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Oxidative stress in human immunodeficiency virus infection

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Abstract. Infection by the human immunodeficiency virus (HIV-1) causes chronic ongoing inflammation in HIV-1 seropositive individuals as shown by high plasma levels of inflammatory cytokines and production of reactive oxygen intermediates (ROIs). One source of ROIs is provided from the very early stages of HIV infection by activated polymorphonuclear neutrophils. Tat, the viral protein, is also specifically responsible for an endogenous cellular increase of ROI. In this review we also evaluate the effects of this oxidative stress on various biological parameters such as immune response and survival of T lymphocytes, virus transcription and replication. It was clearly demonstrated in ex vivo experiments that the oxidative stress due to infection itself participates in $CD4^+$ T lymphocyte depletion by increasing their rate of apoptosis and particularly of Fas-induced apoptosis. This oxidative stress also facilitates NF-κB-dependent activation of HIV transcription. In vitro studies suggest that the early steps of HIV activation from its quiescent state might be subsequently facilitated by this oxidative environment, whereas already active replication is not influenced. The data presented here lead to a better understanding of the consequences of oxidative stress on the pathophysiology of HIV infection and also enable us to evaluate the potential use of antioxidant molecules as therapeutic agents against AIDS.

Key words. Oxidative stress; HIV infection; HIV replication; HIV transcription; antioxidants.

HIV infection induces oxidative stress

HIV-1 infection causes a chronic ongoing inflammation shown by high plasma levels of inflammatory cytokines [1] and production of reactive oxygen intermediates in HIV-1 seropositive individuals. This oxidative stress occurring at the onset of the disease was shown by a decrease in the concentration of the main antioxidant molecules such as plasma and lymphocyte glutathione [2–4] or lymphocyte [5] and plasma [6] thioredoxin. These reports were consistent with the observation of an increase in plasma concentration of the byproduct of lipid peroxidation, malondialdehyde (MDA) [7, 8]. This oxidative stress was shown to be related to the constitutive production of H_2O_2 by neutrophils at all stages of the disease, even in the early stages when the number of CD4 T cells is still high (fig. 1) [9]. This overproduction of H_2O_2 is accompanied by other signs of cell activation such as expression of adhesion molecules like CD11b or actin polymerisation. This study was realized, using flow cytometry, in conditions permitting analysis of H_2O_2 production directly in the blood and avoiding any artefactual activation linked to neutrophil isolation [9]. In addition, HIV-1 infected cells undergo endogenous oxidative stress related to inhibiting effect of the viral protein Tat on activity of manganese superoxide dismutase (MnSOD) [10, 11], leading to an increase in endogenous ROI. This process of induction of endogenous oxidative stress, dependent on the expression of a viral protein, is thus very specific for HIV infection. Since the role of HIV infection in inducing oxidative stress from exogenous (polymorphonuclear

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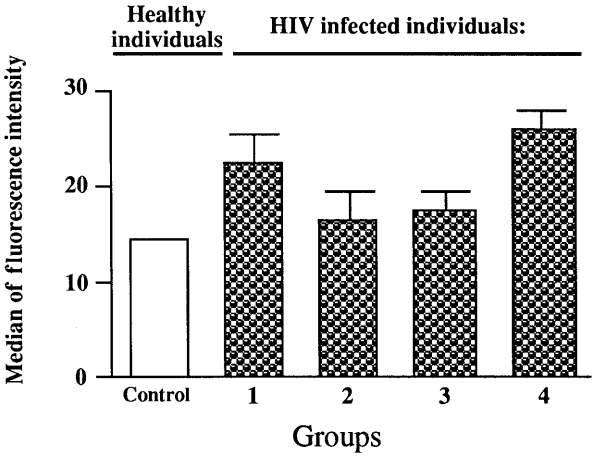

Figure 1. H_2O_2 production by neutrophils detected in the blood of HIV infected individuals. The distinct stages of infection are defined according to the Centers for Disease Control and Prevention (CDC): group 1 (n = 10) corresponds to asymptomatic patients with more than 500 $CD4^+$/μl, group 2 (n = 10) to asymptomatic patients with fewer than 500 $CD4^+$/μl, group 3 (n = 12) to ARC (IDS-related complex) patients and group 4 (n = 10) to AIDS patients. The controls are healthy individuals (n = 10). Neutrophils were incubated with dichlorofluorescein diacetate for 15 min at 37 °C. The fluorescence of its oxidized product (dichlorofluoresceine) was detected using flow cytometry at 530 nm. These data are adapted from Elbim et al. [9].

neutrophils) and endogenous (Tat) sources has been well established, it is important to evaluate the influence of this oxidative stress on the pathophysiology of HIV infection.

