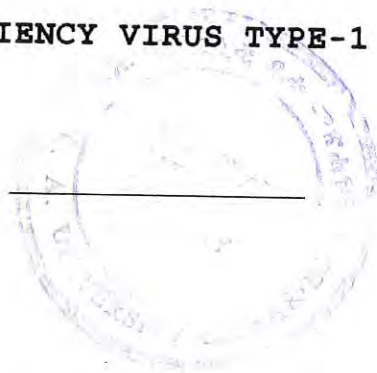


MED

**ASSESSMENT OF *IN VITRO* CYTOKINE RESPONSE TO HUMAN
IMMUNODEFICIENCY VIRUS TYPE-1 ANTIGENS**



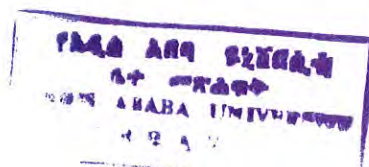
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ADDIS ABABA UNIVERSITY

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OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY

By

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DECEMBER, 1992.



DEDICATED TO MY MOTHER

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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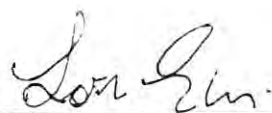
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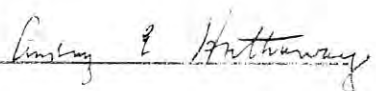
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ABBREVIATIONS

ADCC	antibody-dependent cellular cytotoxicity
AIDS	acquired immunodeficiency syndrome
CTL	cytotoxic T lymphocytes
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
gp	glycoprotein
GM-CSF	granulocyte monocyte-colony stimulating factor
HIV	human immunodeficiency virus
IFN	interferon
Ig	immunoglobulin
IL	interleukin
LPS	lipopolysaccharide
mAb	monoclonal antibody
MHC	major histocompatibility complex
NK	natural killer
PBMC	peripheral blood mononuclear cells
PHA	phytohaemagglutinin
TNF	tumor necrosis factor

A B S T R A C T

The study of mononuclear cell responses after human immunodeficiency virus type-1 (HIV-1) activation may help in providing some insight into the understanding of the pathogenic mechanisms of HIV infection or acquired immunodeficiency syndrome (AIDS)-related disorders. The secretion of three cytokines, interleukin-6 (IL-6), tumor necrosis factor (TNF)-alpha, and interferon (IFN)-gamma by peripheral blood mononuclear cells (PBMC) was determined after *in vitro* stimulation with heat-inactivated HIV-1 antigens, using cytokine-specific monoclonal antibodies and an indirect immunofluorescence technique. HIV-1 antigen stimulation of PBMC from healthy donors did not induce the intracellular accumulation of IL-6, TNF-alpha, or IFN-gamma. Similarly, cells from asymptomatic HIV-infected subjects did not result in cytokine production either spontaneously or following stimulation with HIV antigen. After activation with mitogen or antigen, cells from HIV-infected persons produced amounts of IL-6, TNF-alpha and IFN-gamma comparable to that of cells from healthy individuals. Furthermore, co-stimulation of normal PBMC with HIV-antigen plus *L. donovani* resulted in intracellular accumulation of IL-6 and TNF-alpha comparable to that of cells that were activated with *Leishmania* antigen alone. Heat-inactivated HIV-1 antigen does not appear to induce or modulate cytokine production by PBMC. A defect in cytokine production is also accompanied by inhibition of PPD-induced lymphoproliferative responses. Elevated levels of serum cytokines have been demonstrated in patients with HIV infection indicating their role in the pathogenesis of HIV-associated infections. Therefore, data from our results may partly support the idea that the cause of the abnormally increased cytokine levels in the sera of HIV-infected subjects

might be due to a variety of opportunistic pathogens that these patients contract. As cytokines have been shown to up-regulate HIV replication, our data additionally demonstrate a role for opportunistic infections in cytokine induced transactivation of HIV-1 and disease progression.

I. INTRODUCTION AND LITERATURE REVIEW

AIDS is a name given to a syndrome that is caused by an infection with a virus belonging to the retrovirus family (Fauci 1988). The disease was first described in 1981 in homosexual men with opportunistic infections (Gottlieb et al 1981, Masur et al 1981, Siegal et al 1981). Subsequently cases among persons with hemophilia, blood transfusion recipients, and intravenous drug abusers were reported (Curran 1983). Two years after the first report of AIDS, a cytopathic retrovirus was isolated from patients with the disease (Barre-Sinoussi et al 1983, Gallo et al 1984, Levy et al 1984). The etiologic agent was originally named as "lymphadenopathy-associated virus" (LAV) (Barre-Sinoussi et al 1984), "human T cell lymphotropic virus type III" (HTLV-III) (Gallo et al 1984), and "AIDS-associated retrovirus" (ARV) (Levy et al 1984). Currently, it has been named "human immunodeficiency virus" (HIV) by an International nomenclature committee (Coffin et al 1986). This virus is now referred to as HIV-1 to differentiate it from a related but distinct retrovirus named HIV-2, that has been isolated from West African patients (Clavel et al 1986).

Although depletion and functional impairment of T4⁺ lymphocytes play a pivotal role in the pathogenesis of HIV-1 induced immunosuppression, the precise mechanisms whereby HIV-1 infection leads to the immunosuppression and the clinical manifestations characteristic of the disorder are complex and not completely understood. Furthermore, there is lack of information regarding the nature of protective humoral and cellular immunity against HIV. Investigations carried out to elucidate the immunopathogenesis and the immune response to HIV should

help in designing and developing drugs or vaccines to prevent and/or cure HIV infections, and will have important implications for understanding the regulatory mechanisms involved in the human immune system.

1:THE VIRUS

HIV is an RNA retrovirus belonging to the lentivirus family (Gallo et al 1984, Levy et al 1984, Coffin et al 1986). It is a small enveloped virus that contains double and single-stranded RNA as its genomic material. The viral genome consists of *gag*, *pol* and *env* genes, that code for the various structural and functional proteins, and at least five other genes involved in the regulation of viral gene expression (Chen 1986). The virus particle contains an inner core that encloses the viral genome, as well as enzymes required for early replication events. The inner core is itself surrounded by a capsid protein and a lipid envelope encloses the capsid. A virus matrix protein is inserted into the inner surface of the membrane. An integral membrane protein, the envelope glycoprotein, protrudes through the membrane and forms the outer surface of the virus particle (Fig 1).

2:REPLICATIVE CYCLE OF THE VIRUS

Viral infection begins when a virus surface envelope glycoprotein, Gp120, interacts with a host cell bearing the CD4 molecule. The CD4 surface molecule is the receptor for the virus; it has a high affinity for the viral envelope (Dalglish et al 1984, Klatzman & Gluckmann 1984, McDougal et al 1985b). Certain subsets of monocytes and macrophages also express the CD4

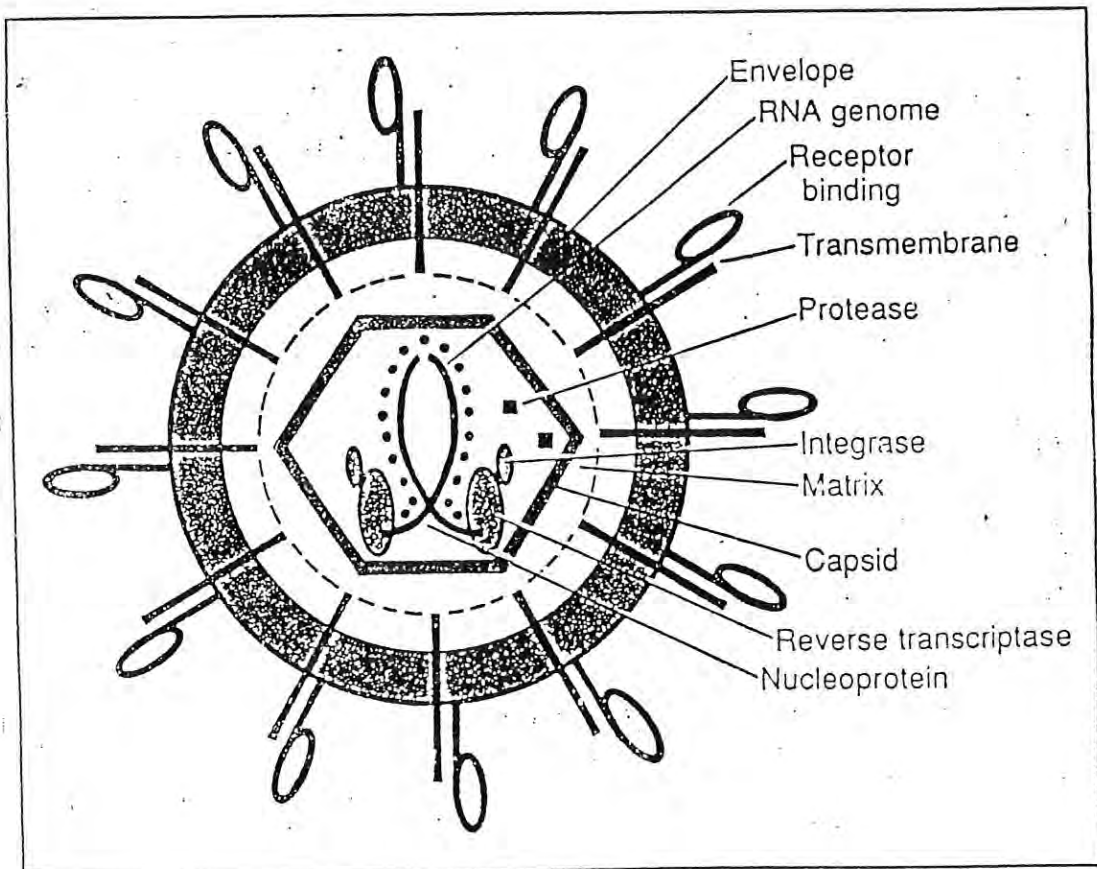


Figure 1. The basic structure of the human immunodeficiency virus (adapted from Meissner and Coffin 1989).

molecule (Sattentau & Weiss 1988), and the cells can bind and be infected by HIV (Levy et al 1985, Ho et al 1986, Nicholson et al 1986). Fusion of the virus with cell surface molecules results in successful cell entry (Stein et al 1987). There is some evidence that the envelope glycoprotein of the HIV is responsible for mediating the fusion reaction (Sodroski et al 1986, Lifson et al 1986, Kowalski et al 1991). However, other mechanisms, such as Fc-receptor or complement-receptor mediated phagocytosis may facilitate virus entry and infection (Robinson et al 1988, Bolognesi 1989, Mann et al 1990). Following entry, the viral genome is uncoated in the cytoplasm of the host cell. The viral RNA is then used as a template and is transcribed into double-stranded DNA, which is catalyzed by the viral associated enzyme, reverse transcriptase (Varmus 1988). The viral DNA enters the nucleus where it is inserted into the host cell's genome. The integrated viral DNA is now called provirus. The enzyme integrase is responsible for inserting the viral genome into the host cell DNA. Restricted gene expression of the incorporated HIV provirus leads to latent infection (Harper et al 1986, Varmus 1988), and it is believed that some factors are required to specifically initiate HIV gene expression prior to the development of progressive disease (McDougal et al 1985b, Varmus 1988). After activation, the integrated proviral DNA is utilized in the infected cell for the transcription of viral RNA (Clements 1985). Translation and processing of viral mRNA and their products is followed by assembly and budding of mature progeny from the membrane of the infected host cell (Ho et al 1987).

3:IMMUNOPATHOGENIC MECHANISMS OF HIV INFECTION

3.1:ROLE OF T4 LYMPHOCYTES

The key point for the observed immunosuppression in HIV-infection is the selective depletion in the helper subset of T-lymphocytes (McDougal et al 1985b). CD4⁺ lymphocytes are involved in the induction of most immunologic responses either directly or indirectly and this is effected for the most part by the ability of the T-helper cells to secrete a variety of soluble factors, collectively termed "cytokines", that have either tropic or inductive effects on their target cells (Balkwill & Burke 1989). Therefore, conditions that cause selective CD4⁺ lymphocyte depletion would result in a decrease in the inductive signals to the different arms of the immune response and subsequently would lead to progressive systemic immunosuppression, and the development of a wide variety of opportunistic infections or unusual neoplasms.

3.1.1:Quantitative CD4 T lymphocyte depletion

Although a defect in the helper subset of T lymphocytes is known to be associated with a generalized immunosuppression, the precise mechanisms by which HIV causes a decrease in the number of circulating CD4⁺ T cells is not fully known. Several different mechanisms have been proposed in an attempt to elucidate the pathogenesis of CD4⁺ T cell killing.

Many reports have indicated that HIV may destroy CD4⁺ T cells as a result of direct infection and replication. The proposed mechanisms include: (a) cell membrane destruction due to massive viral budding (Leonard et al 1988); (b) induction of terminal differentiation and shortened life span of T cells

(Zagury et al 1986); (c) accumulation of unintegrated retroviral DNA (Shaw et al 1984, Pauza & Galindo 1989); (d) infection of CD4⁺ T cell precursors (Fauci 1988); and (e) intracellular complexing of CD4 and HIV-gp 120 (Hoxie et al 1986).

Although HIV is believed to cause direct cytopathic effects of infected T4 cells, only 1 in 10⁴ to 1 in 10⁵ of the circulating helper cells in HIV infected individuals are positive for the viral genome (Harper et al 1986, Schnittman et al 1989). Therefore, HIV may not only cause CD4⁺ T cell depletion as a result of direct infection, but also HIV-induced indirect cytopathic mechanisms of uninfected cells may contribute to the state of wide spread immunosuppression. These include: (a) auto-immune phenomenon (Ziegler & Stites 1986, Klatzman & Gluckmann 1986, Lanzavecchia et al 1988); (b) antibody dependent cellular cytotoxicity involving anti gp120 antibodies (Weinhold et al 1989, Katz et al 1988); (c) secretion by HIV infected cells of soluble factors that are toxic to T4 cells (Ruddle 1986); and (d) syncytia formation of infected cells with uninfected ones (Sodroski et al 1986, Lifson et al 1986).

