

# **A Key Role for HIV-1-Activated NADPH Oxidase in the Pathogenesis of AIDS**

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## **Abstract**

HIV-1 infection is associated with increased systemic oxidative stress; within HIV-1-infected cells, this promotes propagation of the virus by boosting activation of NF-kappaB, a key transcription factor for the LTR. The ability of Tat and gp120 to activate NADPH oxidase in various cell lines suggests that this enzyme complex may be the chief source of this oxidative stress. Moreover, there is reason to suspect that activated NADPH oxidase is a key mediator of the increased apoptosis that depletes the CD4+ lymphocyte population as AIDS progresses, and may also play a role in the induction of AIDS dementia. In HIV-1-infected patients treated with HAART, activation of NADPH oxidase in the vasculature and possibly adipocytes may be a mediator of the metabolic syndrome and increased cardiovascular risk associated with such therapy. These observations take on practical significance in light of recent evidence that phycocyanobilin (PCB) from spirulina can function as a nutraceutical inhibitor of NADPH oxidase. Furthermore, spirulina contains polysaccharides that can boost the ability of antigen-presenting cells to promote a Th1 immune response – characteristically impaired in AIDS. The observation that the prevalence of HIV-1 infection is anomalously low (for sub-Saharan Africa) among populations near Lake Chad which regularly consume spirulina, should further encourage studies to evaluate the therapeutic and preventive potential of spirulina or PCB with respect to HIV-1 infection. Low-cost strategies for HIV-1 control involving spirulina, melatonin, selenium, salicylate, and silymarin can be envisioned, and might be of particular utility in impoverished Third World populations.

## **Oxidant Stress Promotes Propagation of HIV-1**

For reasons that require further clarification, HIV-1 infection is typically associated with increased systemic oxidant stress.<sup>1-6</sup> Within cells that are HIV-1 infected, such stress would be expected to promote transcription of the the HIV-1 genome, inasmuch as oxidants induce and up-regulate activation of NF-kappaB transcription factors; NF-kappaB –preferably the p50-p65 heterodimer – binds to kappaB response elements in the LTR promoter, and plays an obligate role in the transcription of HIV-1 genes.<sup>7-9</sup> These considerations suggest that the ability of HIV-1 to evoke oxidant stress is of selective advantage to the virus, and likely has been conserved during the virus' evolution.

There is recent evidence that the impact of oxidants on NF-kappaB activity can be mediated by protein kinase D (PKD); following two serine phosphorylations mediated by PKC-delta and a tyrosine phosphorylation mediated by c-Abl, PKD associates with and phosphorylates IkappaB kinase-beta; the latter then phosphorylates IkappaB, routing it toward proteasomal degradation and thereby freeing NF-kappaB to migrate to the nucleus

and regulate transcription.<sup>10-12</sup> Upstream, this process is triggered by oxidant-mediated activation of c-Src, which in turn activates c-Abl. The latter not only provides the tyrosine phosphorylation of PKD, but also activates PKC-delta, which then provides the requisite serine phosphorylations of PKD.

One likely source of oxidant stress in HIV-1 infection is NADPH oxidase. In various cell lines, the HIV-1 proteins Tat, gp120, and gp160 – which circulate in free form or as components of intact blood-borne virions – have been shown to activate NADPH oxidase.<sup>13-19</sup> In particular, a phagocyte-type NADPH oxidase is expressed in T lymphocytes,<sup>20</sup> and both Tat and gp160 have been shown to trigger its activation in these cells.<sup>19</sup> NADPH oxidase may be viewed as the chief source of pathogenic oxidant stress in the majority of disease states;<sup>21</sup> it would therefore not be surprising if it played a leading role in the oxidant stress associated with HIV-1 infection. Although the route by which Tat activates NADPH oxidase is not clear, it is notable that Tat activates both PKC-betaII and PKC-delta in monocytes;<sup>22</sup> these would be expected to phosphorylate p47phox, an essential step in NADPH activation.<sup>23</sup> PKC-delta activation could also contribute to NF-kappaB activation, as outlined above.