Consequence of oxidative stress on the immune response

The hallmark of HIV infection is immunodeficiency with progressive $CD4^+$ T lymphocyte depletion. One important issue is whether the oxidative stress induced by the infection itself might participate in this immunodeficiency.

In vivo studies have shown that oxidative stress might lead to immunodeficiency at the cellular and humoral levels. For instance, exposure of mice to normobaric oxygen resulted in a decrease of the number of B cells which produce antibodies after immunization, in a delayed-type hypersensibility response and in a decrease of T cell proliferation in response to mitogens [12, 55].

The participation of oxidative stress in lymphocyte depletion during HIV infection may result from different mechanisms such as impairment of proliferation, as suggested in animal models, but also from apoptosis. Indeed, ex vivo studies have shown that T lymphocytes from HIV-infected individuals exhibit a higher rate of apoptosis than lymphocytes from normal subjects [13–15] and that this rate of apoptosis is significantly decreased by the addition of antioxidant compounds [16].

When activated, peripheral blood T lymphocytes are induced to express Fas/APO-1/CD95 receptors which mediate apoptosis upon binding to Fas ligand [17–19]. Surface expression of Fas was also found to be higher on $CD4^+$ and $CD8^+$ T lymphocytes from HIV-infected individuals [20, 21]. Furthermore, these lymphocytes from HIV-infected individuals undergo apoptosis in vitro in response to antibody stimulation (cross-linking) of Fas at a much higher frequency than uninfected controls. This suggests that HIV-infected individuals are primed in vivo to undergo apoptosis in response to Fas stimulation. Fas signalling then may be responsible for T lymphocyte functional defects and depletion observed in HIV disease. HIV-1 Tat and gp120 were also shown to accelerate induction of Fas-mediated T cell apoptosis [22]. The cellular antioxidant thioredoxin was shown to protect U937 cells against apoptosis triggered by anti-Fas mAb [23]. This is in agreement with in vitro studies showing that the increase of the reduced glutathione, the most abundant intracellular thiol antioxidant, obtained by the use of *N*-acetylcysteine (NAC) or other thiol compounds are able to inhibit apoptosis triggered by Fas ligation [24].

Conversely, in monocytic cells U937 capable of sustaining a chronic infection in vitro, an increase of reduced glutathione over the constitutive level of uninfected cells was observed. This was accompanied by a refractory state of these cells to tumor necrosis factor (TNF) or phorbol myristate acetate (PMA) [25]. This adaptative mechanism might be a key component of resistance to apoptosis and of viral persistence in macrophages which are known to constitute a reservoir for the virus [26]. Work is in progress in our laboratory to characterize the redox status of primary macrophages during the establishment of chronic infection.

In conclusion, these data provide evidence that infection-induced oxidative stress contributes to $CD4^+$ T lymphocyte depletion by increasing their rate of apoptosis, particularly Fas-induced apoptosis. Adaptative mechanisms in some cells (U937 in vitro) or drugs which maintain the level of cellular antioxidants counteract this induction of apoptosis.

Consequence of oxidative stress on HIV transcription

Furthermore, the progressive and irreversible decline of $CD4^+$ T cells observed during HIV-1 infection is clearly associated with a progressive increase of plasma viral load [27, 28]. In order to better understand AIDS pathogenesis, it was therefore important to find out how the oxidative stress induced by the infection itself might influence the expression of the viral genome.

HIV responds to transcriptional stimuli similar to those leading to the induction of a series of cellular genes

Table 1. BHA leads to an increase in total thiol content accompanied by a dose-dependent decrease of TNF-induced LTR transactivation in a Jurkat cell clone stably expressing luciferase activity driven by HIV-1 LTR. TNF does not modify thiol content, even though it induces a high level of LTR transactivation. Luciferase activity was measured in cell lysates and expressed as RLU (relative light units)/10^6 cells. Thiol content was determined using a previously described technique [55] and expressed in nmol/10^6 cells. These data are adapted from Israël et al. [40].

