed as by-products during a variety of biochemical reactions and this steady-state formation of pro-oxidants are normally balanced by a similar rate of consumption by antioxidants. Oxidative stress results from imbalance between formation and neutralization of pro-oxidants resulting in accumulation of pro-oxidants with potential harmful consequences.<sup>1</sup> Enhanced oxidative stress has been implicated in the pathogenesis of several clinical disorders such as heart and brain ischemic diseases, neurodegenerative disorders, various autoimmune diseases and seems to be involved in carcinogenesis and aging.<sup>1-5</sup> Several lines of evidence suggest that enhanced oxidative stress also plays a pathogenic role in human immunodeficiency virus (HIV) infection. Thus, a number of reports have found impaired antioxidant defence, particularly manifested by disturbed glutathione metabolism in HIV-infected patients.<sup>5-9</sup> Moreover, enhanced oxidative stress may be involved in the pathogenesis of impaired T cell responsiveness and enhanced T cell apoptosis during HIV infection, and may also play a role in the development of certain HIV-related clinical disorders such as malignancies and HIV-related encephalopathy.<sup>5,10,11</sup>

An important pathophysiological consequence of increased intracellular oxidative stress is endogenous DNA damage.<sup>12</sup> Several types of oxidative DNA lesions have been reported, including strand breaks, baseless sugars (AP-sites) and oxidized base residues, with 7,8-dihydro-8-oxoguanine (8-oxoG) and 5-hydroxycytosine (5-ohC) representing the most frequent mutagenic base lesions.<sup>13-15</sup> DNA repair mechanisms have evolved specifically to counteract the biological effects of DNA damage. Of particular importance for removal of oxidative damage is the base excision repair pathway, which is initiated by

the action of DNA glycosylases removing different types of modified bases by cleavage of the *N*-glycosylic bond.<sup>16,17</sup> Five DNA glycosylases for removal of oxidative base residues, hNth1, hOgg1, Neil1, Neil2 and Neil3 (hFpg2) have been cloned and characterized in human cells.<sup>18-28</sup> hNth1, Neil1 and Neil2 remove oxidized pyrimidines such as 5-ohC, whereas hOgg1 is removing oxidized purines such as 8-oxoG. However, Neil1 appear to be a backup function for hOgg1 for removal of 8-oxoG. All five enzymes have been shown to remove cytotoxic imidazole-ring fragmented formamidopyrimidine (faPy) residues which represent blocks to replication.

Based on the potential pathogenic role of enhanced oxidative stress in HIV infection we examined 8-oxoG levels, as a marker of oxidative DNA damage, in relation to DNA glycosylase activity for repair of oxidative damage in T cell subsets from HIV-infected patients and healthy controls. We found increased level of 8-oxoG in DNA in CD4<sup>+</sup> T cells from HIV-infected patients, along with a reduced capacity for repair of oxidized base residues in these cells.

## **Materials and methods**

### **Patients and controls**

Fifteen HIV-infected patients were consecutively recruited for the study (Table 1). The patients were clinically classified according to the revised criteria from Center for Disease Control and Prevention (CDC) in AIDS patients (CDC group C, n=7) and non-AIDS patients (CDC group A+B, n=8). Patients with ongoing acute or exacerbation of chronic infection at the time of blood collection were not included. Eleven of the patients received antiretroviral therapy with nucleoside analog(s), but except for one sub-study

(see below), none received highly active antiretroviral therapy (HAART). None had initiated or changed therapy during the last 5 months. None of the patients had abnormal liver or kidney function, were abusing drugs or alcohol or were taking any regular medication except for nucleoside analog(s) as described above. Controls in the study were 13 healthy HIV-seronegative sex- and age-matched healthy blood donors (Table 1). Approval was obtained from the National Hospital in Oslo institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