3.1.2:Qualitative/functional T4 lymphocyte defects

Although quantitative depletion of CD4⁺ T cells is the most prominent immunological abnormality in HIV-infected individuals, there is growing evidence that HIV can cause a qualitative or functional impairment of immune cells in the absence of a cytopathic effect. Mononuclear cells from AIDS patients fail to proliferate in response to specific antigens (Lane et al 1985a, Miedema et al 1990, Teeuwssen et al 1990). In addition to HIV induced suppression of T cell responses that result from infection of the cells, it has been shown that *in vitro*

exposure of mononuclear cells to specific HIV gene products in the absence of infection of the cell is sufficient to inhibit antigen-specific responses (Ruegg & Strand 1990, Nong et al 1991). The mechanism(s) by which HIV or its envelope proteins impair T cell function include: (a) blocking of the normal interaction of the CD4 molecule with class II MHC molecules on the antigen-presenting cell (Gay et al 1987, Doyle & Strominger 1987); (b) disruption of the normal cellular post-receptor signal transduction pathways (Horak et al 1990, Cefai et al 1990, Hofmann et al 1990); (c) interference with the synthesis or expression of CD4 (Folks et al 1985, Hoxie et al 1985); (d) down-regulation of cellular genes, such as IL-2, IL-2 receptor and T cell receptor genes, involved in T cell activation (Rosenberg & Fauci 1989, Willard-Gallo et al 1990), and (e) selective infection of CD29⁺ memory cells that are primarily involved in antigen-specific responses (van Noesel et al 1990).

One of the mechanisms recently proposed to account for both qualitative and quantitative helper T-cell defects in HIV-infected patients is the induction by HIV of T4 cell suicide or programmed T cell death (Ameison & Capron 1991).

3.2:ROLE OF OTHER CELL TYPES

The T-lymphocyte which expresses a high concentration of CD4 surface molecules is the principal cell target in the peripheral blood that is infected with HIV (Schnittman et al 1989, McElrath et al 1989). However, other cell types that are CD4⁺ (Levy et al 1985, Gartner et al 1986, Ho et al 1986, Nicholson et al 1986, Rosenberg & Fauci 1989, Macatonia et al 1990) as well as CD4⁺(B-cells) (Salahuddin et al 1987, Levy et al 1985) are also subject to infection with HIV. Furthermore, CD4⁺ cells (such as CD8⁺ &

NK-cells) which are not known to be infected with HIV show profound functional defects in persons infected with HIV (Rook et al 1983, Rook et al 1985b, Shearer et al 1985). The mechanisms responsible for the dysfunction of these cell lineages have not been determined. However, a defect in the inductive signal due to quantitative and functional defects in T4⁺ cells may explain, in part, the functional abnormalities of these cells.

3.2.1: Role of monocytes/macrophages

In contrast to HIV infection of CD4⁺ T cells, infection of monocytes/macrophages is relatively refractory to the cytopathic effects of the virus (Gartner et al 1986). The reason why these cells are resistant to the cytopathic effects of the HIV-1 is not clear, but low levels of expression of the CD4 cell surface molecule is one possible reason. Furthermore, budding of virus in these cells occurs not at the outer cell membrane, but intracellularly into the Golgi organelle (Gendelman et al 1988) and this could be another reason why the virus is not cytopathic to the monocyte cell lineage. The virus is not only able to survive within these cells, but is also able to replicate more efficiently inside the cell (Gartner et al 1986, Gendelman et al 1988). This observation has the implication that HIV-infected cells of the monocyte line can serve as a potential reservoir for HIV replication and dissemination to other parts of the body, such as the lung and brain (Koenig et al 1986). Furthermore, by infecting these cells, HIV is able to evade immune surveillance mechanisms (Gendelman et al 1988). Conversely, HIV-infection of the monocyte/ macrophage cell lineage can result in a number of functional abnormalities of

these cells, such as defective chemotaxis (Smith et al 1984), phagocytosis (Pinching et al 1983), and intracellular killing activities (Roux-Lombard et al 1986), and decreased accessory cell function (Ennen et al 1990).

3.2.2: Role of CD8⁺ T lymphocytes

Although CD8⁺ T lymphocytes have been demonstrated in the peripheral blood of HIV-infected individuals (Walker et al 1987, Plata et al 1987) they exhibit functional abnormalities because of defects in HIV-specific cytolytic activity (Hoffenbach et al 1989) or diminished capacity to undergo clonal expansion (Margolick et al 1985). CD8⁺ lymphocytes have the capacity for antiviral effector function and have been shown to suppress HIV replication in vitro (Walker et al 1986). These results indicate that functional abnormalities of the CD8⁺ T lymphocyte population may contribute to clinical progression in HIV-infected persons.

3.2.3: Role of B-cells

B-cell function is also impaired in patients with HIV-infection. AIDS patients characteristically develop polyclonal B cell activation (Lane et al 1983, Pahwa et al 1985), B cell lymphomas (Ziegler et al 1984), lymphadenopathy (Lane et al 1983), autoimmune phenomena (Kloster et al 1984, Williams et al 1984, McDougal et al 1985a), and impaired B cell responses (Lane et al 1983). Furthermore, B-cell hyperactivity may contribute to enhancement of spread of HIV by secreting cytokines, such as IL-6 and TNF-alpha, which have been shown to enhance viral replication (Poli et al 1990a, 1990 b), by enhanced Fc-receptor (Bolognesi 1989, Laurence et al 1990) or complement-receptor (Robinson et al 1988) mediated phagocytosis of HIV, or by induction of

suppressor mechanisms that depress T-cell functions (Shearer et al 1985).

3.2.4: Role of NK cells

Even though the number of circulating NK cells is not significantly diminished in HIV-infection, including those with AIDS, their cytotoxic capability is reduced (Rook et al 1983, Rook et al 1985a, 1985b), most probably because of a defect in the post-binding transmembrane signal normally required for activation (Katzman & Lederman 1986).

3.3: ACTIVATION OF LATENT HIV INFECTION

One of the unique features of the HIV is the ability of proviral DNA to persist in a quiescent state without viral gene expression (Hoxie et al 1985, Harper et al 1986, Varmus 1988). The ability of the virus genome to persist in the host cell without active expression accounts in part for the long latent period of HIV-1 infection. Therefore, active viral replication requires an activation signal (McDougal et al 1985b, Varmus 1988). Studies have demonstrated that HIV replication could be induced in latently infected cells following exposure of the cells to mitogens (McDougal et al 1985b, Zagury et al 1986), soluble antigens (Margolick et al 1987, Pomerantz et al 1990), heterologous viruses (Gendelman et al 1986, Fauci 1987), UV-light (Stanley et al 1989), heat (Stanley et al 1990), and cytokines (Poli et al 1990a, 1990b, Rosenberg & Fauci 1990, Mellors et al 1991). Such activating signals may also stimulate uninfected T-cells, which in turn renders the cells more susceptible to infection. Thus, it is likely that any perturbation of the immune system that leads to

the proliferation of latently or chronically infected cells may promote active viral replication and subsequent development of progressive disease.

3.4: CYTOKINE RESPONSES TO HIV AND THEIR ROLE IN THE PATHOGENESIS OF HIV INFECTION

It has been well documented that a complex network of cytokines are involved in the regulation of immune responses, hematopoiesis, and inflammatory reactions (Balkwill & Burke 1989). Most of the cytokines have pleiotropic (multiple) and redundant (overlapping) effects. Moreover, these cytokines are shown to be produced not only by immunocompetent cells, but also by a wide variety of other cell lineages. Cells produce such cytokines in response to stimuli, such as various infections or tissue damage. Furthermore, the expression of one cytokine is influenced by other cytokine(s) (Akira et al 1990).

Elevated levels of cytokines have been found in the sera and cerebro-spinal fluid of patients with HIV infection (Lahdevirta et al 1988, Reddy et al 1988, Fuchs 1989, Gallo et al 1989, Breen et al 1990, Kobayashi et al 1990, Sydow et al 1991). Likewise, increased levels of cytokines are produced by mononuclear cells from HIV infected subjects (Wright et al 1989, Roux-Lombard et al 1989). Although, the direct involvement of the virus in its ability to induce cytokines has been a subject of controversy, cumulative data have demonstrated a more extensive role for cytokines as important mediators in the pathogenesis of HIV infection and many of the disorders associated with it. In particular the role of cytokines in the activation of latently infected cells and transition to productive infection is well documented (McDougal et al 1985b, Varmus 1988). The hypothesis

that cytokines may be involved in the activation of HIV was supported by the observation that T cell activating factors, such as mitogens and antigens, were able to induce HIV gene expression (McDougal et al 1985b, Zagury et al 1986, Margolick et al 1987, Clouse et al 1989, Pomerantz et al 1990). Of the cytokines, IL-6, TNF-alpha, and GM-CSF have been shown to play major roles in up-regulating HIV gene expression (Rosenberg & Fauci 1990, Poli et al 1990a, 1990b, Mellors et al 1991, Koyanagi et al 1988). These cytokines are able to induce HIV expression either by transcriptional or post-transcriptional mechanisms which are similar to the molecular mechanisms by which certain cytokines regulate the immune system (Rosenberg & Fauci 1990, Poli et al 1990a). Therefore, it seems more likely that HIV uses the same cytokines that modulate immune responses for its own advantage to regulate its own gene expression.

Cytokines may also contribute to HIV-associated immunosuppression via other mechanisms, such as by enhancing HIV-mediated giant cell formation (Matsuyama et al 1989, Vyakarnam et al 1990), by inducing cytotoxicity of HIV-infected cells (Wong et al 1988), and by inhibiting T-cell responses (Zhou et al 1991). Furthermore, polyclonal B-cell activation (Breen et al 1990), neuropsychiatric abnormalities (Lee et al 1989), malignancies (Ruszczak et al 1987, Ensoli et al 1992), and other symptoms, like fever and cachexia (Lehdevirta et al 1988) are all associated with AIDS and have been linked to the production of cytokines in these patients.

4:IMMUNE RESPONSE TO HIV IN MAN

There are some reports of individuals with documented exposure to HIV, such as the wives of HIV-infected hemophiliacs,

who remained uninfected, while others with similar exposure seroconverted rapidly (Smiley et al 1988). However, conditions influencing disease course and clinical sequelae following HIV infection are unclear. Many factors such as, virulence of the virus isolate, the size of viral inoculum, the presence of other coinfecting pathogens, route of viral entry and the nutritional status may influence the outcome of infection with HIV. The capacity of the individual's immune system to contain or eliminate the virus may be the major determinant of the clinical outcome (Sher et al 1992). Therefore, the various parts of the immune system may participate in the body's attempt to mount effective and protective immune responses against HIV infection.

4.1:HUMORAL RESPONSES

Infection with HIV leads to viral DNA integration and latent infection of T cells, monocytes and other cells bearing the CD4 receptor molecule. Antibodies to multiple viral proteins develop 3-12 weeks following infection and characteristically there is a long period of time before the development of full-blown AIDS. However, despite the presence of large quantities of neutralizing antibodies in sera of these patients (Robert-Guroff et al 1988) there is no evidence that antibodies to HIV protect the infected individual from developing AIDS. The reason why antibodies produced against viral proteins are inadequate in preventing HIV propagation and clinical progression may be because HIV is subject to frequent antigenic variation (Fisher et al 1988).

4.2:CELLULAR RESPONSES

The development of cellular cytotoxic immune responses, such

as antibody-dependent cellular cytotoxicity (ADCC), natural-killer (NK) cell and cytotoxic T lymphocyte (CTL) responses, may play a more critical role than neutralizing antibody formation in providing protection against HIV infection.

It has been demonstrated that sera from HIV-positive subjects can mediate ADCC activity in vitro (Rook et al 1987, Ojo-Amaize et al 1987) and sera derived from AIDS patients demonstrate less activity as compared to asymptomatic HIV-positive individuals, indicating that the level of ADCC-activity may correlate inversely with the severity of the disease (Rook et al 1987). However, global defects in immune function in the advanced disease state, may be the reason for the observed decrease in the level of activity.

NK cells from seronegative and seropositive individuals have been shown to mediate cytolytic responses against HIV-infected cells (Ruscetti et al 1986, Rook et al 1985a). The activity of these cells appeared to correlate with the patients clinical status in that cells from healthier individuals demonstrated greater activity (Bonavida et al 1986). NK cell activity from HIV-infected patients can be enhanced with IL-2 in vitro and in vivo (Rook et al 1983, Rook et al 1985a,b), although the importance of these cells in preventing the spread of HIV is unknown.

Major histocompatibility complex (MHC)-restricted CTLs directed against HIV may play a critical role in limiting viral spread. CTLs detected in the circulation of HIV-infected individuals have been shown to lyse target cells expressing HIV or HIV-associated proteins (Walker et al 1987, Plata et al 1987). Furthermore, CTLs have been shown to suppress HIV

replication in vitro (Walker et al 1986).

However, until now the importance of both humoral- and cellular-specific immune responses in preventing HIV infection is not well defined. Although, the data support the notion that effective immunity is able to limit the spread of HIV for a long period, why the immune system is not able to clear the virus completely to prevent the development of AIDS is still a mystery. It is well known that non-specific immune responses to viral infections are required to prevent the spread of infection before specific immunological defence mechanisms are activated (Larrick & Wright 1992). As infected cells are a major source of virus transmission, inability of the HIV to initiate responses, such as the generation of cytokines, at the site of entry may, be the reason why the virus is not totally cleared. Following viral entry into susceptible cells, the virus remains latent with restricted gene expression so that the host's immune system cannot recognize it. Repeated activation of such latently infected cells would also lead to persistent viral replication and sustained viral dissemination even in the presence of the host's CTL. Direct transmission of the virus from cell to cell, such as by formation of syncytia with a neighbouring cell that does not contain the virus, or sanctuary of the virus in certain cell types, such as the monocyte cell line, will protect the virus from the destructive effects of humoral immune mechanisms. Furthermore, because of frequent changes in the envelope antigen of individual viral particles, immune responses generated are not effective and additional progressive defects in the quantity and function of CD4⁺ lymphocytes will ultimately lead to disease progression.

5:EPIDEMIOLOGY OF HIV INFECTION

AIDS is now a major cause of morbidity and mortality throughout the World. HIV infection is transmitted by sexual contact, by infected blood or blood products, and perinatally from mother to infant (Fauci et al 1984, Curran 1985). World wide, a total of 8-10 million people are believed to be infected with HIV and the number of AIDS cases is 500,000. In Africa alone, the WHO estimates about 6 million people to be infected by the virus (Palca 1991). In Ethiopia, currently there are 3411 patients with AIDS and the number of HIV-infected persons is estimated to be about 350,000 (Personal communication, AIDS task force, Ministry of health, 1992). Furthermore, the epidemiological pattern of HIV-1 infection in Africa is different from the rest of the World in that HIV-1 spreads mainly through heterosexual intercourse and affects men and women equally (Clumeck et al 1984). The reason for these differences could be due to differences in individual viral strains (Benn et al 1985, Ayehunie et al 1990) or due to other factors, such as differences in the host response, sexual behaviour, exposure to other sexually transmitted diseases, and number of accompanying pathogens.

