A CRITICAL ANALYSIS OF THE HIV-T4-CELL-AIDS HYPOTHESIS

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Knowledge is one. Its division into subjects is a concession to human weakness. - Halford John Mackinder

ABSTRACT

The data generally accepted as proving the HIV theory of AIDS, HIV cytopathy, destruction of T4 lymphocytes, and the relationsip between T4 cells, HIV and the acquired immune deficiency clinical syndrome are critically evaluated. It is concluded these data do not prove that HIV preferentially destroys T4 cells or has any cytopathic effects, neither do they demonstrate that T4 cells are preferentially destroyed in AIDS patients, or that T4 cell destruction and HIV are either necessary or sufficient prerequisites for the development of the clinical syndrome.

INTRODUCTION

With few exceptions by workers who either reject it (Duesberg, 1987, 1992; Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos et al., 1989a; Papadopulos-Eleopulos, Turner & Papadimitriou, 1992a, 1993b), or who postulate the necessity for cofactors (Lemaitre et al., 1990; Root-Bernstein, 1993), the currently accepted HIV theory of AIDS pathogenesis states that:

1. HIV causes destruction of T4 (helper) lymphocytes, that is, acquired immune deficiency, AID;

2. AID leads to the appearance of Kaposi's sarcoma (KS), Pneumocystis carinii pneumonia (PCP) and certain other "indicator" diseases which constitute the clinical syndrome, S.

For this to constitute a valid theory of AIDS pathogenesis the minimum requirements are:

1. HIV, is both necessary and sufficient for destruction of T4-cells;

2. Decrease in T4 lymphocytes (AID) is both necessary and sufficient for the appearance of the clinical syndrome, S;

3. All AIDS patients are infected with HIV.

Evidence will be presented which shows that the HIV/AIDS hypothesis as stated above, cannot be considered proven by the data presently available. Reference will be made to an oxidative theory (Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos, Turner & Papadimitriou, 1992a, 1992b) which claims that the immunological abnormalities seen in AIDS patients, including decreased numbers of T4 lymphocytes, as well as the clinical syndrome, are induced by oxidising agents and not HIV.

CYTOPATHIC EFFECTS OF HIV

According to Gallo and his colleagues, "HIV has been shown to have a direct cytopathic effect" (cell killing effect) on CD4+ cells, firstly by Montagnier and his colleagues in 1983, and then by him (Gallo) and his colleagues in a series of four papers published in Science in 1984 (Shaw et al., 1988). However, in the 1983 paper where Montagnier and his colleagues describe the isolation of HIV from a homosexual patient with lymphadenopathy, no evidence is presented regarding the biological effects of HIV (Barre-Sinoussi et al., 1983). Although Gallo claims that in the four Science papers (Gallo et al., 1986) he and his colleagues "provided clearcut evidence that the aetiology of AIDS and ARC was the new lymphotropic retrovirus, HTLV-III", no such data were presented. (Papadopulos-Eleopulos et al., 1993b) Reference to the cytopathic effects is made only in the first paper where it was stated "The virus positive cultures consistently showed a high proportion of round giant cells containing numerous nuclei", (syncytia) (Popovic et al., 1984). The cultures described in that paper utilised clones of the HT cell line; however, it subsequently became known that the HT line used by Gallo is in fact HUT78, (Rubinstein, 1990) a cell line established from a patient with mature T4-cell leukaemia (Gazdar et al., 1980; Gallo, 1986). It has been shown however, that other cell lines established from patients with mature T4-cell leukaemia have multinucleated giant cells (Poiesz et al., 1980) and therefore, one may expect to find giant cells containing numerous nuclei in the HT (clones) cell cultures even in the absence of HIV. At present, evidence also exists showing that other cells permissive for HIV, monocyte-derived macrophages, "in the absence of infection", form syncytia during cultivation (Collman et al., 1989).

Later, Gallo expressed the view that syncytial formation and direct cell killing are unlikely to be the major pathway for cell loss. In addition, cells infected by several viruses produce extensive syncytia without cytopathy (Shaw et al., 1988).

In 1985, Gallo and his colleagues (Gallo et al., 1985) showed that in mitogenically stimulated lymphocyte cultures from AIDS patients or in cultures from healthy donors "infected" with HIV, there is a decrease in the total number of viable cells. However:

(i) the decrease in viable cells begins before a significant increase in reverse transcriptase activity (RT), that is, HIV expression;

(ii) the rate of cell loss remains the same even when the expression of HIV (RT), is maximum. These suggest that the cause of the decrease in viable cells may not be HIV. Since then other researchers have shown that:

(a) "lymphocytes may be productively infected in the absence of cell death" (Hoxie et al., 1985);

(b) the presence or absence of the cytopathic effects is a function of the cell type (cell line), culture conditions (presence or absence of interleukin-2 (IL-2),

presence or absence of serum, fibrinogen, fibronectin, alpha-globulin), and the origin of the HIV preparation (von Briesen et al., 1987; Ushijima et al., 1992);

(c) early in 1986, Zagury, Gallo and their colleagues reported that: "T4 lymphocytes from normal donors infected by HTLV-III in vitro, as well as HTLV-III-infected primary T4 cells from AIDS patients, have been difficult to maintain in culture for longer than 2 weeks, and it has often been assumed that the virus has a direct cytolytic effect on these cells". However, by avoiding PHA stimulation and by reducing the number of cells per millilitre of culture medium from 105-106 to 103-104, they were able to "grow the infected cells for 50-60 days" without cellular degeneration which, according to them, was due to "the lack of further antigenic stimulation and, presumably, the reduced concentrations of toxic substances released by the mature cells" (Zagury et al., 1986);

(d) cytopathy does not always correlate with RT activity, that is, HIV expression. "In fact, there was sometimes an inverse correlation in CEM cells, with the high RT isolates exhibiting a slower inhibition of cell division and reduction of viability than the low RT-producing viruses" (Cloyd & Moore, 1990).

In other words, the correlation between HIV production and decreased cellular viability is not as the HIV hypothesis predicts, especially if, as is presently accepted, that "Although the effect of HIV on the immune system resembles autoimmune disease, it is driven by persistent active, viral expression" (Weiss, 1993). Despite all these data, consensus still prevails that HIV infection leads to a "quantitative decrease in the TH-cell population that will lead to acquired immune deficiency syndrome (AIDS)" (Ameisen & Capron, 1991) [TH/T4]. However, no agreement exists as to the mechanism by which HIV kills T4 cells.

According to Claude Ameisen and Andre Capron from the Pasteur Institute, not one of the mechanisms "proposed to account for these TH-cell defects, including: (1) immune suppression, or its opposite, hyperactivation and exhaustion of the TH cells, (2) inhibitory signals mediated by HIV viral or regulatory gene products, (3) autoimmune responses, (4) selective infection and destruction of memory TH cells, (5) syncytia formation between infected and uninfected cells, and (6) inappropriate immune killing of uninfected cells", is satisfactory.

Instead, in 1991 they put forward the hypothesis "that a single unique mechanism, activation-induced T-cell death [also known as programmed cell death (PCD) or apoptosis] can account for both the functional and numerical abnormalities of T4 cells from HIV infected patients...We propose that the simplest explanation of TH-cell defects leading to AIDS is that HIV infection leads to an early priming of TH cells for a suicide process upon further stimulation. In HIV infected patients, circulating gp120, gp120-antibody immune complexes or anti-CD4 autoantibodies, that all bind CD4, may represent appropriate candidates for the priming of T cells for a PCD response following activation" (Ameisen & Capron, 1991). In support of their theory they reported that stimulation of peripheral blood mononuclear cells (PBMC) of asymptomatic HIV infected individuals with pokeweed mitogen (PWM) or staphylococcal enterotoxin B (SEB), "was followed by cell death", whereas no death was observed at 48h in the unstimulated cells. Cell death was only observed in the CD4+ enriched population and not in the CD8+ lymphocytes. Cell death was not found in unstimulated or stimulated PBMC from HIV- negative individuals (Groux et al., 1991; Groux et al., 1992). However, to date, "no evidence for circulating soluble gp120 has yet been reported" (Capon & Ward, 1991), or for gp120- antibody immune complexes in AIDS patients. Furthermore, although in the following years, researchers from many institutions published data confirming the apoptotic death of PBMC cultures from HIV infected individuals, their data seem to contradict both Ameisen and Capron's experimental findings as well as their proposed mechanism of HIV induced apoptosis:

1. Addition of anti-gp120 or anti-CD4 monoclonal antibodies (MCA) to HIV infected cultures permitted sustained high levels of viral replication, but blocked apoptosis and cell death (Terai et al., 1991; Laurent-Crawford et al., 1992);

2. Experiments performed on cultures with or without stimulation showed "both CD4+ and CD8+ cells from HIV-infected individuals die as a result of apoptosis" (Meyaard et al., 1992).