TNF addition	BHA addition	Luciferase activity RLU/10^6 cells	Thiol content nm/10^6 cells
−	0	92	14.0
+	0	2266	12.2
+	100	2137	13.7
+	200	1841	18.4
+	300	1166	19.4
+	400	214	24.7
+	500	43	25.7

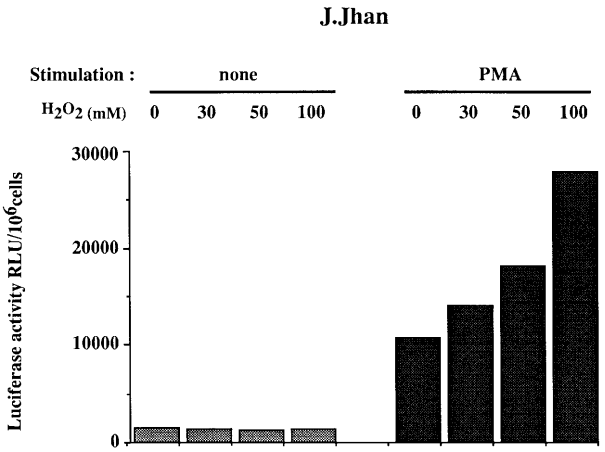

Figure 2. H_2O_2 alone does not significantly modify the constitutive level of luciferase activity, but potentiates the effect of PMA. J.Jhan cells, transiently transfected with the plasmid 3 Enh-TK-Luc which carries 3 'HIV-enhancer' motifs (6 κB sites) upstream of a minimum promoter (TK: thymidine kinase of herpes simplex virus) driving the luciferase gene, were exposed to H_2O_2 (30–100 μM) alone or in association with PMA (20 ng/ml) from 24 to 30 h after transfection. Luciferase activity was measured in cell lysates and expressed as RLU/10^6 cells. These data are adapted from Israël et al. [40].

during T cell activation. The pivotal event is the activation of NF-κB transcription factor (for review see [29]). Activation of this factor was shown to be one of the main requirements in the early stages of viral replication in T lymphocytes. The event necessary to efficiently trigger NF-κB-induced viral transcription is recognition of a specific antigen [30, 31]. NF-κB is composed of two subunits (p50 and p65) and is retained in an inactive form in the cytoplasm by interaction with inhibitory molecules designated IkBs (for reviews see [32–35]). Under various stimuli mediated through a number of cell surface molecules, such as receptors to antigens or to cytokines (for review see [29]), this factor is translocated into the nucleus. There, it interacts with two specific cis-acting sequences present within the long terminal repeat sequence (LTR) of HIV-1 [36].

Various antioxidant molecules have been shown to block cellular activation events [37–39]. Since HIV transcriptional activation is closely related to cellular activation events, we tested the hypothesis that phenolic, lipid-soluble, chain-breaking antioxidants that act at the membrane level, such as nordihydroquairetic acid (NDGA), α-tocopherol (vitamin E), and particularly butylated hydroxyanisole (BHA) would decrease NF-κB-dependent PMA- or TNF-induced HIV-LTR activation. Since the main targets of the virus are $CD4^+$ T lymphocytes and monocytes/macrophages, we used both lymphoblastoid T (J.Jhan) and monocytic (U937) cell lines and we showed that these free radical scavengers inhibit HIV-LTR transactivation by blocking NF-κB activation (table 1) [40]. Besides the phenolic antioxidants, various antioxidants such as dithiocarbamates [41], NAC or other glutathione precursors [4, 42–44] were also demonstrated to decrease HIV transcription by blocking NF-κB activation induced by PMA or TNF.

We also examined the mechanism involved in the influence of the redox status on the modulation of NF-κB activation in lymphoblastoid T cell lines. It was suggested that all stimuli which activate NF-κB induce ROIs that act as second messengers to mediate NF-κB activation and LTR transactivation. Subsequently, antioxidant molecules, which scavenge ROIs, should prevent NF-κB activation [41]. Since thiols, and particularly glutathione, constitute one of the major antioxidant systems, we approached the mechanism of the BHA-mediated inhibitory effect by a correlative study of NF-κB activation and of the cellular thiol content in various experimental conditions of stimulation by TNF or PMA or of inhibition by BHA as shown in table 1 [40]. We showed that modification of the redox status of Jurkat cells by BHA lead, as expected, to an accumulation of thiols within the cells accompanied by a decrease of TNF induced LTR transactivation in a dose-dependent manner. TNF by itself barely modified this level of thiols even though it led to LTR transactivation. This indicates that production of ROIs was not a step necessary to induce NF-κB activation. This conclusion was strengthened by the fact that even oxidative stress (H_2O_2 or hyperoxic treatments) did not lead to NF-κB activation in most hematopoietic cell lines, as we (fig. 2) [40] and others [45, 46] have shown. However, H_2O_2 synergizes with a signal such as PMA (fig. 2) [40]. In conclusion, oxidative stress facili-