6:CLINICAL MANIFESTATIONS OF HIV INFECTION

Infection with HIV results in diverse clinical manifestations, ranging from an asymptomatic carrier state to life threatening opportunistic infections and malignancies. Clinical manifestations, however, may vary according to the individuals age, race, geographical location, and behavioral history.

The majority of HIV infected persons have an asymptomatic primary stage. However, in some individuals a condition characterized by acute mononucleosis-like symptoms and termed "acute retroviral syndrome" has been reported (Cooper et al 1985) at the time of seroconversion. Ten to thirty percent of seropositive individuals develop attributes of severe immunosuppression within 3-5 years of infection. However, in certain cases, HIV-infection is associated with a high prevalence of persistent generalized lymphadenopathy before development of full-blown AIDS and this has been suggested as a prodromal state prior to the development of AIDS. There is also a condition called AIDS-related complex which is characterized by fever, weight loss and lymphadenopathy. An increasing proportion of these patients will develop AIDS over time.

Patients with full-blown AIDS have their immune system severely compromised and are at high risk of infection by opportunistic organisms to which the patients have been currently or previously exposed. Among the opportunistic organisms, *Pneumocystis carinii* is the most common pathogen, but infections with Cytomegalovirus, Epstein-Barr and Herpes simplex viruses are common. Fungi such as *Candida*, *Aspergillus* and *Cryptococcus*, and protozoa like *Toxoplasma* can also occur. Additionally, patients with AIDS are at risk of developing malignancies, such as Kaposi's sarcoma and Non-Hodgkin's lymphoma. Serious complications involving the nervous system also appear in about 30-60 % of cases with AIDS. In Africa, however, the most typical presentation of terminal HIV-infection is probably "slim-disease", a symptom complex of chronic diarrhoea and wasting (Serwadda et al 1985). *Cryptosporidia* are frequently isolated from a number of these patients. Furthermore, tuberculosis in all

of its forms and unusual presentations has been identified as a very common HIV-related pathogen (Harries 1990).

The diagnosis of HIV infection is best accomplished by detecting specific antibodies against viral antigens serologically. Direct detection of viral antigens or genome is also possible, but it is more expensive. The most sensitive, efficient and practical approach used most commonly to diagnose HIV is the technique that uses Enzyme-Linked Immunosorbent Assay (ELISA) and then confirming positive result by Western-blot analysis.

7:THERAPY OF HIV INFECTION

Although there are many potential ways to interfere with HIV infection, expression and spread, until now there is no effective vaccine that can prevent infection with HIV and no drug that can cure patients. There are many problems to overcome the development of either an effective vaccine or drug. One problem presented is the high degree of envelope antigen heterogeneity seen among different variants of HIV-1. Another problem is that the viral life cycle is so intimately connected with cellular processes and selective toxicity with antiviral drugs is made very difficult. In this respect, treatment with azidodeoxythymidine is associated with serious side effects, despite the fact that the drug has proved to be of some success (Surbone et al 1988). Appropriate treatment of associated opportunistic infections or malignancies depends on the nature of the disease concerned.

Therefore, the only currently effective means of controlling HIV-1 is to avoid infection in the first place. Infections

associated with transfusion of blood or blood products have almost been eliminated by screening of blood. Health education is important in persuading people to change the social behaviour that increases the risk of acquiring infection, such as intravenous drug abuses, sexual promiscuity, and some traditional practices.

II. AIMS OF THE STUDY

- A. To determine whether heat-inactivated HIV-1 antigens induce cytokine production *in vitro* in peripheral blood mononuclear cells obtained from healthy donors.
- B. To determine whether heat-inactivated HIV-1 antigens induce cytokine production *in vitro* in peripheral blood mononuclear cells obtained from subjects with asymptomatic HIV-1 infection.
- C. To determine the capacity of antigens, other than HIV-1, to modulate the immune response of cells activated with HIV-1 antigen.
- D. By answering the above questions to try to give some insights into the potential immunopathogenic role of cytokines in HIV-related immunosuppression.

III. METHODOLOGY

1. ANTIGEN PREPARATION

Purified protein derivative (PPD) (Statens Serum Institute, Copenhagen, Denmark), lipopolysaccharide (LPS) from *E. coli* serotype 0128:B12 (Sigma, St Louis, MO), and phytohaemagglutinin (PHA) (Sigma, St Louis, MO) were used as positive controls in cytokine generation. PHA and PPD were also used as positive controls in lymphocyte transformation tests.

HIV-1 strains isolated from PBMC from Ethiopian and Swedish patients were used in the experiment. HIV isolates from culture supernatant (with $0.5-1.0 \times 10^4$ cpm/ml reverse transcriptase activity) were prepared by heat-inactivation of the isolates at 56°C for 1 hour. The isolates were stored at -70°C until used. We used inactivated HIV-1 antigen because of the observation that HIV-1 is able to mediate immunosuppressive effects (Pahwa et al 1985, Weinhold et al 1989) in the absence of viral infection.

Leishmania donovani promastigotes (obtained from Armauer Hansen Research Institute, Addis Ababa, Ethiopia) maintained in continuous culture were used in the second phase of the experiment. Live *Leishmania* promastigotes used in the experiment were harvested at their stationary phase of growth. Killed *Leishmania* promastigotes were prepared by heat-inactivation at 56°C for 1 hour and promastigotes prepared this way were 98% not-viable i.e. did not display motility.

2. PBMC PREPARATION

Samples were obtained from healthy donors and persons with asymptomatic HIV-infection. Informed consent was obtained from

all donors. Diagnosis of HIV infection was made by ELISA and confirmed by Western-blot. Then PBMC were separated by Ficoll-gradient centrifugation (Pharmacia LKB, Uppsala, Sweden) following the method of Boyum (1968). Cells were washed three times and resuspended in RPMI-1640 medium (Gibco, Life Technologies, UK), that was supplemented with 10% heat-inactivated (56° C for 30 min) fetal calf serum (FCS) (Gibco, Paisley, Scotland), penicillin (100u/ ml), streptomycin (100 ug/ml), and L-glutamine (2 mM/ml). The culture medium was subjected to ultrafiltration before use, through 0.20 um-pore size filters (Nalge company, Rochester, NY) to remove potential contaminating bacteria which could have been a source of endotoxin. The number of cells per ml was determined by counting in a haemocytometer chamber and their viability assessed by trypan-blue exclusion test.

3.ANTIGEN/MITOGEN STIMULATION FOR INDUCTION OF CYTOKINES

Cells resuspended at a concentration of 1×10^6 /ml were put into a 96-well flat-bottomed microtiter plate (Corning Glass Works, Corning, NY) at a volume of 200 ul per well. To these were added either LPS (1 ug/ ml), or PHA (2 ug/ml), or PPD (10 ug/ml), or heat-inactivated HIV-antigens (ranging from 25-100 ul/ml).

In order to investigate the potential role of co-factors that accompany infections with HIV in their ability to influence cytokine responses, in a second set of experiments, we also included the following groups of antigens for stimulation: promastigotes of live *Leishmania donovani* (cell/parasite ratio 1:20), or *Leishmania donovani* plus HIV-antigen. In all experiments negative controls were included and received medium

alone. Plates were then incubated at 37°C in humidified air containing 5% CO₂.

4. CYTOKINE ASSAY

Assay for cytokines was performed at the appropriate time following initiation of culture, using a method described previously (Sander et al 1991). Cells were washed twice in balanced salt solution (BSS) (Gibco, Paisley, Scotland) supplemented with 0.01 M Hepes buffer and put onto adhesion glass slides (Bio-rad lab. GMBH, Munich, Germany) and incubated at room temperature in a humidified chamber for 10 min. Unbound cells were removed by washing and fixation of anchored cells was performed by phosphate buffered 4% paraformaldehyde (paraformaldehyde 40 g/l, NaH₂PO₄·xH₂O 16.833 g/l, NaOH 3.85 g/l and glucose 5.4 g/l, pH 7.4) at room temperature for 5 min. Cells were then washed with BSS. At this stage cells were either stained immediately or kept for days at +4° C until stained.

To detect cytokine producing cells, each reaction field of the slide was incubated with cytokine-specific mAbs, namely: anti IL-6 mAb, BSF2, mouse IgG1; anti TNF-alpha mAb, TNF-E, mouse IgG1; and anti IFN-gamma mAb, IDIK, (kindly provided by Dr. J. Andersson, Stockholm University, Sweden), that was diluted in BSS with 0.1% saponin (USB, Cleveland, Ohio), at 37° C for 15 min. For controls irrelevant- antibody (mouse IgG1) was used as a primary antibody instead of the cytokine-specific mAbs. After washing, cells were incubated at room temperature for another 30 minutes with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG1 (Caltag Lab., San Francisco, CA) as secondary antibody. All antibodies were ultracentrifuged before use to remove aggregates. After further washing, a mounting medium made of carbonate/

Bicarbonate buffered glycerol (1:1 w/w) containing 2% 1.4 Diazobicyclo 2.2 octane (Sigma, St Louis, MO) was added to each reaction field of the slide in order to reduce the fading of FITC (Sander et al 1991). The stained cells were then examined with a fluorescence microscope (Leitz, Germany) equipped with a 50W mercury lamp. Cells were considered positive when a characteristic perinuclear staining of the Golgi organelle was seen (fig.2) and at least 200 cells were counted before a percentage of positively staining cells was calculated. In the study, the absolute values of positively stained cells per total varied between the different donors, however the differences between controls and activated cultures were consistent throughout.

5. LYMPHOPROLIFERATION ASSAY

Mononuclear cells resuspended in RPMI medium supplemented with 10% FCS and antibiotics, were cultured in triplicate at 2×10^5 cells per well in a 96-well flat-bottomed tissue culture plate at a volume of 200 μ l per well. Cells were cultured either without antigen, or with *L.donovani*, or with *L.donovani* plus HIV-antigen, or PHA, or PPD (as in above), or PPD plus HIV-antigen, and incubated at 37°C in 5% CO₂ in air. Cultured cells were harvested after 7 days and during the last 18 hours prior to culture termination, ³H-thymidine (Amersham, UK) was added at 1 μ Ci per well. Cells were collected onto filter paper discs with a semiautomatic harvesting device (Skatron, Lierbyen, Norway) and discs were then dried at 60° C for 30 min and placed in a scintillation cocktail (Beckman instruments, Inc., Galway, Ireland). The level of radioactive thymidine incorporation was determined by an LS 5000 TD Beckman beta counter (Beckman instruments, Inc., Fullerton, CA).

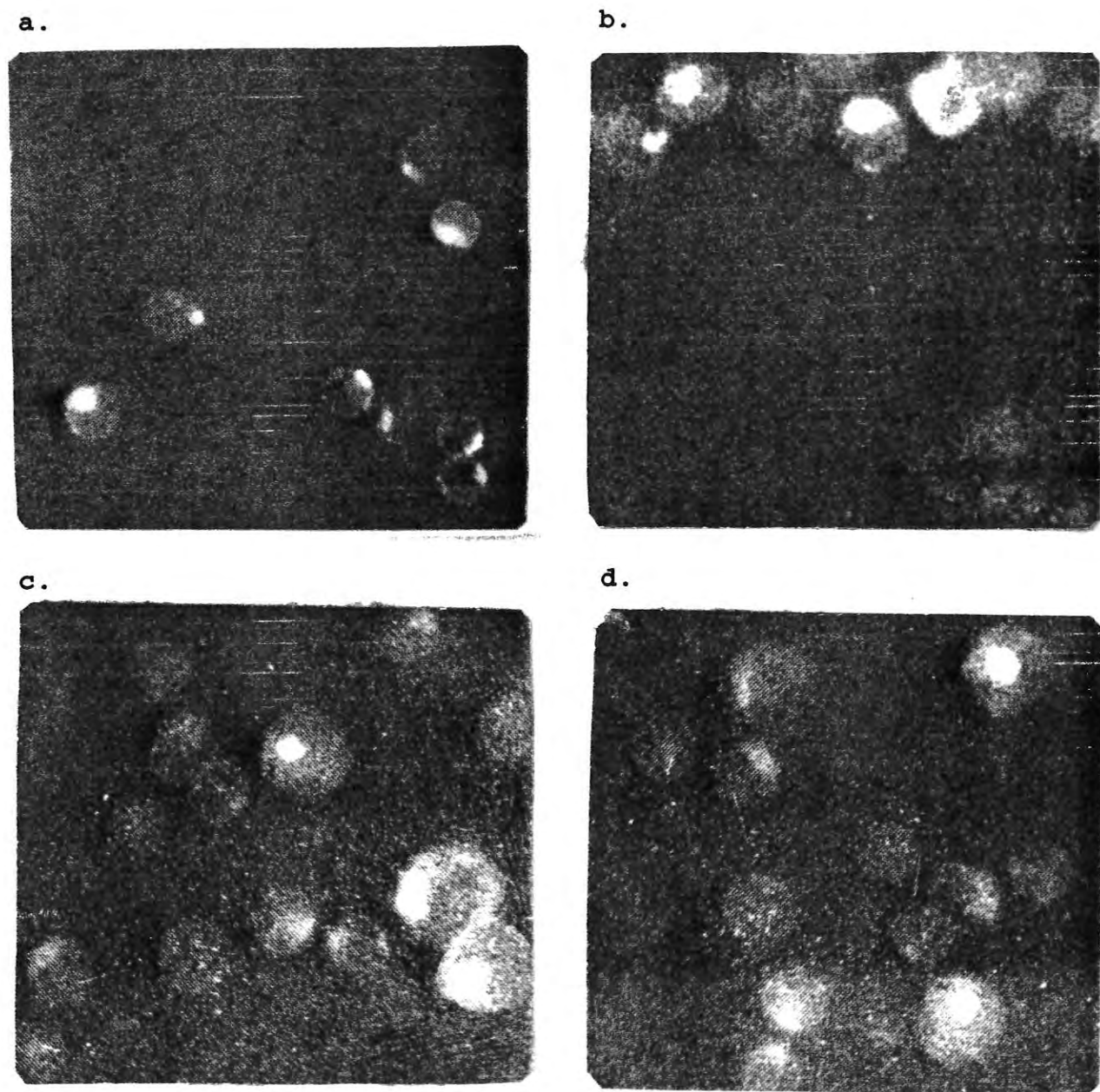


Figure 2. Immunofluorescence staining of cytokine producing cells. a) IL-6 producing PBMC activated with LPS. b) IFN-gamma producing cells stimulated with PHA. c) TNF-alpha positive cells after stimulation with *L.donovani*. d) IL-6 production in cells co-stimulated with HIV-1 antigen and *L.donovani*. Note the localized Golgi-staining for intracellular cytokines.