In a 1991 paper, published in Virology (Laurent-Crawford et al., 1991), Montagnier and his colleagues showed that:

> (a) in acutely HIV infected CEM cultures in the presence of mycoplasma removal agent, cell death (apoptosis) is maximum at 6-7 days post infection, "whereas maximal virus production occurred at Days 10-17"--that is, maximum effect precedes maximum cause;
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> (b) in chronically infected CEM cells and the monocytic line, U937, no apoptosis was detected although "These cells produced continuously infectious virus";
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> (c) in CD4 lymphocytes isolated from a normal donor, stimulated with PHA and infected with HIV in the presence of IL-2, apoptosis becomes detectable 3 days post infection and clearly apparent at 4 days. "Intriguingly, on the 5th day" apoptosis "became detectable in uninfected, PHA stimulated cells". Figure 9, where the data are presented, shows approximately the same degree of "apoptotic events" in the PHA cultures at 5 days as in the PHA+HIV cultures on the 4th day "post infection".

They concluded: "These results demonstrate that HIV infection of peripheral blood mononuclear cells leads to apoptosis, a mechanism which might occur also in the absence of infection due to mitogen treatment of these cells... Interestingly, HIV infection of such mitogen stimulated cells resulted in a slight acceleration of the first signs of apoptosis, thus indicating the intrinsic effect of HIV infection" (Laurent-Crawford et al., 1991).

The conclusion that HIV has an "intrinsic effect" on PCD can be questioned on several grounds:

1. The "slight acceleration of the first signs of apoptosis" in the stimulated HIV infected cultures, as compared to the non-HIV infected stimulated cultures, may not be due to HIV but to the many non-HIV factors present in "HIV" inocula, including:

   > (a) Mycoplasmas and other infectious agents;
   >
   > (b) The many cellular proteins present in the "HIV preparation" (Henderson et al., 1987);
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   > (c) PHA, present in the cultures from which the "HIV preparation" was derived;

2. That HIV is not the cause of apoptosis is also indicated by the fact that in chronically infected cell lines in which virus is continuously produced, apoptosis is not detected;

3. That HIV may play no role in apoptosis is also suggested by the presently accepted mechanism of apoptosis. Apoptosis occurs both in healthy and in pathological conditions, is frequently prominent amongst the proliferating cells of lymphoid germinal centres, and can be enhanced by numerous agents including radiation, cytotoxic drugs, corticosteroids and the calcium ionophore A23187 (Kerr & Searle, 1972; Don et al., 1977; Wyllie et al., 1980; Wyllie et al., 1984).

Apoptosis is cellular death characterised by morphological criteria: cellular condensation, DNA fragmentation, and plasma membrane "blebbing" leading to the release of "apoptic bodies" which vary widely in size and some of which contain pyknotic chromatin surrounded by intact membranes (Kerr & Searle, 1972; Don et al., 1977; Wyllie et al., 1980; Wyllie et al., 1984). These changes are thought to be induced by increased concentration of Ca++ which in its turn induces contraction of the cytoskeleton whose main components are known to be the ubiquitous proteins, actin and myosin (Jewell et al., 1982; Cohen & Duke, 1984; McConkey et al., 1988; McConkey et al., 1989; Reed, 1990).

However, evidence exists indicating that intracellular Ca++ concentration and contraction of the actin-myosin system (cellular condensation), are induced by perturbances in the cellular redox state (Papadopulos-Eleopulos et al., 1985; Papadopulos-Eleopulos et al., 1989b). In fact, for more than a decade, evidence has existed showing that oxidising agents, including all mitogenic (activating) agents, can induce: reversible cellular changes; cellular activation; malignant transformation; mitogen unresponsive cells; or cellular death, including death by apoptosis. The ultimate outcome depends on the concentration of the agent, its rate of application, the initial state of the cells and the cellular milieu (See reference (Papadopulos-Eleopulos, 1982)).

More recent data confirm the fact that the intracellular free Ca++ concentration is regulated by the cellular redox state. Oxidation leads to an increased, and reduction to a decreased, Ca++ concentration (Trimm et al., 1986). Cellular surface blebbing (Jewell et al., 1982; Lemasters et al., 1987; Reed, 1990), chromatin condensation (Pellicciari et al., 1983), and apoptosis (Morris et al., 1984) are the direct result of cellular oxidation in general and of cellular sulphydryl groups in particular. This is supported by Montagnier's group's recent finding that apoptosis can be inhibited by reducing agents (Rene et al., 1992). (In fact, at present, Montagnier (Gougeon & Montagnier, 1993) agrees with our view that anti-oxidants should be used for treatment of HIV/AIDS patients (Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos et al., 1989a; Turner, 1990; Papadopulos-Eleopulos et al., 1992a; Papadopulos-Eleopulos et al., 1992b)). At present it is also known that:

> (a) for the expression of HIV phenomena (RT, virus-like particles, antigen/antibody reactions), activation (mitogenic stimulation) is a necessary requirement (Klatzmann & Montagnier, 1986; Ameisen & Capron, 1991; Papadopulos-Eleopulos et al., 1992b);
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> (b) activation (stimulation) is induced by oxidation (Papadopulos-Eleopulos, 1982; Papadopulos-Eleopulos et al., 1992b); Since both AIDS cultures and AIDS patients are exposed to mitogens (activating agents), all of which are oxidising agents (Papadopulos-Eleopulos, 1988), both apoptosis and the phenomena upon which the presence of HIV is based (viral-like particles, RT, antigen/antibody reactions (WB), "HIV-PCR- hybridisation"), may all be the direct result of oxidative stress and therefore their specificity questionable (Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos et al., 1992a; Papadopulos-Eleopulos et al., 1992b).

As far back as January 1985 Montagnier wrote, "....replication and cytopathic effect of LAV can only be observed in activated T4 cells. Indeed, LAV infection of resting T4 cells does not lead to viral replication or to expression of viral antigen on the cell surface, while stimulation by lectins or antigens of the same cells results in the production of viral particles, antigenic expression and the cytopathic effect" (Klatzmann & Montagnier, 1986). One year later Gallo and his colleagues wrote: "the expression of HTLV-III was always preceded by the initiation of interleukin-2 secretion, both of which occurred only when T-cells were immunologically [PHA] activated. Thus, the immunological stimulation that was required for IL-2 secretion also induced viral expression, which led to cell death" (Zagury et al., 1986). Thus, relatively early after the appearance of AIDS it was known that HIV is not sufficient for the appearance of the cytopathic effects. For some unknown reason, up till 1991 very little (or no) data was

presented regarding the effects of the activating agents themselves on cell survival. However, in the above discussed 1991 Virology paper, Montagnier and his colleagues showed that activation, in the absence of HIV, can induce the same cytopathic effects. In other words, Montagnier and his colleagues have shown that HIV is neither necessary nor sufficient for the induction of the cytopathic effects observed in HIV infected cultures. Thus, the presently available evidence from the in vitro studies does not prove that HIV has direct cytopathic effects on any T-cells, T4 or T8. The cytopathic effects observed in the cultures are most likely caused by the many activating (oxidising) agents to which the cultures are exposed. Even if HIV were shown to have cytopathic effects, since it is accepted that "The hallmark of AIDS is a selective depletion of CD4-bearing helper/inducer" lymphocytes (Shaw et al., 1988), the available evidence must show that T4 cells are preferentially destroyed in individuals at risk of developing the clinical syndrome.