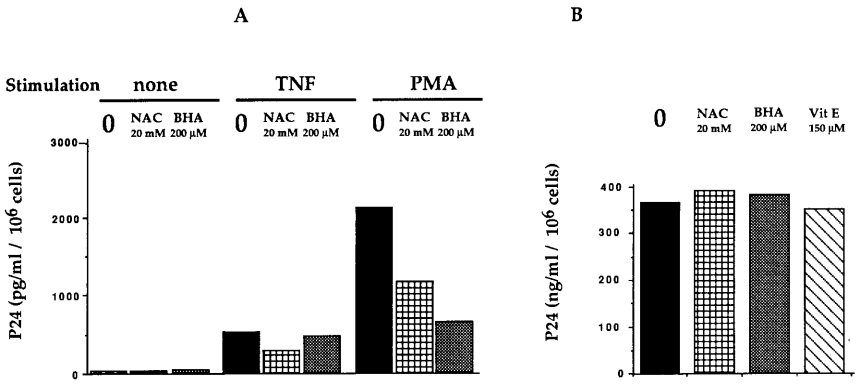

Figure 3. BHA or NAC partially decrease TNF- or PMA-induced HIV replication in U1 cells (*A*) but do not modify HIV replication in chronically infected U937 cells (*B*). Panel A: U1 cells were left unstimulated or stimulated for 6 h with TNF (500 U/ml) or PMA (20 ng/ml) in the presence of NAC (30 mM) or BHA (300 µM) for 6.5 h; Panel B: Chronically infected cells were left untreated or treated respectively with NAC (20 mM), BHA (200 µM) or vitamin E (150 µM) for 19 h. Each bar represents the p24 level expressed in pg/ml/10^6 U1 cells (*A*) or in ng/ml/10^6 U937 cells (*B*) measured in the supernatant of cells 6.5 h (U1) or 24 h (U927) after the various treatments. These data are taken from one representative experiment out of five. These data are adapted from Aillet et al. [25].

tates but does not mediate NF-κB activation and LTR transactivation. These cofactors are probably constitutively present and are sufficient to permit the full expression of the TNF signal in most hematopoietic cell lines. This might not be the case in peripheral blood lymphocyte subsets, since these cells respond to ultra-violet (UV) or H_2O_2 by inducing NF-κB activation [46].

We hypothesize that BHA, on blocking constitutive membrane peroxidation as an ROI scavenger at the level of the plasma membrane, leads to impairment of the TNF signalling pathway, possibly by modifying either the structure of the TNF receptor or the activity of enzymes located at the plasma membrane and implicated in this signalling pathway. This event could be one of the modifications taking place at the plasma membrane during oxidative stress, like the phosphorylation of the epidermal growth factor receptor triggered by UV radiation [47] or phosphorylation of tyrosine kinases [48] such as ZAP 70 [46] mediated by CD3, or $P56^{lck}$ [49]. The targets of antioxidant molecules on the plasma membrane leading to impairment of TNF signalling have not yet been identified.

Consequence of oxidative stress on HIV replication

Since oxidative stress activates NF-κB-dependent transactivation of HIV-LTR, we examined the dependence of NF-κB activation for replication in two human monocytic cell systems of the same origin, but exhibiting different levels of HIV replication: the U1 subclone previously characterized as a model of latency [50] and chronically infected U937 which actively replicate the virus. We investigated whether the suppression of NF-κB activation by the two prototypic antioxidants, namely NAC and BHA, would counteract HIV replication in both stages of HIV activation: namely, from its quiescent state in U1 cells and from its already replicating state in chronically infected U937.

We showed that BHA and NAC both counteract TNF- and PMA-induced NF-κB activation in the U1 cell line and suppress the constitutive NF-κB activity permanently induced [51] by the virus itself in chronically infected U937 cells [40]. Inhibition of TNF- or PMA-induced NF-κB activity was accompanied by a partial decrease of induction of HIV replication in U1 cells (as measured by p24 antigen concentration), whereas suppression of NF-κB activity in chronically infected U937 cells did not lead (at least not within the 48 h of the study) to any detectable decrease in HIV replication level (fig. 3) [25]. These data also suggest that participation of NF-κB in viral replication is different in the two systems. In the latent system, activation of NF-κB is necessary for induction of HIV replication, whereas NF-κB constitutively present in the nucleus of the actively replicative U937 cells does not seem to be important when an active replication has been established for a long time. It is likely that, under these conditions, HIV Tat, the transactivator encoded by the virus, plays a major role in virus replication, and BHA and NAC cannot counteract its strong transactivating effect [25].