6. STATISTICAL ANALYSIS

The student's t - test was used to determine the significance of differences . The results are expressed as mean values plus or minus the standard deviation.

IV. RESULTS

Cytokine response by normal PBMC stimulated with HIV-antigens:

Heat-inactivated HIV-1 antigens did not induce IL-6, TNF-alpha, or IFN-gamma production from peripheral blood mononuclear cells obtained from healthy donors, as compared to the response of cells that were stimulated with control antigen or mitogen (fig. 3). Similarly, the lack of a direct stimulatory effect was also observed when cells were cultured with different concentrations of the HIV-antigen, ranging from 25ul/ml to 100ul/ml, and also at various times following initiation of culture (table 1).

Cytokine response of PBMC from asymptomatic HIV-infected subjects:

To determine whether PBMC from HIV-infected persons might be more responsive to modulation by HIV-antigen, we examined also the cytokine response by cells from HIV-seropositive, but asymptomatic individuals. Similarly, the inactivated HIV-1 antigen did not stimulate the patients' PBMC to produce IL-6, TNF-alpha, or IFN-gamma (fig 4). Furthermore, antigens or mitogens did not significantly change (i.e. enhance or decrease) the levels of these cytokines from the same cells when compared to the response by PBMC from healthy donors. The cells from these subjects also did not exhibit constitutive cytokine production (fig 4).

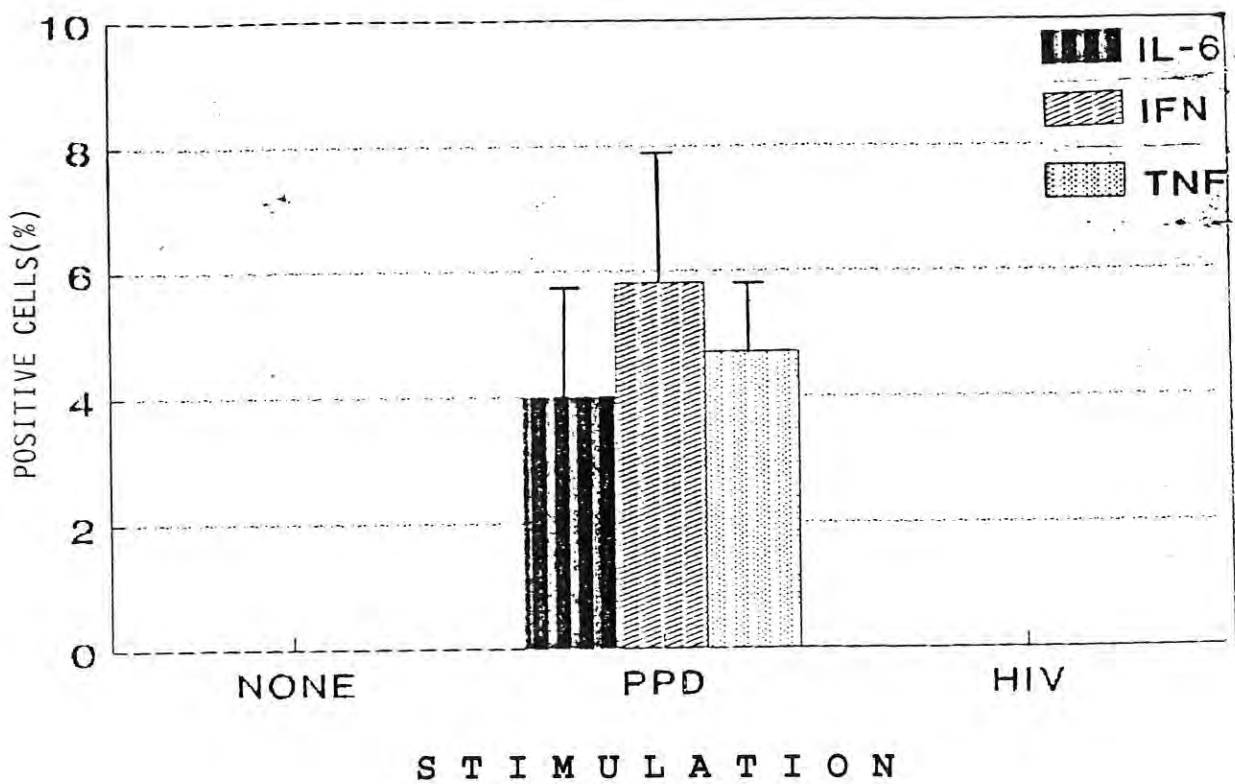


Figure 3. Cytokine response by normal peripheral blood mononuclear cells (PBMC) to HIV-1 antigen after 48 hr of incubation. Data represent mean $\pm$ SD from five separate experiments. $P < 0.05$ when Purified Protein Derivative (PPD) stimulated cultures were compared to unstimulated or HIV-stimulated cultures.

Table 1. Kinetics of intracellular cytokine accumulation by PBMC from normal individuals to various concentrations of HIV-antigen.

		Positive staining cells(%)				
Incub. time (hrs)	Cytokine	None	*PPD or PHA	HIV-ag 25 ul	HIV-ag 50ul	HIV-ag 100ul
6	IL-6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	TNF	0.0±0.0	1.8±1.1	0.0±0.0	0.0±0.0	0.0±0.0
	IFN	0.0±0.0	1.4±0.7	0.0±0.0	0.0±0.0	0.0±0.0
12	IL-6	0.0±0.0	1.3±0.4	0.0±0.0	0.0±0.0	0.0±0.0
	TNF	0.0±0.0	3.2±1.2	0.0±0.0	0.0±0.0	0.0±0.0
	IFN	0.0±0.0	2.9±0.5	0.0±0.0	0.0±0.0	0.0±0.0
24	IL-6	0.0±0.0	1.8±0.4	0.0±0.0	0.0±0.0	0.0±0.0
	TNF	0.0±0.0	6.4±1.2	0.0±0.0	0.0±0.0	0.0±0.0
	IFN	0.0±0.0	8.8±1.1	0.0±0.0	0.0±0.0	0.0±0.0
48	IL-6	0.0±0.0	5.1±0.4	0.0±0.0	0.0±0.0	0.0±0.0
	TNF	0.0±0.0	4.8±0.6	0.0±0.0	0.0±0.0	0.0±0.0
	IFN	0.0±0.0	6.3±0.8	0.0±0.0	0.0±0.0	0.0±0.0
72	IL-6	0.0±0.0	3.2±0.4	0.0±0.0	0.0±0.0	0.0±0.0
	TNF	0.0±0.0	2.1±0.8	0.0±0.0	0.0±0.0	0.0±0.0
	IFN	0.0±0.0	2.6±0.4	0.0±0.0	0.0±0.0	0.0±0.0

* PPD used for IL-6 induction.

PHA used for TNF and IFN induction.

Data are mean ± SD of results from two donors.

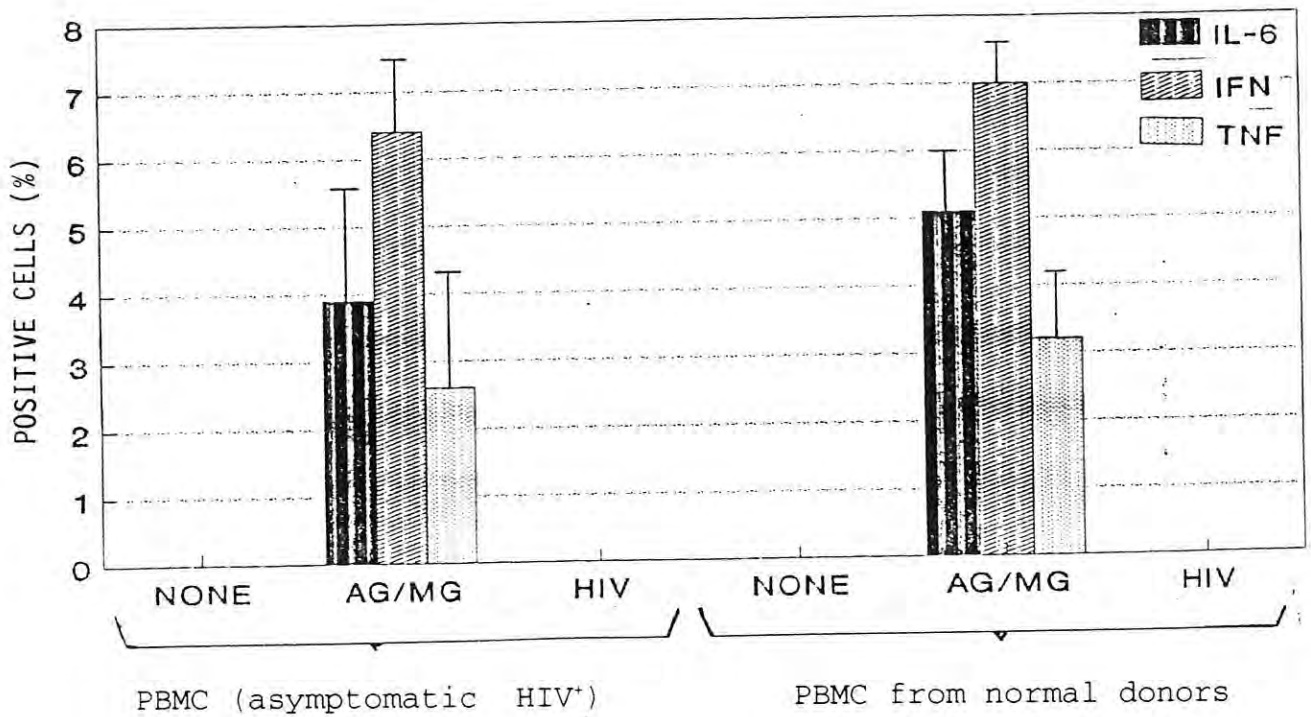


Figure 4. Cytokine response of PBMC after 48 hr of incubation. Results are expressed as mean $\pm$ SD from four separate experiments. AG = antigen:PPD; MG = mitogen:PHA. $P > 0.01$ when cells from asymptomatic HIV-subjects were compared to cells from normal donors following activation with PPD or PHA.

Effects of *L.donovani* antigens on the cytokine production by PBMC from healthy subjects activated with HIV-1 antigens:

To determine the effects of *L.donovani* on cytokine production by cells stimulated with HIV antigen, we also examined the cytokine response by normal cells co-stimulated with both antigens. HIV-1 antigen alone did not stimulate the intracellular accumulation of cytokines within a 12-h exposure time (fig 5). However, cells stimulated by leishmanial antigens alone were positive for staining with IL-6 and TNF-alpha, but not IFN-gamma mAbs. Furthermore, there was no significant change ($P>0.05$) in the production of these cytokines when cells were co-stimulated with both *Leishmania* and HIV antigens as compared to the production of cytokines by cells stimulated with *Leishmania* alone. This lack of a modulatory effect of HIV was also observed at different periods following initiation of culture (fig 6a & b). These results demonstrate that the IL-6 and TNF-alpha produced are due to the effects of *L.donovani* but not of HIV-1 antigen, as cells exhibited a positive response when stimulated with *Leishmania* alone, a negative response when stimulated to HIV alone. In addition there was no modulatory effect of *Leishmania* antigens on cytokine production by cells co-stimulated with HIV.

To exclude the possibility that the observed cytokine response to live *L.donovani* might be caused by endotoxin contaminating the parasite preparation, we examined the ability of killed leishmanial antigens to induce cytokine production. Unlike, live *L.donovani* promastigotes, killed leishmanial antigens were not able to induce IL-6 or TNF-alpha production, possibly because of the heat lability of the stimulatory

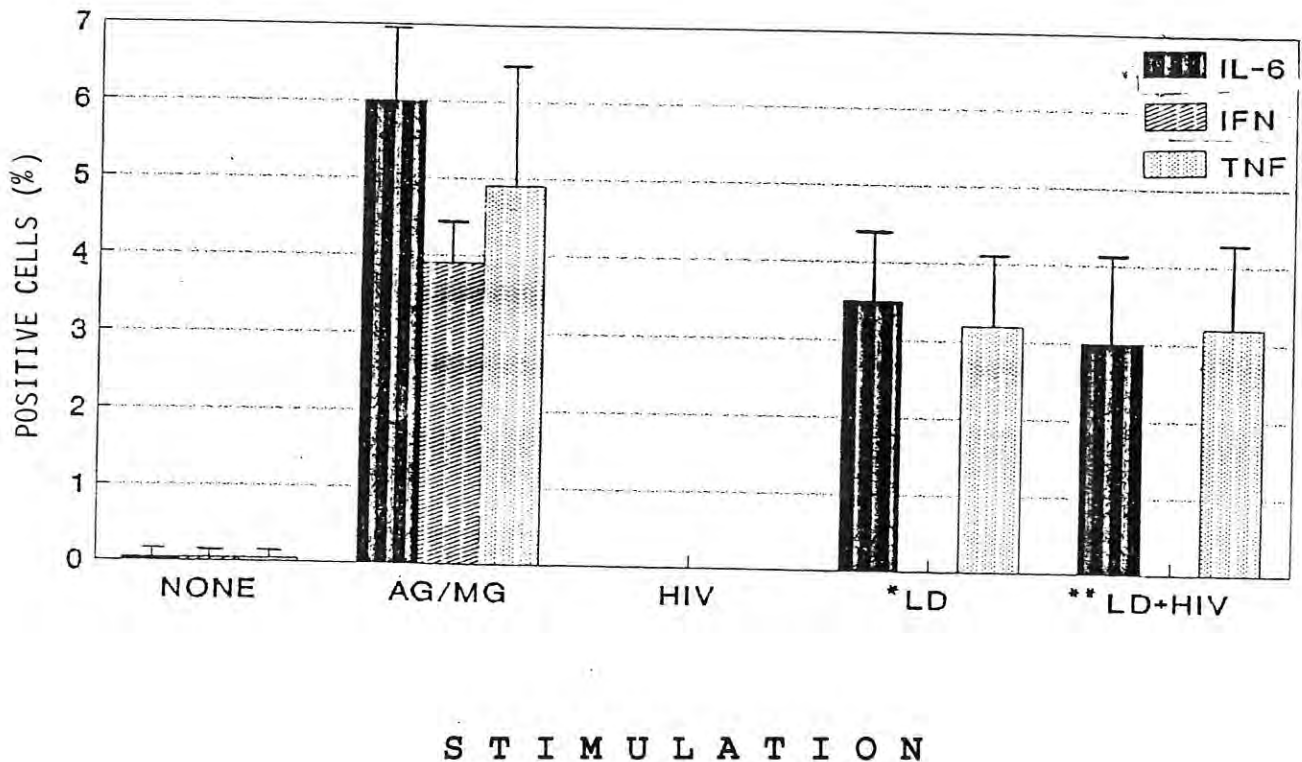


Figure 5. Cytokine response of normal PBMC co-stimulated with *Leishmania donovani* (LD) and HIV-1 antigen after 12 hr of incubation. Data are expressed as mean $\pm$ SD from seven different donors. AG = antigen:LPS; MG = mitogen:PHA, which were used as positive controls.

