

Oxidative Stress, HIV and AIDS

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As long ago as 1983, one of us (E.P.-E.) proposed that oxidative mechanisms are of critical significance in the genesis of AIDS (acquired immune deficiency syndrome). A prediction of this hypothesis was that the mechanisms responsible for AIDS could be reversed by the administration of reducing agents, especially those containing sulphhydryl groups (SH groups). The discovery of HIV resulted in a broadening of this hypothesis in that it considered oxidative stress as a principal mechanism in both the development of AIDS and expression of HIV (Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos et al., 1989). However, the general acceptance of the HIV hypothesis of AIDS completely overshadowed this alternative hypothesis, and although many other scientists have questioned the role of HIV in the causation of AIDS (Duesberg, 1987; Root-Bernstein, 1990) Robert Gallo and most AIDS researchers consider HIV to be the sole "sine qua non" cause of AIDS.

Notwithstanding, some flaws, especially recently, have appeared which cast serious doubt on the prevailing HIV/AIDS hypothesis. Luc Montagnier, the discoverer of HIV, is presently of the opinion that cofactors are necessary for the appearance of AIDS (Lemaitre et al., 1990). It has been accepted by researchers at the CDC that KS (Kaposi's sarcoma), the first and most specific of the AIDS indicator diseases, for which the explanation of the HIV hypothesis was put forward by Gallo in 1982, is not caused directly or indirectly by HIV (Beral et al., 1990). On the other hand, recent empirical observations from three seemingly unrelated areas of AIDS research are in agreement with the hypothesis that oxidative mechanisms play a critical role in HIV expression and AIDS development.

(1) Pompidou et al. (1985a) and more recently researchers from many other institutions (Lang et al., 1988; Brewton et al., 1989; Reisinger et al., 1990; Hersh et al., 1991) have shown that a reducing agent, diethyl dithiocarbonate, previously used as an immunomodulator, and inhibitor of tumour promotion, may be useful in improving the immune response in HIV infected individuals and in preventing and treating AIDS. Other reducing agents have also been found to have similar effects (Schulof et al., 1986; Wu et al., 1989).

(2) In 1989, Eck et al. measured the level of acid soluble-SH groups in plasma and the intracellular concentration of reduced glutathione (GSH) in peripheral blood mononuclear cells (PBMC) and monocytes in HIV-infected patients: both were found to be significantly decreased. Following the above report, Buhl et al. (1989) determined the glutathione concentration (reduced, oxidised and total) in plasma and lung epithelial lining fluid of symptom-free HIV seropositive individuals: in both tissues, both the reduced and total GSH concentration was found to be significantly decreased.

(3) In 1985, Pompidou et al. (1985b) and more recently many other researchers including Anthony Fauci have shown that reducing agents suppress the expression of HIV (Scheib et al., 1987; Bitterlich et al., 1989; Kalebic et al., 1991).

Because of the possible therapeutic implications of reducing agents in AIDS patients it is important to have a basic understanding as to why:

- reducing agents suppress the expression of HIV;

- asymptomatic HIV-infected individuals and AIDS patients have decreased sulphydryl and total glutathione levels.

HIV expression and reducing agents

The answer to the first question is encompassed in basic retroviral research conducted over half a century. It is well known that all cells contain retroviral genomic sequences (Martin et al., 1981 ; Callahan et al., 1989; Nakamura et al., 1991). Recently French researchers suggested that human DNA also contains sequences which are homologous with the HIV genome (Parravicini et al., 1988). Many eminent retrovirologists, including Weiss, did not exclude the possibility that retroviruses with gene sequences not originally present in cells may arise during the lifetime of the animal by duplication and/or recombination of endogenous proviruses or even by rearrangement of cellular DNA, caused by many factors including the pathogenic process itself, and that retroviruses may be the effect and not the cause of the disease (Weiss et al., 1971).

According to Temin (1974) who shared the Nobel prize with Baltimore for the discovery of reverse transcriptase (RT) and who, from the time of its discovery considered the enzyme to be constituent of all cells not just retroviruses, the genome of a retrovirus (ribodeoxyvirus) may arise by rearrangement of the normal cell genome by the following mechanism. "A section of a cell genome becomes modified in successive DNA(w) to RNA(-) to DNA transfers until it becomes a ribodeoxyvirus genome. First, these sequences evolve as part of a cellular genome. After they have escaped as a virus they evolve independently as a virus genome. The time may be millions of years in germ-line cells and days in somatic cells". In fact, Temin and Baltimore (1972) did not exclude the possibility that, in at least some cases, particles which band at 1.16 g/ml contain RT and have morphological characteristics similar to retroviruses, may be nothing more than cellular fragments. Irrespective of the mechanism it is a fact, firmly established from basic retroviral research, that retroviruses can appear even in virus-free cultures with a rate that can be accelerated a million-fold by radiation, infection with other viruses and mitogens (Weiss et al., 1971 Aaronson et al., 1971).

Of particular relevance to the present discussion is the fact that all mitogenic agents including radiation exert their biological effect by oxidation of cellular sulphydryl groups (Papadopulos-Eleopulos, 1982).