### **Isolation of cells**

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque-Ficoll (Lymphoprep; Nycomed, Oslo, Norway) gradient centrifugation within 45 minutes, and further positive selection of CD4<sup>+</sup> and CD8<sup>+</sup> T cells was done at 4°C as previously described.<sup>7</sup> Briefly, PBMC were mixed with Dynabeads (Dyna, Oslo, Norway) coated with appropriate antibodies (anti-CD4, Dynabeads M-450 CD4; anti-CD8, Dynabeads M-450 CD8) in a cell-to-bead ratio of 1:10. The mixture was incubated in a test tube on a rocking platform for 30 minutes and rosetting cells isolated by application of a magnet. After five consecutive washes in cold phosphate-buffered saline/bovine serum albumin (Calbiochem, La Jolla, CA), the positively selected cells (cell pellet) were immediately stored in liquid nitrogen. Storage of cells with immunomagnetic beads did not influence glycosylase or 8-oxoG levels. The purity of cell populations was >98% as assessed by staining of cytopsin preparations of positively selected cells by the alkaline phosphatase-antialkaline phosphatase procedure.<sup>7</sup>

### **Preparation of nuclear DNA and analyses of 8-oxoG by high-performance liquid chromatography-electrochemical detection (HPLC-ECD)**

Extraction of DNA and hydrolyses to nucleosides by nucleaseP<sub>1</sub> and alkaline phosphatase were performed as described elsewhere.<sup>29</sup> To reduce oxidation during the preparation of DNA, TEMPO (2,2,6,6-tetramethylpiperidine-*N*-oxyl) was added to all solutions at 100 µM immediately before use. 8-Hydroxy-2'-deoxyguanosine and 2'-deoxyguanosine were separated by HPLC and analyzed by ECD (+300 mV) and UV light (290 nm). Results were expressed as the ratio of 8-oxoG/10<sup>6</sup> bp in each DNA sample.

### **Preparation of cellular extracts**

Cell pellets were resuspended in 25 µl 84 % sucrose/10 mM ethyleneglycol tetraacetic acid. After 10 minutes incubation on ice the cells were frozen and thawed three times with subsequent centrifugation (15000g for 15 minutes) to remove cell debris.

Supernatant was stored at -20°C.

### **Assays for DNA glycosylase activity**

All enzyme activities were assayed in a reaction buffer containing 70 mM morpholinopropanesulfonic acid (MOPS), pH 7.5, 1 mM ethylenediamide tetra-acetic acid (EDTA), 5% glycerol and 1 mM dithiothreitol, and the mixtures were incubated at 37°C for 30 minutes. For analyzing faPy removal *N*-[<sup>3</sup>H]methyl-*N'*-nitrosoourea (18Ci/mmol) was used to prepare poly(dG-dC) DNA containing faPy residues (5000 dpm/µg DNA).<sup>30</sup> FaPy DNA glycosylase activity was measured in a total volume of 50 µl containing 0.4 µg faPy-DNA substrate. For analyzing DNA glycosylase activity for repair of alkylating damage the cell extracts were mixed with 0.3 µg of [methyl <sup>3</sup>H] methylated DNA (containing 5-10 pmol of methylated bases) in a total volume of 50 µl.

### **Assays for cleavage of 8-oxoguanine and 5-hydroxycytosine containing DNA.**

Duplex DNA containing a single 8-oxoG residue at position 10 (5'-ATCACCGGC[8-oxoG]CCACACGAGCTG) opposite C and duplex DNA containing a single 5-OHC residue at position 22 (AATTGCGATCTAGCTCGCCAG [5OHC]AGCGACCTTA TCTGATGA) were purified by 20% non denaturing polyacrylamide gel electrophoresis. For all substrates, the 5' ends were <sup>32</sup>P-labeled by T4 polynucleotide kinase and [ $\gamma$ -<sup>32</sup>P]adenosine triphosphate (3000Ci/mmol; Amersham, Aylesbury, UK). The reaction mixtures contained 50 fmol substrates and protein extracts as indicated in a total volume of 10  $\mu$ l. After incubation at 37°C for 30 minutes, the reaction products were separated on 20 % polyacrylamide/7 M urea denaturing gels (Hydrolink, FMC, AT Biochem, Malvern, PA) with 1xTris-Borate-EDTA and radiolabeled fragments finally visualized by PhosphorImaging [Molecular Dynamics (model 445 SI), Sunnyvale, CA].