HIV AND THE T4 CELLS

Using MCA for serial measurement of CD4 and CD8 expressing lymphocytes in mitogenically stimulated HIV infected cultures, it has been shown that in cultures prepared such that the majority (>95%) of lymphocytes are purified T4 cells, there is a progressive disappearance of CD4 expressing cells. This observation was interpreted by Gallo and others "that HTLV-III has a cytopathic effect on OKT4-positive (OKT4+) cells" (Fisher et al., 1985). However, according to Klatzmann, Montagnier and other French researchers "this phenomenon could not be related to the cytopathic effect" of HIV but is "probably due to either modulation of T4 molecules at the cell membrane or steric hindrance of antibody-binding sites" (Klatzmann et al., 1984a Klatzmann et al., 1984b). That is, the decrease in T4 cells is not due to destruction of cells but due to a decrease in MCA binding to their surface. Nevertheless, the above data were interpreted as evidence for selective infection and killing of T4 cells by HIV, and together with the fact that "we knew of no agents, aside from a family of human T- lymphotropic retroviruses that we had discovered three years earlier and named human T-cell leukaemia (lymphotropic) virus (HTLV), that demonstrated such tropism to a subset of lymphocytes", was presented as one of two arguments in support of the HIV hypothesis of AIDS (Gallo et al., 1985). (The other argument was based on the perceptions that AIDS was a new disease and the epidemiology was consistent with an infectious cause).

However:

(a) HIV cultures/co-cultures are stimulated with such oxidising agents as PHA, ConA, radiation, PMA, polybrene and IL-2;

(b) these agents at relatively low concentration can induce decrease in CD4 expressing cells, in the absence of HIV (Acres et al., 1986; Hoxie et al., 1986; Zagury et al., 1986; Scharff et al., 1988), without killing T4 cells.

(c) in 1986, Zagury, Gallo and their associates (Zagury et al., 1986), prepared T-cell cultures (which contained 34% CD4+ cells), from normal donors. Cultures were stimulated with PHA and were (i) "infected" with HIV; (ii) left uninfected. Control cultures remained both unstimulated and uninfected. After 2 days of culture, the proportion of CD4+ cells in the stimulated-uninfected and stimulated-infected cultures was 28% and 30% respectively, while at 6 days the number was 10% and 3%; the controls not changing significantly. Thus, HIV is not necessary for the disappearance of CD4 expressing cells, as measured by the use of MCA in "HIV infected" stimulated cultures. The stimulants can induce the effect in the absence of "HIV". Furthermore, the decrease in T4 cells may not be due to destruction of T4 cells but to a decrease in the number of cells binding MCA.

Even if the in vitro evidence shows that HIV is a cytopathic retrovirus and that it preferentially infects and kills T4 lymphocytes, evidence must exist that the same effect takes place in vivo, that is, patients infected with HIV have diminished numbers of T4 cells which is caused by preferential infection and killing of these cells by HIV.

Following the frequent diagnosis of KS, PCP and other opportunistic infections (OI) in gay men and intravenous (IV) drug users, it was realised, when T lymphocytes of these patients were reacted with MCA to the CD4 antigen, the number of CD4 antigen bearing cells is diminished. This led to a diagnosis of "acquired immune deficiency" defined as a decrease in T4 cell number, which was thought then and now to be due to the death of T4 cells. This finding, together with the then known fact that patients who were treated with the so called immunosuppressive drugs or who suffered from "immunosuppressive illness" had relatively high frequencies of KS and OI, led to the conclusion that the high frequencies of these diseases in gay men, IV users as well as haemophiliacs amongst others, were the direct result of suppressed cellular immunity (immunosuppression) defined by diminished numbers of T4 helper cells (cell-mediated immunodeficiency). In 1982, the Center for Disease Control (CDC) defined a case of AIDS as "illnesses in a person who 1) has either biopsy-proven KS or biopsy-or culture-proven life-threatening opportunistic infection, 2) is under age 60, and 3) has no history of either immunosuppressive underlying illness or immunosuppressive therapy" (CDC, 1982). The claim by Gallo and his colleagues in 1984 that AIDS is caused by HIV led the CDC to redefine AIDS. In 1985 the CDC defined AIDS as:

> "I. one or more of the opportunistic diseases listed below (diagnosed by methods considered reliable) that are at least moderately indicative of underlying cellular immunodeficiency; and
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> II. absence of all known underlying causes of cellular immunodeficiency (other than LAV/HTLV-III infection) and absence of all other causes of reduced resistance reported to be associated with at least one of those opportunistic diseases.

Despite having all the above, patients are excluded as AIDS cases if they have negative result(s) on testing for serum antibody to LAV/HTLV-III, do not have a positive culture for LAV/HTLV-III, and have both a normal or high number of T- helper (OKT4 or LEU3) lymphocytes and a normal or high ratio of T-helper to T-suppressor (OKT8 or LEU2) lymphocytes. In the absence of test results, patients satisfying all other criteria in this definition are included as cases" (WHO, 1986).

This definition presupposes that proof exists or can be obtained that HIV is the sole cause of the acquired immune deficiency (decreased T4) which, in turn, leads to the appearance of the clinical syndrome. Such a proof can only be obtained by the administration of PURE HIV to healthy humans or, as Montagnier (Vilmer et al., 1984) pointed out in 1984, "Definite evidence will require an animal model in which such viruses could induce a disease similar to AIDS". At present no animal AIDS model exists and of course it is not ethical to administer HIV, pure or otherwise, to humans (Papadopulos-Eleopulos et al., 1993a). In the absence of the above one must, at the very least, have (indirect) evidence that:

> (a) in HIV positive individuals, at least by the time diseases attributed to HIV infection such as persistent generalised lymphadenopathy (PGL) and AIDS-related complex (ARC) have appeared, there is an abnormally low T4 cell number;
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> (b) in patients defined as AIDS cases the decrease in T4 cells follows and does not precede "HIV infection", as evidenced by a positive HIV antibody test;
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> (c) patients before, during or after seroconversion have not been exposed to any agents known to cause immunosuppression;

(d) following seroconversion there must be a steady decrease in T4 cell numbers. However, three years after seroconversion the majority of HIV positive individuals continue to have normal T4 cell counts (Detels et al., 1988). Even in the presence of PGL and other "constitutional symptoms of HIV-related diseases", a significant number of patients continue to have normal T4 cell numbers (T4/T8 ratio). In some individuals, seroconversion is followed by an increase, not a decrease in T4 cells (Detels et al., 1988; Natoli et al., 1993).

When AIDS was first diagnosed in gay men and IV drug users, but before the discovery of HIV, epidemiological data, some of which appeared in the Morbidity and Mortality Weekly Reports published by the CDC, rapidly accumulated which showed that in the 1970's, individuals from the AIDS risk groups suffered from many infectious and non-infectious diseases unrelated to AIDS. Data was recently presented from the Multicenter AIDS Cohort Study (Hoover et al., 1993) (MACS) which shows that HIV seropositive gay men "at least 1.67-3.67 years prior to a clinical diagnosis of AIDS", as well as HIV seronegative gay men, although the frequency in the latter is lower, suffer from a wide variety of complaints including fatigue, shortness of breath, night sweats, rash, cough, diarrhoea, headaches, thrush, skin discolouration, fever, weight loss, sore throat, depression, anaemia and sexually transmitted diseases. Evidence which existed at the beginning of the AIDS era, or which has accumulated since, shows that some of the diseases which occurred in these individuals, or the agents which caused them, including Epstein-Barr virus and CMV, are immunosuppressive (Papadopulos-Eleopulos, 1988). Many of the agents used in treatment, including corticosteroids and some antibiotics, as well as the recreational drugs used by both gay men and drug users, are also known to be immunosuppressive. From the start of the epidemic, the CDC was aware that approximately 50% of gay men used nasal cocaine and about the same proportion smoked marijuana. Nitrite use was considered practically ubiquitous.