*P < 0.01 compared to unstimulated cells.

**P > 0.05 compared to LD stimulated cells.

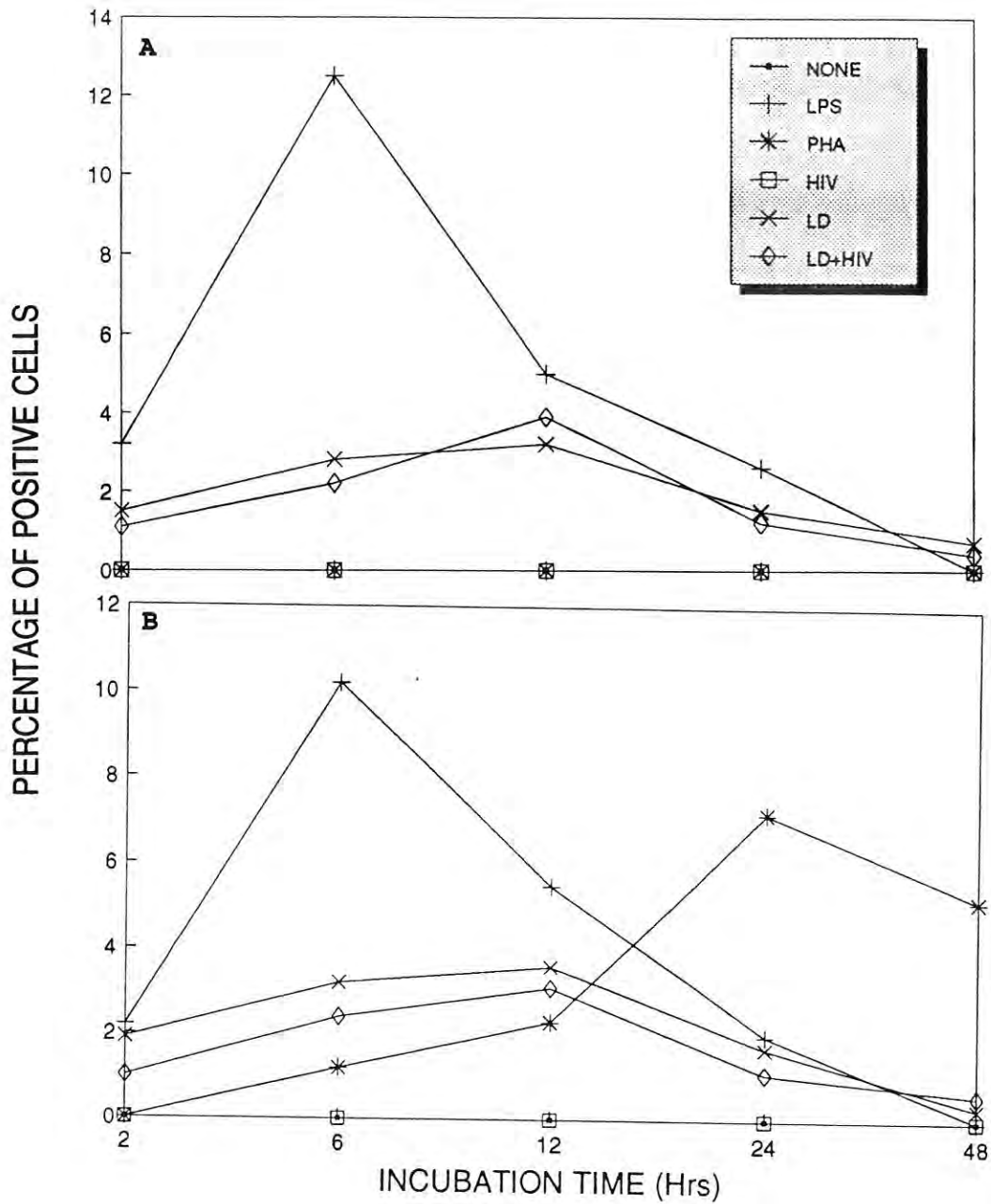


Figure 6. Kinetics of IL-6. (A) and TNF-alpha (B) production by PBMC from healthy donors stimulated without or with LPS, or PHA, or HIV-1 antigen, or LD, or LD plus HIV-1 antigen. Response obtained from a representative experiment.

molecule (table 2). Therefore, this experiment supports the observation that cytokine inductions of IL-6 and TNF-alpha is by the parasite itself rather than endotoxin, because heating does not abolish the ability of bacteria to induce cytokines (Yachie et al 1992).

Lymphoproliferative responses of normal PBMC to various antigens:

Heat-inactivated HIV-1 antigen was able to inhibit PPD-induced lymphoproliferative response of PBMC in a dose-dependent manner (fig. 7). As shown in table 3, of the seven individuals tested, cells from two individuals did not respond by proliferation to *L.donovani*. Addition of HIV-1 antigens to the cultures abolished the proliferative response to *L.donovani* (decrease by 73%, $P < 0.001$). All the individuals tested had strong PPD response *in vitro*.

Table 2. Cytokine production by PBMC stimulated with killed *L.donovani* after 12 hrs.

CYTOKINES	POSITIVE CELLS (%)			
	NONE	LPS	*LL	**KL
IL-6	0.0 $\pm$ 0.0	4.1 $\pm$ 0.1	2.5 $\pm$ 0.4	0.0 $\pm$ 0.0
TNF- α	0.0 $\pm$ 0.0	3.4 $\pm$ 0.7	2.8 $\pm$ 0.3	0.0 $\pm$ 0.0

Data are results expressed as mean $\pm$ SD of three experiments.

*P<0.05 when compared with response from unstimulated cultures or from cells stimulated with KL.

* LL=Live *Leishmania*.

**KL=Killed *Leishmania*.

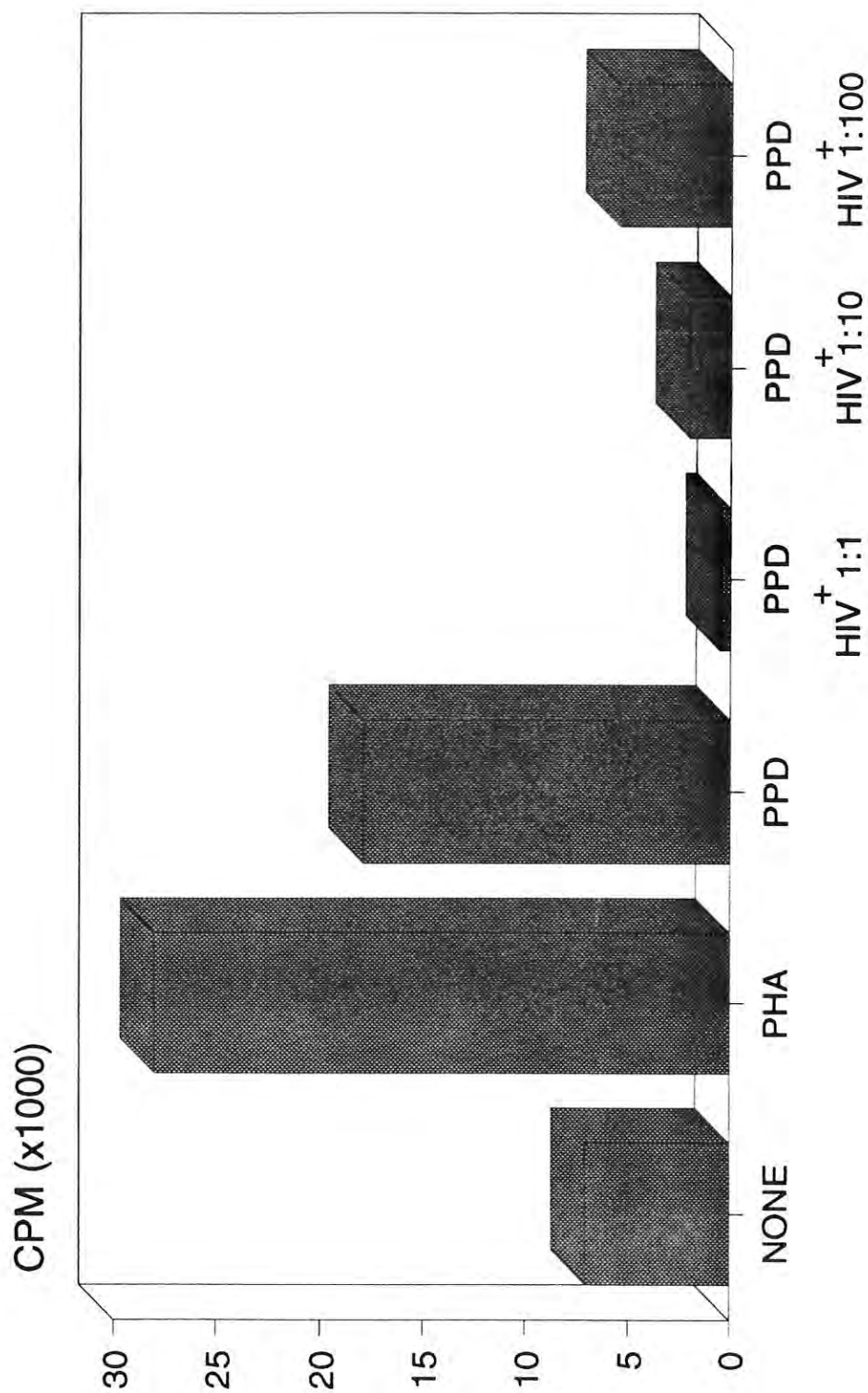


Figure 7. Inhibition of PPD-induced lymphoproliferation by HIV-1 antigen in a dose-dependent manner. Data from a representative experiment.

Table 3. Lymphoproliferative response by PBMC from healthy subjects to various antigens.

EXPERIM. NUMBER	A N T I G E N S			
	NONE	PPD	* ¹ LD	**LD + HIV
1	205 $\pm$ 20	² ND	463 $\pm$ 402	189 $\pm$ 43
2	796 $\pm$ 507	19546 $\pm$ 1134	2132 $\pm$ 117	656 $\pm$ 371
3	823 $\pm$ 86	24807 $\pm$ 1265	3763 $\pm$ 157	179 $\pm$ 24
4	524 $\pm$ 415	12159 $\pm$ 1466	1660 $\pm$ 713	416 $\pm$ 264
5	486 $\pm$ 113	14539 $\pm$ 2108	1197 $\pm$ 292	418 $\pm$ 77
6	1940 $\pm$ 140	23241 $\pm$ 2058	2420 $\pm$ 359	806 $\pm$ 47
7	399 $\pm$ 30	15137 $\pm$ 963	769 $\pm$ 71	192 $\pm$ 47

Results are expressed as mean cpm $\pm$ SD of triplicate cultures.

*P<0.001 when compared with cells stimulated by PPD.

**P<0.001 when compared with cells stimulated by *L. donovani* alone.

¹LD: *Leishmania donovani*

²ND: Not Done.

V. DISCUSSION

In this study, we examined the ability of heat-inactivated HIV antigens to modulate the production of cytokines by PBMC from healthy as well as asymptomatic HIV-positive individuals, using cytokine-specific mAbs and an indirect immunofluorescence technique. Other more widely used methods of cytokine detection have some drawbacks. Bioassays are rarely monospecific and the results are influenced by the presence of inhibitory factors. ELISA or RIA techniques are highly specific but do not distinguish biologically active and inactive substances. Furthermore, these assays determine the extracellular presence of a secreted cytokine, reflecting the net outcome of produced, absorbed and degraded cytokine. Techniques based on cytokine mRNA determination are highly sensitive, but the technique is fairly time consuming and the presence of cytokine mRNA does not imply that it will be translated. Therefore, we decided to study cytokine production at a single cell level using an immunofluorescence technique. This is a rapid, highly sensitive and specific method. Furthermore, the technique offers a unique opportunity to study the synthesis of multiple cytokines in individual cells and the phenotype of the producer cells by performing multiple-colour staining. Shortcomings of this technique are that the presence of intracellular cytokines may not be related to the amount secreted and that the method is, so far, semiquantitative, since cytokine contents in individual cells can not yet be quantified (Sander et al 1991).

Infection with HIV is associated with selective depletion in the CD4⁺ T lymphocyte subpopulation (McDougal et al 1985). However, the range of immunosuppression observed in these

patients can not be explained simply as a result of direct cytopathic effects of HIV on CD4⁺ cells, because only a small proportion of the surviving peripheral T4 cells in HIV-infected patients are positive for the viral genome (Harper et al 1986, Schnittman et al 1989). Therefore, immunosuppression associated with HIV-1 infection is probably the result of multiple factors. Cytokines have been shown to play an important role in the regulation of immune responses, and thus examining cytokine responses following stimulation of cells with HIV may provide some insight into the understanding of immunopathogenic mechanisms involved in HIV infection.