### **Miscellaneous**

HIV RNA levels were measured in EDTA plasma by quantitative reverse PCR (Amplicor HIV Monitor, Roche Diagnostic Systems, Branchburg, NY; detection limit 50 copies/mL). Plasma levels of tumor necrosis factor (TNF) $\alpha$  were measured by enzyme immunoassay (BioSource, Camarillo, CA).

### **Statistical analysis**

When comparing three groups of individuals, the Kruskal-Wallis test was used *a priori*. If a significant difference was found, Mann-Whitney *U* test (two-tailed) was used to determine the differences between each pair of groups. For comparisons within the same individuals, the Wilcoxon matched pairs test was used. Coefficients of correlation were

calculated by the Spearman's rank test. If not otherwise quoted, data are given as medians and 25<sup>th</sup>-75<sup>th</sup> percentiles. P-values (two-sided) are considered significant when <0.05.

## **Results**

### **Accumulation of 8-oxoG in the DNA of CD4<sup>+</sup> and CD8<sup>+</sup> T cells from HIV-infected patients**

We have previously reported markedly disturbed intracellular redox balance in CD4<sup>+</sup> T cells from HIV-infected patients.<sup>7</sup> To examine if such disturbances were associated with enhanced oxidative DNA damage, we analysed 8-oxoG levels in genomic DNA of CD4<sup>+</sup> and CD8<sup>+</sup> T cells from 5 AIDS patients, 4 non-AIDS HIV-infected patients and 7 healthy controls. As shown in Figure 1, CD4<sup>+</sup> T cells from HIV-infected patients were characterized by significantly raised 8-oxoG contents, with particularly high levels in AIDS patients (~6-fold increase). In contrast, 8-oxoG levels were similar in CD8<sup>+</sup> T cells from HIV-infected patients and controls (Figure 1). In fact, rather than increased, the 8-oxoG level in the CD8 subset tended to decrease in the non-AIDS patients compared with healthy controls, although the difference did not reach statistical significance (p=0.07, Figure 1). In HIV-infected patients, 8-oxoG levels in CD4<sup>+</sup> T cells were inversely correlated with numbers of HIV RNA copies (r=-0.77, p<0.03) and positively correlated with TNF $\alpha$  (r= 0.70, p<0.05) levels in plasma.

### **DNA glycosylase activity in CD4<sup>+</sup> and CD8<sup>+</sup> T cells during HIV infection**

We next analysed if accumulation of 8-oxoG in CD4<sup>+</sup> T cells was accompanied by any alterations in DNA glycosylase activity for repair of oxidative damage. FaPy is a common substrate for DNA glycosylases involved in repair of oxidative DNA lesions and

removal of faPy was measured in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from 15 HIV-infected patients and 13 healthy controls. Notably, while a slight but significant decrease in removal of faPy was found in CD4<sup>+</sup> T cells from non-AIDS patients, CD4<sup>+</sup> T cells in AIDS patients had profoundly decreased faPy activity reaching only ~20% of activity in healthy controls (Figure 2). In contrast, faPy excision in CD8<sup>+</sup> T cells were either markedly raised (~3.5-fold increase, non-AIDS patients) or similar (AIDS patients) to levels in healthy controls (Figure 2). In HIV-infected patients, the decrease in faPy activity in CD4<sup>+</sup> T cells was inversely correlated with numbers of HIV RNA copies ( $r=-0.53$ ,  $p<0.05$ ) and positively correlated with TNF $\alpha$  ( $r=0.61$ ,  $p<0.01$ ) levels in plasma. In contrast to faPy excision, DNA glycosylase activity for repair of alkylating damage showed no significant differences in these T cell subsets between HIV-infected patients and controls (Figure 2), suggesting a specific down-regulation of DNA glycosylases repairing oxidative DNA damage in HIV-infected patients.