Thus activation of HIV transcription by oxidative stress due to HIV infection might occur preferentially in the first steps of HIV replication from its quiescent state. In contrast, ongoing replication in cells which chronically replicate the virus might not be influenced by the redox status of the cells, since HIV transcription is then independent of NF-κB.

Potential use of antioxidants as therapeutic agents against AIDS

On the basis of the data collected here, we discuss the potential use of antioxidant molecules in the treatment of HIV-infected individuals.

A potential therapeutic use of NAC in the treatment of HIV-infected patients was first proposed on the basis of its inhibitory effect on virus replication in normal circulating lymphocytes infected in vitro with HIV-1 and stimulated with mitogens [42] or in decreasing virus replication in infected cell lines [43, 44]. However, we think that anti-HIV therapies might aim, at the same time, at inhibiting induced HIV replication in latent cells and permanent replication in cells which actively multiply the virus, since in vivo the integrated provirus may either remain latent, especially in normal circulating lymphocytes, or be actively transcribed, as in lymphoid organs [52, 53] or tissue macrophages [26, 53]. Our results obtained in vitro suggest that one of the limitations of antioxidant therapy in vivo might be that sustained replication in reservoir cells such as tissue macrophages might not be impaired by antioxidant compounds.

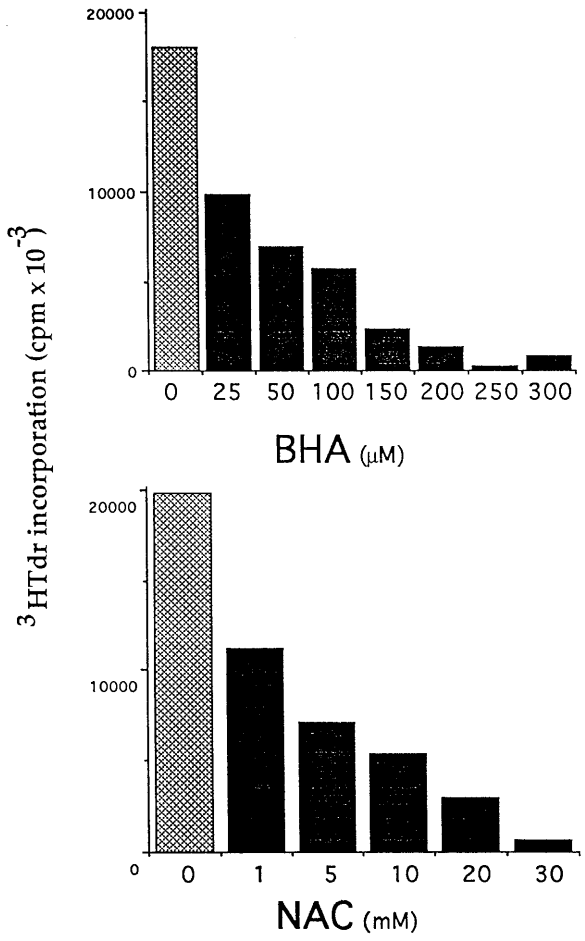

Figure 4. Effects of BHA and NAC on rhIL-2-induced proliferation of human PBMCs. The PBMCs were cultured with rhIL-2 (30 ng/ml) for 72 h. At the start of the culture, 20 μl of either culture medium or BHA (final concentrations ranging from 25 to 300 μM) or NAC (final concentrations ranging from 1 to 30 μM) were added. Cells were pulsed with (^{3}H) TdR 6 h before the end of the culture. These data are adapted from Aillet et al. [25].

Another concern is that the concentrations needed to prevent NF-κB activation would be difficult to obtain in vivo, and if obtained, might have deleterious effects on immune functions such as TNF production or peripheral blood lymphocytes proliferation. Furthermore, NAC or BHA were shown to inhibit interleukin 2-induced lymphocyte proliferation even at concentrations lower than that necessary to impair HIV replication (fig. 4) [25].