Results from our study demonstrated that heat-inactivated HIV-1 does not induce IL-6, TNF-alpha or IFN-gamma production in vitro by PBMC from healthy individuals nor in cells from asymptomatic HIV-1-infected persons. Furthermore cells from asymptomatic HIV-1 subjects did not show cytokine production spontaneously. These data are consistent with other studies that showed that activation with HIV-1 or its envelope glycoprotein failed to induce detectable cytokine activity or mRNA in macrophages (Molina et al 1990, Munis et al 1990, Gendelman et al 1990, Dooley et al 1991, Rieckman et al 1991, Goldfeld et al 1991). Early immune responses to viral infections, such as induction of cytokines, is required for intracellular killing of pathogens and preventing the spread of virus infection before specific immunological defence mechanisms can be mounted (Larrick & Wright 1992). Therefore, these data may partly explain the inability of HIV-1 to induce such immune responses during the early stages of infection and remain latent without being eliminated.

Our data also provide evidence that heat-inactivated HIV-1 antigen was able to inhibit PPD-induced lymphoproliferative responses of PBMC from healthy donors. This result is in agreement with the observations of other investigators which demonstrated that HIV-1 is able to mediate significant *in vitro* immunosuppression in the absence of infection (Pahwa et al 1985, Weinhold et al 1989). A defect in cytokine production accompanied with immunosuppression suggest that circulating envelope glycoproteins in individuals infected with HIV may down regulate certain cellular genes that are critical in regulating the immune system.

Previous studies have demonstrated elevated levels of circulating cytokines in the sera of patients with HIV-infection (Lahdevirta et al 1988, Reddy et al 1988, Fuchs et al 1989, Gallo et al 1989, Breen et al 1990, Kobayashi et al 1990, Sydow et al 1991). This is of particular relevance because increased levels of circulating cytokines are also present in a number of infections with organisms that commonly infect HIV positive individuals during their stage of profound immunosuppression (Cerami & Beutler 1988). However, the direct involvement of HIV in modulating cytokine production has not been fully explored. Some studies have shown that HIV or its envelope protein is able to induce normal human mononuclear cells to produce cytokines (Ratner et al 1987, Merrill et al 1989, Nakajima et al 1989, Vyakarnam et al 1990). On the other hand, cumulative data from other studies demonstrated that stimulation of normal cells by HIV or its envelope protein failed to induce cytokine production (Molina et al 1990, Munis et al 1990, Gendelman et al 1990, Dooley et al 1991, Rieckman et al 1991, Goldfeld et al 1991). Similarly, defective production of the

cytokine IFN-gamma was observed in patients with HIV infection or AIDS (Nokta & Pollard 1990, Biglino et al 1991). These differences in results could be due to differences in methodology, or differences in the individual virus isolates used, or differences in response among different cells. However, a form of cytokine exists as a preformed inducible surface protein that is released by activated cells (Kriegler et al 1988) and thus it is possible that the virus induces the release of this protein, accounting for the reports by some investigators which showed increased cytokine production in the supernatant of mononuclear cell cultures. The method used in this study detects intracellularly accumulated cytokines, and it is in accordance with other studies that failed to detect cytokine mRNA following stimulation with HIV (Goldfeld et al 1991, Molina et al 1990).

The cause of the abnormally elevated cytokine levels in many HIV-positive patients, therefore, may be a result of accompanying infections by opportunistic organisms to which these persons have been currently or previously exposed. To further elucidate this hypothesis, we examined the cytokine response of cells following co-stimulation with HIV antigen and *Leishmania donovani*. Cells stimulated by *L.donovani* alone were positive for intracellular IL-6 and TNF-alpha, and there was no significant change in the production of these cytokines by cells that were co-stimulated with *L.donovani* and HIV. These results indicate that HIV antigen alone did not induce cytokine production and that activation of cells with HIV antigens does not impair the production of IL-6 or TNF-alpha when they are induced by leishmanial antigens. This is consistent with the finding that endotoxin induction of IL-6 and/or TNF-alpha production appears to be unaffected by concurrent activation with HIV

or its envelope protein (Munis et al 1990, Molina et al 1990, Dooley et al 1991).

It is therefore possible that associated opportunistic infections in HIV-infected or AIDS-patients may partly account for the observed increased levels of circulating cytokines in the sera of these patients. This idea is further supported by the publication of conflicting reports regarding circulating serum levels of cytokines during the different stages of HIV infection. Some investigators showed that patients with AIDS or AIDS-related complex have increased serum levels of TNF-alpha compared to levels in subjects with asymptomatic HIV infection (Lahdevirta et al 1988, Reddy et al 1988, Murray et al 1988, Sydow et al 1991). Others, however, reported results which are the reverse (Amman et al 1987, Haas et al 1987). It is therefore possible that differences in the nature of the opportunistic pathogens in different patients with AIDS may in part account for such results. In this respect it is also worthwhile to note that differences in the clinical presentation of HIV-infected patients in Africa and other western countries may be partly accounted for by the differences in the type of secondary infections which they contract. Opportunistic organisms such as, *Pneumocystis carinii*, *Cytomegalovirus* and *atypical mycobacteria* are commonly encountered in European and North American patients, while most African patients with AIDS present with the so called "Slim-disease" in which organisms like *Cryptosporidia* and *Mycobacterium tuberculosis* are frequently isolated (Serwadda et al 1985, Harries 1990).

Generally it is speculated that some of the pathogenic sequelae associated with AIDS, such as polyclonal B-cell stimulation, may be linked to secondary infections (Pahwa et al

1985, Pahwa et al 1986). It is therefore possible that many of the abnormal pathological manifestations associated with AIDS, such as fever, anaemia, weight loss and polyclonal B-cell activation, may also be linked to sustained overproduction of cytokines (TNF and IL-6) induced by previously or currently acquired opportunistic pathogens. Furthermore, cytokines have been shown to enhance HIV replication and expression in T- cells and macrophages (Poli et al 1990a, 1990b). Thus our data provide additional evidence for a possible role of opportunistic organisms, such as *Leishmania donovani* and others, in cytokine mediated activation of latently HIV-infected cells by their ability to induce such cytokines. Furthermore, cells stimulated with both *Leishmania* and HIV exhibited decreased proliferative responses, aside from the suppressive effect of leishmanial antigen. These findings of augmented suppressive effects of HIV-antigen may account partly for the reactivation of *Leishmania* in latently infected cells. This hypothesis is supported by the observation that an increasing number of patients with AIDS are found to suffer also from visceral leishmaniasis, although they have no documented history of leishmaniasis. On the basis of these observations it has been suggested that visceral leishmaniasis should be considered as an opportunistic infection in these patients (Montalban et al 1987, Montalban et al 1989).



CONCLUSIONS

1. Stimulation of PBMC from normal individuals with heat-inactivated HIV-1 antigen did not induce IL-6, TNF-alpha, or IFN-gamma production. There was a significant difference when compared to cells stimulated with control mitogens or antigens.
2. PBMC from asymptomatic HIV-infected individuals did not show intracellular accumulation of IL-6, TNF-alpha, or IFN-gamma either spontaneously or after *in vitro* stimulation with HIV-1 antigen. Furthermore, cells from these patients produced cytokine levels comparable to that of cells from healthy subjects when stimulated with mitogens or heterologous antigen.
3. Inability of HIV to induce cytokine production during the initial stages of infection might be associated with the development of latent or chronic infection.
4. Co-stimulation of cells with HIV-1 antigen and *L. donovani* resulted in cytokine production which is similar to the production of cytokine following stimulation with *Leishmania* alone. This observation indicates that HIV-1 antigen does not modulate cytokine production in cells stimulated by other antigens.
5. Co-stimulation of cells with HIV-1 and *L. donovani* resulted in a significant decrease in the lymphoproliferative response, indicating that the immune response is severely depressed.
6. The cause of abnormally elevated cytokines in the sera of HIV-

infected patients may be due to the result of infection with opportunistic organisms.

7. Secondary infections may play a critical role in disease progression by their ability to induce cytokines and activate latent HIV infection in an autocrine or paracrine manner.
8. Persons residing in areas where certain parasitic diseases, like leishmaniasis, are endemic might be more susceptible to HIV infection.
9. Future studies should determine the pattern of disease progression of HIV infection in patients with tropical diseases.

REFERENCES

- Akira S, Hirano T, Taga T & Kishimoto T. Biology of multifunctional cytokines: IL-6 & related molecules (IL-1 & TNF). *FASEB J.* 4:2860-67, 1990.
- Ameisen JC & Capron A. Cell dysfunction & depletion in AIDS: the programmed cell death hypothesis. *Immunol Today* 12:102-05, 1991.
- Amman AJ, Palladino M, Volberding P, Abrams D, Martin N & Conant M. TNF-alpha & beta in AIDS & AIDS-related complex. *J Clin Immunol* 7:481-85, 1987.
- Ayenunie S, Sonnerborg A, Johansson B, Fehninger TE, Zewdie DW, Yemane-Berhan T, Petros B, Abens J, Britton S & Strannegard O. Differences in PCR reactivity between HIV proviruses from individuals from Ethiopia & Sweden. *J Acq I Def S* 3:975-80, 1990.
- Balkwill FR & Burke F. The cytokine network. *Immunol Today* 10:299-304, 1989.
- Barre-Sinoussi F, Cherman JC, Rey F, Nugeyne MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rosenbaum W & Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for AIDS. *Science* 220:868-71, 1983.
- Benn S, Rutledge R, Folks T, Gold J, Baker L, McCormick J, Feorino P, Piot P, Quinn T & Martin M. Genome heterogeneity of AIDS retroviral isolates from North America & Zaire. *Science* 230:949-51, 1985.
- Biglino A, Surbone A, Lipani F, Capello N, Forno B, Pollono AM, Busso M & Pegliese A. Spontaneous release of IFN as a predictor of clinical evolution in HIV-positive subjects. *Infection* 19:11-16, 1991.

- Bolognesi DP. Do antibodies enhance the infection of cells by HIV ? *Nature (London)* 340:431-32, 1989.
- Bonavida B, Katz J & Gottlieb M. Mechanisms of defective NK cell activity in patients with AIDS & AIDS-related complex. I. Defective trigger on NK cells for NKCF production by target cells, & partial restoration by IL-2. *J Immunol* 137:1157-63, 1986.
- Boyum A. Separation of lymphocytes from blood & bone marrow. *Scan J Clin Lab Invest* 21:7-16, 1968.
- Bowen DL, Lane HC & Fauci AS. Immunopathogenesis of the acquired immunodeficiency syndrome. *Ann Intern Med* 103:704-09, 1985.
- Breen EC, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, Kishimoto T & Martinez-maza O. Infection with HIV is associated with elevated IL-6 levels & production. *J Immunol* 144:480-84, 1990.
- Cefai D, Debre P, Kaczorek M, Idziorek T, Autran B & Bismuth G. HIV-1 gp120 & gp160 specifically inhibit the CD4/T cell antigen receptor phosphoinositide transduction pathways. *J Clin Invest* 86:2117-24, 1990.
- Cerami A & Beutler B. The role of cachectin/TNF in endotoxic shock & cachexia. *Immunol Today* 9:28-31, 1988.
- Chen IS. Regulation of AIDS virus expression. *Cell* 47:1-2, 1986.
- Clause KA, Robbins PB, Fernie PB, Ostrove JM & Fauci AS. Viral antigen stimulation of the production of human monokines capable of regulating HIV-1 expression. *J Immunol* 143:470-75, 1989.
- Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C & Rouzioux C. Isolation of a new human retrovirus from west African patients with AIDS. *Science* 233:343-46, 1986.
- Clements JE. Hypothesis on the molecular basis of nononcogenic

- retroviral diseases. *Rev Infect Dis* 7:68-74, 1985.
- Clumek N, Sonnet J & Taelman H. AIDS in African patients. *N Engl J Med* 310:492-97, 1984.
- Coffin J, Haase A, Levy JA, Montagnier L, Oroszlan S, Teich N, Temin H, Toyoshima K, Varmus H, Vogt P & Weiss R. Human immunodeficiency virus. *Science*(letter) 232:697, 1986.
- Cooper DA, Gold J, MacLean P, Donovan PB, Finlayson R, Barnes TG, Michelmores HM, Brooke P & Penny R. Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. *Lancet* i:537-40, 1985.
- Curran JW. AIDS-two years later(editorial). *N Engl J Med* 309: 609-11, 1983.
- Curran JW, Morgan WM, Hardy AM, Jaffe HW, Darrow WW & Doudle WR. The epidemiology of AIDS:current status & future prospects. *Science* 229:1352-57, 1985.
- Dalgleish AG, Beverley PC, Clapham PR, Crawford DH, Greaves MF & Weiss RA. The CD4(T4) antigen is an essential component of the receptor of the AIDS retrovirus. *Nature(London)* 312:763-67, 1984.
- Dooley DP, Cox RA & Looney DJ. Effect of a recombinant HIV gp160 vaccine on monokine production. *Clin Exp Immunol* 83:192-96, 1991.
- Doyle C & Strominger JL. Interaction between CD4 & class II MHC molecules mediates cell adhesion. *Nature(London)* 330:256-59, 1987.
- Ennen J, Seipp I, Norley SG & Kurth R. Decreased accessory cell function of macrophages after infection with HIV-1 in vitro. *Eur J Immunol* 20:2451-56, 1990.
- Ensoli B, Barillari G & Gallo RC. Cytokines & growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Immunol Rev* 127:147-55, 1992.