### **Levels of DNA glycosylase activity for repair of 8-oxoG and 5-ohC in T cell subsets during HIV infection**

While the assay for measuring DNA glycosylase activity for repair of alkylating damage is specific for the human Aag DNA glycosylase, the faPy assay may detect activity of five different DNA glycosylases for repair of oxidative damage; hOgg1, hNth1, Neil1, Neil2 and Neil3. We therefore examined removal of 8-oxoG and 5-ohC to further distinguish between these DNA glycosylases. Notably, the removal of 8-oxoG in CD4<sup>+</sup> T cells of AIDS patients showed only ~15% of the activity in CD4<sup>+</sup> T cells from healthy controls suggesting a decrease in the major 8-oxoG DNA glycosylase activity (i.e., hOgg1)(Figure 3). Excision of 5-ohC in CD4<sup>+</sup> T cells was also decreased in AIDS

patients (~70% of activity in healthy controls) suggesting a moderate reduction also in one or more of the three glycosylases reported to remove 5-ohC (hNth1, Neil1 and Neil2). Furthermore and in accordance with our findings for faPy activity, CD8<sup>+</sup> T cells from non-AIDS patients showed a 3-fold increase in 8-oxoG removal and a 4-fold increase in 5ohC removal comparing CD8<sup>+</sup> T cells from healthy controls, indicating an enhancement of hOgg1 activity and an up-regulation of one or several of the DNA glycosylases excising oxidized pyrimidines (hNth1, Neil1 and Neil2). In contrast, although CD8<sup>+</sup> T cells from AIDS patients showed no changes in faPy removal, we found a 3-fold increase in 5-ohC excision comparing healthy controls (Figure 3). This could indicate a DNA glycosylase activity with affinity for 5-ohC but not faPy. The 8-oxoG activity in CD8<sup>+</sup> T cells from AIDS patients was not altered (Figure 3).

Except for 11 patients who were receiving nucleoside analog(s) (see Methods), none of the patients were taken any regular medications. Although these 11 patients did not differ from the other patients with regard to the measured parameters (data not shown), we can not exclude some influence from the use of these medications.

### **DNA glycosylase activity during HAART**

HAART strongly reduces levels of plasma HIV RNA with concomitant increases in T cell counts and beneficial effects on clinical symptoms and mortality.<sup>31</sup> To further examine the relationship between HIV infection, oxidative DNA damage and DNA glycosylases, we examined DNA glycosylase activity for removal of faPy and alkylating DNA damage in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from 7 HIV-infected patients before and 6 months after initiating HAART. All patients received an HIV protease inhibitor (indinavir, 800 mg thrice a day) in combination with two nucleoside analogs [zidovudine

(250 mg) and lamivudine (150 mg), twice a day]. In response to HAART there was a marked fall in HIV RNA copies in plasma [maximal decrease:  $3.02(1.92-3.98)\log_{10}$ ,  $p<0.05$ ] and a marked increase in  $CD4^+$  [maximal increase:  $150(50-215)\times 10^6/L$ ,  $p<0.05$ ] and  $CD8^+$  T cell [maximal increase:  $260(30-415)\times 10^6/L$ ,  $p<0.05$ ] counts in peripheral blood. Concomitantly, there was a significant rise in faPy activity in  $CD4^+$  T cells (Figure 4), inversely correlated with the decrease in plasma viral load ( $r=0.68$ ,  $p<0.05$ ). However, there was no normalization compared with faPy activity in healthy controls (Figure 4). Moreover, even if faPy activity in  $CD8^+$  T cells was raised before initiating therapy, HAART induced a further increase in activity with particularly enhancing effect in those with the lowest faPy levels at baseline (Figure 4). In comparison, DNA glycosylase activity for repair of alkylating damage showed no changes during HAART in either  $CD4^+$  or  $CD8^+$  T cells (data not shown). Finally, we analysed 8-oxoG levels in T cell subsets of 4 AIDS patients who had received HAART for 1 year. Notably, while  $CD4^+$  T cells in AIDS patients not receiving HAART had markedly enhanced levels of 8-oxoG in genomic DNA (Figure 1),  $CD4^+$  T cells from AIDS patients on successful HAART had 8-oxoG levels within the range of healthy controls [ $34.0(29.5-36.0)$  8-oxoG per  $10^6$  dG versus  $40.5(27.8-48.0)$  8-oxoG per  $10^6$  dG, patients and controls, respectively].