Another approach proposed the use of low concentrations of antioxidants and particularly cysteine derivatives to correct low thiol content (particularly glutathione) in HIV-infected patients, with beneficial effects on CD4 lymphocyte survival [54]. However, low concentrations of antioxidants might have opposite effects to those observed with high concentrations, resulting in NF-κB activation. These bimodal effects were shown on HIV transcription, using concentrations lower than 20 mM for vitamin E [25] and 1 mM for BHA (data not shown), and by others with dithiocarbamate derivatives [41]. The effect of these molecules on PBMC (peripheral blood mononuclear cells) proliferation is also bimodal, depending on the concentrations used. Stimulation was observed with low concentrations of thiols, for instance. The bimodal effect of antioxidant molecules is another limitation on the use of these molecules in AIDS therapy.

In conclusion, these considerations warrant prudence in the design of antioxidant-based anti-HIV therapies.

1 Emilie D., Fior R., Jarrousse B., Marfaing K. A., Merrien D., Devergne O. et al. (1994) Cytokines in HIV infection [Review]. Int. J. Immunopharmacol. **16:** 391–396
2 Eck H. P., Gmunder H., Hartmann M., Petzoldt D., Daniel V. and Droge W. (1989) Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol. Chem. Hoppe Seyler **370:** 101–108
3 Buhl R., Jaffe H. A., Holroyd K. J., Wells F. B., Mastrangeli A., Saltini C. et al. (1990) Glutathione deficiency and HIV [Letter]. Lancet **335:** 546
4 Staal F. J., Roederer M., Israelski D. M., Bubp J., Mole L. A., McShane D. et al. (1992) Intracellular glutathione levels in T cell subsets decrease in HIV-infected individuals. AIDS Res. Hum. Retroviruses **8:** 305–311
5 Masutani H., Naito M., Takahashi K., Hattori T., Koito A., Takatsuki K. et al. (1992) Dysregulation of adult T-cell leukemia-derived factor (ADF)/thioredoxin in HIV infection: loss of ADF high-producer cells in lymphoid tissues of AIDS patients. AIDS Res. Hum. Retroviruses **8:** 1707–1715
6 Nakamura H., Derosa S., Roederer M., Anderson M. T., Dubs J. G., Yodoi J. et al. (1996) Elevation of plasma thioredoxin levels in HIV-infected individuals. Int. J. Immunol. **8:** 603–611
7 Sonnerborg A., Carlin G., Akerlund B. and Jarstrand C. (1988) Increased production of malondialdehyde in patients with HIV infection. Scand. J. Infect. Dis. **20:** 287–290

8 Revillard J. P., Vincent C. M., Favier A. E., Richard M. J., Zittoun M. and Kazatchkine M. D. (1992) Lipid peroxidation in human immunodeficiency virus infection [Letter]. J. Acquir. Immune Defic. Syndr. **5:** 637–638

9 Elbim C., Prevot M. H., Bouscarat F., Franzini E., Chollet M. S., Hakim J. et al. (1994) Polymorphonuclear neutrophils from human immunodeficiency virus-infected patients show enhanced activation, diminished fMLP-induced L-selectin shedding and an impaired oxidative burst after cytokine priming. Blood **84:** 2759–2766

10 Flores S. C., Marecki J. C., Harper K. P., Bose S. K., Nelson S. K. and McCord J. M. (1993) Tat protein of human immunodeficiency virus type 1 represses expression of manganese superoxide dismutase in HeLa cells. Proc. Natl. Acad. Sci. USA **90:** 7632–7636

11 Westendorp M. O., Frank R., Ochsenbauer C., Stricker K., Dhein J., Walczak H. et al. (1995) Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature **375:** 497–500

12 Levacher P. M., Gougerot P. M., Rouveix B., Kraus L. and Pocidalo J. J. (1983) Characterization of immunological depression in mice exposed to normobaric oxygen. Clin. Exp. Immunol. **54:** 580–586

13 Ameisen, J. C., Groux H. and Capron A. (1992) Programmed death (apoptosis) of T CD4 lymphocytes and AIDS pathogenesis. C R Acad Sci **314:** 47–50

14 Finkel T. H., Tudor W. G., Banda N. K., Cotton M. F., Curiel T., Monks C. et al. (1995) Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nature Med. **1:** 129–134

15 Gougeon M. L., Lecoeur H., Dulioust A., Enouf M. G., Crouvoiser M., Goujard C. et al. (1996) Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. J. Immunol. **156:** 3509–3520