### **NADPH Oxidase as a Mediator of HIV-1 Cytopathology**

NADPH oxidase may also be a mediator of the excessive apoptosis that depletes the pool of CD4+ lymphocytes as AIDS progresses. Tat, via its impact on oxidant generation, has been shown to markedly potentiate activation-induced cell death (AICD), a phenomenon that is accelerated in AIDS and suspected to contribute notably to CD4+ depletion.<sup>24-26</sup> In previously activated T cells, engagement of the T cell receptor and its accessory receptor CD4 generates two signals – an increase in free intracellular calcium and a burst of oxidant stress.<sup>26</sup> The downstream effects of these signals – which include activation of NF-kappaB, Egr, and NF-AT<sup>27;28</sup> – lead to increased transcription and expression of Fas ligand, which can then trigger Fas-mediated apoptosis in T cells (which constitutively express Fas); activated T cells which concurrently express the Fas-inhibitory protein FLICE are however spared from activation-induced death.<sup>29;30</sup> Both the calcium and oxidant signals play an obligate role in triggering this process.<sup>26</sup>

Krammer and colleagues have recently reported that apoptotic T cell death evoked by T cell receptor engagement (as mimicked by treatment with anti-CD3 antibodies) is greatly potentiated by pre-incubation with Tat, which boosts the strength of the evoked oxidative signal.<sup>26</sup> Moreover, engagement of CD4 alone – which triggers calcium influx – is sufficient to induce Fas ligand-mediated apoptotic cell death in cells pretreated with Tat. The authors note that, in HIV-1-infected patients, CD4 activation could be mediated by gp120; thus, Tat and gp120 could collaborate in inducing AICD in lymphocytes even without antigen-dependent stimulation. The increased cell death associated with Tat pretreatment may reflect, in part, the fact that the oxidative stress mediated by Tat sensitizes T cells to Fas ligand-mediated apoptosis.<sup>31</sup>

In the T lymphocytes of chimpanzees, Tat fails to evoke oxidative stress or potentiate AICD.<sup>32</sup> Perhaps this explains why, even though HIV-1 produces productive infections

in this species, it fails to induce an immunodeficiency state or deplete CD4+ lymphocytes.

Activation of NADPH oxidase may also play a role in some of the ancillary syndromes associated with AIDS, such as AIDS-induced dementia. The ability of gp120 to induce apoptosis in cultured human primary neurons has been traced to activation of neural NADPH oxidase.<sup>17</sup> Moreover, activated microglia are suspected to contribute to neuronal death in this syndrome,<sup>33-35</sup> and one of the most prominent neurotoxins produced by such microglia is peroxynitrite, whose production requires activation of microglial NADPH oxidase.<sup>36;37</sup>

### **Bilirubin to the Rescue**

Recently, Devadas and Dhawan have reported that treatment of HIV-1-infected monocytes with the heme oxygenase-1 (HO-1) inducer hemin dose-dependently suppresses expression of HIV-1 mRNA and proteins in these cells.<sup>38</sup> This effect was presumably mediated by HO-1 induction, since concurrent exposure to an HO-1 inhibitor. Moreover, preinduction of HO-1 substantially suppressed the infectibility of monocytes and T cells exposed to a range of clinical HIV-1 strains. This phenomenon was also demonstrated in vivo – in nude mice infused with human peripheral blood mononuclear cells, and subsequently infected with HIV-1, hemin treatment markedly lowered serum levels of p24, a marker to HIV-1 protein synthesis.

Free bilirubin, whose intracellular production is induced by HO-1 activity, is believed, along with carbon monoxide, to mediate the protective effects of HO-1.<sup>39</sup> The profound antioxidant activity of free bilirubin has recently been traced to its ability to inhibit NADPH oxidase in low nanomolar concentrations.<sup>21;40-42</sup> Thus, it is reasonable to suspect that inhibition of NADPH oxidase – resulting in reduced activation of NF-kappaB - is a key mediator of the protection afforded by HO-1 to HIV-1 exposed cells. Of course, the possibility that the carbon monoxide produced by HO-1 activity likewise contributes to this protection cannot be excluded – albeit the chief physiological target of CO is guanylate cyclase, and there appears to be little evidence that cGMP modulates HIV-1 infection.