## **Discussion**

Several studies have reported enhanced oxidative stress in HIV infection possibly playing a pathogenic role in this disorder.<sup>10</sup> In the present study we show that HIV-infected patients, and particularly those with advanced disease, are characterized by enhanced oxidative DNA damage in  $CD4^+$  T cells as assessed by increased 8-oxoG accumulation.

Notably, this increase in oxidative DNA damage was accompanied by a marked decline in DNA glycosylase activity for repair of oxidative base lesion in CD4<sup>+</sup> T cells. In contrast, CD8<sup>+</sup> T cells of HIV-infected patients, with 8-oxoG levels similar or decreased compared with those in healthy controls, showed enhanced capacity to repair oxidative damage. Finally, during HAART there was a rise in glycosylase activity in CD4<sup>+</sup> T cells accompanied by a near normalisation of 8-oxoG levels in these cells. Although we can not totally exclude some influence from any undiagnosed and clinical asymptomatic co-infection on our results, our data suggest that impaired base excision repair of oxidative damage, with subsequent accumulation of oxidative DNA base lesions in CD4<sup>+</sup> T cells, may play a pathogenic role in HIV infection.

The mechanisms leading to enhanced oxidative stress in CD4<sup>+</sup> T cells during HIV infection are complex. Increased activity of inflammatory cytokines (e.g. TNF $\alpha$ ) and altered intracellular glutathione redox status, characterizing HIV-infected patients,<sup>9</sup> may promote oxidative DNA damage in these cells. However, our findings of an inverse correlation between viral load and glycosylase activity and near normalization of repair capacity during HAART, suggest that direct HIV-related mechanisms may be involved in the dysregulation of oxidative DNA damage/repair activity during HIV infection. Thus, TNF $\alpha$  and ROS can increase transcription of viral proteins by activating the nuclear factor  $\kappa$ B.<sup>32</sup> The HIV proteins Tat and gp120 may again directly enhance oxidative stress in T cells possibly involving TNF dependent mechanisms,<sup>33</sup> representing a pathogenic loop promoting inflammation, enhanced oxidative stress and HIV replication. Moreover, the HIV protein *vpr*, which may arrest T cells and induce subsequent apoptosis, has been found to incorporate catalytically active uracil DNA glycosylase into HIV viron particle

further supporting a link between HIV replication and DNA repair mechanisms.<sup>34</sup> In fact, this glycosylase, which initiates base excision repair of deaminated cytosine, is important for modulation of the virus mutation rate.<sup>35</sup> It is therefore tempting to hypothesize that the dysbalance between glycosylase activity and oxidative DNA damage in CD4<sup>+</sup> T cells during HIV infection could reflect potent interactions between viral proteins, enhanced oxidative stress and increased activity of inflammatory cytokines overwhelming DNA repair mechanisms.