Montagnier and his associate David Klatzmann were the first to draw attention to the fact that LAV infection of T4 cells in vitro does not lead to HIV expression unless the cells are stimulated. "Infection of resting T4 cells does not lead to viral replication or to expression of viral antigens on the cell surface, while stimulation by lectins or antigens of the same cells results in production of viral particles, antigenic expression and the cytopathic effect" (Klatzmann and Montagnier, 1986). Gallo also expressed the view that without "activation" the T4 cells do not express virus (Zagury et al., 1986). But, apparently, they did not realise that oxidative phenomena are implicated in human T-cell stimulation (Sekkat et al., 1988).

As early as 1984 it was realised that in vivo HIV genomic sequences are not always detected in tissues obtained from patients with ARC and AIDS or, when found, the "signal" is low. According to Gallo and his colleagues "this low signal intensity could also be explained by the presence of a virus distantly homologous to HTLV-III in these cells" (Shaw et al., 1984).

Anthony Fauci and his colleagues, on comparing the evidence obtained from the study of macrophages in vivo and in vitro, concluded: "These data indicate that the ability to isolate in vitro macrophage tropic strains of HIV does not reflect in vivo infection of circulating monocytes, but is related to phenomena of in vitro selection or adaptation" (Massari et al., 1990).

Furthermore, (a) to date, with perhaps one exception, no two identical HIV have been isolated, not even from the same person; in one case where two sequential isolates were made 16 months apart, none of the provirus in the first isolate was found in the second (Saag et al., 1988); (b) the genetic data obtained in vitro does not correlate with the data obtained in vivo - "To culture is to disturb" (Meyerhans et al., 1989); (c) many, if not all, of the proviruses detected in vivo and in vitro are defective.

This data led researchers at the Pasteur Institute and their associates to conclude that (1) "the task of defining HIV infection in molecular terms will be difficult", (2) "virus isolated from PBMC may be produced by the complementation of defective genes or by recombination between two of them" (Meyerhans et al., 1989; Wain-Hobson, 1989). Be this as it may, of particular relevance to the present discussion is the fact that:

- a) HIV has been isolated only from in vitro cultures;
- b) no HIV can be isolated, unless the cultures, one way or the other, are subjected to oxidative stress, even although the tissue from AIDS patients is already oxidised; it may be then that oxidative stress is of pivotal significance in the detection of all retroviruses including HIV. If oxidation is a prerequisite for HIV expression, it follows that reducing agents will have the opposite effect: HIV will not be expressed in their presence.

Oxidative factors in AIDS patients

AIDS patients suffer from many opportunistic microorganisms. Like all cells, these microorganisms require reducing equivalents, including SH, for division and survival (Papadopulos-Eleopulos, 1982) which they obtain to the detriment of body tissues. In AIDS patients, a decrease in the level of SH may also result from malnutrition and diarrhoea. However, opportunistic infections, diarrhoea and malnutrition cannot account for the low level of GSH and acid-soluble SH found in HIV-positive, symptom-free, well-nourished homosexuals and haemophiliacs.

Since viral production also requires thiols, which they obtain from the host, it may be reasonable to assume that the decreased SH level in HIV-positive individuals may be the result of HIV infection, as has already been proposed for SIV-infected monkeys (Eck et al., 1991). However, because for both HIV and SIV expression, oxidative stress is a prerequisite, this cannot be the case, i.e. oxidation cannot be both the cause and the effect of HIV expression (Papadopulos-Eleopulos et al., 1991).

At first sight it appears that there is no common factor, apart from HIV infection, linking the various AIDS risk groups. However, homosexuals are exposed to relatively high levels of nitrites and anally deposited sperm, drug abusers to opiates and nitrites, haemophiliacs to factor VIII. All these are known potent oxidising agents which oxidise many cellular reducing equivalents such as NADPH and all sulphhydryl groups, including those of cysteine (acid-soluble thiols) (Papadopulos-Eleopulos, 1988).

In normal tissue almost all glutathione is found intracellularly in the reduced form (GSH) where it is also synthesised from glutamic acid, cysteine and glycine, in the presence of ATP and magnesium. Cysteine which is the rate-limiting amino acid cannot be substituted by its oxidised form, cystine. Oxidation of cysteine (acid-soluble SH) is also known to decrease cellular ATP and magnesium concentration (Tateishi and Higashi, 1978; Siliprandi et al., 1987). Malnutrition and diarrhoea may also lead to cysteine, magnesium and ATP deficiency.

As a result of the decrease in cysteine, ATP and magnesium concentration, the synthesis of glutathione will be inhibited. The oxidising agents to which the AIDS risk groups are exposed would also directly oxidise GSH to GSSG. GSSG is efficiently excreted from cells (Sies and Akerbrum, 1984). Glutathione exported across the cell membrane interacts with gamma-glutamyl transpeptidase, an enzyme which catalyses the breakdown of glutathione by transferring the gamma-glutamyl group to an acceptor.

It should be noted that: cystine is one of the best acceptors for the gamma-glutamyl group; with exception of the kidney and pancreas, the highest activity of the enzyme is in the epididymis and seminal vesicles; the highest concentration of its soluble form, apart from urine and pancreatic juice, is in seminal fluid (Meister and Anderson, 1983). Thus, the systemic decrease of glutathione concentration in HIV seropositive individuals may result from both, decrease in synthesis and increased degradation. The oxidative stress to which the AIDS patients are subjected would lead to cellular anomalies in many cells,

including lymphocytes, resulting in opportunistic infection, immunological abnormalities and neoplasia.

All this argues in favour of oxidation as being a critical factor in the pathogenesis of AIDS and HIV expression.

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