16 Olivier R., Lopez O., Mollerau M., Dragic T., Guetard D. and Montagnier L. (1994) Prevention of early cell death in peripheral blood lymphocytes of HIV infected individuals by an antioxidant N Acetyl-Cysteine, In: Oxidative Stress, Cell Activation and Viral Infection, vol. 1, pp. 323–332, Pasquier, C. and Olivier, R. (eds), Birkhäuser Verlag, Basel

17 Alderson M. R., Tough T. W., Davis S. T., Braddy S., Falk B., Schooley K. A. et al. (1995) Fas ligand mediates activation-induced cell death in human T lymphocytes. J. Exp. Med. **181:** 71–77

18 Brunner T., Mogil R. J., LaFace D., Yoo N. J., Mahboubi A., Echeverri F. et al. (1995) Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature **373:** 441–444

19 Dhein J., Walczak H., Baumler C., Debatin K. M. and Krammer P. H. (1995) Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature **373:** 438–441

20 Katsikis P. D., Wunderlich E. S., Smith C. A., Herzenberg L. A. and Herzenberg L. A. (1995) Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. J. Exp. Med. **181:** 2029–2036

21 Boudet F., Lecoeur H. and Gougeon M. L. (1996) Apoptosis associated with ex vivo down-regulation of Bcl-2 and up-regulation of Fas in potential cytotoxic $CD8^+$ T lymphocytes during HIV infection. J. Immunol. **156:** 2282–2293

22 Westendorp M. O., Shatrov V. A., Schulze O. K., Frank R., Kraft M., Los M. et al. (1995) HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. EMBO J. **14:** 546–554

23 Matsuda M., Masutani H., Nakamura H., Miyajima S., Yamauchi A., Yonehara S. et al. (1991) Protective activity of adult T cell leukemia-derived factor (ADF) against tumor necrosis factor-dependent cytotoxicity on U937 cells. J. Immunol. **147:** 3837–3841

24 Deas O., Dumont C., Mollereau B., Metivier D., Pasquier C., Bernardpomier G. et al. (1997) Thiol-mediated inhibition of fas and cd2 apoptotic signaling in activated human peripheral T cells. Int. J. Immunol. **9:** 117–125

25 Aillet F., Gougerot P. M., Virelizier J. L. and Israel N. (1994) Appraisal of potential therapeutic index of antioxidants on the basis of their in vitro effects on HIV replication in monocytes and interleukin 2-induced lymphocyte proliferation. AIDS Res. Hum. Retroviruses **10:** 405–411

26 Vazeux R., Brousse N., Jarry A., Henin D., Marche C., Vedrenne C. et al. (1987) AIDS subacute encephalitis. Identification of HIV-infected cells. Am. J. Pathol. **126:** 403–410

27 Ho D. D., Moudgil T. and Alam M. (1989) Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N. Engl. J. Med. **321:** 1621–1625

28 Saksela K., Stevens C., Rubistein P. and Baltimore D. (1994) Human immunodeficiency virus type 1 mRNA expression in peripheral blood cells predicts disease progression independently of the numbers of $CD4^+$ lymphocytes. Proc. Natl. Acad. Sci. USA **91:** 1104–1108

29 Kopp E. B. and Ghosh S. (1995) NF-kappa B and rel proteins in innate immunity. Adv. Immunol. **58:** 1–27

30 Hazan U., Thomas D., Alcami J., Bachelerie F., Israel N., Yssel H. et al. (1990) Stimulation of a human T-cell clone with anti-CD3 or tumor necrosis factor induces NF-kappa B translocation but not human immunodeficiency virus 1 enhancer-dependent transcription. Proc. Natl. Acad. Sci. USA **87:** 7861–7865

31 Weissman D., Barker T. D. and Fauci A. S. (1996) The efficiency of acute infection of $CD4^+$ T cells is markedly enhanced in the setting of antigen-specific immune activation. J. Exp. Med. **183:** 687–692

32 Baeuerle P. A. and Henkel T. (1994) Function and activation of NF-kappa B in the immune system. Annu. Rev. Immunol. **12:** 141–179

33 Siebenlist U., Fransozo G. and Brown K. (1994) Structure, regulation and function of NF-κB. Annu. Rev. Cell Biol. **10:** 405–430

34 Finco T. S. and Baldwin A. S. (1995) Mechanistic aspects of NF-kappa B regulation: the emerging role of phosphorylation and proteolysis. Immunity **3:** 263–272

35 Israel A. (1995) A role for phosphorylation and degradation in the control of NF-kappa B activity. Trends Genet. **11:** 203–205