The regulation of the various DNA glycosylases involved in repair of oxidative damage in man has not been fully clarified. However, hNth1 transcription was shown to increase during early and mid S-phase of cell cycle<sup>36</sup> suggesting that increased 5ohC DNA glycosylase activity in CD8<sup>+</sup> T cells during HIV infection may correlate with the enhanced spontaneous T cell proliferation observed in these patients.<sup>37</sup> In CD4<sup>+</sup> T cells such a stimulus may be counteracted and overshadowed by mechanisms as discussed above. In contrast to hNth1, the hOgg1 gene lacks TATA or CAAT boxes suggesting that Ogg1 is a housekeeping gene with stable expression during cell cycle.<sup>38</sup> However, the results are somewhat conflicting, possibly reflecting different regulation in different cell types. Thus, raised 8-oxoG levels and ischemia have been reported to enhance Ogg1 activity in human colorectal carcinoma cells and in mouse brain, respectively.<sup>39,40</sup> Moreover, while acute ischemia and oxidative stress may promote up-regulation of DNA glycosylase activity, persistently enhanced oxidative stress and inflammation as in HIV infection, may potentially down-regulate or “consume” the activity of these enzymes. Nevertheless, the mechanisms for regulation of DNA glycosylases for repair of oxidative base lesions in T cell subsets during HIV infection are at present unclear.

In the present study we show that DNA glycosylases for repair of oxidative base lesions are differently regulated in CD4<sup>+</sup> and CD8<sup>+</sup> T cells during HIV infection. In fact, rather than decreased activity, CD8<sup>+</sup> T cells in non-AIDS patients were characterized by enhanced faPy, 5-ohC and 8-oxoG DNA glycosylase activity, accompanied by a non-significant decrease in 8-oxoG level in these cells. This phenomenon could possibly reflect that while HIV infection is characterized by a functional and numerical decline in CD4<sup>+</sup> T cells, the CD8<sup>+</sup> T cells is characterized by increased numbers and enhanced activity at least in non-AIDS patients.<sup>41</sup>

Our findings suggest that CD4<sup>+</sup> T cells in HIV-infected patients may lack the ability to sufficiently repair DNA damage induced by oxidative stress that may have several consequences. Mutant forms of hOgg1 have been found in lung and kidney tumors.<sup>42</sup> A very recent report suggests that reduced Neil1 activity arising from mutations and reduced expression may be involved in pathogenesis of a subset of gastric cancers.<sup>43</sup> It is possible that the imbalance between oxidative DNA damage and repair activity in CD4<sup>+</sup> T cells from AIDS patients might contribute to the increased frequency of malignancies in these patients. Moreover, while long-time exposure to moderately increased oxidative DNA damage may pre-dispose to malignancies, markedly enhanced oxidative DNA damage combined with decreased repair activity as found in CD4<sup>+</sup> T cells from HIV-infected patients, may lead to functional impairment of these cells by slowing cell cycle progression upon activation and by enhancing apoptosis, possibly contributing to the pathogenesis of T cell deficiency in these patients. Finally, the HIV genome is characterized by a high frequency of mutation that at least partly is due to the high rate of HIV replication and the low fidelity of the reverse transcriptase gene. These mutations

which involve a high frequency of base misincorporations, may lead to a several-fold decrease in sensitivity to one or more antiretroviral drugs and is at present an increasing challenge in HAART.<sup>44</sup> Several oxidative base lesions are strongly premutagenic if they remain in DNA during replication. 5-ohC and 8-oxoG are stable oxidation products with strong miscoding properties, which produce G→T transversions<sup>45</sup> and C→T transitions/C→A transversions,<sup>46</sup> respectively. Therefore, it is possible that accumulation of mutagenic base lesions as a result of enhanced oxidative stress and impaired capacity for repair of oxidative DNA damage in CD4<sup>+</sup> T cells in HIV-infected patients, also may pre-dispose for elevated mutation frequency in the HIV genome of these cells.

. In conclusion, the present study further supports a role for oxidative stress in the pathogenesis of HIV infection by demonstrating a marked imbalance between accumulation of oxidative DNA damage and the capacity to repair such lesions in CD4<sup>+</sup> T cells (favouring enhanced DNA damage). These findings may represent a previous unrecognised mechanism involved in the numerical and functional impairment of CD4<sup>+</sup> T cells during HIV infection.