That the immunosuppression found in AIDS patients is not caused by HIV is indicated by the fact that individuals from the AIDS risk groups may have low T4 cell numbers (T4/T8 ratio), even in the presence of a persistently negative HIV antibody test (Drew et al., 1985; Novick et al., 1986; Donahoe et al., 1987; Detels et al., 1988). Although one such study showed "reduced proliferative response to the T cell mitogen PHA in AIDS...PHA responses in symptomless HIV infection, with or without lymphadenopathy, were also significantly reduced compared to heterosexual controls. However seronegative homosexuals had similarly reduced PHA responses. Thus, in symptomless infection, HIV does not appear to cause more impairment than seen in their uninfected peers...Our findings re-emphasise the importance of using seronegative peer group controls in studies on HIV infection" (Rogers et al., 1989). In considering the data from haemophiliacs, a group of British researchers, including the well known retrovirologist Robin Weiss, concluded in 1985: "We have thus been able to compare lymphocyte subset data before and after infection with HTLV- III. It is commonly assumed that the reduction in T-helper- cell numbers is a result of the HTLV-III virus being tropic for T-helper-cells. Our finding in this study that T-helper- cell numbers and the helper/suppressor ratio did not change after infection supports our previous conclusion that the abnormal T-lymphocyte subsets are a result of the intravenous infusion of factor VIII concentrates per se, not HTLV-III infection" (Ludlam et al., 1985).

In relation to patients with haemophilia A, von Willebrand's disease and "hypertransfused patients with sickle cell anaemia" Kessler et al found that: "Repeated exposure to many blood products can be associated with development of T4/T8 abnormalities" including "significantly reduced mean T4/T8 ratio compared with age and sex-matched controls" (Kessler et al., 1983). In 1984, Tsoukas et al observed that amongst a group of 33 asymptomatic haemophiliacs receiving factor VIII concentrates, 66% were immunodeficient "but only half were seropositive for HTLV-III", while "anti-HTLV-III antibodies were also found in the asymptomatic subjects with normal immune function". They summarised their

findings as follows: "These data suggest that another factor (or factors) instead of, or in addition to, exposure to HTLV-III is required for the development of immune dysfunction in haemophiliacs" (Tsoukas et al., 1984).

By 1986 researchers from the CDC concluded: "Haemophiliacs with immune abnormalities may not necessarily be infected with HTLV-III/LAV, since factor concentrate itself may be immunosuppressive even when produced from a population of donors not at risk for AIDS" (Jason et al., 1986) (factor concentrate - factor VIII). In 1985 Montagnier (Montagnier, 1985) wrote: "This [clinical AID] syndrome occurs in a minority of infected persons, who generally have in common a past of antigenic stimulation and of immune depression before LAV infection", that is, Montagnier recognised that in the AIDS risk groups, AID appears before "HIV infection" [LAV-HIV]. A recent study of IV drug users in New York (Des Jarlais et al., 1993) showed that "The relative risk for seroconversion among subjects with one or more CD4 count <500 cells/uL compared with HIV-negative subjects with all counts >500 cells/uL was 4.53". A similar study in Italy (Nicolosi et al., 1990) showed that "low number of T4 cells was the highest risk factor for HIV infection", that is, decrease in T4 cells is a risk factor for seroconversion and not vice versa. The observations that T4 decrease precedes a positive antibody test ("HIV infection"), is additional (Papadopulos-Eleopulos et al., 1993a) evidence that factors other than HIV lead to both T4 decrease and positive "HIV" antibody tests.

Thus gay men, IV users and haemophiliacs, have "known underlying causes of cellular immunodeficiency (other than LAV/HTLV-III infection)", and therefore, according to the 1985 CDC AIDS definition, these individuals cannot be AIDS cases. The finding in individuals belonging to the above groups of a decreased T4 cell number and decreased T4/T8 ratio, even if due to killing of T4 cells and not to "modulation of T4 molecules at the cell membrane or steric hindrance of antibody-binding sites", cannot be interpreted as being caused by HIV. Nonetheless, from 1981 to the present, gay men, IV users and haemophiliacs form the vast majority of AIDS cases.

From the beginning, it was realised that in AIDS patients the decrease in T4 lymphocytes is accompanied by an increase in T8 lymphocytes while the total T cell population remains relatively constant. This has recently been confirmed by Margolick et al who showed that the decline in T4 cells in HIV positive individuals is accompanied by a T8 increase "with kinetics that mirrored the loss of CD4+ cells, resulting in a CD8 polarization" (Margolick et al., 1993; Stanley & Fauci, 1993).

This finding has been neglected until recently when a theory has been put forward to explain how infection of even a small proportion of T4 cells, (perhaps 1/1000) can have this effect. This theory states that "loss of either CD4+ or CD8+ T cells is detected by the immune system only as a decrease in CD3+ T cells. The compensatory response to such a selective decrease, then, is to generate both CD4+ and CD8+ T cells in order to bring the total CD3+ T cells back to a normal level. The consequence of this nonselective T cell replacement after a selective depletion of one T cell subset would be an alteration in the CD4 to CD8 ratio after normalization of the total T cell count with a polarization toward the subset that had not been initially depleted...repeated events of selective CD4+ T-cell killing will result in higher and higher CD8+ T- cell count and lower and lower CD4+ T-cell count" (Adleman & Wofsy, 1993; Margolick et al., 1993; Stanley & Fauci, 1993) However, a brief look at the history of the discovery of the T4 and T8 cells and the presently available data show that the above theory may not be valid.

In 1974, a group of researchers from the National Cancer Institute USA observed that when normal lymphocytes were cultured with T-cells from hypogammaglobulinaemic patients in the presence of PWM, the synthesis of immunoglobulin (antibodies) by the normal lymphocytes was depressed by 84% to 100%. They put forward the hypothesis "that patients with common variable hypogammaglobulinemia have circulating suppressor T lymphocytes

that inhibit B-lymphocyte maturation and immunoglobulin synthesis" (Waldman et al., 1974).

Subsequently, it was shown that ConA stimulated T cells from healthy animals "can under appropriate circumstances perform helper, suppressor, and killer functions" (Jandinski et al., 1976). By 1977 many studies of the cellular basis of the immune response had indicated that T cells have both suppressive and helper activities and it was concluded that "these activities are specialized functions of distinct subclasses of T cells", which could be distinguished by cell- surface components thought to be specific to each subclass (Cantor & Boyse, 1977). In the late 1970s the discrimination and separation of these two subclasses were facilitated by the development of MCA to cell-surface antigens considered specific for each subclass, the subclasses being given the name T4-helper and T8-suppressor cells (Reinherz et al., 1979). By 1980 it was generally accepted that:

> (a) in humans the CD4 antigen and the CD8 antigen are expressed on helper and suppressor T cell subsets respectively. "Each T-cell subclass has a unique set of biological properties and immunologic functions" (Cantor & Boyse, 1977). "T4+ T cells provide helper function for optimal development of cytotoxicity in cell-mediated lympholysis...In addition, the T4+ subset produces a variety of helper factors that induce B cells to secrete immunoglobulin and all lymphocyte subpopulations (T,B and null) to proliferate". The T8 subset "suppresses the proliferative response of other T cells and B-cell immunoglobulin production and secretion" (Reinherz et al., 1981).
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> (b) "cells of these two subclasses do not give rise to one another...they represent products of separate subclasses of thymus dependent maturation", that is, "although both T4+ and T5+ subsets arise from a common progenitor cell within the thymus, they diverge during ontogeny and result in separate subsets" [T5-T8].
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> (c) "stimulation of T cells by conventional antigens, histocompatibility antigens and mitogens results in the formation of suppressor T cells" (Cantor & Boyse, 1977; Reinherz et al., 1980; Reinherz et al., 1981). The conclusion in (a) and (b) are at odds with evidence published in the 1980s.