Remarkably, there are two previous reports that bilirubin or its precursor biliverdin can inhibit the infectivity or pathogenicity of HIV-1 in cells cultures.<sup>43;44</sup> These authors however focused on the possibility that these agents were either inhibiting reverse transcriptase or interacting directly with HIV-1 virions to block entry into target cells.

### **Phycocyanobilin – A Nutraceutical Inhibitor of NADPH Oxidase**

Very recently, phycocyanobilin (PCB), a biliverdin derivative that functions as a chromophore in cyanobacteria (or blue-green algae) and can constitute up to 0.6% of the dry mass of these organisms, has been shown to inhibit NADPH oxidase in human cell cultures with a potency comparable to that of biliverdin (Inoguchi T, personal communication, and ref 21). This effect presumably reflects the fact that, within cells,

the ubiquitously expressed biliverdin reductase can convert PCB to phycocyanorubin, a homolog of bilirubin.<sup>45</sup> The fact that oral administration of spirulina (or of phycocyanin – the holoprotein that includes PCB as a covalently-bound chromophore) induces a wide range of potent anti-inflammatory effects in rodent studies strongly suggests that spirulina-bound PCB can be absorbed following ingestion and act as a systemic inhibitor of NADPH oxidase activity.<sup>46-50</sup> This has obvious implications for clinical control – and possibly prevention – of HIV-1 infection if NADPH oxidase does indeed play a prominent role in the pathogenesis of AIDS.

In this regard, it is intriguing to note the observation of Teas et al. that the prevalence of HIV-1 infection is anomalously low (about 3%, quite low for sub-Saharan Africa) in Chad – where the Kanembu tribe traditionally consumes 1-2 tablespoons of spirulina daily; this organism grows wild in Lake Chad.<sup>51</sup> However, these authors focus on evidence that spirulina contains immunostimulatory polysaccharides that potentially could promote improved Th1 activity in HIV-1-infected patients. Indeed, these polysaccharides have been shown to stimulate monocytes via TLR2 receptors;<sup>52</sup> as a result, the ability of antigen-presenting cells to promote a Th1 response is boosted, leading to increased production of IL-12 and interferon-gamma – cytokines that are suppressed in AIDS.<sup>25;53</sup> Similar immunostimulatory effects were noted in human volunteers given hot water extracts of spirulina orally.<sup>54</sup> Thus, it is reasonable to anticipate that spirulina could exert two independent but complementary effects in HIV-1 infection – an antioxidant effect, mediated by PCB, that could diminish the propagation and pathogenicity of HIV-1, and an immunostimulatory effect, mediated by polysaccharides, that could provide some compensation for the suppressive impact of HIV-1 on Th1 immunity. Of interest in this regard is a report that IL-12 protects T lymphocytes from AICD.<sup>55</sup>

Alternative or adjunctive approaches to down-regulating NADPH oxidase activity in HIV-1 infected individuals could include oral administration of biliverdin or PCB as nutraceuticals, or administration of agents that either induce HO-1 (such as hemin)<sup>38</sup> or that boost plasma levels of free autogenous bilirubin by inhibiting the glucuronosyl transferase that conjugates it in the liver (thereby mimicking Gilbert syndrome).<sup>21</sup>

### **NADPH Oxidase May Mediate Cardiovascular Risk Associated with HAART**

Consideration should also be given to the likelihood that NADPH oxidase activation is a mediator of the increased cardiovascular risk associated with long-term HAART therapy.<sup>56;57</sup> Indeed, patients receiving this therapy are at increased risk for metabolic syndrome, and activation of NADPH oxidase in vascular endothelium is suspected to play a key role in the atherogenesis and increased coronary risk associated with this syndrome.<sup>58-61</sup> Moreover, there is recent evidence that activation of NADPH oxidase in hypertrophied adipocytes mediates the insulin resistance and altered adipocytokine production by these cells in insulin resistance syndrome associated with obesity;<sup>62;63</sup> the possibility that the protease inhibitors which induce lipodystrophy and metabolic syndrome could activate NADPH oxidase in adipocytes merits evaluation. Indeed, there is a recent report that oxidant stress is markedly increased in adipocytes exposed to nelfinavir.<sup>64</sup>