- Fauci AS. AIDS:immunopathogenic mechanisms & research strategies. *Clin Res* 35:503-10,1987.
- Fauci AS,Macher AM,Lono DL,Rook A,Masur H & Gelmann EP. AIDS: epidemiologic,clinical,immunologic & therapeutic considerations. *Ann Intern Med* 100:92-106,1984.
- Fauci AS. The human immunodeficiency virus:infectivity & mechanisms of pathogenesis. *Science* 239:617-22,1988.
- Fisher AG,Ensoli B,Looney D,Rose A,Gallo RC,Saag MS,Shaw GM,Hahn BH & Wong-Staal F. Biologically diverse molecular variants within a single HIV-1 isolate. *Nature(London)* 334:444-47, 1988.
- Folks T,Benn S,Rabson A,Theodore T,Hoggan MD,Martin M,Lightfoot M & Sell K. Characterization of a continuous T-cell line susceptible to the cytopathic effects of AIDS-associated retrovirus. *Proc Natl Acad Sci USA* 82:4539-43,1985.
- Folks TM,Justement J,Kinter A,Dinareello CA & Fauci AS. Cytokine induced expression of HIV-1 in a chronically infected promonocyte cell line. *Science* 238:800-02,1987.
- Fuchs D,Hausen A,Reibnegger G,Werner ER,Werner-Felmayer G,Dierich MP & Wachter H. IFN-gamma concentrations are increased in sera from individuals infected with HIV type 1. *J Acq I Def S* 2:158-62,1989.
- Gallo P,Frei K,Rordorf C,Lazdins J,Tarolato B & Fontana A. HIV-1 infection of the CNS:an evaluation of cytokines in cerebrospinal fluid. *J Neuroimmunol* 23:109-16,1989.
- Gallo RC,Sallahudin SZ,Popovic M,Shearer GM,Kaplan M,Haynes BF, Palker TJ,Redfield R,Oleske J,Safai B,White G,Foster P & Markham PD. Frequent detection & isolation of cytopathic retroviruses(HTLV-III) from patients with AIDS & at risk for AIDS. *Science* 224:500-03,1984.
- Gan H,Newman G,Tobin E,Remold H & Mellors J. Effects of HIV-1

- infection on the expression of LPS & IFN-gamma induced activation markers in human macrophages. *FASEB J* 4:A-2265, 1990.
- Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC & Popovic M. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 233:215-19, 1986.
- Gay D, Maddon P, Sekaly R, Talle MA, Godfrey M, Long E, Goldstein G, Chess L, Axel R, Kappler J & Marrak P. Functional interaction between human T-cell protein CD4 & the MHC HLA-DR antigen. *Nature (London)* 328:626-29, 1987.
- Gendelman HE, Friedman RM, Joe S, Baca LM, Turpin JA, Dueksler G, Meltzer M & Dieffenbach C. A selective defect of IFN-alpha production in HIV-infected monocytes. *J Exp Med* 172:1433-42, 1990.
- Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke DS, Skillman D & Meltzer MS. Efficient isolation & propagation of HIV on recombinant colony stimulating factor-1 treated monocytes. *J Exp Med* 167:1428-31, 1988.
- Gendelman HE, Phelps W & Feigenbaum L. Transactivation of the HIV terminal repeat sequence by DNA viruses. *Proc Natl Acad Sci USA* 83:9759-63, 1986.
- Goldfeld AE, Birch-Limberger K, Schooley RT & Walker BD. HIV-1 does not induce TNF-alpha or IFN-beta gene transcription. *J Acq I Def S* 4:41-47, 1991.
- Gottlieb MS, Schroff R, Schanker HM, Weisman J, Fan PT & Saxon A. *Pneumocystis carinii* pneumonia & mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 305:1425-31, 1981.
- Hass JG, Riethmuller G & Ziegler-Heitbrock H. Monocyte phenotype & function in patients with the AIDS & AIDS related

- disorders. *Scand J Immunol* 26:371-79,1987.
- Harper ME, Marselle LM, Gallo RC & Wong-Staal F. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes & peripheral blood from infected individuals by in situ hybridization. *Proc Nat Acad Sci USA* 83:772-76, 1986.
- Harries AD. Tuberculosis & HIV infection in developing countries. *Lancet* 335:387-90,1990.
- Ho DD, Pomerantz RJ & Kaplan JC. Pathogenesis of infection with HIV. *N Engl J Med* 317:278-86,1987.
- Ho DD, Rota TR & Hirsch MS. Infection of monocyte/macrophages by human T lymphotropic virus type III. *J Clin Invest* 77:1712-15,1986.
- Hoffenbach A, Langlade-Demoyen P, Dadaglio G, Vilmer E, Michel F, Mayaud C, Autran B & Plata F. Unusually high frequencies of HIV-specific CTL in humans. *J Immunol* 142:452-62,1989.
- Hofmann B, Nishanian P, Baldwin RL, Insixiengmay P, Nel A & Fahey JL. HIV inhibits the early steps of lymphocyte activation, including initiation of inositol phospholipid metabolism. *J Immunol* 145:3699 -705,1990.
- Horak ID, Popovic M, Horak EM, Lucas PJ, Gress RE, June CH & Bolen JB. No T cell tyrosine protein kinase signalling or Ca^{2+} mobilization after CD4 association with HIV-1 or HIV-1 gp120. *Nature (London)* 348:557-60,1990.
- Hoxie JA, Alpers JD, Rackowski JL, Huebner K, Haggarty BS, Cedarbaum AJ & Reed JC. Alterations in T4 (CD4) protein & mRNA synthesis in cells infected with HIV. *Science* 234:1123-27,1986.
- Hoxie JA, Haggarty BS, Rackowski JL, Pillsbury N & Levy JA. Persistent noncytopathic infection of normal human T

- lymphocytes with AIDS-associated retrovirus. *Science* 229: 1400-02, 1985.
- Katz JD, Nishanian P & Mitsuyasu R. Antibody-dependent cellular cytotoxicity (ADCC)-mediated destruction of HIV-coated CD4⁺ T lymphocytes by AIDS effector cells. *Clin Immunol* 8:453-58, 1988.
- Katzman M & Lederman MM. Defective postbinding lysis underlies the impaired NK activity in factor V III-treated, human T lymphotropic virus type III sero positive hemophiliacs. *J Clin Invest* 77:1057-62, 1986.
- Klatzman D, Champagne E, Chamaret S, Gruest J, Guetard D, Hercend T, Gluckman JD & Montagnier L. T lymphocyte T4 molecule behaves as the receptor for human retroviral LAV. *Nature (London)* 312:767-68, 1984.
- Klatzman D & Gluckman JD. HIV infection: facts & hypothesis. *Immunol Today* 7:291-96, 1986.
- Kloster BE, Tomar RH & Spira TJ. Lymphocytotoxic antibodies in the AIDS. *Clin Immunol Immunopathol* 30:330-35, 1984.
- Kobayashi S, Hamamoto Y, Kobayashi N & Yamamoto N. Serum level of TNF-alpha in HIV-infected individuals. *AIDS* 4:169-70, 1990.
- Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA & Fausi AS. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 233:1089-93, 1986.
- Koyanagi Y, O'Brien WA, Zhao JQ, Golde DW, Gasson JC & Chen ISY. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. *Science* 241:1673-75, 1988.
- Kowalski M, Bergeron K, Dorfman T, Haseltine W & Sodroski J. Attenuation of HIV-1 cytopathic effect by a mutation affecting the transmembrane envelope glycoprotein. *J Virol* 65:281-91, 1991.

- Kriegler M, Perez C, DeFay K, Albert I & Lu SD. A novel form of TNF/cachectin in a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* 53:45-53, 1988.
- Lahdevirta J, Maury CPJ, Teppo AM, Repo H. Elevated levels of circulating cachectin/TNF in patients with AIDS. *Am J Med* 85: 289-91, 1988.
- Lane HC, Depper JM, Greene WC, Whalen G, Waldmann TA & Fauci AS. Qualitative analysis of immune function in patients with the AIDS. *N Engl J Med* 313:79-84, 1985a.
- Lane HC, Masur H, Edgar LC, Whalen G, Rook AH & Fauci AS. Abnormalities of B-cell activation & immune regulation in patients with AIDS. *N Engl J Med* 309:453-58, 1983.
- Lanzavecchia A, Roosnek E, Gregory T, Berman P & Abrignani S. T cells can re-present antigens such as HIV gp120 targeted to their own surface molecules. *Nature (London)* 334:530-32, 1988.
- Larrick JW & Wright SC. Cytokine modulation of viral infections. Kunkel SL & Remick DG (eds.). *New York: Marcel Dekker Inc.*, pp. 181-95, 1992.
- Laurence J, Saunders A, Early E & Salmon JE. HIV-infection of monocytes: relationship to Fc-gamma receptors & antibody-dependent viral enhancement. *Immunology* 70:338-43, 1990.
- Lee MR, Ho DD & Gurney ME. Functional interaction & partial homology between HIV & interleukin. *Science* 237:1047-51, 1987.
- Leonard R, Zagury D & Desportes I. Cytopathic effect of HIV in T4 cells is linked to the last stage of virus infection. *Proc Natl Acad Sci USA* 85:3570-74, 1988.
- Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro J & Oshiro LS. Isolation of lymphocytopathic retrovirus from San Francisco patients with AIDS. *Science* 225:840-42, 1984.

- Levy JA, Shimabukuro J, McHugh T, Casavant C, Stites D & Oshiro L.
AIDS-associated retrovirus (ARV) can productively infect other cells besides human T helper cells. *Virology* 147:441-48, 1985.
- Lifson JD, Feinberg MR, Reyes GR, Rabin L, Basiripour B, Chakrabarti S, Moss B, Wong-Staal F, Steimer KS & Engelman EG. Induction of CD4-dependent cell fusion by HTLV-III/LAV envelope glycoprotein. *Nature (London)* 323:725-28, 1986.
- Macatonia SE, Lau R, Patterson S, Pinching AJ & Knight SC.
Dendritic cell infection, depletion & dysfunction in HIV-infected individuals. *Immunology* 71:38-45, 1990.
- Mann DL, Gartner S, LeSane F, Buchow H & Popovic M. HIV-1 transmission & function of virus-infected monocytes/macrophages. *J Immunol* 144:2152-58, 1990.
- Masur H, Michellis MA, Greene JB, Onorato I, Vande Stouwe RA, Holzman RS, Wormser G, Brettman L, Lange M, Murray HW & Cunningham-Rundles S. An outbreak of community acquired *Pneumocystis carinii* pneumonia: initial manifestations of cellular immune dysfunction. *N Eng J Med* 305:1431-38, 1981.
- Matsuyama T, Yoshiyama H, Hamamoto Y, Yamamoto N, Soma GI, Muzino D & Kobayashi N. Enhancement of HIV replication & giant cell formation by TNF. *AIDS Res Hum Retroviruses* 5:139-46, 1989.
- Margolick JB, Volkman DJ, Folks TM & Fausi AS. Amplification of HTLV-III/LAV infection by antigen induced activation of T cells & direct suppression by virus of lymphocyte blastogenic response. *J Immunol* 138:1719-23, 1987.
- McDougal JS, Hubbard M & Nicholson JKA. Immune complexes in the acquired immunodeficiency syndrome. *J Clin Immunol* 5:130-38, 1985a.
- McDougal JS, Mawle A, Cort SF, Nicholson JKA, Cross DG, Scheppler-Campbell JA, Hicks D & Sligh J. Cellular tropism of the human retrovirus HTLV III/LAV. I. Role of T cell activation &

- expression of the T4 antigen. *J Immunology* 135:3151-62,1985b.
- McElrath MJ, Pruett JE & Cohn ZA. Mononuclear phagocytes of blood & bone marrow: comparative roles as viral reservoirs in HIV-type 1 infections. *Proc Natl Acad Sci USA* 86:675-79,1989.
- Meissner C & Coffin J. The retroviruses & AIDS. Gardner JN (ed) *Baltimore:Williams & Wilkins* P.437,1989.
- Mellors JW, Griffith BP, Ortiz MA, Landry ML & Ryan JL. TNF-alpha/ Cachectin enhances HIV type 1 replication in primary macrophages. *J Infect Dis* 163:78-82,1991.
- Merrill JE, Koyanagi Y & Chen ISY. IL-1 & TNF-alpha can be induced from mononuclear phagocytes by HIV-1 binding to the CD4 receptor. *J Virol* 63:4404-08,1989.
- Miedema F, Tersmette M & van Lier RA. AIDS pathogenesis: a dynamic interaction between HIV & the immune system. *Immunol Today* 11:293-97,1990.
- Mikovits JA, Raziuddin, Gonda M, Ruta M, Lohrey NC, King HF & Ruscetti FW. Negative regulation of HIV replication in monocytes. Distinctions between restricted & latent expression in cells. *J Exp Med* 171:1705-20,1990.
- Molina JM, Schnidler R, Ferriani R, Sakaguchi M, Vannier E, Dinarello CA & Groopman JE. Production of cytokines by peripheral blood monocytes/macrophages infected with HIV-1. *J Infect Dis* 161:888-93,1990.
- Montalban C, Martinez-Fernandez R, Calleja JL, Garcia-Diaz JD, Rubio R, Dronda F, Moreno S, Yebra M, Barros C, Cobo J, Martinez MC Ruiz F & Costa JR. Visceral leishmaniasis (Kala-azar) as an opportunistic infection in patients infected with HIV in Spain. *Rev Infect Dis* 11:655-60,1989.
- Montalban C, Sevilla F, Moreno A, Nash R, Celma ML & Munoz RF. Visceral leishmaniasis as an opportunistic infection in the AIDS. *J Infect* 15:247-50,1987.

- Munis JR, Richman DD & Kornbluth RS. HIV-1 infection of macrophages in vitro neither induces TNF/cachectin gene expression nor alters TNF/cachectin induction by LPS. *J Clin Invest* 85:591-9, 1990.
- Murray HW, Scavuzzo DA, Kelly CD, Rubin BY & Roberts RB. T4⁺ cell production of IFN-gamma & the clinical spectrum of patients at risk for & with AIDS. *Arch Int Med* 148:1613-16, 1988.
- Nakajima K, Martinez-Maza O, Hirano T, Breen EC, Nishanian PG, Salazar-Gonzalez GF, Fahey JL & Kishimoto T. Induction of IL-6 (BCSF-2/IFN-beta) production by HIV. *J Immunol* 142:531-36, 1989.
- Nicholson JKA, Gross GD, Callaway CS & McDougal JS. *In vitro* infection of human monocytes with HTLV-III/LAV. *J Immunol* 137:323-29, 1986.
- Nokta MA & Pollard RB. Patterns of IFN-gamma production by PBMC from patients with HIV. *J Interferon Res* 10:173-81, 1990.
- Nong Y, Kandil O, Tobin EH, Rose RM & Remold HG. The HIV core protein P²⁴ inhibits IFN-gamma induced increase in the human monocyte-like cell line THP1. *Cell Immunol* 132:10-16, 1991.
- Ojo-Amaize EA, Nishanian P, Keith DE, Houghton RL, Heitjan DF, Fahey JL & Giori JV. Antibodies to HIV in human sera induce cell-mediated lysis of HIV-infected cells. *J Immunol* 139:2458-63, 1987.
- Pahwa S, Pahwa R, Good R, Gallo RC & Saxinger C. Stimulatory & inhibitory influences of HIV on normal B lymphocytes. *Proc Natl Acad Sci USA* 83:9124, 1986.
- Pahwa S, Pahwa R, Saxinger C, Gallo RC & Good R. Influence of the HTLV/LAV on functions of human lymphocytes: evidence for immunosuppressive effects & polyclonal B-cell activation by banded viral preparations. *Proc Natl Acad Sci USA* 82:8198, 1985.