In 1989 it was shown that when "monocytes adhered to plastic (but not when cultured on Teflon), a significant decrease in CD4 expression was observed between 1 and 24 h post-adherence. CD4 expression could not be detected in macrophages adhered to plastic for 5 days by using four anti-CD4 monoclonal antibodies in flow cytometry or direct immunofluoresence. Conversely, an increasing proportion of adherent cells expressed LeuM3 and OKM5 surface antigens over the 5 days". It was also shown that:

> (a) "The down-regulation of CD4 was post-translational";
>
> (b) unlike monocytes cultured on Teflon, the adherence of monocytes to plastic resulted in superoxide anion generation, that is, oxidative stress (Kazazi et al., 1989).

In the early 1980s, many researchers found that under certain conditions, while the number of T4 cells decreases, the number of T8 cells increases and the total number of cells remains constant or even increases. In 1982 Birch et al showed that incubation of T lymphocytes with adenosine or impromidine, (an H2 histamine agonist), leads to a decrease in the number of T-cells expressing the CD4 antigen and to an increase in the number of T-cell expressing the CD8 antigen whilst the sum (T4 + T8) remains constant (Birch et al., 1982). In an experiment conducted in the same year by Burns et al (Burns et al., 1982), normal human peripheral blood lymphocytes from different subjects were grown in conditioned medium containing IL-2, and, after varying periods of time in culture, the cells were tested by indirect immunofluorescence for OKT4 and OKT8. The "conditioned medium" (CM) consisted of "cell-free supernatant passed through a bacterial filter" from 7-day cultures of PHA

stimulated leucocytes obtained from patients with hemochromatosis. "For some experiments CM was freed of residual PHA by passage over a thyroglobulin-Sepharose column". They found that "...the cell population progressively increased in size to large blasts...but most striking was the rapid change in the OKT4:OKT8 ratio of cells within the population, from 60:40 to 40:60...The change in the surface phenotype of the major population also occurred in cultures maintained in medium containing IL2 which had been freed of PHA". They also found that the "change in phenotype of the culture as a whole took place very rapidly, often within one day", by 3 weeks the ratio OKT8:OKT4 was about 70:30, and that the "change did not appear to be simply the preferential outgrowth of OKT8+ cells", but to a "possible change in phenotype of cultured human lymphoblasts, from OKT4 to OKT8" (Burns et al., 1982). One year later in 1983, Zagury, an eminent HIV researcher (Zagury et al., 1983) and Gallo collaborator, and his colleagues, selected normal human T cells for in vitro cloning according to the expression of T4, T8 or T10 antigens on individual cells. The individual cells were cultured in the presence of TCGF (IL-2) "Preparations deprived of PHA", and "an irradiated lymphoid cell filler- layer". They summarised their findings as follows: "Clones were produced from each of these cells irrespective of the antigenic phenotype of the parental cell. The cloned progeny manifested, in many cases, shifts in antigen expression. Thus, T4+T8- cells gave clones expressing predominantly T4-T8+ and vice versa. The clonal expression of T4 and T8 seemed to be mutually exclusive. Antigenic shifts were recorded also in clones derived from T4-T8-T10- cells, resulting in T10+ clones which were also either T4+ or T8+ and from T4+T8-T10+ cloned cells yielding clones of either T4+ or T8+ cells. Testing functional properties we found that NK activity was mediated not only by T10+ cells but also, in some cases, by T4+ and T8+ cells. Moreover, TCGF production, which may reflect helper activity, was mediated not only by T4+ cells. Only the cytotoxic (CTL) activity seems to be confined to the T8 phenotype. Thus, it appears that T antigens, which seemed to be molecular markers of differentiation, are not markers for terminal differentiation and do not always reflect defined functional properties" (Zagury et al., 1983).

Given the in vitro evidence that:

> (1) HIV is neither necessary nor sufficient for the observed decrease in T4 cell numbers;
>
> (2) T4 cells can change into T8 cells while the sum of T4 + T8 remains constant;
>
> (3) stimulation of T cells by PHA, ConA, radiation, PMA and polybrene all of which are oxidising agents leads to "down regulation" of CD4 and change of T4 to T8; and the evidence that:
>
> > (i) individuals from the AIDS risk groups are exposed to many oxidising agents including well known mitogens;
> >
> > (ii) in individuals at risk for developing AIDS the decrease in T4 cell number is paralleled by an increase in T8 cells (decrease in the T4/T8 ratio), while the total T cell numbers remains constant;
> >
> > (iii) in individuals belonging to the main AIDS risk groups the above changes can be observed in the absence of HIV,
> >
> > one must conclude that:
> >
> > > (a) the decrease in the T4 cell numbers and increase in T8 cell numbers in "HIV infected" cultures and individuals is due to agents other than HIV; HIV is neither necessary nor sufficient for the induction of the above phenomenon;

(b) in vivo the above changes may not be due to a selective destruction of T4 cells and increased proliferation of T8 cells, but loss of T4 surface markers and acquisition of T8 surface markers.

T4 AND THE CLINICAL SYNDROME

The HIV/AIDS researchers consider T4 decrease as being the "hallmark" and "gold standard" of HIV infection and AIDS (Shaw et al., 1988; Levacher et al., 1992). In fact, in the most recent (1992) CDC AIDS definition, an AIDS case can be defined solely on serological, (positive HIV antibody test), and immunological (T4 cell count less than 200 X 106/L), evidence (CDC, 1992). The new definition also requires that "the lowest accurate, but not necessarily the most recent, CD4+ T- lymphocyte count should be used" to define an AIDS case (CDC, 1992). However, ample evidence exists that T4 cell decrease can be induced by many factors, some trivial, such as sun bathing and solarium exposure, a decrease which can persist for at least two weeks after exposure has ceased (Hersey et al., 1983; Walker & Lilleyman, 1983). T4 cell counts "can vary widely between labs or because of a person's age, the time of day a measurement is taken, and even whether the person smokes" (Cohen, 1992). That many factors can affect the T4 cell number is reflected by their large variation in HIV positive patients. In one such study, patient measurements repeated by one laboratory within 3-days showed a "minimum CD4+ cell count of 118 cells/mm3 and a maximum CD4+ cell count of 713 cells/mm3" (Malone et al., 1990). In the MACS, consisting of 4954 "homosexual/bisexual men", it was stressed that physicians and patients should be "aware that a measured CD4 cell count of 300X106/L really may mean it is likely that the "true" CD4 cell state is between 178 and 505X106/L. Thus there is no certainty this person's "true CD4" is less than 500X106/L or that it is greater than 200X106/L" (Hoover et al., 1992). It is important to note that these variations were obtained despite the fact that the CD4 measurements were undertaken in laboratories which "are carefully standardized in an ongoing quality control program".

In a study (Brettle et al., 1993) which examined the impact of the 1993 CDC AIDS definition on the annual number of AIDS cases as compared to the 1987 definition, it was found that if the definition was based on:

(i) the "first of two consecutive CD4 cell counts < or equal to 200 X 106/L", the number of AIDS cases doubled;

(ii) one abnormal CD4 count, the number of AIDS cases trebled.

Researchers at the University of California at Los Angeles School of Medicine found that 5% of healthy persons seeking life insurance had abnormal T4 cells counts, and that "In a subgroup of patients, the low T-cell numbers or ratios appear to be stable findings". They concluded: "In the absence of a history of a specific infection or illness or major abnormalities on a physical examination, it is not worthwhile to attempt to find a specific cause for the abnormality of T- cell subsets...A uniform approach to this problem throughout the medical community will help alleviate patients' anxiety and reduce the concern of the insurance industry about this relatively common problem" (Rett et al., 1988).

If LAS, ARC, and the AIDS indicator diseases such as KS and PCP are the consequence of T4 cell depletion then all groups of people who have a low T4 cell count, irrespective of cause, should have high frequencies of opportunistic infections and neoplasms. Conversely, all patients with AIDS indicator diseases should have abnormally low T4 cells.