## Adjunctive Measures

More generally, it is hardly novel to suggest the use of nutraceutical antioxidants in HIV-1 infection; various antioxidants have been shown to inhibit NF-kappaB activation and thus HIV-1 expression in infected cells in vitro.<sup>9;65;66</sup> Two antioxidants with particular clinical potential in this regard are melatonin and selenium. The former, via receptor-dependent mechanisms, boosts the expression of antioxidant enzymes as well as glutathione in many types of cells.<sup>67-69</sup> However, melatonin has the further merit that it boosts the ability of antigen-presenting cells to produce IL-12 and thereby stimulate a Th1 response.<sup>70-72</sup> Moreover, for reasons that remain obscure, melatonin secretion tends to fall off precipitously as AIDS progresses – a phenomenon that is accompanied by a substantial reduction in plasma IL-12 levels.<sup>73</sup> It seems likely that melatonin replacement could provide some immunostimulatory benefit to AIDS patients, particularly those in whom endogenous melatonin production is declining.

With respect to selenium, it is well known that adequate selenium nutrition is required for optimal activity of the several antioxidant enzymes, including the various isoforms of glutathione peroxidase as well as thioredoxin reductase.<sup>74</sup> However, selenium also has a high-dose immunostimulant effect, not mediated by selenium-dependent enzymes, that boosts expression of IL-2 receptors in lymphocytes and NK cells.<sup>75-77</sup> Each of these effects would be expected to be helpful during HIV-1 infection, and indeed several clinical studies have demonstrated that moderate-dose selenium supplementation (typically 200 mcg daily) has a very favorable impact on clinical course in AIDS patients, whether or not they are concurrently treated with HAART.<sup>78;79</sup>

As noted, activation of NF-kappaB is a key mediator of the pernicious impact of oxidant stress during HIV-1 infection. Thus, additional practical measures for suppressing NF-kappaB activation may be appropriate in the management of this infection.<sup>9</sup> In this regard, both salicylate (in the high doses traditionally used to treat inflammatory arthritis) and silymarin may have clinical potential.<sup>80-82</sup> Topical salicylate has been reported to block the inductive impact of uv-B exposure on HIV-1 expression in skin samples obtained from HIV-positive patients.<sup>83</sup> Salicylate may also have potential for prevention of the cachectic loss of muscle protein often seen in late stage AIDS patients, which likely is mediated by NF-kappaB activation in skeletal muscle fibers.<sup>84</sup>

It should be borne in mind that both NADPH oxidase and NF-kappaB play key roles in immune function. The goal in HIV-1 management should therefore be to down-regulate their activities, not to eliminate them! However, using these as targets in HIV-1 management has its advantages – whereas drugs that target viral proteins have the drawback that viral mutations can render them ineffective, agents which target normal physiological pathways can be expected to have durable efficacy.<sup>9</sup>

## Overview

The possibility that NADPH oxidase is a key mediator of the pathogenicity of HIV-1 – and its corollary that ingestion of spirulina (or PCB or biliverdin) may have utility in the management or possibly prevention of HIV-1 infection – could have exciting practical implications. Evidently, spirulina or PCB could be used as a relatively low-cost adjuvant to HAART therapy, aiding clinical control while also likely reducing the long-term adverse cardiovascular effects of such therapy. But such measures might take on greater significance in Third World populations which have had limited access to HAART for financial reasons. Indeed, if initial pilot trials can confirm that spirulina or PCB have a favorable impact on the course of HIV-1 infection, it would be of interest to explore the impact and feasibility of spirulina ingestion as a strategy for controlling HIV-1 in sub-Saharan Africa or other Third World populations at high risk. As Teas' suggests, perhaps spirulina could prove to be the "poor man's HAART".<sup>51</sup> Other low-cost agents such as melatonin, selenium, salicylate, and silymarin would likely complement its efficacy.

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