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## Figure legends

**Figure 1.** Accumulation of 8-oxoG in (A) CD4<sup>+</sup> and (B) CD8<sup>+</sup> T cells from HIV-infected patients. Nuclear DNA was isolated from T cell subsets of AIDS patients (n=5), non-AIDS HIV-infected patients (n=4) and healthy controls (n=7). Levels of 8-oxoG were quantified by HPLC-ECD after enzymatic DNA hydrolysis. \* p<0.05 versus healthy controls; # p<0.05 versus non-AIDS patients. Data are given as medians and 25<sup>th</sup>-75<sup>th</sup> percentiles.

**Figure 2.** FaPy DNA glycosylase activity (A and B) and alkylbase DNA glycosylase activity (C and D) in extracts (ng) of CD4<sup>+</sup> and CD8<sup>+</sup> T cells from HIV-infected patients and controls. Extracts (ng) isolated from AIDS patients (n=7), non-AIDS HIV-infected patients (n=8) and healthy controls (n=13) were incubated with 0,4 µg <sup>3</sup>H-labelled faPy-poly(dG-dC) DNA or alkylated calf thymus DNA. Horizontal lines represent median values.

**Figure 3.** 5-ohC DNA glycosylase activity (A) and 8-oxoG DNA glycosylase activity (B) in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from HIV-infected patients. Extracts (ng), isolated from AIDS-patients (n=7), non-AIDS HIV-infected patients (n=8) and healthy controls (n=13), were incubated with 50 fmol duplex oligo containing a single 8-oxoG or 5-ohC residue. The cleavage products were analysed by 20% denaturing PAGE and PhosphoImager scanning. \* p<0.05 and \*\* p<0.01 versus healthy controls. # p<0.05 versus AIDS patients. Data are given as medians and 25<sup>th</sup>-75<sup>th</sup> percentiles.

**Figure 4.** Fapy DNA glycosylase activity in CD4<sup>+</sup> (A) and CD8<sup>+</sup> (B) T cells from HIV-infected patients during HAART. Extracts (ng), isolated from 7 HIV-infected patients

before and after 6 months of HAART, were incubated with 0,4  $\mu\text{g}$   $^3\text{H}$ -labelled faPy-poly(dG-dC) DNA. The shaded area indicates ranges in 13 healthy controls.

Table 1. Characteristics of the study group.

	Non-AIDS HIV- infected patients (n=8)	AIDS patients (n=7)	Controls (n=13)
Age (years), median and ranges	38(32-52)	37(34-56)	37(29-61)
Males/females	6/2	5/2	9/4
CD4 <sup>+</sup> T cells (10 <sup>6</sup> /l)	280 (95-430)	45 (10-110)	760 (540-980)
CD8 <sup>+</sup> T cells (10 <sup>6</sup> /l)	710 (410-1170)	280 (110-430)	400 (210-550)
TNF $\alpha$ (pg/ml)	25 (12-37)	65 (40-98)	8 (0-12)
HIV RNA copies/ml plasma (x10 <sup>3</sup> )	44.2 (22.9-108.6)	510.9 (210.0-1200.0)	-

Data are given as medians and 25<sup>th</sup>-75<sup>th</sup> percentiles if not otherwise stated.

T cell subset counts were analysed in peripheral blood and TNF $\alpha$  levels in plasma.

Figure 1.

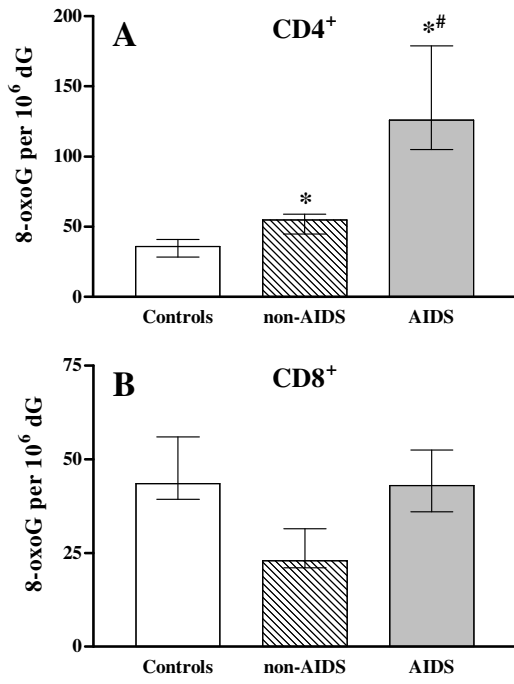


Figure 2.