In a study on the effects of blood transfusion on patients with thalassaemia major, researchers at the Cornell University Medical Center and the Sloan-Kettering Institute for Cancer Research observed decreased T4 cell numbers and inverted T4/T8 ratios associated with the transfusions, but no increase in KS or PCP, and concluded that "...studies which define

transfusion related AIDS on the basis of analyses with monoclonal antibodies must be viewed with caution" (Grady et al., 1985). Although patients with alcoholic liver disease do not develop KS, PCP and other AIDS indicator diseases more often than usual, they have both immune deficiency and positive HIV antibody tests leading researchers from the Veterans Administration Medical Centre to stress the importance of recognising these facts: "...lest these patients be falsely labelled as having infection with the AIDS virus and suffer the socioeconomic consequences of this diagnosis" (Mendenhall et al., 1986).

Patients who have malaria have severe immunoregulatory disturbances including decrease in T4 cells. A significant number of these patients also test positive for HIV but they do not develop the AID clinical syndrome, leading Volsky et al to conclude, "exposure to HTLV-III/LAV or the related retrovirus and the occurrence of severe immunoregulatory disturbances may not be sufficient for the induction of AIDS" (Volsky et al., 1986).

The MACS in the USA showed that "even in the absence of treatment, close to 25, 15 and 10% of men were alive and asymptomatic 4, 5 and 6 years after first CD4+ <200 X 106/L measurement" (Hoover, 1993). In the same study comparing HIV positive individuals who within five years progressed to AIDS (Group A) with that those who did not (Group B), it was found that: "receptive anal intercourse both before and after seroconversion with different partners was reported more frequently by men with AIDS. The ratio of the differences in this sexual activity between groups A and B was higher at 12 (2.3) and 24 (2.6) months after seroconversion than before seroconversion (2.0)". It was concluded that "sexually transmitted co-factors, preseroconversion and/or postseroconversion...augment (or determine) the rate of progression to AIDS" (Phair et al., 1992). However, since:

> (a) sexually transmitted infectious agents are bi- directionally transmitted, that is, from the active to the passive partner and vice-versa;
>
> (b) in the above study the only sexual act directly related to the progression to AIDS was passive anal intercourse (unidirectionally); one would have to conclude that the "co-factors that augment (or determine)" progression to AIDS are non-infectious. These findings are in agreement with the oxidative theory of AIDS which claims that both HIV phenomena (RT, virus-like particles, antigen/antibody reactions, "HIV-PCR") and AIDS are caused by the many oxidative agents (including semen), to which the AIDS risk groups are exposed (Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos et al., 1989a; Papadopulos-Eleopulos et al., 1992a; Papadopulos-Eleopulos et al., 1992b) [PCR/polymerase chain reaction].

According to Canadian researchers, "In TB as well as in lepromatous leprosy, an immunosuppressive state will frequently develop in the host. This state is characterised by T lymphopenia with a decreased number of T helper cells and an inverted T-helper/T-suppressor cell ratio ...immunosuppression induced by the infection with M.tuberculosis can persist for life, even when TB is not progressive" (Lamoureux et al., 1987). Yet these patients do not have high frequencies of KS, PCP or other AIDS indicator diseases. In other words, decrease in T4 cells is not sufficient for the AIDS indicator diseases to appear. This is also supported by evidence from animal studies. Experimental depletion of T4 cells in mice used as models for systemic lupus erythematosus in humans did not lead to increased frequencies of neoplasms, nor did mice "develop infectious complications, even though they were housed without special precautions". In fact mice with low T4 cell numbers had "prolonged life" (Wofsy & Seaman, 1985) It is also of interest that despite the indispensable role attributed to T4 and T8 lymphocytes in antibody production (helper and suppressor respectively), AIDS patients in the presence of low numbers of T4 cells and high numbers of T8 cells, have increased levels of serum gammaglobulins, and are not hypogammaglobulinaemic as might be expected. Also, although human umbilical cord T-cells produce suppressor factors(s), the factor(s) is produced by T8- (T4+) not T8+ cells

(Cheng & Delespesse, 1986). Thus, T4 and T8 cells do not seem to possess the generally accepted functions attributed to them. According to the HIV theory of AIDS pathogenesis, "The Human Immunodeficiency Virus (HIV), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), has the capability of selectively infecting and ultimately incapacitating the immune system whose function is to protect the body against such invaders. HIV-induced immunosuppression results in a host defense defect that renders the body highly susceptible to "opportunistic" infections and neoplasms" (Fauci, 1988).

Decrease of T4 cells to approximately 200X106/L leads to the development of "constitutional symptoms", and to less than 100X106/L to "Opportunistic diseases" (Pantaleo et al., 1993). If this is the case then:

1. In all individuals with "constitutional symptoms", OI and neoplasms, the T4 cell number should be abnormally low;

2. The decrease in T4 cells should precede the development of the clinical symptoms since: (a) the cause must precede the effect; (b) for many neoplastic and infectious diseases, there is evidence that the diseases themselves and the agents used to treat them may induce immune suppression including decreased numbers of T4 lymphocytes and reversal of T4/T8 ratios.

This is not the case even for the most serious and characteristic of the AIDS diseases, KS and PCP. In the MACS it was reported that:

> (a) "...persistent generalised lymphadenopathy was common but unrelated to immunodeficiency", and "Although seropositive men had a significantly higher mean number of involved node groups than the seronegative men (5.7 compared with 4.5 nodes, $p<0.005$), the numerical difference in the means is not striking".
>
> (b) weight loss, diarrhoea, fatigue, fever, which constitute the "wasting" syndrome, (which at present is an AIDS indicator disease), night sweats, herpes zoster, herpes simplex (another AIDS indicator disease), oral thrush, fungal skin infections and haematological abnormalities, were present in both seronegative and seropositive individuals, although some of them were present at higher frequencies in the latter group. A relationship was found between thrush, anaemia, fever and neutropenia and T4 cell deficiency. However, "the clinical abnormalities were considerably better at reflecting concurrent CD4 lymphocyte depression than the low CD4 lymphocyte counts were at determining clinical involvement" (Kaslow et al., 1987). These observations are just as compatible with the hypothesis that T4 lymphocyte deficiency is the result and not the cause of the observed clinical abnormalities.

KS, the main reason for which the retroviral hypothesis was put forward, was initially postulated to be caused by infection of normal cells with the retrovirus. When, late in 1984 it became clear that the KS cells were not infected with HIV, it was generally accepted that the disease was caused by HIV indirectly, that is, as a consequence of T4 cell decrease. At present, it is generally believed that KS is caused by "a specific sexually transmitted etiologic agent" (Beral et al., 1990; Weiss, 1993) other than HIV, but "immune suppression (both in AIDS and in transplant patients) is the dominant cofactor for subsequent disease" (Weiss, 1993). However, unlike the Unites States CDC and most AIDS centres around the world, for the Walter Reed Army Institute of Research "...the presence of opportunistic infections is a criterion for the diagnosis of AIDS, but the presence of Kaposi's sarcoma is omitted because the cancer is not caused by immune suppression..." (Redfield & Burke, 1988) In a study by a group of researchers from Amsterdam regarding the relationship between the T4 cell number and the development of the clinical syndrome, KS was excluded "Because Kaposi's sarcoma may manifest at higher CD4+ lymphocyte counts than other AIDS- defining conditions"

(Schellekens et al., 1992). This is not surprising since by the beginning of the AIDS era, the immune surveillance hypothesis of carcinogenesis had been already refuted (Kinlen, 1982). In fact, the presently available data indicate that KS in all individuals, including gay men, may be caused by a non-infectious agent (Papadopulos-Eleopulos et al., 1992a). Even in the early stages of the AIDS era, it was reported that KS in gay men appeared following corticosteroid administration (which was administered for diseases totally unrelated to HIV or AIDS) and resolved when the drug was discontinued (Schulhafer et al., 1987; Gill et al., 1989). Thus the HIV/AIDS hypothesis cannot account for the very disease for which it was originally put forward.