36 Nabel G. and Baltimore D. (1987) An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature **326:** 711–713

37 Novogrodsky A., Ravid A., Rubin A. L. and Stenzel K. H. (1982) Hydroxyl radical scavengers inhibit lymphocyte mitogenesis. Proc. Natl. Acad. Sci. U.S.A. **79:** 1171–1174

38 Chaudhri G., Clark I. A., Hunt N. H., Cowden W. B. and Ceredig R. (1986) Effect of antioxidants on primary alloantigen-induced T cell activation and proliferation. J. Immunol. **137:** 2646–2652

39 Dornand, J. and Gerber M. (1989) Inhibition of murine T-cell responses by anti-oxidants: the targets of lipo-oxygenase pathway inhibitors. Immunology **68:** 384–391

40 Israël N., Gougerot-Pocidalo M.-A., Aillet F. and Virelizier J. L. (1992) Redox status of cells influences constitutive or induced NF-kappa B translocation and HIV long terminal repeat activity in human T and monocytic cell lines. J. Immunol. **149:** 3386–3393

41 Schreck R., Albermann K. and Baeuerle P. A. (1992) Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic. Res. Commun. **17:** 221–237

42 Roederer M., Staal F. J., Raju P. A., Ela S. W., Herzenberg L. A. and Herzenberg L. A. (1990) Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. Proc. Natl. Acad. Sci. USA **87:** 4884–4888

43 Kalebic T., Kinter A., Poli G., Anderson M. E., Meister A. and Fauci A. S. (1991) Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester and N-acetylcysteine. Proc. Natl. Acad. Sci. USA **88:** 986–990

44 Mihm S., Ennen J., Pessara U., Kurth R. and Droge W. (1991) Inhibition of HIV-1 replication and NF-kappa B activity by cysteine and cysteine derivatives. AIDS **5:** 497–503

45 Molitor J. A., Ballard D. W. and Greene W. C. (1991) Kappa B-specific DNA binding proteins are differentially inhibited by enhancer mutations and biological oxidation. New Biol. **3:** 987–996
46 Schieven G. L., Mittler R. S., Nadler S. G., Kirihara J. M., Bolen J. B., Kanner S. B. et al. (1994) ZAP-70 tyrosine kinase, CD45 and T cell receptor involvement in UV- and H_2O_2-induced T cell signal transduction. J. Biol. Chem. **269:** 20718–20726
47 Sachsenmaier C., Radler P. A., Zinck R., Nordheim A., Herrlich P. and Rahmsdorf H. J. (1994) Involvement of growth factor receptors in the mammalian UVC response. Cell **78:** 963–972
48 Devary Y., Gottlieb R. A., Smeal T. and Karin M. (1992) The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell **71:** 1081–1091
49 Nakamura K., Hori T., Sato N., Sugie K., Kawakami T. and Yodoi J. (1993) Redox regulation of a src family protein tyrosine kinase p56lck in T cells. Oncogene **8:** 3133–3139
50 Folks T. M., Justement J., Kinter A., Schnittman S., Orenstein J., Poli G. et al. (1988) Characterization of a promonocyte clone chronically infected with HIV and inducible by 13-phorbol-12-myristate acetate. J. Immunol. **140:** 1117–1122
51 Bachelerie F., Alcami J., Arenzana S. F. and Virelizier J. L. (1991) HIV enhancer activity perpetuated by NF-kappa B induction on infection of monocytes. Nature **350:** 709–712
52 Pantaleo G., Graziosi C., Butini L., Pizzo P. A., Schnittman S. M., Kotler D. P. et al. (1991) Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc. Natl. Acad. Sci. U.S.A. **88:** 9838–9842
53 Embretson J., Zupancic M., Ribas J. L., Burke A., Racz P., Tenner R. K. et al. (1993) Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature **362:** 359–362
54 Herzenberg L. A., Derosa S. C., Dubs J. G., Roederer M., Anderson M. T., Ela S. W. et al. (1997) Glutathione deficiency is associated with impaired survival in hiv disease. Proc. Natl. Acad. Sci. U.S.A. **94:** 1967–1972
55 Gougerot-Pocidalo, M.-A., Fay M., Roche Y., Lacombe P. and Marquetty C. (1985) Immune oxidative injury induced in mice exposed to normobaric O2: effects of thiol compounds on the splenic cell sulfhydryl content and Con A proliferative response. J. Immunol. **135:** 2045–2051