- Palca J. The sobering geography of AIDS. *Science* 252:372-73, 1991.
- Pauza CD & Galindo J. Persistent HIV-type 1 infection of monoblastoid cells leads to accumulation of self-integrated viral DNA & to production of defective virions. *J Virol* 63: 3700-07, 1989.
- Pinching A, Jeffries D, Donaghy M, Munday P, McManus T, Moshatael O, Parking J & Harris J. Studies of cellular immunity in male homosexuals in London. *Lancet* 2:126-30, 1983.
- Plata F, Autran B, Martins LP, Wain-Habson S, Raphael M, Mayaud C, Denis M, Guillon JM & Debre P. AIDS-virus specific CTL in lung disorders. *Nature (London)* 328:348-51, 1987.
- Poli G, Bressler P, Kinter A, Duh E, Timmer WC, Rabson A, Justement JS, Stanley S & Fauci AS. IL-6 induces HIV expression in infected monocytic cells alone or in synergy with TNF-alpha by transcriptional & posttranscriptional mechanisms. *J Exp Med* 172:151-58, 1990a.
- Poli G, Kinter A, Justement JS, Kehrl JH, Bressler P, Stanley S & Fauci AS. TNF-alpha functions in an autocrine manner in the induction of HIV expression. *Proc Natl Acad Sci USA* 87:782-85, 1990b.
- Pomerantz RJ, Feinberg MB, Trono D & Baltimore D. LPS is a potent monocyte/macrophage specific stimulator of HIV type 1 expression. *J Exp Med* 172:253-61, 1990.
- Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC & Cleary P. The brain in AIDS: CNS HIV-1 infection & AIDS dementia complex. *Science* 239:586-92, 1988.
- Ratner L, Polmer SH, Paul N & Ruddle N. Cytotoxic factors secreted by cells infected by HIV type 1. *AIDS Res Hum Retroviruses* 3:147-55, 1987.
- Reddy MM, Sorrell SJ, Lange M & Grieco MH. TNF & HIV P²⁴ antigen

- levels in serum of HIV-infected populations. *J Acq I Def S* 1:436-40,1988.
- Rieckman P, Poli G, Kehrl JH & Fauci AS. Activated B lymphocytes from HIV-infected individuals induce virus expression in infected T-cells & promonocytic cell line, U1. *J Exp Med* 173: 1-5, 1991.
- Robert-Guroff M, Goedert JJ, Naugle CJ, Jennings AM, Blattner WA & Gallo RC. Spectrum of HIV-1 neutralizing antibodies in a cohort of homosexual men: results of a 6 year prospective study. *AIDS Res Hum Retroviruses* 4:343-50, 1988.
- Robinson WE Jr, Montefiori DC & Mitchell WM. Antibody-dependent enhancement of HIV-type-1 infection. *Lancet* i:790-94, 1988.
- Rook AH, Hooks JJ, Quinnan GV, Lane HC, Manischewitz JF, Macher AM, Masur H, Fauci AS & Djeu JY. IL-2 enhances the NK cell activity of AIDS patients through a gamma-IFN-independent mechanism. *J Immunol* 134:1503-07, 1985a.
- Rook AH, Lane HC, Folks T, McCoy S, Alter H & Fauci AS. Sera from HTLV-III/LAV antibody positive individuals mediate ADCC against HTLV-III/LAV-infected T cells. *J Immunol* 138:1064-67, 1987.
- Rook AH, Manischewitz JD, Frederick WR, Epstein JS, Jackson L, Gelmann E, Steis R, Masur H & Quinnan GV. Deficient HLA-restricted cytomegalovirus-specific cytotoxic T-cells & NK cells in patients with the AIDS. *J Infect Dis* 152:627-30, 1985b.
- Rook AH, Masur H, Lane HC, Frederic W, Kasahara T, Macher A, Djeu J, Manischewitz J, Jackson L, Fauci AS & Quinan G. IL-2 enhances the depressed NK & CMV-specific cytotoxic activities of lymphocytes from patients with the AIDS. *J Clin Invest* 72: 398-403, 1983.
- Rosenberg ZF & Fauci AS. Immunopathogenic mechanisms of HIV

- infection:cytokine induction of HIV-expression. *Immunol Today* 11:176-80,1990.
- Rosenberg ZF & Fauci AS. The immunopathogenesis of HIV infection. *Adv Immunol* 47:377-431,1989.
- Roux-Lombard P,Aladjem D,Balavoine JF,Chofflon M,Despont JP, Hirschel B,Jeannet M,Kapanci Y,Lang R,Toccanier MF,Voinier B,Wilhelm JA,Dayer JM & Cruchaud A. Altered function of peripheral blood monocytes in homosexual males & intravenous drug users with persistent generalised lymphadenopathy. *Eur J Clin Invest* 16:262-70,1986.
- Roux-Lombard P,Modoux C,Cruchaud A & Dayer JM. Purified blood monocytes from HIV-1 infected patients produce high levels of TNF-alpha & IL-1. *Clin Immunol Immunopathol* 50:374-84, 1989.
- Ruddle NH. Lymphotoxin production in AIDS. *Immunol Today* 7:8-9, 1986.
- Ruegg CI & Strand M. Inhibition of protein kinase-C & anti-CD3-induced Ca^{2+} influx in Jurkat T cells by a synthetic peptide with sequence identity to HIV-1 gp41. *J Immunol* 144:3928-35, 1990.
- Ruschetti FW,Mikovits JA,Kalyanaraman VS,Overton R,Stevenson H, Stromberg K,Herberman RB,Farrar WL & Ortaldo JR. Analysis of effector mechanism against HTLV-I & HTLV-III/LAV infected lymphoid cells. *J Immunol* 136:3619-24,1986.
- Ruszczak Z,Mayer Da Silva A & Orfanos CE. Angioproliferative changes in clinically non-involved,perilesional skin in AIDS associated with Kaposi's sarcoma. *Dermatologica* 175:270,1987.
- Salahuddin SZ,Ablashi DV,Hunter EA,Gonda MA,Sturzenegger S, Markham PD & Gallo RC. HTLV-III infection of EBV-genome positive B-lymphoid cells with or without detectable T4 antigens. *Int J Cancer* 30:198-202,1987.

- Salahuddin SZ, Rose RM, Groopman JE, Markham PD & Gallo RC. HTLV-III infection by human alveolar macrophages. *Blood* 68:281-84, 1986.
- Sander B, Andersson J & Andersson U. Assessment of cytokines by immunofluorescence & the paraformaldehyde-saponin procedure. *Immunol Rev* 119:65-93, 1991.
- Sattentau OJ & Weiss RA. The CD4 antigen: physiological ligand & HIV receptor. *Cell* 52:631-33, 1988.
- Schnittman SM, Psallidopoulos MC, Lane HC, Thomson L, Baseler M, Massari F, Fox CH, Salzman NF & Fauci AS. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* 245:305-08, 1989.
- Serwadda D, Mugerwa RD, Sewankambo NK, Lwegaba A, Carswell JW, Kirya GB, Bayley AC, Downing RG, Tedder RS, Clayden SA, Weiss RA & Dalgleish AG. Slim disease: a new disease in Uganda & its association with HTLV-III infection. *Lancet* ii; 849-52, 1985.
- Shaw GM, Hahn BH, Arya SK, Groopman JE, Gallo RC & Wong-Staal F. Molecular characterization of HTLV-III in the acquired immunodeficiency syndrome. *Science* 226:1165-71, 1984.
- Shearer GM, Salahuddin SZ, Markham PD, Joseph LJ, Payne SM, Kriebel P, Bernstein DC, Biddison WE, Sarngadharan MG & Gallo RC. Prospective study of CTL responses to influenza & antibodies to HTLV-III in homosexual men. Selective loss of an influenza specific, HLA-restricted CTL response in HTLV-III positive individuals with symptoms of AIDS & in a patient with AIDS. *J Clin Invest* 76:1699-704, 1985.
- Sher A, Gazzinelli RT, Oswald IP, Clerici M, Kullberg M, Pearce EJ, Berzofsky JA, Mosmann TR, James SL, Morse HC & Shearer GM. Role of T-cell derived cytokines in the downregulation of immune responses in parasitic & retroviral infections. *Immunol Rev* 127:183-204, 1992.

- Siegal FP, Lopez C, Hammer CS, Brown AE, Kornfeld SJ, Gold J, Hassett J, Hirschman SZ, Cunningham-Rundles C, Adelsbery BR, Parkham DM, Siegal M, Cunningham-Rundles S & Armstrong D. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative Herpes-simplex lesions. *N Eng J Med* 305: 1439-44, 1981.
- Smiley ML, White II GC & Becherer P. Transmission of HIV to sexual partners of hemophiliacs. *Am J Hematol* 28:27-32, 1988.
- Smith PD, Ohura K, Masur H, Lane H, Fauci AS & Wahl SM. Monocyte functions in the AIDS. Defective chemotaxis. *J Clin Invest* 74:2121-28, 1984.
- Sodroski J, Goh WC, Rosen C, Tartar A, Portetelle D, Burney A & Haseltine WA. Replication & cytopathic potential of HTLV/LAV with sor gene deletions. *Science* 231:1549-53, 1986.
- Stanley SK, Bressler PB, Poli G & Fauci AS. Heat shock induction of HIV production from chronically infected promonocyte & T cell lines. *J Immunol* 145:1120-26, 1990.
- Stanley SK, Folks TM & Fauci AS. Induction of expression of HIV in a chronically infected promonocytic cell line by ultraviolet irradiation. *AIDS Res Hum Retroviruses* 5:375-84, 1989.
- Stein B, Gouda S, Lifson J, Penhallow R, Bensch K & Engleman E. P^H -independent HIV entry into CD4⁺ T cells via virus envelope fusion to the plasma membrane. *Cell* 49:659-68, 1987.
- Surbone A, Yarchoan R, McAtee N, Blum MR, Maha M, Allain JP, Thomas RV, Mitsuya H, Lehrman SN, Leuther M, Pluda JM, Jacobsen FK, Kessler HA, Myers CE & Broder S. Treatment of AIDS & ARC with a regimen of azidothymidine & acyclovir. *Ann Intern Med* 108:534-40, 1988.
- Sydow MV, Sonnerborg A, Gaines H & Strannegard O. IFN-alpha & TNF-alpha in serum of patients in various stages of HIV-1

- infection. *AIDS Res Hum Retroviruses* 7:375-80,1991.
- Teewsen VJ, Siebelink KH, de Wolf F, Goudsmit J, Uytdehaag FG & Osterhaus AD. Impairment of in vitro immune responses occurs within 3 months after HIV-1 seroconversion. *AIDS* 4:77-81, 1990.
- van Noesel CJ, Gruters RA, Terpstra FG, Schellekens PT, van Lier RA & Miedema F. Functional & phenotypic evidence for a selective loss of memory T in asymptomatic HIV-infected men. *J Clin Invest* 86:293-99,1990.
- Varmus H. Retroviruses. *Science* 231:382-85,1988.
- Vyakarnam A, McKeating J, Meager A & Beverly PC. TNF (alpha, Beta) induced by HIV-1 in PBMC potentiate virus replication. *AIDS* 4:21-27,1990.
- Walker BD, Chakrabarti S, Moss B, Baradis TJ, Flynn T, Durno AG, Blumberg RS, Kaplan JC, Hirsch MS & Schoolay RT. HIV specific CTL in seropositive individuals. *Nature(London)* 328:345-48, 1987.
- Walker CM, Moody DJ, Stites DP & Levy JA. CD8⁺ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* 234:1563-66,1986.
- Weinhold KJ, Lyerly HK, Stanley SD, Austin AA, Matthews TJ & Bolognesi DP. HIV-1 gp120-mediated immunosuppression & lymphocyte destruction in the absence of viral infection. *J Immunol* 142:3091-97,1989.
- Williams RC Jr, Masur H & Spira TJ. Lymphocyte reactive antibodies in AIDS. *J Clin Immunol* 4:118-23,1984.
- Willard-Gallo KE, Van de Keere F & Kettmann R. A specific defect in CD3 gamma-chain gene transcription results in loss of T-cell receptor/CD3 expression late after HIV infection of a CD4⁺ T-cell line. *Proc Natl Acad Sci USA* 87:6713-17,1990.
- Wong GHW, Krowka JF, Stites DP & Goeddel DV. In vitro anti-HIV

- activities of TNF-alpha & IFN-gamma. *J Immunol* 140:120-24, 1988.
- Wright SC, Jewette A, Mitsuyasu R & Bonavida B. Spontaneous cytotoxicity & TNF production by peripheral blood monocytes from AIDS patients. *J Immunol* 141:99-104, 1988.
- Yachie A, Takano N, Ohta K, Uehara T, Fujita SI, Miyawaki T & Taniguchi N. Defective production of IL-6 in very small premature infants in response to bacterial pathogens. *Immun Infect* 60:749-53, 1992.
- Zagury D, Bernard J, Leonard R, Cheynier R, Feldman M, Sarin PS & Gallo RC. Long-term cultures of HTLV-III-infected T cells: a model of cytopathology of T-cell depletion in AIDS. *Science* 231:850-53, 1986.
- Zhou D, Munster A & Winchurch RA. Pathologic concentrations of IL-6 inhibit T cell responses via induction of activation of TGF-beta. *FASEB J* 5:2582-85, 1991.
- Ziegler JL, Beckstead JA, Volberding PA, Abrams DI, Levine AM, Lukes RJ, Gill PS, Burkes RL, Meyer PR, Metroka CE, Mouradian J, Moore A, Riggs SA, Butler JJ, Cabanillas FC, Hersch E, Newell GR, Laubenstein LJ, Knowles D, Odajnyk C, Raphael B, Koziner B, Urmacher C & Clarkson BD. Non-Hodgkins lymphoma in 90 homosexual men: relation to generalized lymphadenopathy & the AIDS. *N Engl J Med* 311:565-70, 1984.
- Ziegler JL & Stites DP. Hypothesis: AIDS is an autoimmune disease directed at the immune system & triggered by a lymphotropic retrovirus. *Clin Immunol Immunopathol* 41:305-13, 1986.

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been dully acknowledged.

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