In a study of 145 patients, 97% of whom were homosexuals, with biopsy proven PCP at St. Vincent's Hospital and Medical Centre, New York, 17% of AIDS patients had a T4 cell count higher than 500/mm3, and a further 14% between 301-500/mm3, "in addition, patients with T4-T8 ratio greater than 1.0 and those with total T4 lymphocyte counts greater than 500/mm3 cells did not show improved survival compared with patients with abnormal values....the degree of suppression did not influence mortality (Kales et al., 1987). Researchers from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute, studied 100 HIV-infected patients "who had 119 episodes of pulmonary dysfunction within 60 days after CD4 lymphocyte determinations". T4 cells were less than 200X106/L before 46 of 49 episodes of PCP, 8 of 8 episodes of CMV pneumonia, 7 out of 7 Cryptococcal neoformans pneumonia, 19 of 21 episodes of Mycobacterium avium-intracellulare pneumonia, 6 of 8 [pulmonary] KS and in 30 out of 41 non-specific interstitial pneumonia. However, "Before the 119 episodes of pulmonary dysfunction were diagnosed in this study, the HIV- infected patients had manifested the following clinical HIV- related disorders: no disorders (4 episodes), Kaposi's sarcoma without opportunistic infections (68 episodes), life-threatening opportunistic infection (44 episodes), other AIDS- related conditions (11 episodes)". In addition before the diagnosis of the pulmonary episodes the patients had received: "zidovudine (36 episodes), interferon (23 episodes), recombinant interleukin-2 (3 episodes), cytotoxic chemotherapy (16 episodes), dideoxycytidine (6 episodes), muramyl tripeptide (1 episode), suramin (6 episodes), heteropolyanion 23 (5 episodes), zidovudine plus interferon (5 episodes), nonablative bone marrow transplantation (4 episodes). Twenty-two episodes occurred in patients who had been receiving neither experimental therapy nor zidovudine" (Masur et al., 1989). These data may be interpreted as showing that in some types of "pulmonary dysfunction", most cases (but not all) appear to be preceded by a CD4 count <200X106/L. However, given the well known fact that malignant neoplasms, infectious diseases and the administration of chemotherapeutic agents may themselves cause immunosuppression (Serrou, 1974; Oxford, 1980; Reinherz et al., 1980; Rubin et al., 1981; Thomas, 1981; Weigle et al., 1983; Williams et al., 1983; Kempf & Mitchell, 1985; Feldman et al., 1989), it is equally plausible to argue that both "pulmonary dysfunction" and the low CD4 cell counts observed in patients were the result of their recent past illnesses and previous exposure to prescribed and illicit drugs and other factors.

In a recent study it was found that 3 patients who developed PCP within 8-14 days of "symptomatic, primary HIV infection", had normal T4 cell numbers and T4/T8 ratios 50-90 days before they became symptomatic. During the symptomatic phase the T4 cell count dropped to 62-91 cells/uL. However, "Within four months of symptom onset, their CD4 counts and CD4/CD8 ratios returned to normal". In two of the patients, a bisexual man and a gay man, "HIV-1 antibodies were detectable by EIA and WB" 30 days after these two individuals became symptomatic [EIA-ELISA].

"Twenty-nine to forty-eight months after acquiring HIV-1 infection", all three patients still had normal T4 cell numbers and were asymptomatic. The authors concluded "profound CD4 lymphocytopenia can revert to normal without antiretroviral therapy" and stressed "it is important that such cases are not misdiagnosed as AIDS" (Vento et al., 1993). That no relationship exists between OI and T4 depletion was confirmed in a recent study where it was

shown that "The appearance of OI and wasting syndrome was independent of T4 cells count" (Alejandro et al., 1991), as well as other studies which show that the OI may appear in the presence of normal T4 cell numbers (Stagno et al., 1980; Martinez et al., 1991; Felix et al., 1992).

In conclusion, decrease in the number of T4 lymphocytes irrespective of how it is induced, that is, by destruction of the T4 cells or by a phenotypic change, and of its cause, is neither necessary nor sufficient for the appearance of KS and OI including PCP, that is, of the clinical syndrome.

HIV AND AIDS

If HIV is either necessary and sufficient, or necessary but not sufficient for the appearance of AIDS, then the minimum requirement is that the virus be present in all cases. Three methods are used to demonstrate the presence of HIV: antibody tests, viral "isolation", and PCR. At present, "the applications of PCR in the evaluation of HIV-1 seropositive individuals are not completely defined" (Conway, 1990). Although PCR has a very high sensitivity, the test is not standardised and its reproducibility and specificity have not been determined. The limited data presently available suggest that PCR is neither reproducible nor specific (Fox et al., 1989; Conway, 1990; Dickover et al., 1990; Long, Komminoth & Wolfe, 1992), even when the serological status and not HIV, as should be the case, is used as a gold standard (Defer et al., 1992). Furthermore, since the specificity of the primers used in the PCR assay ultimately relate to the material originating from "HIV isolates", the test specificity can be no more meaningful (regarding the presence in AIDS patients of an exogenous retrovirus), than "HIV isolation". However, HIV has never been isolated as an independent particle separate from everything else. In fact, by isolation is meant, at best, detection of two or more of the following phenomena:

> (a) reverse transcriptase, either in the cultures/co-cultures or in material derived from these cultures including nucleic acids and proteins which in sucrose density gradients bands at a density of 1.16 gm/ml;
>
> (b) proteins either in the cultures/co-cultures or banding at 1.16 gm/ml and which react with AIDS patient sera;
>
> (c) virus-like particles in the cultures. Lately, for many researchers including Montagnier (Learmont et al., 1992; Henin et al., 1993), detection in cultures/co-cultures of only p24 or reverse transcription is considered synonymous with "HIV isolation". The finding of the above phenomena cannot be considered synonymous with "HIV isolation". They can be used only for viral detection, and then if and only if, they have first been proven specific for the virus. Not one of the above phenomena is specific to HIV or even to retroviruses (Papadopulos-Eleopulos, Turner and Papadimitriou, 1993a). Furthermore, and most importantly, HIV cannot be isolated unless the cultures are subjected to oxidative stress (mitogenic stimulation, activation). However:
>
> 1. The normal human genome contains many copies of endogenous retroviral sequences (proviruses), "including a complex family of HIV-1 related sequences" (Horwitz et al., 1992), a "large fraction" of which "may exist within a host cell as defective genomic fragments. The process of recombination however may allow for their expression as either particle or synthesis of a new protein(s)" (Weiss et al., 1982; Varmus & Brown, 1989; Cohen, 1993; Lower & Lower, 1993; Minassian et al., 1993);
>
> 2. Cultivation of normal "non-virus" producing cells leads to retroviral production (expression), "the failure to isolate endogenous viruses from certain species may

> reflect the limitations of in vitro cocultivation techniques" (Todaro et al., 1976). The expression can be accelerated and the yield increased by exposing the cultures to mitogens, mutagens or carcinogens, co-cultivation techniques and cultivation of cells with supernatant from non-virus producing cultures (Toyoshima & Vogt, 1969; Aaronson et al., 1971; Hirsch et al., 1972). For HIV isolation, in most instances, all the above techniques are employed. Thus, even if "true" (Popovic et al., 1984) retroviral isolation can be achieved from the AIDS cultures/co-cultures, it would be difficult if not impossible to be certain that the retrovirus in question is an exogenous retrovirus. For such evidence to be accepted as proof of the existence of HIV, the activation of an endogenous provirus or a provirus assembled by recombination of endogenous retroviral and cellular sequences would need to be rigorously excluded. For example, in many cases of "HIV isolation", the human leukaemic cell lines CEM or HT(H9) are co-cultured with tissue from AIDS patients which is assumed to be "infected with HIV". The finding of two or more of the following: (i) reverse transcription; (ii) proteins which react with patient sera either in the co-cultures or the material which bands at 1.16 gm/ml; (iii) retrovirus-like particles in the culture; is considered as proof of the isolation from the patient of a retrovirus (HIV) which infected the CEM or HT (H9) cells.