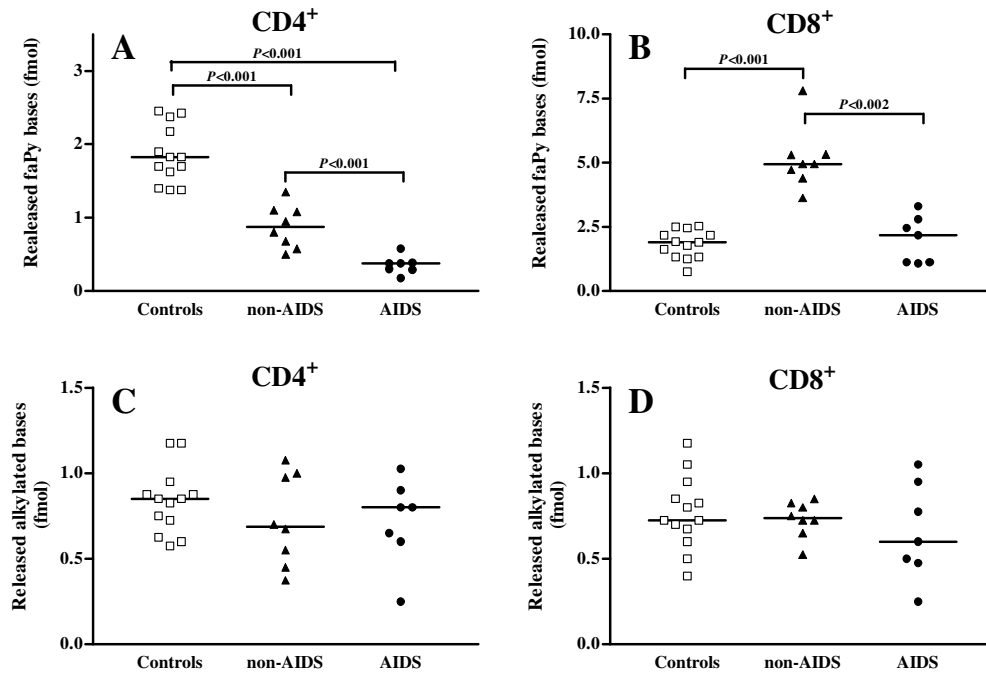


Figure 3.

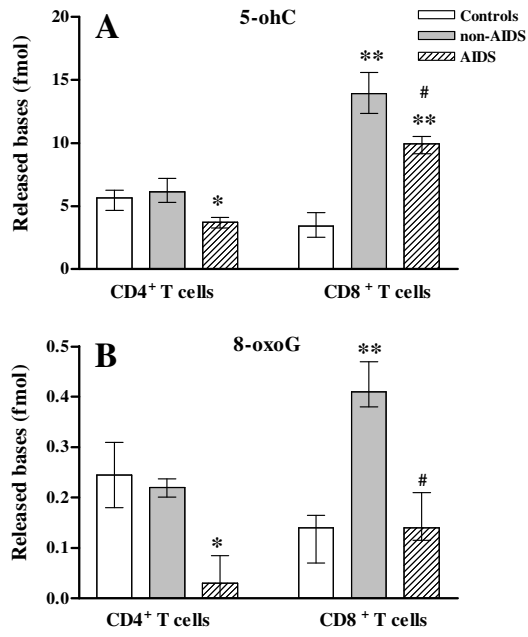


Figure 4.