However, when CEM (CEM-SS) cells "otherwise negative for known human retrovirus", are stimulated with the mutagen ethyl- methyl-sulfonate (EMS), "Large, syncytia-like cells reminiscent of those which appear after a retrovirus infection were observed 5-6 days after treatment...Cell-free supernatants from CEM-SS cells heavily treated with EMS were able to induce a transmissible retrovirus infection in Jurkat and Molt 3 cells...All attempts to identify viral expression in the unmutagenized parental cells by EM, RT activity, or immunohistochemical methods were negative" (Minassian et al., 1993) [EM/electron microscopy]. It has already been stated that the HT cell line originated from a patient with adult T4 cell leukaemia, a disease which Gallo claims is caused by another retrovirus, HTLV-I. If this is the case, CEM and HT (H9) cultures would have retrovirus which, under the right conditions, would be expressed even if the patient tissues did not contain "HIV". Be this as it may, neither PCR nor "HIV isolation" have ever been used to demonstrate a causal relationship between HIV and AIDS.

At present, as was the case in 1984, the claim that a "causal relation between HIV and AIDS is compelling" is based on the epidemiological relationships between a positive "HIV antibody" test and AIDS (Weiss, 1993). One of these tests, the Western blot (WB), is considered to be both nearly 100% sensitive and specific, and is used as a gold standard for the other tests. Despite knowledge that cellular constituents and/or fragments of the same buoyant density as retroviral particles may contaminate the supernatants of cell cultures (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a), material for the WB is obtained by density gradient centrifugation of the supernatant from "HIV infected" cell cultures or even cell lysates, the latter being the case in the first "HIV isolation" (Barre-Sinoussi et al., 1983), and subsequently in other laboratories (Essex et al., 1985; Albert et al., 1988; Levinson & Denys, 1988). Material which bands at 1.16 gm/ml is considered to represent pure HIV and consequently the proteins found at this density are considered to be HIV antigens. For the Western blot, these proteins are electrophoretically separated according to molecular weight and charge. The separated proteins are then transferred on to nitrocellulose strips by electroblotting. When sera are added and the strips developed, coloured bands appear representing sites of protein/antibody reactions. Each band is designated by a small "p" for protein, followed by its molecular weight in thousands. Although the material which bands at 1.16gm/ml is considered to represent pure HIV, many of the proteins which band at this density are accepted to be cellular proteins (Henderson et al., 1987), including proteins which react with patient sera: "Sera from some AIDS patients bound a lot of cellular protein. In ELISA this problem was overcome by comparing the serum binding to the viral antigen

with binding to a lysate of uninfected lymphocytes. This binding was apparent in the RIPA and only sera which specifically precipitated the p25 [p24] were regarded as positive" [RIPA/radioimmune precipitation assay] (Brun-Vezinet et al., 1984; Burke, 1989). Even the proteins which are considered to be HIV proteins may not be so (Papadopulos-Eleopulos et al., 1993a; Papadopulos-Eleopulos et al., 1993b). For example, the p41 band which is considered by most AIDS researchers as one of the most specific HIV proteins, is regarded by Montagnier's group as being cellular actin (Barre-Sinoussi et al., 1983). Furthermore, the pattern of reaction, including that of the bands considered to represent HIV proteins varies, from patient to patient and in the same patient from time to time. Because of this, criteria for the interpretation of the WB are necessary. Yet, even today, 10 years after the discovery of HIV, there are no national USA or international agreed criteria as to what constitutes a positive WB pattern. Some institutions have more "stringent" criteria than others to define a positive WB. When the WB pattern does not satisfy the definition for a positive test for a given institution, but displays reactive bands, representing either cellular or "HIV proteins", the test is considered to be indeterminate, (WBI). A WB which has no reactive bands, representing either "HIV" or cellular proteins, is considered by all institutions as negative (Lundberg, 1988).

For some time evidence has existed showing that:

(a) when the least "stringent" criteria used to define a positive WB are [p24 or p31/32 and (p41 or p120/160)], only approximately 80% of AIDS patients test positive for HIV and this decreases to less than 50% when the most "stringent" [p24 and p31/32 and (p41 or p120/160)] criteria are used. The remaining AIDS patients have either an indeterminate or a negative test (Lundberg, 1988). Conversely, according to the USA Consortium for Retrovirus Serology Standardization, 127/1306 (10%) of sera from individuals at "low risk" of HIV infection, which "includes specimens from blood donor centers" have a positive WB even when the most "stringent" criteria are used to define a positive test (Lundberg, 1988). (The Consortium authors did not comment on the significance of the occurrence of such stringently positive tests in low risk individuals).

(b) WBI are very common in non-AIDS patients. For example, 42% of patients transfused with HIV negative blood have WBI results. In about 30% of these patients, the WBI contains the p24 band, the band considered by Montagnier's group to be the most specific HIV band (Genesca et al., 1989). (In fact at present, for many researchers, the detection of p24 in AIDS cultures/co-cultures is synonymous with "HIV isolation"). These results lead some HIV researchers to conclude that "WBI patterns are exceedingly common in randomly selected donors and recipients and such patterns do not correlate with the presence of HIV-1 or the transmission of HIV-1" (Genesca et al., 1989).

(c) the specificity of an antibody test must be determined by the use of a gold standard. The only valid gold standard for the HIV antibody tests is HIV itself. However, to date, nowhere in the AIDS scientific literature has there been any report whatsoever of the use of "Human Immunodeficiency Virus" itself as a gold standard for the verification of the sensitivity and specificity of the HIV antibody tests. In fact, this may not be presently possible since, even if one considers the phenomena detected in AIDS cultures/co-cultures to be HIV and the methods used to represent unequivocal isolation, in the best laboratories, and with no efforts spared, "HIV can be isolated" only from 17-80% of HIV positive individuals (Chiodi et al., 1988; Learmont et al., 1992). Since no gold standard has been used to confirm the specificity of the WB results, the probability cannot be excluded that both WBI and WB results do not indicate HIV infection and transmission, but are the result of cross- reaction with antibodies directed against non-HIV antigens. This is especially the case in AIDS

patients and in individuals at risk of AIDS, since both groups possess a vast array of antibodies directed against many antigenic determinants (Matsiota et al., 1987; Calabrese, 1988). Thus, a positive "HIV antibody test" ought to be regarded as a non-specific marker for the development of AIDS in the high AIDS risk groups, and should not be regarded as a diagnostic and epidemiological tool for HIV infection (Papadopulos-Eleopulos et al., 1993a). Notwithstanding, if:

(i) the sensitivity and specificity of the WB is nearly 100% as it is generally accepted;

(ii) only 50-80% (depending on which criteria are used to define a positive WB) of AIDS patients test positive; then between 20-50% of AIDS patients are not infected with HIV.

Lately, some of the best known HIV researchers (Moore & Ho, 1992) have accepted that the clinical syndrome, including its most specific and frequent manifestation, KS and PCP, may appear in the absence of HIV, that is, in patients in whom all HIV tests including the WB and PCR, are negative. For example, in 1991, Jacobs et al (Jacobs et al., 1991) reported that at the New York Hospital-Cornell Medical Center during a three month period, they diagnosed PCP in five adults. Two out of three patients tested for T-lymphocyte subsets had T4>40% and all had normal T4/T8 ratios. "Cultures of peripheral-blood mononuclear cells for retrovirus were negative" in 4/5 patients, (the 5th apparently was not tested). The HIV-1,2 antibody tests were negative in all cases. One year later workers from the same institution and three other centres had "identified five other individuals from the New York City area (four who have known risk factors for HIV infection), with profound CD4 depletion and clinical syndromes consistent with definitions of the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex. None had evidence of HIV-1, 2 infection, as judged by multiple serologies over several years, standard viral co-cultures for HIV p24 Gag antigen, and proviral DNA amplification by polymerase chain reaction" (Laurence et al., 1992). Similar cases have recently been reported from other institutions including the CDC (Afrasiabi et al., 1986; Pankhurst & Peakman, 1989; Safai et al., 1991; Seligmann et al., 1991; Sirianni et al., 1991; CDC, 1992; Hishida et al., 1992; Tijhuis et al., 1993).

The available data do not support the presently accepted hypothesis that HIV is either necessary or sufficient for the pathogenesis of AIDS, and thus it would seem logical to consider alternative theories (Papadopulos-Eleopulos, 1988; Duesberg, 1992).

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